CHAPTER 12

Intracellular Compartments and Protein Sorting

IN THIS CHAPTER

Unlike a bacterium, which generally consists of a single intracellular compartment surrounded by a plasma membrane, a eukaryotic cell is elaborately subdivided into functionally distinct, membrane-enclosed compartments. Each compartment, or **organelle**, contains its own characteristic set of enzymes and other specialized molecules, and complex distribution systems transport specific products from one compartment to another. To understand the eukaryotic cell, it is essential to know how the cell creates and maintains these compartments, what occurs in each of them, and how molecules move between them.

Proteins confer upon each compartment its characteristic structural and functional properties. They catalyze the reactions that occur there and selectively transport small molecules into and out of the compartment. For membrane-enclosed organelles in the cytoplasm, proteins also serve as organelle-specific surface markers that direct new deliveries of proteins and lipids to the appropriate organelle.

An animal cell contains about 10 billion (10^{10}) protein molecules of perhaps 10,000 kinds, and the synthesis of almost all of them begins in the **cytosol**, the space of the cytoplasm outside the membrane-enclosed organelles. Each newly synthesized protein is then delivered specifically to the organelle that requires it. The intracellular transport of proteins is the central theme of both this chapter and the next. By tracing the protein traffic from one compartment to another, one can begin to make sense of the otherwise bewildering maze of intracellular membranes.

THE COMPARTMENTALIZATION OF CELLS

In this brief overview of the compartments of the cell and the relationships between them, we organize the organelles conceptually into a small number of discrete families, discuss how proteins are directed to specific organelles, and explain how proteins cross organelle membranes.

All Eukaryotic Cells Have the Same Basic Set of Membrane-enclosed Organelles

Many vital biochemical processes take place in membranes or on their surfaces. Membrane-bound enzymes, for example, catalyze lipid metabolism; and oxidative phosphorylation and photosynthesis both require a membrane to couple the transport of H^+ to the synthesis of ATP. In addition to providing increased membrane area to host biochemical reactions, intracellular membrane systems form enclosed compartments that are separate from the cytosol, thus creating functionally specialized aqueous spaces within the cell. In these spaces, subsets of molecules (proteins, reactants, ions) are concentrated to optimize the biochemical reactions in which they participate. Because the lipid bilayer of cell membranes is impermeable to most hydrophilic molecules, the membrane of an organelle must contain membrane transport proteins to import and export specific metabolites. Each organelle membrane must also have a mechanism for importing, and incorporating into the organelle, the specific proteins that make the organelle unique.

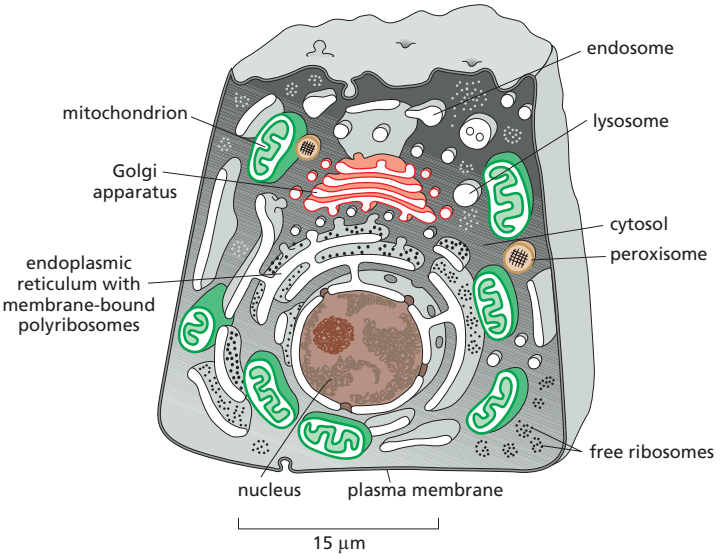


Figure 12–1 The major intracellular compartments of an animal cell. The cytosol *(gray),* endoplasmic reticulum, Golgi apparatus, nucleus, mitochondrion, endosome, lysosome, and peroxisome are distinct compartments isolated from the rest of the cell by at least one selectively permeable membrane (see Movie 9.2).

Figure 12–1 illustrates the major intracellular compartments common to eukaryotic cells. The *nucleus* contains the genome (aside from mitochondrial and chloroplast DNA), and it is the principal site of DNA and RNA synthesis. The surrounding **cytoplasm** consists of the cytosol and the cytoplasmic organelles suspended in it. The cytosol constitutes a little more than half the total volume of the cell, and it is the main site of protein synthesis and degradation. It also performs most of the cell's *intermediary metabolism*—that is, the many reactions that degrade some small molecules and synthesize others to provide the building blocks for macromolecules (discussed in Chapter 2).

