In addition to the presence of a nucleus, eukaryotic cells have a variety of membrane-enclosed organelles within their cytoplasm. These organelles provide discrete compartments in which specific cellular activities take place, and the resulting subdivision of the cytoplasm allows eukaryotic cells to function efficiently in spite of their large size—at least a thousand times the volume of bacteria.

Because of the complex internal organization of eukaryotic cells, the sorting and targeting of proteins to their appropriate destinations are considerable tasks. The first step of protein sorting takes place while translation is still in progress. Proteins destined for the endoplasmic reticulum, the Golgi apparatus, lysosomes, the plasma membrane, and secretion from the cell are synthesized on ribosomes that are bound to the membrane of the endoplasmic reticulum. As translation proceeds, the polypeptide chains are transported into the endoplasmic reticulum where protein folding and processing take place. From the endoplasmic reticulum, proteins are transported in vesicles to the Golgi apparatus where they are further processed and sorted for transport to endosomes, lysosomes, the plasma membrane, or secretion from the cell. Some of these organelles also participate in the sorting and transport of proteins being taken up from outside the cell (see Chapter 13). The endoplasmic reticulum, Golgi apparatus, endosomes, and lysosomes are thus distinguished from other cytoplasmic organelles by their common involvement in protein processing and connection by vesicular transport. About one-third of cellular proteins are processed in the endoplasmic reticulum, highlighting the importance of this pathway in cell physiology.

The Endoplasmic Reticulum

The endoplasmic reticulum (ER) is a network of membrane-enclosed tubules and sacs (cisternae) that extends from the nuclear membrane throughout the cytoplasm (Figure 10.1). The entire endoplasmic reticulum is enclosed by a continuous membrane and is the largest organelle of most eukaryotic cells. Its membrane may account for about half of all cell membranes, and the space enclosed by the ER (the lumen, or cisternal space) may represent about 10% of the total cell volume. As discussed below, there are three contiguous membrane domains within the ER that perform different functions within the cell. The rough ER, which is covered by ribosomes on its outer (cytosolic)
surface, and the transitional ER, where vesicles exit to the Golgi apparatus, both function in protein processing. The smooth ER is not associated with ribosomes and is involved in lipid, rather than protein, metabolism.

**The endoplasmic reticulum and protein secretion**

The role of the endoplasmic reticulum in protein processing and sorting was first demonstrated by George Palade and his colleagues in the 1960s (Figure 10.2). These investigators studied the fate of newly synthesized proteins in specialized cells of the pancreas (pancreatic acinar cells) that secrete digestive enzymes into the small intestine. Because most of the protein synthesized by these cells is secreted, Palade and coworkers were able to study the pathway

![Diagram of protein secretion](image)

**FIGURE 10.2 The secretory pathway** Pancratic acinar cells, which secrete most of their newly synthesized proteins into the digestive tract, were labeled with radioactive amino acids to study the intracellular pathway taken by secreted proteins. After a 3-minute incubation with radioactive amino acids (a “pulse”), autoradiography revealed that newly synthesized proteins were localized to the rough ER. Following further incubation with nonradioactive amino acids (a “chase”), proteins were found to move from the ER to the Golgi apparatus and then, within secretory vesicles, to the plasma membrane and cell exterior.

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taken by secreted proteins simply by labeling newly synthesized proteins with radioactive amino acids in a procedure known as a “pulse-chase” experiment. The location of the radiolabeled proteins within the cell was then determined by autoradiography and electron microscopy, revealing the cellular sites involved in the events leading to protein secretion. After a brief exposure of pancreatic acinar cells to radioactive amino acids (a “pulse”), newly synthesized proteins were detected in the rough ER, which was therefore identified as the site of synthesis of proteins destined for secretion. If the cells were then incubated for a short time in media containing nonradioactive amino acids (a “chase”), the radiolabeled proteins were detected in the Golgi apparatus. Following longer chase periods, the radiolabeled proteins traveled from the Golgi apparatus to the cell surface in **secretory vesicles**, which then fused with the plasma membrane to release their contents outside of the cell.

These experiments defined a pathway taken by secreted proteins—the **secretory pathway**: rough ER → Golgi → secretory vesicles → cell exterior. Further studies extended these results and demonstrated that this pathway is not restricted to proteins destined for secretion from the cell. Portions of it are shared by proteins destined for other compartments. Plasma membrane and lysosomal proteins also travel from the rough ER to the Golgi and then to their final destinations. Still other proteins travel through the initial steps of the secretory pathway but are then retained and function within either the ER or the Golgi apparatus.

The entrance of proteins into the ER thus represents a major branch point for the traffic of proteins within eukaryotic cells (**Figure 10.3**). Proteins destined for secretion or incorporation into the ER, Golgi apparatus, lysosomes, or plasma membrane are initially targeted to the ER, as are nuclear and peroxisomal membrane proteins. In mammalian cells most proteins are transferred into the ER while they are being translated on membrane-bound ribosomes. In contrast,
proteins destined to remain in the cytosol or to be incorporated into mitochondria, chloroplasts, or the interior of the nucleus or peroxisomes are synthesized on free ribosomes and released into the cytosol when their translation is complete.

**Targeting proteins to the endoplasmic reticulum**

Proteins can be translocated into the ER either during their synthesis on membrane-bound ribosomes (cotranslational translocation) or after their translation has been completed on free ribosomes in the cytosol (post-translational translocation). In mammalian cells, most proteins enter the ER cotranslationally, whereas both cotranslational and posttranslational pathways are used in yeast. The first step in the cotranslational pathway is the association of the ribosome-mRNA complex with the ER. Ribosomes are targeted for binding to the ER membrane by the amino-acid sequence of the polypeptide chain being synthesized, rather than by intrinsic properties of the ribosome itself. Free and membrane-bound ribosomes are functionally indistinguishable, and protein synthesis generally initiates on ribosomes that are free in the cytosol. Ribosomes engaged in the synthesis of proteins that are destined for secretion are then targeted to the endoplasmic reticulum by a **signal sequence** at the amino terminus of the growing polypeptide chain. These signal sequences are short stretches of hydrophobic amino acids that are cleaved from the polypeptide chain during its transfer into the ER lumen.

The general role of signal sequences in targeting proteins to their appropriate locations within the cell was first elucidated by studies of the import of secretory proteins into the ER. These experiments used *in vitro* preparations of rough ER, which were isolated from cell extracts by density-gradient centrifugation (Figure 10.4). When cells are disrupted, the ER breaks up into small vesicles called **microsomes**. Because the vesicles derived from the rough ER are covered with ribosomes, they can be separated from similar vesicles derived from the smooth ER or from other membranes (e.g., the plasma membrane). In particular, the large amount of RNA within ribosomes increases the density of the membrane vesicles to which they are attached, allowing purification of vesicles derived from the rough ER (rough microsomes) by equilibrium centrifugation in density gradients.

![FIGURE 10.4 Isolation of rough ER](image)

When cells are disrupted, the ER fragments into small vesicles called microsomes. The microsomes derived from the rough ER (rough microsomes) are lined with ribosomes on their outer surface. Because ribosomes contain large amounts of RNA, the rough microsomes are denser than smooth microsomes and can be isolated by equilibrium density-gradient centrifugation.
Günter Blobel and David Sabatini first proposed in 1971 that the signal for ribosome attachment to the ER might be an amino acid sequence near the amino terminus of the growing polypeptide chain. This hypothesis was supported by the results of in vitro translation of mRNAs encoding secreted proteins, such as immunoglobulins (Figure 10.5). If an mRNA encoding a secreted protein was translated on free ribosomes in vitro, it was found that the protein produced was slightly larger than the normal secreted protein. If microsomes were added to the system, however, the in vitro translated protein was incorporated into the microsomes and cleaved to the correct size. These experiments led to a more detailed formulation of the signal hypothesis, which proposed that an amino terminal signal sequence targets the polypeptide chain to the microsomes and is then cleaved by a microsomal protease. Many subsequent findings have substantiated this model, including recombinant DNA experiments demonstrating that addition of a signal sequence to a normally nonsecreted protein is sufficient to direct the incorporation of the recombinant protein into the rough ER.

The mechanism by which secretory proteins are targeted to the ER during their translation (the cotranslational pathway) is now well understood. The signal sequences span about 15–40 amino acids, including a stretch of 7–12 hydrophobic residues, usually located at the amino terminus of the polypeptide chain (Figure 10.6). As they emerge from the ribosome, signal sequences are...
**The Context**

How are specific polypeptide chains transferred across the appropriate membranes? Studies in the 1950s and 1960s indicated that secreted proteins are synthesized on membrane-bound ribosomes and transferred across the membrane during their synthesis. However, this did not explain why ribosomes—engaged in the synthesis of secreted proteins—attach to membranes, while ribosomes synthesizing cytosolic proteins do not. A hypothesis to explain this difference was first suggested by Günter Blobel and David Sabatini in 1971. At that time, they proposed that (1) mRNAs to be translated on membrane-bound ribosomes contain a unique set of codons just 3′ of the translation initiation site, (2) translation of these codons yields a unique sequence at the amino terminus of the growing polypeptide chain (the signal sequence), and (3) the signal sequence triggers attachment of the ribosome to the membrane. In 1975 Blobel and Dobberstein reported a series of experiments that provided critical support for this notion. In addition, they proposed “a somewhat more detailed version of this hypothesis, henceforth referred to as the signal hypothesis.”

**The Experiments**

Myelomas are cancers of B lymphocytes that actively secrete immunoglobulins, so they provide a good model for studies of secreted proteins. Previous studies in Cesar Milstein’s laboratory had shown that the proteins produced by in vitro translation of immunoglobulin light-chain mRNA contain about 20 amino acids at their amino terminus that are not present in the secreted light chains. This result led to the suggestion that these amino acids direct binding of the ribosome to the membrane. To test this idea, Blobel and Dobberstein investigated the synthesis of light chains by membrane-bound ribosomes from myeloma cells.

As expected from earlier work, in vitro translation of light-chain mRNA on free ribosomes yielded a protein that was larger than the secreted light chain (see figure). In contrast, in vitro translation of mRNA associated with membrane-bound ribosomes from myeloma cells yielded a protein that was the same size as the normally secreted light chain. Moreover, the light chains synthesized by ribosomes that remained bound to microsomes were resistant to digestion by added proteases, indicating that the light chains had been transferred into the microsomes.

These results indicated that an amino-terminal signal sequence is removed by a microsomal protease as growing polypeptide chains are transferred across the membrane. The results were interpreted in terms of a more detailed version of the signal hypothesis. As stated by Blobel and Dobberstein, “the essential feature of the signal hypothesis is the occurrence of a unique sequence of codons, located immediately to the right of the initiation codon, which is present only in those mRNAs whose translation products are to be transferred across a membrane.”