About half the total area of membrane in a eukaryotic cell encloses the labyrinthine spaces of the *endoplasmic reticulum* (*ER*). The *rough ER* has many ribosomes bound to its cytosolic surface. Ribosomes are organelles that are not membrane-enclosed; they synthesize both soluble and integral membrane proteins, most of which are destined either for secretion to the cell exterior or for other organelles. We shall see that, whereas proteins are transported into other membrane-enclosed organelles only after their synthesis is complete, they are transported into the ER as they are synthesized. This explains why the ER membrane is unique in having ribosomes tethered to it. The ER also produces most of the lipid for the rest of the cell and functions as a store for Ca^{2+} ions. Regions of the ER that lack bound ribosomes are called *smooth ER*. The ER sends many of its proteins and lipids to the *Golgi apparatus*, which often consists of organized stacks of disc-like compartments called *Golgi cisternae*. The Golgi apparatus receives lipids and proteins from the ER and dispatches them to various destinations, usually covalently modifying them *en route*.

Mitochondria and *chloroplasts* generate most of the ATP that cells use to drive reactions requiring an input of free energy; chloroplasts are a specialized version of *plastids* (present in plants, algae, and some protozoa), which can also have other functions, such as the storage of food or pigment molecules. *Lysosomes* contain digestive enzymes that degrade defunct intracellular organelles, as well as macromolecules and particles taken in from outside the cell by endocytosis. On the way to lysosomes, endocytosed material must first pass through a series of organelles called *endosomes*. Finally, *peroxisomes* are small vesicular compartments that contain enzymes used in various oxidative reactions.

In general, each membrane-enclosed organelle performs the same set of basic functions in all cell types. But to serve the specialized functions of cells, these organelles vary in abundance and can have additional properties that differ from cell type to cell type.

On average, the membrane-enclosed compartments together occupy nearly half the volume of a cell (**Table 12–1**), and a large amount of intracellular membrane is required to make them. In liver and pancreatic cells, for example, the

endoplasmic reticulum has a total membrane surface area that is, respectively, 25 times and 12 times that of the plasma membrane (**Table 12–2**). The membrane-enclosed organelles are packed tightly in the cytoplasm, and, in terms of area and mass, the plasma membrane is only a minor membrane in most eukaryotic cells (**Figure 12–2**).

The abundance and shape of membrane-enclosed organelles are regulated to meet the needs of the cell. This is particularly apparent in cells that are highly specialized and therefore disproportionately rely on specific organelles. Plasma cells, for example, which secrete their own weight every day in antibody molecules into the bloodstream, contain vastly amplified amounts of rough ER, which is found in large, flat sheets. Cells that specialize in lipid synthesis also expand their ER, but in this case the organelle forms a network of convoluted tubules. Moreover, membrane-enclosed organelles are often found in characteristic positions in the cytoplasm. In most cells, for example, the Golgi apparatus is located close to the nucleus, whereas the network of ER tubules extends from the nucleus throughout the entire cytosol. These characteristic distributions depend on interactions of the organelles with the cytoskeleton. The localization of both the ER and the Golgi apparatus, for instance, depends on an intact microtubule array; if the microtubules are experimentally depolymerized with a drug, the Golgi apparatus fragments and disperses throughout the cell, and the ER network collapses toward the cell center (discussed in Chapter 16). The size, shape, composition, and location are all important and regulated features of these organelles that ultimately contribute to the organelle's function.

TABLE 12–1 Relative Volumes Occupied by the Major Intracellular Compartments in a Liver Cell (Hepatocyte)

Intracellular compartment	Percentage of total cell volume
Cytosol	54
Mitochondria	22
Rough ER cisternae	9
Smooth ER cisternae plus Golgi cisternae	6
Nucleus	6
Peroxisomes	1
Lysosomes	1
Endosomes	1

Evolutionary Origins May Help Explain the Topological Relationships of Organelles

To understand the relationships between the compartments of the cell, it is helpful to consider how they might have evolved. The precursors of the first eukaryotic cells are thought to have been relatively simple cells that—like most bacterial and

TABLE 12–2 Relative Amounts of Membrane Types in Two Kinds of Eukaryotic Cells

Membrane Type	Percentage of total cell membrane	
	Liver hepatocyte*	Pancreatic exocrine cell*
Plasma membrane	2	5
Rough ER membrane	35	60
Smooth ER membrane	16	<1
Golgi apparatus membrane	7	10
Mitochondria Outer membrane Inner membrane	 7 32	 4 17
Nucleus Inner membrane	 0.2	 0.7
Secretory vesicle membrane	Not determined	3
Lysosome membrane	0.4	Not determined
Peroxisome membrane	0.4	Not determined
Endosome membrane	0.4	Not determined

*These two cells are of very different sizes: the average hepatocyte has a volume of about 5000 μm^3 compared with 1000 μm^3 for the pancreatic exocrine cell. Total cell membrane areas are estimated at about 110,000 μm^2 and 13,000 μm^2, respectively.