**The Impact**

The selective transfer of proteins across membranes is critical to the maintenance of the membrane-enclosed organelles of eukaryotic cells. To maintain the identity of these organelles,
recognized and bound by the **signal recognition particle (SRP)** consisting of six polypeptides and a small cytoplasmic RNA (**SRP RNA**). The SRP binds the ribosome as well as the signal sequence, inhibiting further translation and targeting the entire complex (the SRP, ribosome, mRNA, and growing polypeptide chain) to the rough ER by binding to the **SRP receptor** on the ER membrane (**Figure 10.7**). Binding to the receptor releases the SRP from both the ribosome and the signal sequence of the growing polypeptide chain. The ribosome then binds to a protein translocation complex or **translocon** in the ER membrane, and the signal sequence is inserted into a membrane channel.

Key insights into the process of translocation through the ER membrane came from determination of the translocon structure by Tom Rapoport and

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**Figure 10.7** Cotranslational targeting of secretory proteins to the ER  
**Step 1:** As the signal sequence emerges from the ribosome, it is recognized and bound by the signal recognition particle (SRP).  
**Step 2:** The SRP escorts the complex to the ER membrane where it binds to the SRP receptor.  
**Step 3:** The SRP is released, the ribosome binds to the translocon, and the signal sequence is inserted into the membrane channel, opening the translocon.  
**Step 4:** Translation resumes and the growing polypeptide chain is translocated across the membrane.  
**Step 5:** Cleavage of the signal sequence by signal peptidase releases the polypeptide into the lumen of the ER.
his colleagues in 2004. In both yeast and mammalian cells, the translocons through the ER membrane are complexes of three transmembrane proteins called the Sec61 proteins (Figure 10.8). The yeast and mammalian translocon proteins are closely related to the plasma membrane proteins that translocate secreted polypeptides in bacteria, demonstrating a striking conservation of the protein secretion machinery in prokaryotic and eukaryotic cells. Insertion of the signal sequence opens the translocon by moving a plug away from the translocon channel. This allows the growing polypeptide chain to be transferred through the translocon as translation proceeds. Thus the process of protein synthesis directly drives the transfer of growing polypeptide chains through the translocon and into the ER. As translocation proceeds, the signal sequence is cleaved by signal peptidase and the polypeptide is released into the lumen of the ER.

Many proteins in yeast, as well as a few proteins in mammalian cells, are targeted to the ER after their translation is complete (posttranslational translocation) rather than being transferred into the ER during synthesis on membrane-bound ribosomes. These proteins are synthesized on free cytosolic ribosomes, and their posttranslational incorporation into the ER does not require the SRP. Instead, their signal sequences are recognized by distinct receptor proteins (the Sec62/63 complex) associated with the translocon in

**FIGURE 10.8 Structure of the translocon** The translocon consists of three transmembrane subunits, shown in green, purple, and red. (A) Lateral view of the translocon inserted into the ER membrane. (B) Cross-sectional view from the cytosol, showing the plug in the translocon channel. (From B. van den Berg et al., 2004. *Nature* 427: 36.)
the ER membrane (Figure 10.9). Cytosolic Hsp70 and Hsp40 chaperones are required to maintain the polypeptide chains in an unfolded conformation so they can enter the translocon, and another Hsp70 chaperone within the ER (called BiP) is required to pull the polypeptide chain through the channel and into the ER. BiP acts as a ratchet to drive the posttranslational translocation of proteins into the ER, whereas the cotranslational translocation of growing polypeptide chains is driven directly by the process of protein synthesis.

**Insertion of proteins into the ER membrane**

Proteins destined for secretion from the cell or residence within the lumen of the ER, Golgi apparatus, endosomes, or lysosomes are translocated across the ER membrane and released into the lumen of the ER as already described. However, proteins destined for incorporation into the plasma membrane or the membranes of these compartments are initially inserted into the ER membrane instead of being released into the lumen. From the ER membrane, they proceed to their final destination along the same pathway as that of secretory proteins: ER → Golgi → plasma membrane or endosomes → lysosomes. However, these proteins are transported along this pathway as membrane components rather than as soluble proteins.

Integral membrane proteins are embedded in the membrane by hydrophobic sequences that span the phospholipid bilayer (see Figure 2.25).
The membrane-spanning portions of these proteins are usually α-helical regions consisting of 20 to 25 hydrophobic amino acids. The formation of an α-helix maximizes hydrogen bonding between the peptide bonds, and the hydrophobic amino acid side chains interact with the fatty acid tails of the phospholipids in the bilayer. However, different integral membrane proteins vary in how they are inserted (Figure 10.10). For example, whereas some integral membrane proteins span the membrane only once, others have multiple membrane-spanning regions. In addition, some proteins are oriented in the membrane with their amino terminus on the cytosolic side; others have their carboxy terminus exposed to the cytosol. These orientations of proteins inserted into the ER, Golgi, lysosomal, and plasma membranes are established as the growing polypeptide chains are translocated into the ER. The lumen of the ER is topologically equivalent to the exterior of the cell, so the domains of plasma membrane proteins that are exposed on the cell surface correspond to the regions of polypeptide chains that are translocated into the ER lumen (Figure 10.11).

The most straightforward mode of insertion into the ER membrane results in the synthesis of transmembrane proteins oriented with their carboxy termini exposed to the cytosol (Figure 10.12). These proteins have a normal amino terminal signal sequence, which is cleaved by signal peptidase during translocation of the polypeptide chain across the ER membrane through the translocon. They are then anchored in the membrane by a membrane-spanning α-helix in the middle of the protein. This hydrophobic transmembrane sequence, called a stop-transfer sequence, signals a change in the translocon channel. The membrane-spanning helices of the translocon open laterally, allowing the hydrophobic transmembrane domain of the protein to exit the translocon into the lipid bilayer. Further translocation of the polypeptide chain across the ER membrane is thus blocked, so the carboxy terminal portion of the growing polypeptide chain remains in the...
**FIGURE 10.11 Topology of the secretory pathway**  The lumens of the endoplasmic reticulum and Golgi apparatus are topologically equivalent to the exterior of the cell. Consequently, those portions of polypeptide chains that are translocated into the ER are exposed on the cell surface following transport to the plasma membrane.
The insertion of these transmembrane proteins into the membrane thus involves the sequential action of two distinct elements: a cleavable amino terminal signal sequence that initiates translocation across the membrane, and a hydrophobic transmembrane stop-transfer sequence that anchors the protein in the membrane.

Proteins can also be anchored in the ER membrane by internal hydrophobic transmembrane signal sequences that are not cleaved by signal peptidase (Figure 10.13). These internal signal sequences are recognized by the SRP and brought to the translocon as already discussed. Rather than being cleaved by signal peptidase, these signal sequences act as transmembrane $\alpha$ helices that exit the translocon and anchor proteins in the ER membrane. Importantly, internal signal sequences can be oriented so as to direct the translocation of either the amino or carboxy terminus of the polypeptide chain across the membrane. Therefore, depending on the orientation of the signal sequence, proteins inserted into the membrane by this mechanism can have either their amino or carboxy terminus exposed to the cytosol.

Proteins that span the membrane multiple times are inserted as a result of an alternating series of internal signal sequences and transmembrane stop-transfer sequences. For example, an internal signal sequence can result in membrane insertion of a polypeptide chain with its amino ter-
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minus on the cytosolic side (Figure 10.14). If a stop-transfer sequence is then encountered, the polypeptide will form a loop in the ER lumen, and protein synthesis will continue on the cytosolic side of the membrane. If a second signal sequence is encountered, the growing polypeptide chain will again be inserted into the ER, forming another looped domain on the cytosolic side of the membrane. This can be followed by yet another stop-transfer sequence and so forth, so that an alternating series of signal and stop-transfer sequences can result in the insertion of proteins that span the membrane multiple times, with looped domains exposed on both the luminal and cytosolic sides.

**FIGURE 10.13** Insertion of membrane proteins with internal noncleavable signal sequences Internal noncleavable signal sequences can lead to the insertion of polypeptide chains in either orientation in the ER membrane. (A) The signal sequence directs insertion of the polypeptide such that its amino terminus is exposed on the cytosolic side. The remainder of the polypeptide chain is translocated into the ER as translation proceeds. The signal sequence is not cleaved, instead acting as a membrane-spanning sequence that anchors the protein in the lipid bilayer with its carboxy terminus in the lumen of the ER. (B) Other internal signal sequences are oriented to direct the transfer of the amino-terminal portion of the polypeptide across the membrane. Continued translation results in a protein that spans the ER membrane with its amino terminus in the lumen and its carboxy terminus in the cytosol. Note that this orientation is the same as that resulting from insertion of a protein that contains a cleavable signal sequence followed by a stop-transfer sequence (see Figure 10.12).

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As discussed below, most transmembrane proteins destined for other compartments in the secretory pathway are delivered to them in transport vesicles. However, proteins destined for the nuclear envelope (which is continuous with the ER) move laterally in the plane of the membrane rather than being transported by vesicles. Recent studies suggest that inner nuclear membrane proteins (such as emerin or LBR; see Chapter 9) contain specific transmembrane sequences that signal their transport to the inner nuclear membrane, where they are retained by interactions with nuclear components such as lamins or chromatin.

**FIGURE 10.14 Insertion of a protein that spans the membrane multiple times** In this example, an internal signal sequence results in insertion of the polypeptide chain with its amino terminus on the cytosolic side of the membrane. A membrane-spanning stop-transfer sequence then signals lateral exit from the translocon, causing the polypeptide chain to form a loop within the lumen of the ER; translation continues in the cytosol. A second internal signal sequence reopens the channel, triggering reinsertion of the polypeptide chain into the translocon and forming a loop in the cytosol. The process can be repeated many times, resulting in the insertion of proteins with multiple membrane-spanning regions.

As discussed below, most transmembrane proteins destined for other compartments in the secretory pathway are delivered to them in transport vesicles. However, proteins destined for the nuclear envelope (which is continuous with the ER) move laterally in the plane of the membrane rather than being transported by vesicles. Recent studies suggest that inner nuclear membrane proteins (such as emerin or LBR; see Chapter 9) contain specific transmembrane sequences that signal their transport to the inner nuclear membrane, where they are retained by interactions with nuclear components such as lamins or chromatin.