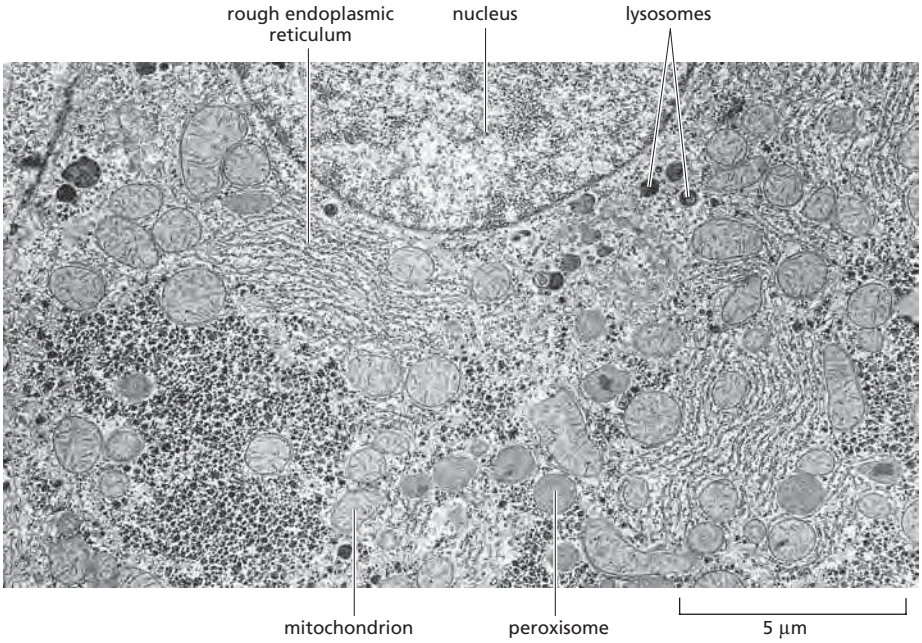


Figure 12–2 An electron micrograph of part of a liver cell seen in cross section. Examples of most of the major intracellular organelles are indicated. (Courtesy of Daniel S. Friend.)

archaeal cells—have a plasma membrane but no internal membranes. The plasma membrane in such cells provides all membrane-dependent functions, including the pumping of ions, ATP synthesis, protein secretion, and lipid synthesis. Typical present-day eukaryotic cells are 10–30 times larger in linear dimension and 1000–10,000 times greater in volume than a typical bacterium such as *E. coli*. The profusion of internal membranes can be regarded, in part, as an adaptation to this increase in size: the eukaryotic cell has a much smaller ratio of surface area to volume, and its plasma membrane therefore presumably has too small an area to sustain the many vital functions that membranes perform. The extensive internal membrane systems of a eukaryotic cell alleviate this problem.

The evolution of internal membranes evidently went hand-in-hand with the specialization of membrane function. A hypothetical scheme for how the first eukaryotic cells, with a nucleus and ER, might have evolved by the invagination and pinching off of the plasma membrane of an ancestral cell is illustrated in **Figure 12–3**.This process would create membrane-enclosed organelles with an interior or **lumen** that is topologically equivalent to the exterior of the cell. We shall see that this topological relationship holds for all of the organelles involved in the secretory and endocytic pathways, including the ER, Golgi apparatus, endosomes, lysosomes, and peroxisomes. We can therefore think of all of these organelles as members of the same topologically equivalent compartment. As we discuss in detail in the next chapter, their interiors communicate extensively with one another and with the outside of the cell via *transport vesicles*, which bud off from one organelle and fuse with another (**Figure 12–4**).

As described in Chapter 14, mitochondria and plastids differ from the other membrane-enclosed organelles because they contain their own genomes. The nature of these genomes, and the close resemblance of the proteins in these organelles to those in some present-day bacteria, strongly suggest that mitochondria and plastids evolved from bacteria that were engulfed by other cells with which they initially lived in symbiosis (see Figures 1–29 and 1–31): the inner membrane of mitochondria and plastids presumably corresponds to the original plasma membrane of the bacterium, while the lumen of these organelles evolved from the bacterial cytosol. Like the bacteria from which they were derived, both mitochondria and plastids are enclosed by a double membrane and they remain isolated from the extensive vesicular traffic that connects the interiors of most of the other membrane-enclosed organelles to each other and to the outside of the cell.

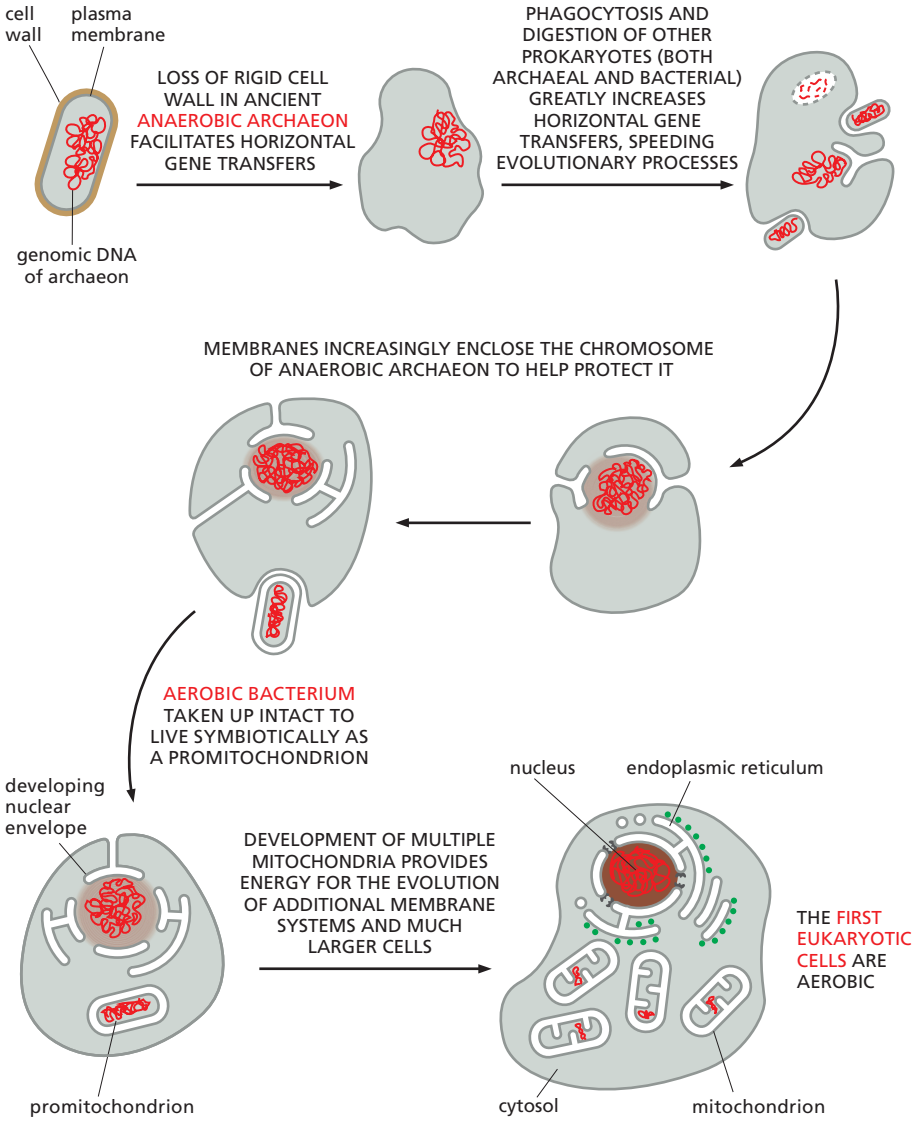


Figure 12–3 One suggested pathway for the evolution of the eukaryotic cell and its internal membranes As discussed in Chapter 1, there is evidence that the nuclear genome of a eukaryotic cell evolved from an ancient archeaon. For example, clear homologs of actin, tubulin, histones, and the nuclear DNA replication system are found in archaea, but not in bacteria. Thus, it is now thought that the first eukaryotic cells arose when an ancient anaerobic archaeon joined forces with an aerobic bacterium roughly 1.6 billion years ago. As indicated, the nuclear envelope may have originated from an invagination of the plasma membrane of this ancient archaeon—an invagination that protected its chromosome while still allowing access of the DNA to the cytosol (as required for DNA to direct protein synthesis). This envelope may have later pinched off completely from the plasma membrane, so as to produce a separate nuclear compartment surrounded by a double membrane. Because this double membrane is penetrated by nuclear pore complexes, the nuclear compartment is topologically equivalent to the cytosol. In contrast, the lumen of the ER is continuous with the space between the inner and outer nuclear membranes, and it is topologically equivalent to the extracellular space (see Figure 12–4). (Adapted from J. Martijn and T.J.G. Ettema, *Biochem. Soc. Trans.* 41: 451–457, 2013.)

The evolutionary schemes just described group the intracellular compartments in eukaryotic cells into four distinct families: (1) the nucleus and the cytosol, which communicate with each other through *nuclear pore complexes* and are thus topologically continuous (although functionally distinct); (2) all organelles that function in the secretory and endocytic pathways—including the ER, Golgi apparatus, endosomes, and lysosomes, the numerous classes of transport intermediates such as transport vesicles that move between them, and peroxisomes; (3) the mitochondria; and (4) the plastids (in plants only).

Proteins Can Move Between Compartments in Different Ways

The synthesis of all proteins begins on ribosomes in the cytosol, except for the few that are synthesized on the ribosomes of mitochondria and plastids. Their subsequent fate depends on their amino acid sequence, which can contain **sorting signals** that direct their delivery to locations outside the cytosol or to organelle surfaces. Some proteins do not have a sorting signal and consequently remain in the cytosol as permanent residents. Many others, however, have specific sorting signals that direct their transport from the cytosol into the nucleus, the ER, mitochondria, plastids, or peroxisomes; sorting signals can also direct the transport of proteins from the ER to other destinations in the cell.