**Protein folding and processing in the ER**

The folding of polypeptide chains into their correct three-dimensional conformations, the assembly of polypeptides into multisubunit proteins, and
the covalent modifications involved in protein processing were discussed in Chapter 8. For proteins that enter the secretory pathway, many of these events occur either during translocation across the ER membrane or within the ER lumen. One such processing event is the proteolytic cleavage of the signal sequence as the polypeptide chain is translocated across the ER membrane. The ER is also the site of protein folding, assembly of multisubunit proteins, disulfide bond formation, N-linked glycosylation, and the addition of glycolipid anchors to some plasma membrane proteins. In fact, the primary role of lumenal ER proteins is to assist the folding and assembly of newly translocated polypeptides.

As already discussed, proteins are translocated across the ER membrane as unfolded polypeptide chains while their translation is still in progress. These polypeptides, therefore, fold into their three-dimensional conformations within the ER, assisted by molecular chaperones that facilitate the folding of polypeptide chains (see Chapter 8). The Hsp70 chaperone, BiP, is thought to bind to the unfolded polypeptide chain as it crosses the membrane and then mediate protein folding and the assembly of multisubunit proteins within the ER (Figure 10.15). Correctly assembled proteins are released from BiP (and other chaperones) and are available for transport to the Golgi apparatus. Abnormally folded or improperly assembled proteins are targets for degradation, as will be discussed later.

The formation of disulfide bonds between the side chains of cysteine residues is an important aspect of protein folding and assembly within the ER. These bonds generally do not form in the cytosol, which is characterized by a reducing environment that maintains most cysteine residues in their reduced (—SH) state. In the ER, however, an oxidizing environment promotes disulfide (S—S) bond formation, and disulfide bonds formed in the ER play important roles in the structure of secreted and cell surface proteins. Disulfide bond formation is facilitated by the enzyme protein disulfide isomerase (PDI) (see Figure 8.27), which is located in the ER lumen.

**Figure 10.15** Protein folding in the ER  The molecular chaperone BiP binds to polypeptide chains as they cross the ER membrane and facilitates protein folding and assembly within the ER.
Proteins are also glycosylated on specific asparagine residues (N-linked glycosylation) within the ER while their translation is still in process (Figure 10.16). As discussed in Chapter 8 (see Figure 8.32), oligosaccharide units consisting of 14 sugar residues are added to acceptor asparagine residues of growing polypeptide chains as they are translocated into the ER. The oligosaccharide is synthesized on a lipid (dolichol) carrier anchored in the ER membrane. It is then transferred as a unit to acceptor asparagine residues in the consensus sequence Asn-X-Ser/Thr by a membrane-bound enzyme called oligosaccharyl transferase. Three glucose residues are removed while the protein is still within the ER, and the protein is modified further after being transported to the Golgi apparatus (discussed later in this chapter). Glycosylation both helps to prevent protein aggregation in the ER and provides signals for subsequent sorting in the secretory pathway.

Some proteins are attached to the plasma membrane by glycolipids rather than by membrane-spanning regions of the polypeptide chain. Because these membrane-anchoring glycolipids contain phosphatidylinositol, they are called glycophosphatidylinositol (GPI) anchors, the structure of which was illustrated in Figure 8.37. The GPI anchors are assembled in the ER membrane. They are then added immediately after completion of protein synthesis to the carboxy terminus of some proteins that are retained in the membrane by a C-terminal hydrophobic sequence (Figure 10.17). The C-terminal sequence of the protein is cleaved and exchanged for the GPI anchor, so these proteins remain attached to the membrane only by their associated glycolipid. Like transmembrane proteins, they are transported to
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their orientation within the ER dictates that GPI-anchored proteins are exposed on the outside of the cell, with the GPI anchor mediating their attachment to the plasma membrane.

Quality control in the ER

Many proteins synthesized in the ER are rapidly degraded, primarily because they fail to fold correctly. Protein folding in the ER is slow and inefficient, and many proteins never reach their correctly folded conformations. Such misfolded proteins are removed from the ER by a process generally referred to as ER-associated degradation (ERAD), in which misfolded proteins are identified, returned from the ER to the cytosol, and degraded by the ubiquitin-proteasome system.

Because they assist proteins in correct folding, chaperones and protein processing enzymes in the ER lumen often act as sensors of misfolded proteins. One well-characterized pathway involves the chaperone, calreticulin,

FIGURE 10.17 Addition of GPI anchors  Glycosylphosphatidylinositol (GPI) anchors contain two fatty acid side chains, an oligosaccharide portion consisting of inositol and other sugars, and ethanolamine (see Figure 8.37 for a more detailed structure). The GPI anchors are assembled in the ER and added to polypeptides anchored in the membrane by a carboxy-terminal membrane-spanning region. The membrane-spanning region is cleaved, and the new carboxy terminus is joined to the NH₂ group of ethanolamine immediately after translation is completed, leaving the protein attached to the membrane by the GPI anchor.
which recognizes the partially-processed oligosaccharides on newly translated glycoproteins and assists the glycoprotein in folding correctly (Figure 10.18). Removal of the terminal glucose residue from the oligosaccharide then releases the glycoprotein from calreticulin and allows it to be recognized by a protein folding sensor, which passes correctly folded glycoproteins on to exit the ER. However, if the glycoprotein is not correctly folded, the folding sensor will add back a glucose residue to the oligosaccharide, allowing it to

**FIGURE 10.18** Glycoprotein folding by calreticulin. As the glycoprotein exits the translocon, two glucose residues are removed, allowing calreticulin to bind and assist in folding. Removal of the remaining glucose residue terminates the interaction with calreticulin, releasing the glycoprotein. A protein-folding sensor then assesses the extent of folding of the glycoprotein by monitoring exposed hydrophobic regions. If none are found, the glycoprotein is correctly folded and passed on to the transitional ER. If the glycoprotein is incorrectly folded, the folding sensor, which is a glucosyltransferase, will add back a glucose residue, allowing the glycoprotein to re-enter the calreticulin chaperone cycle. If too many hydrophobic regions remain exposed and the protein cannot be properly folded, the mannose residues are removed and the protein is targeted back to the cytosol through a ubiquitin ligase complex in the ER membrane. The protein is ubiquitinated at the cytosolic side of this complex and degraded in the proteasome.
cycle back to calreticulin for another attempt at correct folding. Glycoproteins that are severely misfolded and cannot be folded correctly are instead targeted to the ERAD pathway for degradation. These misfolded proteins are transferred to a transmembrane complex with ubiquitin ligase activity. They are then translocated through this complex back to the cytosol, where they are ubiquitinated and degraded in the proteasome (see Figure 8.44).

The level of unfolded proteins in the ER is monitored in order to coordinate the protein-folding capacity of the ER with the physiological needs of the cell. This regulation is mediated by a signaling pathway known as the unfolded protein response (UPR), which is activated if an excess of unfolded proteins accumulate in the ER (Figure 10.19). Activation of the unfolded protein response pathway leads to expansion of the ER and production of

**FIGURE 10.19 Unfolded protein response** Unfolded proteins activate three receptors in the ER membrane. The first, IRE1, cleaves pre-mRNA of a transcription factor (XBP1). This leads to synthesis of XBP1, which translocates to the nucleus and stimulates transcription of UPR target genes. The second receptor, ATF6, is cleaved to release the active ATF6 transcription factor. The third receptor, PERK, is a protein kinase that phosphorylates the translation factor eIF2. This inhibits general translation, reducing the amount of protein entering the ER. It also results in preferential translation of the transcription factor ATF4, which further contributes to the induction of UPR target genes encoding chaperones, enzymes involved in lipid synthesis and ERAD proteins.
additional chaperones to meet the need for increased protein folding, as well as a transient reduction in the amount of newly synthesized proteins entering the ER. If these changes are insufficient to adjust protein folding in the ER to a normal level, sustained activity of the unfolded protein response leads to programmed cell death (see Chapter 17), thereby eliminating cells that are unable to properly fold proteins from the body.

In mammalian cells, the unfolded protein response results from the activity of three signaling molecules or stress sensors in the ER membrane (IRE1, ATF6 and PERK), which are activated by unfolded proteins in the ER lumen (see Figure 10.19). Activation of IRE1 (which is the only sensor in yeast) results in cleavage and activation of the mRNA encoding a transcription factor called XBP1 on the cytosolic side of the ER membrane. The XBP1 transcription factor then induces expression of genes involved in the unfolded protein response, including chaperones, enzymes involved in lipid synthesis, and ERAD proteins. The second sensor, ATF6, is itself a transcription factor, which is sequestered in the ER membrane in unstressed cells. Unfolded proteins in the ER signal the transport of ATF6 to the Golgi apparatus, where it is cleaved to release the active form of ATF6 into the cytosol. ATF6 then translocates to the nucleus and induces the expression of additional unfolded protein response genes. The third sensor, PERK, is a protein kinase that phosphorylates and inhibits translation initiation factor eIF2 (see Figure 8.21). This results in a general decrease in protein synthesis and a reduction in the load of unfolded proteins entering the ER. In addition, inhibition of eIF2 leads to the preferential translation of some mRNAs. As a result, activation of PERK leads to increased expression of a transcription factor ATF4, which further contributes to the induction of genes involved in the unfolded protein response.

The smooth ER and lipid synthesis

In addition to its activities in the processing of secreted and membrane proteins, the ER is the major site at which membrane lipids are synthesized in eukaryotic cells. Because they are extremely hydrophobic, membrane lipids are synthesized in association with already existing cellular membranes rather than in the aqueous environment of the cytosol. Although some lipids are synthesized in association with other membranes, most are synthesized in the ER. They are then transported from the ER to their ultimate destinations in other membranes, either by direct contacts between the smooth ER and trans Golgi network membranes, in vesicles, or by lipid transfer proteins, as will be discussed in Chapter 11.

The membranes of eukaryotic cells are composed of three main types of lipids: phospholipids, glycolipids, and cholesterol. Most of the phospholipids, which are the basic structural components of the membrane, are derived from glycerol. They are synthesized on the cytosolic side of the ER membrane from water-soluble cytosolic precursors (Figure 10.20). Fatty acids are first transferred from coenzyme A carriers to glycerol-3-phosphate by membrane-bound enzymes, and the resulting phospholipid (phosphatidic acid) is inserted into the membrane. Enzymes on the cytosolic face of the ER membrane then convert phosphatidic acid to diacylglycerol and catalyze the addition of
different polar head groups, resulting in formation of phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, and phosphatidylinositol.