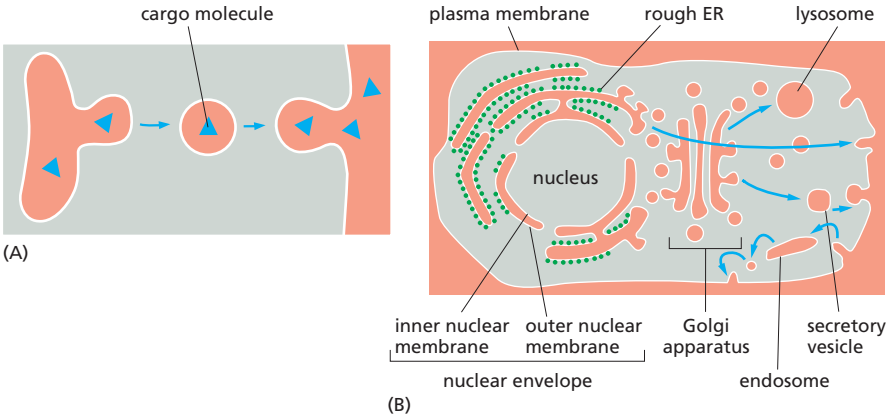


Figure 12–4 Topologically equivalent compartments in the secretory and endocytic pathways in a eukaryotic cell. Compartments are said to be *topologically equivalent* if they can communicate with one another, in the sense that molecules can get from one to the other without having to cross a membrane. Topologically equivalent spaces are shown in *red*. (A) Molecules can be carried from one compartment to another topologically equivalent compartment by vesicles that bud from one and fuse with the other. (B) In principle, cycles of membrane budding and fusion permit the lumen of any of the organelles shown to communicate with any other and with the cell exterior by means of transport vesicles. *Blue arrows* indicate the extensive outbound and inbound vesicular traffic (discussed in Chapter 13). Some organelles, most notably mitochondria and (in plant cells) plastids, do not take part in this communication and are isolated from the vesicular traffic between organelles shown here.

To understand the general principles by which sorting signals operate, it is important to distinguish three fundamentally different ways by which proteins move from one compartment to another. These three mechanisms are described below, and the transport steps at which they operate are outlined in Figure 12–5. We discuss the first two mechanisms (gated transport and transmembrane transport) in this chapter, and the third (vesicular transport, *green arrows* in Figure 12–5) in Chapter 13.

1. In **gated transport**, proteins and RNA molecules move between the cytosol and the nucleus through nuclear pore complexes in the nuclear envelope. The nuclear pore complexes function as selective gates that support the active transport of specific macromolecules and macromolecular assemblies between the two topologically equivalent spaces, although they also allow free diffusion of smaller molecules.
2. In **protein translocation**, transmembrane *protein translocators* directly transport specific proteins across a membrane from the cytosol into a space that is topologically distinct. The transported protein molecule usually must unfold to snake through the translocator. The initial transport of selected proteins from the cytosol into the ER lumen or mitochondria, for example, occurs in this way. Integral membrane proteins often use the same translocators but translocate only partially across the membrane, so that the protein becomes embedded in the lipid bilayer.
3. In **vesicular transport**, membrane-enclosed transport intermediates—which may be small, spherical transport vesicles or larger, irregularly shaped organelle fragments—ferry proteins from one topologically equivalent compartment to another. The transport vesicles and fragments become loaded with a cargo of molecules derived from the lumen of one compartment as they bud and pinch off from its membrane; they discharge their cargo into a second compartment by fusing with the membrane enclosing that compartment (Figure 12–6). The transfer of soluble proteins from the ER to the Golgi apparatus, for example, occurs in this way. Because the

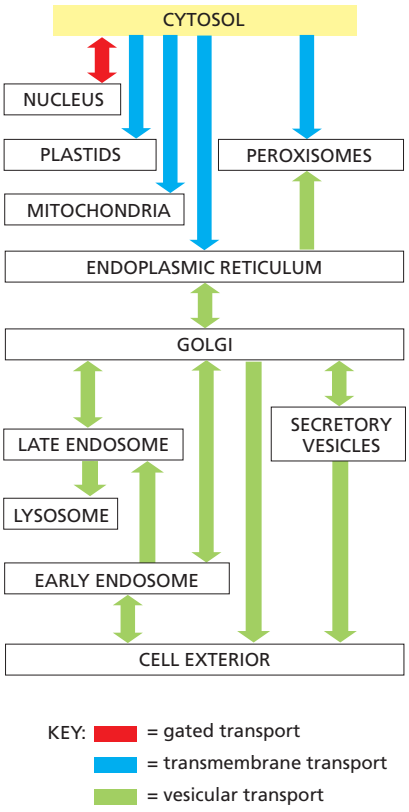


Figure 12–5 A simplified "roadmap" of protein traffic within a eukaryotic cell. Proteins can move from one compartment to another by gated transport *(red)*, protein translocation *(blue)*, or vesicular transport *(green)*. The sorting signals that direct a given protein's movement through the system, and thereby determine its eventual location in the cell, are contained in each protein's amino acid sequence. The journey begins with the synthesis of a protein on a ribosome in the cytosol and, for many proteins, terminates when the protein reaches its final destination. Other proteins shuttle back and forth between the nucleus and cytosol. At each intermediate station *(boxes)*, a decision is made as to whether the protein is to be retained in that compartment or transported further. A sorting signal may direct either retention in or exit from a compartment.

We shall refer to this figure often as a guide in this chapter and the next, highlighting in color the particular pathway being discussed.

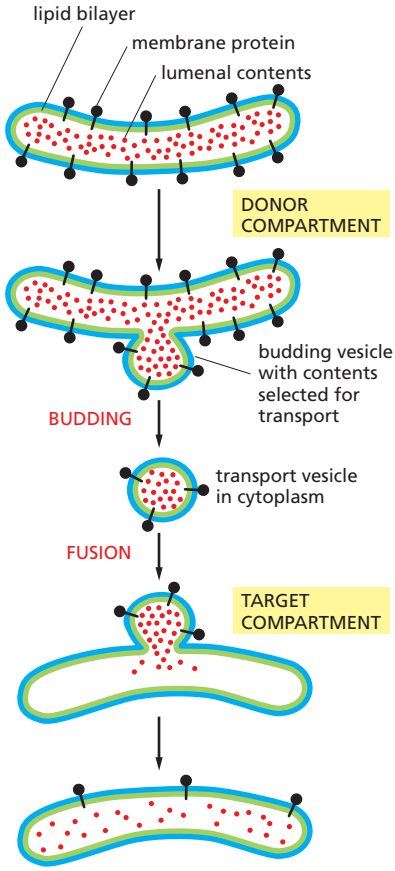


Figure 12–6 Vesicle budding and fusion during vesicular transport. Transport vesicles bud from one compartment (donor) and fuse with another topologically equivalent (target) compartment. In the process, soluble components *(red dots)* are transferred from lumen to lumen. Note that membrane is also transferred and that the original orientation of both proteins and lipids in the donor compartment membrane is preserved in the target compartment membrane. Thus, membrane proteins retain their asymmetric orientation, with the same domains always facing the cytosol.

transported proteins do not cross a membrane, vesicular transport can move proteins only between compartments that are topologically equivalent (see Figure 12–4).

Each mode of protein transfer is usually guided by sorting signals in the transported protein, which are recognized by complementary *sorting receptors*. If a large protein is to be imported into the nucleus, for example, it must possess a sorting signal that receptor proteins recognize to guide it through the nuclear pore complex. If a protein is to be transferred directly across a membrane, it must possess a sorting signal that the translocator recognizes. Likewise, if a protein is to be loaded into a certain type of vesicle or retained in certain organelles, a complementary receptor in the appropriate membrane must recognize its sorting signal.

Signal Sequences and Sorting Receptors Direct Proteins to the Correct Cell Address

Most protein sorting signals involved in transmembrane transport reside in a stretch of amino acid sequence, typically 15–60 residues long. Such **signal sequences** are often found at the N-terminus of the polypeptide chain, and in many cases specialized **signal peptidases** remove the signal sequence from the finished protein once the sorting process is complete. Signal sequences can also be internal stretches of amino acids, which remain part of the protein. Such signals are used in gated transport into the nucleus. Sorting signals can also be composed of multiple internal amino acid sequences that form a specific three-dimensional arrangement of atoms on the protein's surface; such **signal patches** are sometimes used for nuclear import and in vesicular transport.

Each signal sequence specifies a particular destination in the cell. Proteins destined for initial transfer to the ER usually have a signal sequence at their N-terminus that characteristically includes a sequence composed of about 5–10 hydrophobic amino acids. Many of these proteins will in turn pass from the ER to the Golgi apparatus, but those with a specific signal sequence of four amino acids at their C-terminus are recognized as ER residents and are returned to the ER. Proteins destined for mitochondria have signal sequences of yet another type, in which positively charged amino acids alternate with hydrophobic ones. Finally, many proteins destined for peroxisomes have a signal sequence of three characteristic amino acids at their C-terminus.

Table 12–3 presents some specific signal sequences. Experiments in which the peptide is transferred from one protein to another by genetic engineering techniques have demonstrated the importance of each of these signal sequences for protein targeting. Placing the N-terminal ER signal sequence at the beginning of a cytosolic protein, for example, redirects the protein to the ER; removing or mutating the signal sequence of an ER protein causes its retention in the cytosol. Signal sequences are therefore both necessary and sufficient for protein targeting. Even though their amino acid sequences can vary greatly, the signal sequences of proteins having the same destination are functionally interchangeable; physical properties, such as hydrophobicity, often seem to be more important in the signal-recognition process than the exact amino acid sequence.