The synthesis of these phospholipids on the cytosolic side of the ER membrane allows the hydrophobic fatty acid chains to remain buried in the membrane while membrane-bound enzymes catalyze their reactions with water-soluble precursors (e.g., CDP-choline) in the cytosol. Because of this topography, however, new phospholipids are added only to the cytosolic half of the ER membrane (Figure 10.21). To maintain a stable membrane some of these newly synthesized phospholipids must therefore be transferred to the other (lumenal) half of the ER bilayer. This transfer, which requires the passage of a polar head group through the membrane, is facilitated by membrane proteins called flippases. By catalyzing the rapid translocation of phospholipids across the ER membrane, the flippases ensure even growth of both halves of the bilayer. Different families of these enzymes, some of

**FIGURE 10.21 Translocation of phospholipids across the ER membrane**

Because phospholipids are synthesized on the cytosolic side of the ER membrane, they are added only to the cytosolic half of the bilayer. They are then translocated across the membrane by phospholipid flippases, resulting in even growth of both halves of the phospholipid bilayer.
which are specific for particular phospholipids, are found in virtually all cell membranes.

In addition to its role in synthesis of the glycerol phospholipids, the ER also serves as the major site of synthesis of two other membrane lipids: cholesterol and ceramide (Figure 10.22). As discussed later, ceramide is converted to either glycolipids or sphingomyelin (the only membrane phospholipid not derived from glycerol) in the Golgi apparatus. The ER is thus responsible for synthesis of either the final products or the precursors of all the major lipids of eukaryotic membranes. Cholesterol and sphingomyelin are important components of lipid “rafts,” as discussed in Chapter 13.

Smooth ER is abundant in cell types that are particularly active in lipid metabolism. For example, steroid hormones are synthesized (from cholesterol) in the ER, so large amounts of smooth ER are found in steroid-producing cells, such as those in the testis and ovary. In addition, smooth ER is abundant in the liver where it contains enzymes that metabolize various lipid-soluble compounds. These detoxifying enzymes inactivate a number of potentially harmful drugs (e.g., phenobarbital) by converting them to water-soluble compounds that can be eliminated from the body in the urine. The smooth ER is thus involved in multiple aspects of the metabolism of lipids and lipid-soluble compounds.

**Export of proteins and lipids from the ER**

Both proteins and phospholipids travel along the secretory pathway in transport vesicles, which bud from the membrane of one organelle and then fuse with the membrane of another. Thus molecules are exported from the ER in vesicles that bud from the transitional ER and carry their cargo through the ER-Golgi intermediate compartment (ERGIC) and then to the Golgi ap-
paratus (Figure 10.23). Subsequent steps in the secretory pathway involve transport between different compartments of the Golgi and from the Golgi to endosomes, lysosomes, or the plasma membrane. In most cases, proteins within the lumen of one organelle are packaged into budding transport vesicles and then released into the lumen of the recipient organelle following vesicle fusion. Membrane proteins and lipids are transported similarly, and it is significant that their topological orientation is maintained as they travel from one membrane-enclosed organelle to another. For example, the domains of a protein exposed on the cytosolic side of the ER membrane will also be exposed on the cytosolic side of the Golgi and plasma membranes, whereas protein domains exposed on the lumenal side of the ER membrane will be exposed on the lumenal side of the Golgi and on the exterior of the cell (see Figure 10.11).

Most proteins that enter the transitional ER (the first branch point in the secretory pathway) are sorted into vesicles and move through the ER-Golgi intermediate compartment and on to the Golgi. These proteins are marked by sequences that signal their export from the ER (Figure 10.24). Many
transmembrane proteins possess di-acidic or di-hydrophobic amino acid sequences in their cytosolic domains that function as ER export signals. Both GPI-anchored proteins (which are marked for export by their GPI anchors) and lumenal secretory proteins appear to be recognized and sequestered by these transmembrane receptor proteins. Very few ER export signals have been detected on lumenal secretory proteins and their recognition by the transmembrane cargo receptors may depend on the shape of the correctly folded protein. It is also possible that there is a default pathway through which otherwise unmarked proteins in the ER lumen move to the Golgi and beyond.

If proteins that function within the ER lumen (including BiP, protein disulfide isomerase, and other enzymes discussed earlier) are allowed to proceed along the secretory pathway, they will be lost to the cell. To prevent this, these proteins have a targeting sequence Lys-Asp-Glu-Leu (KDEL, in the single-letter code) at their carboxy terminus that directs their retrieval back to the ER. If this sequence is deleted from a protein that normally functions in the ER (e.g., BiP or protein disulfide isomerase), the mutated protein is instead transported to the Golgi and secreted from the cell. Conversely, addition of the KDEL sequence to the carboxy terminus of proteins that are normally secreted blocks their secretion. Some ER transmembrane proteins are similarly marked by short C-terminal sequences that contain two lysine residues (KKXX sequences).

Interestingly, the KDEL and KKXX signals do not prevent ER proteins from being packaged into vesicles and carried to the Golgi. Instead, these signals cause the ER resident proteins to be selectively retrieved from the ER-Golgi intermediate compartment or the Golgi complex and returned to the ER via a recycling pathway (Figure 10.25). Proteins bearing the KDEL and KKXX sequences bind to specific recycling receptors in the membranes of these compartments and are then selectively transported back to the ER.
The KDEL and KKXX sequences are the best-characterized retention/retrieval signals, but there may be others. Other proteins are retrieved because they specifically bind to KDEL-bearing proteins such as BiP. Thus continued movement along the secretory pathway or retrieval back from the Golgi to the ER is the second branch point encountered by proteins being sorted to their correct destinations. Similar branch points arise at each subsequent stage of transport, such as retention in the Golgi versus export to endosomes, lysosomes or the plasma membrane. In each case, specific localization signals target proteins to their correct intracellular destinations.

The Golgi Apparatus

The **Golgi apparatus**, or **Golgi complex**, functions as a factory in which proteins received from the ER are further processed and sorted for transport to their eventual destinations: endosomes, lysosomes, the plasma membrane, or secretion. In addition, as noted earlier, most glycolipids and sphingomyelin are synthesized within the Golgi. In plant cells, the Golgi apparatus further serves as the site at which the complex polysaccharides of the cell wall are synthesized. The Golgi apparatus is thus involved in processing the broad range of cellular constituents that travel along the secretory pathway.

**Organization of the Golgi**

In most cells, the Golgi is composed of flattened membrane-enclosed sacs (cisternae) and associated vesicles (**Figure 10.26**). A striking feature of the Golgi apparatus is its distinct polarity in both structure and function. Proteins from the ER enter at its *cis* face (entry face), which is usually oriented toward the nucleus. They are then transported through the Golgi and exit from its *trans* face (exit face). As they pass through the Golgi, proteins are modified and sorted for transport to their eventual destinations within the cell.

**WEBSITE ANIMATION 10.2**

**Organization of the Golgi**
The Golgi apparatus is composed of flattened membrane-enclosed sacs that receive proteins from the ER, process them, and sort them to their eventual destinations.

---

**FIGURE 10.26** Electron micrograph of a Golgi apparatus The Golgi apparatus consists of a stack of flattened cisternae and associated vesicles. Proteins and lipids from the ER enter the Golgi apparatus at its *cis* face and exit near its *trans* face. (Courtesy of Dr. L. Andrew Staehelin, University of Colorado at Boulder.)
The Golgi consists of multiple discrete compartments, which are commonly viewed as corresponding to four functionally distinct regions: the cis Golgi network, the Golgi stack (which is divided into the medial and trans subcompartments), and the trans Golgi network (Figure 10.27). Distinct processing and sorting events occur in an ordered sequence within different regions of the Golgi complex, and recent investigations visualizing fluorescent-labeled proteins in living cells (discussed later in this chapter) show that specific proteins are processed in different regions of each Golgi cisterna. Proteins from the ER are transported to the ER-Golgi intermediate compartment and then enter the Golgi apparatus at the cis Golgi network where modification of proteins, lipids, and polysaccharides begins. After progress through the medial and trans compartments, where further modification takes place, they move to the trans Golgi network, which acts as a sorting and distribution center, directing molecular traffic to endosomes, lysosomes, the plasma membrane, or the cell exterior.

The mechanism by which proteins move through the Golgi apparatus has been a long-standing area of controversy. However, recent studies have provided considerable support for proteins being carried through compartments of the Golgi within the Golgi cisternae, which gradually mature and progressively move through the Golgi in the cis to trans direction, rather than in transport vesicles (see Figure 10.27). Instead of carrying proteins through the Golgi stacks in the cis to trans direction, the

**FIGURE 10.27 Regions of the Golgi apparatus** Vesicles from the ER fuse to form the ER-Golgi intermediate compartment (ERGIC), and proteins from the ER are then transported to the cis Golgi network. Resident ER proteins are returned from the ERGIC and the cis Golgi network via the recycling pathway. The medial and trans compartments of the Golgi stack correspond to the cisternae in the middle of the Golgi complex and are the sites of most protein modifications. Proteins are then carried to the trans Golgi network where they are sorted for transport to the plasma membrane, secretion, endosomes, or lysosomes. Proteins are carried through the Golgi in the cis to trans direction within the Golgi cisternae, while transport vesicles carry Golgi resident proteins back to earlier compartments for reuse.
transport vesicles associated with the Golgi apparatus function to return Golgi resident proteins back to earlier Golgi compartments for reuse. The importance of this dynamic process for the structure of the Golgi is shown by the disappearance of the Golgi as an organized structure if vesicle transport from the ER is blocked.

**Protein glycosylation within the Golgi**

Protein processing within the Golgi involves the modification and addition of the carbohydrate portions of glycoproteins. One of the major aspects of this processing is the modification of the $N$-linked oligosaccharides that were added to proteins in the ER. As discussed earlier in this chapter, proteins are modified by the addition of an oligosaccharide consisting of 14 sugar residues within the ER (see Figure 10.16). Three glucose residues are removed within the ER and the $N$-linked oligosaccharides of these glycoproteins are then subject to extensive further modifications following transport to the Golgi apparatus (Figure 10.28). As noted in Chapter 8, different glycoproteins are modified in different ways during their passage through the Golgi, depending on both the structure of the protein and on the processing enzymes present in the Golgi complexes of each cell type. Consequently, proteins can emerge from the Golgi with a variety of different $N$-linked oligosaccharides.

The processing of the $N$-linked oligosaccharide of lysosomal proteins differs from that of secreted and plasma membrane proteins. Rather than the initial removal of mannose residues, proteins destined for incorporation into lysosomes are modified by mannose phosphorylation. In the first step of this reaction, $N$-acetylglucosamine phosphates are added

**FIGURE 10.28 Processing of $N$-linked oligosaccharides in the Golgi** The $N$-linked oligosaccharides of glycoproteins transported from the ER are further modified by an ordered sequence of reactions in the Golgi.