Signal sequences are recognized by complementary sorting receptors that guide proteins to their appropriate destination, where the receptors unload their cargo. The receptors function catalytically: after completing one round of targeting, they return to their point of origin to be reused. Most sorting receptors

TABLE 12–3 Some Typical Signal Sequences

Function of signal sequence	Example of signal sequence
Import into nucleus	-Pro-Pro-Lys-Lys-Lys-Arg-Lys-Val-
Export from nucleus	-Met-Glu-Glu-Leu-Ser-Gln-Ala-Leu-Ala-Ser-Ser-Phe-
Import into mitochondria	$^{+}H_3N$-Met-Leu-Ser-Leu-Arg-Gln-Ser-Ile-Arg-Phe-Phe-Lys-Pro-Ala-Thr-Arg-Thr-Leu-Cys-Ser-Ser-Arg-Tyr-Leu-Leu-
Import into plastid	$^{+}H_3N$-Met-Val-Ala-Met-Ala-Met-Ala-Ser-Leu-Gln-Ser-Ser-Met-Ser-Ser-Leu-Ser-Leu-Ser-Ser-Asn-Ser-Phe-Leu-Gly-Gln-Pro-Leu-Ser-Pro-Ile-Thr-Leu-Ser-Pro-Phe-Leu-Gln-Gly-
Import into peroxisomes	-Ser-Lys-Leu-COO^{-}
Import into ER	$^{+}H_3N$-Met-Met-Ser-Phe-Val-Ser-Leu-Leu-Leu-Val-Gly-Ile-Leu-Phe-Trp-Ala-Thr-Glu-Ala-Glu-Gln-Leu-Thr-Lys-Cys-Glu-Val-Phe-Gln-
Return to ER	-Lys-Asp-Glu-Leu-COO^{-}

Some characteristic features of the different classes of signal sequences are highlighted in color. Where they are known to be important for the function of the signal sequence, positively charged amino acids are shown in *red* and negatively charged amino acids are shown in *green*. Similarly, important hydrophobic amino acids are shown in *orange* and important hydroxylated amino acids are shown in *blue*. $^{+}H_3N$ indicates the N-terminus of a protein; COO^{-} indicates the C-terminus.

recognize classes of proteins rather than an individual protein species. They can therefore be viewed as public transportation systems, dedicated to delivering numerous different components to their correct location in the cell.

Most Organelles Cannot Be Constructed *De Novo:* They Require Information in the Organelle Itself

When a cell reproduces by division, it has to duplicate its organelles, in addition to its chromosomes. In general, cells do this by incorporating new molecules into the existing organelles, thereby enlarging them; the enlarged organelles then divide and are distributed to the two daughter cells. Thus, each daughter cell inherits a complete set of specialized cell membranes from its mother. This inheritance is essential because a cell could not make such membranes from scratch. If the ER were completely removed from a cell, for example, how could the cell reconstruct it? As we discuss later, the membrane proteins that define the ER and perform many of its functions are themselves products of the ER. A new ER could not be made without an existing ER or, at least, a membrane that specifically contains the protein translocators required to import selected proteins into the ER from the cytosol (including the ER-specific translocators themselves). The same is true for mitochondria and plastids.

Thus, it seems that the information required to construct an organelle does not reside exclusively in the DNA that specifies the organelle's proteins. Information in the form of at least one distinct protein that preexists in the organelle membrane is also required, and this information is passed from parent cell to daughter cells in the form of the organelle itself. Presumably, such information is essential for the propagation of the cell's compartmental organization, just as the information in DNA is essential for the propagation of the cell's nucleotide and amino acid sequences.

As we discuss in more detail in Chapter 13, however, the ER buds off a constant stream of transport vesicles that incorporate only a subset of ER proteins and therefore have a composition different from the ER itself. Similarly, the plasma membrane constantly buds off various types of specialized endocytic vesicles. Thus, some organelles can form from other organelles and do not have to be inherited at cell division.

Summary

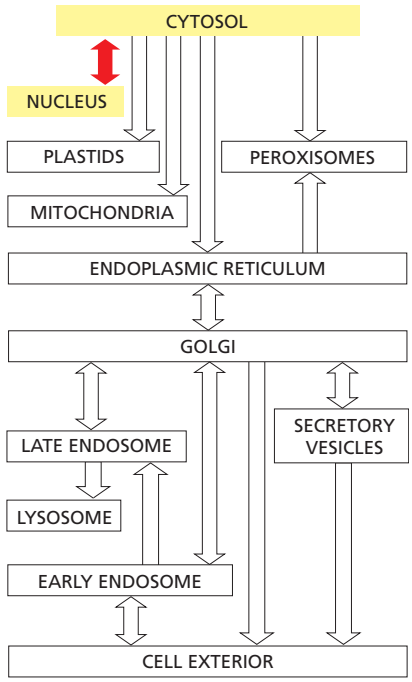
Eukaryotic cells contain intracellular membrane-enclosed organelles that make up nearly half the cell's total volume. The main ones present in all eukaryotic cells are the endoplasmic reticulum, Golgi apparatus, nucleus, mitochondria, lysosomes, endosomes, and peroxisomes; plant cells also contain plastids such as chloroplasts. These organelles contain distinct sets of proteins, which mediate each organelle's unique function.