- **Lysosomal protein**
- **Four mannose residues are removed.**
- **One $N$-acetylglucosamine is added.**
- **Two additional mannose residues are removed.**
- **Fucose and two $N$-acetylglucosamine residues are added.**
- **Three sialic acid residues are added.**
- **Three galactose residues are added.**

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Protein Sorting and Transport

Proteins destined for incorporation into lysosomes are specifically recognized and modified by the addition of phosphate groups to the number 6 position of mannose residues. In the first step of the reaction, \( N \)-acetylglucosamine phosphates are transferred to mannose residues from UDP-\( N \)-acetylglucosamine. The \( N \)-acetylglucosamine group is then removed, leaving a mannose-6-phosphate.

The phosphorylation of mannose residues is thus a critical step in sorting lysosomal proteins to their correct intracellular destinations. The specificity of this process resides in the enzyme that catalyzes the first step in the reaction sequence—the selective addition of \( N \)-acetylglucosamine phosphates to lysosomal proteins. This enzyme recognizes a structural determinant that is present on lysosomal proteins but not on proteins destined for the plasma membrane or secretion. This recognition determinant is not a simple sequence of amino acids; rather, it is formed in the folded protein by the juxtaposition of amino acid sequences from different regions of the polypeptide chain. In contrast to the signal sequences that direct protein translocation to the ER, the recognition determinant that leads to mannose phosphorylation—and thus ultimately targets proteins to lysosomes—depends on the three-dimensional conformation of the folded protein. Such determinants are called **signal patches**, in contrast to the linear targeting signals discussed earlier in this chapter.

Proteins can also be modified by the addition of carbohydrates to the side chains of acceptor serine and threonine residues within specific sequences of amino acids (\( O \)-linked glycosylation) (see Figure 8.33). These modifications take place in the Golgi apparatus by the sequential addition of single sugar residues. The serine or threonine is usually linked directly to \( N \)-acytetylgalactosamine, to which other sugars can then be added. In some cases these sugars are further modified by the addition of sulfate groups.
Lipid and polysaccharide metabolism in the Golgi

In addition to its activities in processing and sorting glycoproteins, the Golgi apparatus functions in lipid metabolism—in particular, in the synthesis of glycolipids and sphingomyelin. As discussed earlier, the glycerol phospholipids, cholesterol, and ceramide are synthesized in the ER. Sphingomyelin and glycolipids are then synthesized from ceramide in the Golgi apparatus (Figure 10.30). Sphingomyelin (the only nonglycerol phospholipid in cell membranes) is synthesized by the transfer of a phosphorylcholine group from phosphatidylcholine to ceramide. Alternatively, the addition of carbohydrates to ceramide can yield a variety of different glycolipids.

Sphingomyelin is synthesized on the luminal surface of the Golgi, but glucose is added to ceramide on the cytosolic side. Glucosylceramide then apparently flips, however, and additional carbohydrates are added on the luminal side of the membrane. Glycolipids are not able to translocate across the Golgi membrane, so they are found only in the luminal half of the Golgi bilayer as is most sphingomyelin. Following vesicular transport they are correspondingly localized to the exterior half of the plasma membrane, with their polar head groups exposed on the cell surface. As will be discussed in Chapter 13, the oligosaccharide portions of glycolipids are important surface markers in cell-cell recognition.

In plant cells, the Golgi apparatus has the additional task of serving as the site where complex polysaccharides of the cell wall are synthesized. As discussed further in Chapter 14, the plant cell wall is composed of three major types of polysaccharides. Cellulose, the predominant constituent, is a

**FIGURE 10.30 Synthesis of sphingomyelin and glycolipids**

Ceramide, which is synthesized in the ER, is converted either to sphingomyelin (a phospholipid) or to glycolipids in the Golgi apparatus. In the first reaction, a phosphorylcholine group is transferred from phosphatidylcholine to ceramide. Alternatively, a variety of different glycolipids can be synthesized by the addition of one or more sugar residues (e.g., glucose).
simple linear polymer of glucose residues. It is synthesized at the cell surface
by enzymes in the plasma membrane. The other cell wall polysaccharides
(hemicelluloses and pectins), however, are complex branched chain molecules
that are synthesized in the Golgi apparatus and then transported in vesicles
to the cell surface. The synthesis of these cell wall polysaccharides is a major
cellular function, and as much as 80% of the metabolic activity of the Golgi
apparatus in plant cells may be devoted to polysaccharide synthesis.

**Protein sorting and export from the Golgi apparatus**

Proteins as well as lipids and polysaccharides are transported from the Golgi
apparatus to their final destinations through the secretory pathway. This
involves the sorting of proteins into different kinds of transport vesicles,
which bud from the *trans* Golgi network and deliver their contents to the
appropriate cellular locations (Figure 10.31). Some proteins are carried from
the Golgi to the plasma membrane, either directly or via recycling endosomes
as an intermediate compartment. Other proteins are transported to the cell
surface by a distinct pathway of regulated secretion, and still others are spe-
cifically targeted to other intracellular destinations, such as late endosomes
and lysosomes in animal cells or vacuoles in yeast.

Proteins that function within the Golgi apparatus must be retained within
that organelle rather than being transported along the secretory pathway. In
contrast to the ER, all of the proteins known to be retained within the Golgi

![FIGURE 10.31 Transport from the
Golgi apparatus](image)

Proteins are sorted in the *trans* Golgi network and transported in vesicles to their final destina-
tions. Proteins can be transported to the plasma membrane either directly
or via recycling endosomes. In addition, proteins can be sorted into dis-
tinct secretory vesicles for regulated secretion. Alternatively, proteins can
be targeted to late endosomes, which develop into lysosomes.
complex are associated with the Golgi membrane rather than being soluble proteins within the lumen. The primary signal responsible for retention of proteins within the Golgi is the length of their transmembrane domains, with short transmembrane domains signaling retention. In addition, like the KKXX sequences of resident ER membrane proteins, signals in the cytoplasmic tails of some Golgi proteins mediate the retrieval of these proteins from subsequent compartments along the secretory pathway.

Transport from the Golgi apparatus to the cell surface can occur by at least three routes (see Figure 10.31). Simplest is direct transport from the trans Golgi network to the plasma membrane, which leads to the continuous secretion of proteins from the cell as well as the incorporation of proteins and lipids into the plasma membrane. In addition, proteins can be transported from the Golgi to the plasma membrane via an intermediate of recycling endosomes, which are one of three types of endosomes in animal cells (discussed later in this chapter).

In addition to these pathways, which lead to continual unregulated protein secretion, some cells possess a distinct regulated secretory pathway in which specific proteins are secreted in response to environmental signals. Examples of regulated secretion include the release of hormones from endocrine cells, the release of neurotransmitters from neurons, and the release of digestive enzymes from the pancreatic acinar cells discussed at the beginning of this chapter (see Figure 10.2). Proteins are sorted into the regulated secretory pathway in the trans Golgi network where they are packaged into specialized secretory vesicles. This sorting appears to be mediated by cargo receptors that recognize signal patches shared by multiple proteins that enter this pathway. These receptor-cargo complexes selectively aggregate in cisternae of the trans Golgi network and are then released by budding as immature secretory vesicles. These vesicles, which are larger than transport vesicles, further process their protein contents and often fuse with each other to form mature secretory vesicles. The mature secretory vesicles then store their contents until specific signals direct their fusion with the plasma membrane. For example, the digestive enzymes produced by pancreatic acinar cells are stored in mature secretory vesicles until the presence of food in the stomach and small intestine triggers their secretion.

A further complication in the transport of proteins to the plasma membrane arises in many epithelial cells, which are polarized when they are organized into tissues. The plasma membrane of such cells is divided into two separate regions, the apical domain and the basolateral domain, which contain specific proteins related to their particular functions. For example, the apical membrane of intestinal epithelial cells faces the lumen of the intestine and is specialized for the efficient absorption of nutrients; the remainder of the cell is covered by the basolateral membrane (Figure 10.32). Distinct domains of the plasma membrane are present not only in epithelial cells but also in other cell types. Thus proteins leaving the trans Golgi network must be selectively transported to these distinct domains of the plasma membrane. This is accomplished by the selective packaging of proteins into transport vesicles targeted for either the apical or basolateral plasma membrane domains, which may take place either in the trans Golgi network or in recycling endosomes. Proteins are targeted to the basolateral domain by short amino acid sequences, such as dileucine (LL) or tyrosine-containing hydrophobic motifs, within their cytoplasmic domains. Protein sorting to apical domains is not yet fully understood, but appears to be directed by targeting signals consisting of carbohydrate modifications rather than amino acid sequences.
The best-characterized pathway of protein sorting in the Golgi is the selective transport of proteins to lysosomes (see Figure 10.31). As already discussed, luminal lysosomal proteins are marked by mannose-6-phosphates that are formed by modification of their N-linked oligosaccharides shortly after entry into the Golgi apparatus. A specific receptor in the membrane of the trans Golgi network then recognizes these mannose-6-phosphate residues. The resulting complexes of receptor plus lysosomal enzyme are packaged into transport vesicles destined for late endosomes that subsequently mature into lysosomes. Lysosomal membrane proteins are targeted by sequences in their cytoplasmic tails rather than by mannose-6-phosphates.

In yeasts and plant cells, both of which lack lysosomes, proteins are transported from the Golgi apparatus to an additional destination: the vacuole (Figure 10.33). Vacuoles assume the functions of lysosomes in these cells as well as perform a variety of other tasks, such as the storage of nutrients and the maintenance of turgor pressure and osmotic balance. In contrast to lysosomal targeting, proteins are directed to vacuoles by short peptide sequences instead of by carbohydrate markers.
The Mechanism of Vesicular Transport

Transport vesicles play a central role in the traffic of molecules between different membrane-enclosed compartments of the secretory pathway. As discussed later in this chapter and in Chapter 13, vesicles are similarly involved in the transport of materials taken up at the cell surface. Vesicular transport is thus a major cellular activity, responsible for molecular traffic between a variety of specific membrane-enclosed compartments. The selectivity of such transport is therefore key to maintaining the functional organization of the cell. For example, lysosomal enzymes must be transported specifically from the Golgi apparatus to lysosomes—not to the plasma membrane or to the ER. Some of the signals that target proteins to specific organelles, such as lysosomes, were discussed earlier in this chapter. These proteins are transported within vesicles, so the specificity of transport is based on the selective packaging of the intended cargo into vesicles that recognize and fuse only with the appropriate target membrane. Because of the central importance of vesicular transport to the organization of eukaryotic cells, understanding the molecular mechanisms that control vesicle packaging, budding, targeting and fusion is a major area of research in cell biology.

Experimental approaches to understanding vesicular transport

Progress toward elucidating the molecular mechanisms of vesicular transport has been made by five distinct experimental approaches: (1) isolation of yeast mutants that are defective in protein transport and sorting; (2) reconstitution of vesicular transport in cell-free systems; (3) biochemical analysis of synaptic vesicles, which are responsible for the regulated secretion of neurotransmitters by neurons; (4) tracing the path of specific GFP fusion proteins through the secretory network; and (5) proteomic analysis of specific secretory compartments. Each of these experimental systems has distinct advantages for understanding particular aspects of the transport process and their exploitation has begun to establish similar molecular mechanisms that regulate secretion in cells as different as yeasts and mammalian neurons.

Yeasts are advantageous in studying the secretory pathway because they are readily amenable to genetic analysis. In particular, Randy Schekman and his colleagues, in 1979, pioneered the isolation of yeast mutants defective in vesicular transport. These include mutants that are defective at various stages of protein secretion (sec mutants), mutants that are unable to transport proteins to the vacuole, and mutants that are unable to retain resident ER proteins. The isolation of such mutants in yeasts led directly to the molecular cloning and analysis of the corresponding genes, thereby identifying a number of proteins involved in various steps of the secretory pathway.

Biochemical studies of vesicular transport using reconstituted systems have complemented these genetic studies and enabled the direct isolation of transport proteins from mammalian cells. The first cell-free transport system was developed in 1980 by James Rothman and colleagues, who analyzed protein transport between compartments of the Golgi apparatus. Similar reconstituted systems have been developed to analyze transport between other compartments, including transport from the ER to the Golgi and transport from the Golgi to secretory vesicles, vacuoles, and the plasma membrane. The development of these in vitro systems has enabled biochemical studies of the transport process and functional analysis of proteins identified by mutations in yeasts, as well as direct isolation of some of the proteins involved in vesicle budding and fusion.
Insights into the molecular mechanisms of vesicular transport have come from studies of synaptic transmission in neurons, a specialized form of regulated secretion. A synapse is the junction of a neuron with another cell, which may be either another neuron or an effector, such as a muscle cell. Information is transmitted across the synapse by chemical neurotransmitters, such as acetylcholine, which are stored within the neuron in synaptic vesicles. Stimulation of the transmitting neuron triggers the fusion of these synaptic vesicles with the plasma membrane causing neurotransmitters to be released into the synapse and stimulating the postsynaptic neuron or effector cell. Synaptic vesicles are extremely abundant in the brain, allowing them to be purified in large amounts for biochemical analysis. Some of the proteins isolated from synaptic vesicles are closely related to proteins that have been shown to play critical roles in vesicular transport by yeast genetics and reconstitution experiments, so biochemical analysis of these proteins has provided important insights into the molecular mechanisms of vesicle fusion.

Recent studies using GFP fusion proteins have allowed transport vesicles carrying specific proteins to be visualized by immunofluorescence as they move through the secretory pathway. In these experiments, cells are transfected with cDNA constructs encoding secretory proteins tagged with green fluorescent protein (GFP) (see Figure 1.28). The progress of the GFP-labeled proteins through the secretory pathway can then be followed in living cells, allowing characterization of many aspects of the dynamics and molecular interactions involved in vesicular transport.

Finally, recent studies have used large-scale proteomic analysis to characterize the proteins present in specific compartments of the secretory pathway. For example, isolation of secretory compartments such as the transitional ER or the trans Golgi network, followed by analysis of their total protein content by mass spectrometry (see Figure 2.31), has allowed the identification of many new proteins involved in cargo selection and packaging.

Cargo selection, coat proteins, and vesicle budding

Transport vesicles that carry secretory proteins from the ER to subsequent compartments are coated with cytosolic coat proteins and thus are called coated vesicles. Initially, the secretory proteins are sorted from proteins targeted for other destinations and from proteins that need to remain behind (see Figure 10.24). The coats assemble as the secretory protein-containing vesicle buds off the donor membrane and are generally removed from the vesicle in the cytosol before it reaches its target (Figure 10.34). Some

FIGURE 10.34 Formation and fusion of a transport vesicle
Membrane proteins and lumenal secretory proteins with their receptors are collected into selected regions of a donor membrane where the formation of a cytosolic coat results in the budding of a transport vesicle. The vesicle is transported by motor proteins along microtubules to its target. During transport the coat is disassembled, and the transport vesicle docks and fuses with the target membrane.
of the remaining proteins allow the vesicles to travel along microtubules to their targets by interacting with specific tubulin-based molecular motors as described in Chapter 12. At the target membrane, the vesicles dock and fuse with the membrane, emptying their lumenal cargo and inserting their membrane proteins into the target membrane.

Three families of vesicle coat proteins have been characterized: clathrin, COPI, and COPII (COP indicates coat protein). Vesicles coated with these different proteins are involved in transport between different compartments of the secretory pathway (Figure 10.35). COPII-coated vesicles carry proteins from the ER to the ER-Golgi intermediate compartment (ERGIC) and on to the Golgi apparatus, budding from the transitional ER and carrying their cargo forward along the secretory pathway. In contrast, COPI-coated vesicles bud from the ERGIC or the Golgi apparatus and carry their cargo backwards, returning resident proteins to earlier compartments of the secretory pathway. Thus, COPI is the coat protein on the retrieval vesicles that return resident ER proteins back to the ER from the ERGIC or the cis Golgi network, and on the vesicles that retrieve Golgi processing enzymes back from the later Golgi.

**FIGURE 10.35** Transport by coated vesicles COPII-coated vesicles carry cargo from the ER to the Golgi, and clathrin-coated vesicles carry cargo outward from the trans Golgi network. Secretory molecules move through the Golgi in the maturing cisternae. Clathrin-coated vesicles also carry cargo back from the plasma membrane to endosomes and other organelles such as the trans Golgi network and lysosomes. COPI-coated vesicles retrieve ER-resident proteins from the ERGIC and cis Golgi and carry Golgi-resident enzymes back from the trans Golgi to earlier Golgi cisternae.
compartments. Finally, **clathrin-coated vesicles** are responsible for transport in both directions between the \textit{trans} Golgi network, endosomes, lysosomes, and the plasma membrane.

The formation of coated vesicles is regulated by small GTP-binding proteins (ARF1 and Sar1) related to Ras and Ran. ARF1 functions in the formation of COPI- and clathrin-coated vesicles budding from the Golgi apparatus and Sar1 in the formation of COPII-coated vesicles budding from the ER. These GTP-binding proteins recruit adaptor proteins that mediate vesicle assembly by interacting both with cargo proteins and with vesicle coat proteins. For example, **Figure 10.36** illustrates the role of ARF1 in the assembly of clathrin-coated vesicles. First, ARF1/GDP binds to proteins in the Golgi membrane, where it is converted to the active GTP-bound form.

**FIGURE 10.36 Formation of a clathrin-coated vesicle**  After being delivered to the \textit{trans} Golgi membrane, ARF/GDP is activated to ARF/GTP by a guanine nucleotide exchange factor (ARF-GEF). ARF/GTP recruits a GGA adaptor protein, which binds to the cytosolic tail of a transmembrane receptor with its lumenal cargo. GGA also recruits a second adaptor protein, AP1, which serves as a binding site for assembly of a clathrin coat. Clathrin consists of three protein chains that associate with each other to form a basketlike lattice that distorts the membrane and drives vesicle budding.
by a guanine nucleotide exchange factor in the membrane. ARF1/GTP then
initiates the budding process by recruiting one adaptor protein that binds
to a transmembrane receptor associated with a luminal cargo protein and a
second adaptor protein that binds clathrin. Clathrin plays a structural role in
vesicle budding by assembling into a basketlike lattice structure that distorts
the membrane and initiates the bud. During transport, the GTP bound to ARF1
is hydrolyzed to GDP and the ARF1/GDP is released from the membrane
to recycle. Loss of ARF1 and the action of uncoating enzymes weakens the
binding of the clathrin coat complex, allowing chaperone proteins in the
cytoplasm to dissociate most of the coat from the vesicle membrane (see
Figure 10.34).

**Vesicle fusion**

The fusion of a transport vesicle with its target involves two types of events.
First, the transport vesicle must recognize the correct target membrane; for
example, a vesicle carrying lysosomal enzymes has to deliver its cargo only
to lysosomes. Second, the vesicle and target membranes must fuse, delivering
the contents of the vesicle to the target organelle. Research over the last
several years has led to the development of a model of vesicle fusion in which
specific recognition between a vesicle and its target (tethering) is mediated
by interactions between proteins on the vesicle and the target membranes,
followed by fusion between the phospholipid bilayers.

Proteins involved in vesicle fusion were initially identified in James Roth-
man’s laboratory by biochemical analysis of reconstituted vesicular transport
systems from mammalian cells. Analysis of the proteins involved in vesicle
fusion in these systems led Rothman and his colleagues to hypothesize that
vesicle fusion is mediated by interactions between specific pairs of trans-
membrane proteins, called **SNAREs**, on the vesicle and target membranes
(v-SNAREs and t-SNAREs, respectively). The formation of complexes be-
tween v-SNAREs and t-SNAREs was proposed to lead to membrane fusion.
This hypothesis was supported by the identification of SNAREs that were
present on synaptic vesicles and by the finding of yeast secretion mutants
that appeared to encode SNAREs required for a variety of vesicle transport
events. For example, transport from the ER to the Golgi in yeast requires
specific SNAREs that are located on both the vesicle and target membranes.

Subsequent research has confirmed that SNAREs are required for vesicle
fusion with a target membrane and that SNARE-SNARE pairing provides
the energy to bring the two bilayers sufficiently close to destabilize them
and fuse. However, the docking, tethering, and fusion of transport vesicles
to specific target membranes is also mediated by small-GTP binding proteins
(Rab proteins) that play a key role in the docking of transport vesicles. More
than 60 different Rab proteins have been identified and shown to function
in specific vesicle transport processes (**Table 10.1**). They function in many
steps of vesicle trafficking, including interacting with SNAREs to regulate
and facilitate the formation of SNARE-SNARE complexes. Individual Rab
proteins or combinations of Rab proteins mark different organelles and
transport vesicles, so their localization on the correct membrane is key to
establishing the specificity of vesicular transport.

To initiate transport vesicle fusion, Rab/GTP on the transport vesicle
interacts with effector proteins and v-SNAREs to assemble a pre-fusion com-
plex (**Figure 10.37**). A different Rab protein on the target membrane similarly
organizes other effector proteins and t-SNAREs. When the transport vesicle
encounters this target membrane, the effector proteins link the membranes
by protein-protein interactions. This tethering of the vesicle to the target

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**Griscelli syndrome** is a rare disease caused by mutations in the gene encoding Rab27a. Rab27a appears to play a key role in the transport of pigment-containing vesicles (melanosomes) to the skin and hair and in the exocytosis of vesicles in T lymphocytes. Patients with Griscelli syndrome exhibit partial albinism (lack of pigment) and are immunodeficient.
membrane stimulates Rab/GTP hydrolysis and allows the v-SNAREs to contact the t-SNAREs. All SNARE proteins have a long central coiled-coil domain like that found in nuclear lamins (see Figure 9.4). As in the lamins, this domain binds strongly to other coiled-coil domains and, in effect, zips the SNAREs together, bringing the two membranes into nearly direct contact. The simplest hypothesis is that this creates instability in the lipid bilayers and they fuse. Following membrane fusion, a protein complex (called the NSF/SNAP complex) disassembles the SNAREs, allowing the SNAREs to be reused for subsequent rounds of vesicle transport. As the energy of SNARE-SNARE interaction drives the fusion of the membranes, energy from hydrolysis of ATP is required to separate the SNAREs.

Membrane fusion is a general process that occurs whenever a transport vesicle fuses with a target membrane. However, specific types of fusion may involve specialized sites on the plasma membrane. One of these is exocytosis, the fusion of a transport vesicle with the plasma membrane, resulting in secretion of the vesicle contents. Many types of exocytosis occur at specific protein complexes, called exocysts, on the plasma membrane. This eight-protein complex is formed by interactions between proteins on

### TABLE 10.1 Rab GTP-Binding Proteins and Their Sites of Action

<table>
<thead>
<tr>
<th>Transport step</th>
<th>Rab proteins involved</th>
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<tbody>
<tr>
<td><strong>Exocytosis</strong></td>
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<tr>
<td>Transitional ER to Golgi</td>
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<tr>
<td>Golgi back to ER</td>
<td>Rab6, Rab6b</td>
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<tr>
<td>Intra-Golgi</td>
<td>Rab1, Rab6, Rab8b</td>
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<td>trans Golgi network to plasma membrane</td>
<td>Rab11a, Rab11b</td>
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<td><strong>Endocytosis</strong></td>
<td></td>
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<tr>
<td>Plasma membrane to early endosome</td>
<td>Rab5a, Rab5b, Rab5c</td>
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<tr>
<td>Early endosome to plasma membrane</td>
<td>Rab4, Rab15, Rab18</td>
</tr>
<tr>
<td>Early endosome to late endosome</td>
<td>Rab7</td>
</tr>
<tr>
<td><strong>Special roles</strong></td>
<td></td>
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<tr>
<td>Exocytosis of secretory granules</td>
<td>Rab8b</td>
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<tr>
<td>Late endosome to trans Golgi network</td>
<td>Rab9, Rab11a, Rab11b</td>
</tr>
<tr>
<td>trans Golgi network to basolateral membrane</td>
<td>Rab8a</td>
</tr>
<tr>
<td>trans Golgi network to apical membrane</td>
<td>Rab21</td>
</tr>
</tbody>
</table>

Examples of the more than 60 mammalian Rab proteins whose locations and presumptive functions are known.

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both the transport vesicle and the target membrane site, regulated by Rab proteins on the vesicle membrane (Figure 10.38). Interaction of these proteins results in efficient targeting of the transport vesicle to a specific location on the plasma membrane.

**Lysosomes**

Lysosomes are membrane-enclosed organelles that contain an array of enzymes capable of breaking down all types of biological polymers—proteins, nucleic acids, carbohydrates, and lipids. Lysosomes function as the digestive system of the cell, serving both to degrade material taken up from outside the cell and to digest obsolete components of the cell itself. In their simplest form, lysosomes are visualized as dense spherical vacuoles, but they can display considerable variation in size and shape as a result of differences in the materials that have been taken up for digestion (Figure 10.39). Lysosomes thus represent morphologically diverse organelles defined by the common function of degrading intracellular material.

**Lysosomal acid hydrolases**

Lysosomes contain about 50 different degradative enzymes that can hydrolyze proteins, DNA, RNA, polysaccharides, and lipids. Mutations in the genes that encode these enzymes are responsible for more than 30 different human genetic diseases, which are called lysosomal storage diseases because undegraded material accumulates within the lysosomes of affected individuals. Most of these diseases result from deficiencies in single lysosomal enzymes.

**FIGURE 10.38 Exocyst assembly and vesicle targeting** Exocysts are complexes of eight different proteins formed during exocytosis from proteins present on both secretory vesicles and specific regions of the plasma membrane. A Rab-family GTP-binding protein regulates assembly of the exocyst complex. Tethering and docking at exocysts results in normal SNARE-mediated membrane fusion.

**FIGURE 10.39 Electron micrograph of lysosomes and mitochondria in a mammalian cell** Lysosomes are indicated by arrows. Notice the variation in size and shape, determined by differences in the materials taken up for digestion.
**The Disease**

Gaucher disease is the most common of the lysosomal storage diseases, which are caused by a failure of lysosomes to degrade substances that they normally break down. The resulting accumulation of nondegraded compounds leads to an increase in the size and number of lysosomes within the cell, eventually resulting in cellular malfunction and pathological consequences to affected organs. Gaucher disease is found primarily in the Jewish population, where it has a frequency of about 1 in 2500 individuals. There are three types of Gaucher disease, which differ in severity and nervous system involvement. In the most common form of the disease (type I), the nervous system is not involved; the disease is manifest as spleen and liver enlargement and development of bone lesions. Many patients with this form of the disease have no serious symptoms, and their life span is unaffected. The more severe forms of the disease (types II and III) are much rarer and found in both Jewish and non-Jewish populations. The most devastating is type II disease, in which extensive neurological involvement is evident in infancy, and patients die early in life. Type III disease, intermediate in severity between types I and II, is characterized by the onset of neurological symptoms (including dementia and spasticity) by about age ten.

**Molecular and Cellular Basis**

Gaucher disease is caused by a deficiency of the lysosomal enzyme glucocerebrosidase, which catalyzes the hydrolysis of glucocerebroside to glucose and ceramide (see figure). This enzyme deficiency was demonstrated in 1965, and the responsible gene was cloned in 1985. Since then more than 30 different mutations responsible for Gaucher disease have been identified. Interestingly, the severity of the disease can be largely predicted from the nature of these mutations. For example, patients with a mutation leading to the relatively conservative amino acid substitution of serine for asparagine have type I disease; patients with a mutation leading to substitution of proline for leucine have more severe enzyme deficiencies and develop either type II or III disease.

The enzyme deficiency in Gaucher disease prevents the hydrolysis of glucocerebroside to glucose and ceramide.

Except for the very rare type II and III forms of the disease, the only cells affected in Gaucher disease are macrophages. Because their function is to eliminate aged and damaged cells by phagocytosis, macrophages continually ingest large amounts of lipids, which are normally degraded in lysosomes. Deficiencies of glucocerebrosidase are therefore particularly evident in macrophages of both the spleen and the liver, consistent with these organs being the primary sites affected in most cases of Gaucher disease.

**Prevention and Treatment**

Gaucher disease is a prime example of a disease that can be treated by enzyme replacement therapy in which exogenous administration of an enzyme is used to correct an enzyme defect. This approach to treatment of lysosomal storage diseases was suggested by Christian de Duve in the 1960s, based on the idea that exogenously administered enzymes might be taken up by endocytosis and transported to lysosomes. In type I Gaucher disease, this approach is particularly attractive because the single target cell is the macrophage. In the 1970s it was discovered that macrophages express cell surface receptors that bind mannose residues on extracellular glycoproteins and then internalize these proteins by endocytosis. This finding suggested that exogenously administered glucocerebrosidase could be specifically targeted to macrophages by modifications that would expose mannose residues. Enzyme prepared from human placenta was appropriately modified, and clinical studies have clearly demonstrated its effectiveness in the treatment of Gaucher disease.

Unfortunately, enzyme replacement therapy for Gaucher disease is expensive. The expense of this treatment puts it far beyond the resources of individual patients and has raised a number of societal issues concerning the cost of expensive drugs for the treatment of rare disorders.

**References**


Lysosomes contain a variety of acid hydrolases that are active at the acidic pH maintained within the lysosome but not at the neutral pH of the cytosol. The acidic internal pH of lysosomes results from the action of a proton pump in the lysosomal membrane, which imports protons from the cytosol coupled to ATP hydrolysis. This pumping requires expenditure of energy in the form of ATP hydrolysis in order to maintain approximately a hundredfold higher H+ concentration inside the lysosome than in the cytosol.

**Endocytosis and lysosome formation**

One of the major functions of lysosomes is the digestion of material taken up from outside the cell by endocytosis, which is discussed in detail in Chapter 13. However, this role of lysosomes relates not only to their function but also to their formation. In particular, lysosomes are formed when transport vesicles from the *trans* Golgi network fuse with a late endosome, which contains molecules taken up by endocytosis at the plasma membrane.

Endosomes represent an intersection between the secretory pathway through which lysosomal proteins and other secreted molecules can be processed, and the endocytic pathway through which extracellular molecules are taken up at the cell surface (Figure 10.41). As noted earlier in this chapter, animal cells have three types of endosomes: early endosomes, recycling endosomes, and late endosomes. Early endosomes are located close to the plasma membrane and receive endocytic vesicles directly from the plasma membrane. They separate molecules targeted for recycling back to the plasma membrane from those destined for degradation in lysosomes. The molecules to be recycled (for example, cell surface receptors, as discussed in Chapter 13) are then passed to recycling endosomes and back to the plasma membrane. In contrast, molecules destined for degradation are transported to late endosomes, which receive lysosomal enzymes from the *trans* Golgi network and either fuse with or mature into lysosomes.

An important change during the early to late endosome transition is the lowering of the internal pH to about 5.5, which plays a key role in the delivery of lysosomal acid hydrolases from the *trans* Golgi network. As discussed earlier, acid hydrolases are targeted to late endosomes by mannose-6-phosphate residues, which are recognized by mannose-6-phosphate receptors in the *trans* Golgi network and packaged into clathrin-coated vesicles. Following removal of the clathrin coat, these transport vesicles fuse with late endosomes, and the acidic internal pH causes the hydrolases to dissociate from the mannose-6-phosphate receptor (see Figure 10.41). The hydrolases are thus released into the lumen of the endosome, while the receptors remain in the membrane and are eventually recycled back to the Golgi. With a full complement of acid hydrolases, late endosomes fuse with or mature into lysosomes, which digest the molecules originally taken up by endocytosis.

For example, Gaucher disease (the most common of these disorders) results from a mutation in the gene that encodes a lysosomal enzyme required for the breakdown of glycolipids. An intriguing exception is I-cell disease, which is caused by a deficiency in the enzyme that catalyzes the first step in the tagging of lysosomal enzymes with mannose-6-phosphate in the Golgi apparatus (see Figure 10.29). The result is a general failure of lysosomal enzymes to be incorporated into lysosomes.

Most lysosomal enzymes are acid hydrolases, which are active at the acidic pH (about 5) that is maintained within lysosomes but not at the neutral pH (about 7.2) characteristic of the rest of the cytoplasm (Figure 10.40). The requirement of these lysosomal hydrolases for acidic pH provides double protection against uncontrolled digestion of the contents of the cytosol; even if the lysosomal membrane were to break down, the released acid hydrolases would be inactive at the neutral pH of the cytosol. To maintain their acidic internal pH, lysosomes must actively concentrate H+ ions (protons). This is accomplished by a proton pump in the lysosomal membrane, which actively transports protons into the lysosome from the cytosol. This pumping requires expenditure of energy.
FIGURE 10.41 Endocytosis and lysosome formation  Molecules are taken up from outside the cell in endocytic vesicles, which fuse with early endosomes. Membrane receptors are recycled to the plasma membrane through recycling endosomes. Early endosomes can mature into late endosomes. Transport vesicles carrying acid hydrolases from the trans Golgi network then fuse with late endosomes, which mature into lysosomes as they acquire a full complement of lysosomal enzymes. The acid hydrolases dissociate from the mannose-6-phosphate receptors when the transport vesicles fuse with late endosomes, and the mannose-6-phosphate receptors are recycled back to the trans Golgi network.
Phagocytosis and autophagy

In addition to degrading molecules taken up by endocytosis, lysosomes digest material derived from two other routes: phagocytosis (discussed in Chapter 13) and autophagy (discussed in Chapter 8) (Figure 10.42). In phagocytosis, specialized cells, such as macrophages, take up and degrade large particles, including bacteria, cell debris, and aged cells that need to be eliminated from the body. Such large particles are taken up in phagocytic vacuoles (phagosomes), which then fuse with lysosomes, resulting in digestion of their contents. The lysosomes formed in this way (phagolysosomes) can be quite large and heterogeneous since their size and shape is determined by the content of material that is being digested.

Lysosomes are also responsible for autophagy, the turnover of the cell’s own components (see Figure 8.46). In contrast to phagocytosis, autophagy is a function of all cells and is critical at certain stages of embryonic development as well as playing an important role in programmed cell death (see Chapter 17). The first step in autophagy appears to be the enclosure of a small area of cytoplasm or a cytoplasmic organelle (e.g., a mitochondrion) in a cytosolic membrane. The resulting vesicle (an autophagosome) then fuses with a lysosome, and its contents are digested (see Figure 10.42).
The Endoplasmic Reticulum

The endoplasmic reticulum and protein secretion: The endoplasmic reticulum is the first branch point in protein sorting. In mammalian cells, proteins destined for secretion, lysosomes, or the plasma membrane are translated on membrane-bound ribosomes and transferred into the rough ER as their translation proceeds.

Targeting proteins to the endoplasmic reticulum: Proteins can be targeted to the ER either while their translation is still in progress or following completion of translation in the cytosol. In mammalian cells, most proteins are translocated into the ER while they are being translated on membrane-bound ribosomes. Ribosomes engaged in the synthesis of secreted proteins are targeted to the endoplasmic reticulum by signal sequences at the amino terminus of the polypeptide chain. Growing polypeptide chains are then translocated into the ER through protein channels and released into the ER lumen by cleavage of the signal sequence.

See Website Animation 10.1

Insertion of proteins into the ER membrane: Integral membrane proteins of the plasma membrane or the membranes of the ER, Golgi apparatus, and lysosomes are initially inserted into the membrane of the ER. Rather than being translocated into the ER lumen, these proteins are anchored by membrane-spanning α helices that stop the transfer of the growing polypeptide chain across the membrane.

Protein folding and processing in the ER: Polypeptide chains are folded into their correct three-dimensional conformations within the ER. The ER is also the site of N-linked glycosylation and addition of GPI anchors.

Quality control in the ER: Many secretory proteins are not folded correctly the first time. Chaperones detect incorrectly folded proteins and recycle them through the folding pathway. Those proteins that cannot be correctly folded are diverted from the secretory pathway and marked for degradation. The unfolded protein response pathway monitors the amount of unfolded protein in the ER and regulates cellular activities to maintain appropriate protein folding capacity.

The smooth ER and lipid synthesis: The ER is the major site of lipid synthesis in eukaryotic cells, and the smooth ER is abundant in cells that are active in lipid metabolism and detoxification of lipid-soluble drugs.

Export of proteins and lipids from the ER: Proteins and lipids are transported in vesicles from the ER to the Golgi apparatus. Targeting sequences mediate the selective packaging of exported proteins into vesicles that transport them to the ERGIC and onto the Golgi. Resident ER proteins are marked by other targeting sequences that signal their return from the Golgi to the ER by a recycling pathway.

The Golgi Apparatus

Organization of the Golgi: The Golgi apparatus functions in protein processing and sorting as well as in the synthesis of lipids and polysaccharides. Proteins are transported from the endoplasmic reticulum to the cis Golgi network. Cisternae from the cis Golgi mature into the Golgi stack, which represents the site of most metabolic activities of the Golgi apparatus. The Golgi stack cisternae with their modified proteins mature into the trans Golgi network, where proteins are sorted and packaged in vesicles for transport to endosomes, the plasma membrane, or the exterior of the cell.

See Website Animation 10.2

SUMMARY

KEY TERMS

endoplasmic reticulum (ER), rough ER, transitional ER, smooth ER, secretory vesicle, secretory pathway

signal sequence, microsome, signal recognition particle (SRP), SRP RNA, SRP receptor, translocon, signal peptidase

protein disulfide isomerase (PDI), glycosylphosphatidylinositol (GPI) anchor

ER-associated degradation (ERAD), unfolded protein response (UPR)

flippase

Golgi apparatus, Golgi complex, cis Golgi network, Golgi stack, trans Golgi network

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SUMMARY

Protein glycosylation within the Golgi: The N-linked oligosaccharides added to proteins in the ER are modified within the Golgi. Those proteins destined for lysosomes are specifically phosphorylated on mannose residues, and mannose-6-phosphate serves as a targeting signal that directs their transport to lysosomes from the trans Golgi network. O-linked glycosylation also takes place within the Golgi.

Lipid and polysaccharide metabolism in the Golgi: The Golgi apparatus is the site of synthesis of glycolipids, sphingomyelin, and the complex polysaccharides of plant cell walls.

Protein sorting and export from the Golgi apparatus: Proteins are sorted in the trans Golgi network for packaging into transport vesicles targeted for secretion, the plasma membrane, endosomes, lysosomes, or yeast and plant vacuoles. In polarized cells, proteins are specifically targeted to the apical and basolateral domains of the plasma membrane.

The Mechanism of Vesicular Transport

Experimental approaches to understanding vesicular transport: The mechanism of vesicular transport has been elucidated through studies of yeast mutants, reconstituted cell-free systems, synaptic vesicles, visualization of protein traffic through the secretory pathway, and proteomic analysis.

Cargo selection, coat proteins, and vesicle budding: The cytoplasmic surfaces of vesicles are coated with proteins that drive vesicle budding. The specific molecules to be transported are selected by complexes of small GTP-binding proteins and adaptor proteins that associate with the coat proteins.

Vesicle fusion: Vesicle binding to the correct target membrane is mediated by interactions between pairs of transmembrane proteins, which lead to membrane fusion. Some types of fusion with the plasma membrane (exocytosis) occur at specific multiprotein complexes called exocysts.

Lysosomes

Lysosomal acid hydrolases: Lysosomes contain an array of acid hydrolases that degrade proteins, nucleic acids, polysaccharides, and lipids. These enzymes function specifically at the acidic pH maintained within lysosomes.

Endocytosis and lysosome formation: Extracellular molecules taken up by endocytosis are transported to early endosomes, which can mature to lysosomes as lysosomal acid hydrolases are delivered from the Golgi.

Phagocytosis and autophagy: Lysosomes are responsible for the degradation of large particles taken up by phagocytosis and for the digestion of the cell’s own components by autophagy.

KEY TERMS

mannose-6-phosphate, signal patch

apical domain, basolateral domain, vacuole

synaptic vesicle

cloathrin, COPI, COPII, COP-coated vesicle, clathrin-coated vesicle

SNARE, Rab, exocyst

lysosome, lysosomal storage disease

endocytosis, endosome

phagocytosis, phagosome, phagolysosome, autophagy, autophagosome

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Questions

1. What was the original experimental evidence for the secretory pathway from rough ER → Golgi apparatus → secretory vesicles → secreted protein?

2. How did in vitro translation of mRNAs provide evidence for the existence of a signal sequence that targets secretory proteins to the rough endoplasmic reticulum?

3. Compare and contrast cotranslational and posttranslational translocation of polypeptide chains into the endoplasmic reticulum.

4. Sec61 is a critical component of the protein channel through the ER membrane. In Sec61 mutant yeast, what is the fate of proteins that are normally localized to the Golgi apparatus?

5. Why are the carbohydrate groups of glycoproteins always exposed on the surface of the cell?

6. What would be the effect of mutating the KDEL sequence of a resident ER protein like BiP? Would this effect be similar or different from that of mutating the KDEL receptor protein?

7. How is a lysosomal protein targeted to a lysosome? What effect would the addition of a lysosome-targeting signal patch have on the subcellular localization of a protein that is normally cytosolic? How would it affect localization of a protein that is normally secreted?

8. What is the predicted fate of lysosomal acid hydrolases in I-cell disease in which cells are deficient in the enzyme required for formation of mannose-6-phosphate residues?

9. What processes result in glycolipids and sphingomyelin being found in the outer—but not the inner—half of the plasma membrane bilayer?

10. A patient comes to your clinic with an accumulation of glucocerebrosides in macrophage lysosomes. What is your diagnosis, and what therapy would you suggest if price is not a limiting factor?

11. Lysosomes contain powerful hydrolytic enzymes, which are transported there from the site of their synthesis in the ER via the Golgi apparatus. Why don’t these enzymes damage the constituents of these organelles?

12. What is the source of energy for fusion between target and vesicle membranes?

References and Further Reading

The Endoplasmic Reticulum


The Mechanism of Vesicular Transport


Lyosomes