Each newly synthesized organelle protein must find its way from a ribosome in the cytosol, where the protein is made, to the organelle where it functions. It does so by following a specific pathway, guided by sorting signals in its amino acid sequence that function as either signal sequences or signal patches. Sorting signals are recognized by complementary sorting receptors, which deliver the protein to the appropriate target organelle. Proteins that function in the cytosol do not contain sorting signals and therefore remain there after they are synthesized.

During cell division, organelles such as the ER and mitochondria are distributed to each daughter cell. These organelles contain information that is required for their construction, and so they cannot be made de novo.

THE TRANSPORT OF MOLECULES BETWEEN THE NUCLEUS AND THE CYTOSOL

The **nuclear envelope** encloses the DNA and defines the *nuclear compartment*. This envelope consists of two concentric membranes, which are penetrated by nuclear pore complexes (**Figure 12–7**). Although the inner and outer nuclear membranes are continuous, they maintain distinct protein compositions. The **inner nuclear membrane** contains proteins that act as binding sites for chromosomes and for the *nuclear lamina,* a protein meshwork that provides structural support for the nuclear envelope; the lamina also acts as an anchoring site for chromosomes and the cytoplasmic cytoskeleton (via protein complexes that span the nuclear envelope). The inner membrane is surrounded by the **outer nuclear membrane**, which is continuous with the membrane of the ER. Like the ER membrane (discussed later), the outer nuclear membrane is studded with ribosomes engaged in protein synthesis. The proteins made on these ribosomes are transported into the space between the inner and outer nuclear membranes (the *perinuclear space*), which is continuous with the ER lumen (see Figure 12–7).



Bidirectional traffic occurs continuously between the cytosol and the nucleus. The many proteins that function in the nucleus—including histones, DNA polymerases, RNA polymerases, transcriptional regulators, and RNA-processing proteins—are selectively imported into the nuclear compartment from the cytosol, where they are made. At the same time, almost all RNAs—including mRNAs, rRNAs, tRNAs, miRNAs, and snRNAs—are synthesized in the nuclear compartment and then exported to the cytosol. Like the import process, the export process is selective; mRNAs, for example, are exported only after they have been properly modified by RNA-processing reactions in the nucleus. In some cases, the transport process is complex. Ribosomal proteins, for instance, are made in the cytosol and imported into the nucleus, where they assemble with newly made ribosomal RNA into particles. The particles are then exported to the cytosol, where they assemble into ribosomes. Each of these steps requires selective transport across the nuclear envelope.

Nuclear Pore Complexes Perforate the Nuclear Envelope

Large and elaborate **nuclear pore complexes** (**NPCs**) perforate the nuclear envelope in all eukaryotes. Each NPC is composed of a set of approximately 30 different proteins, or **nucleoporins**. Reflecting the high degree of internal symmetry, each nucleoporin is present in multiple copies, resulting in 500–1000 protein molecules in the fully assembled NPC, with an estimated mass of 66 million daltons in yeast and 125 million daltons in vertebrates (**Figure 12–8**). Most nucleoporins are

composed of repetitive protein domains of only a few different types, which have evolved through extensive gene duplication. Some of the scaffold nucleoporins (see Figure 12–8) are structurally related to vesicle coat protein complexes, such as clathrin and COPII coatomer (discussed in Chapter 13), which shape transport vesicles; and one protein is used as a common building block in both NPCs and vesicle coats. These similarities suggest a common evolutionary origin for NPCs and vesicle coats: they may derive from an early membrane-bending protein module that helped shape the elaborate membrane systems of eukaryotic cells, and in present-day cells stabilize the sharp membrane bends required to form a nuclear pore.

The nuclear envelope of a typical mammalian cell contains 3000–4000 NPCs, although that number varies widely, from a few hundred in glial cells to almost 20,000 in Purkinje neurons. The total traffic that passes through each NPC is enormous: each NPC can transport up to 1000 macromolecules per second and can transport in both directions at the same time. How it coordinates the bidirectional flow of macromolecules to avoid congestion and head-on collisions is not known.

Each NPC contains aqueous passages, through which small water-soluble molecules can diffuse passively. Researchers have determined the effective size of these passages by injecting labeled water-soluble molecules of different sizes into the cytosol and then measuring their rate of diffusion into the nucleus. Small molecules (5000 daltons or less) diffuse in so fast that we can consider the nuclear envelope freely permeable to them. Large proteins, however, diffuse in much more slowly, and the larger a protein, the more slowly it passes through the NPC. Proteins larger than 60,000 daltons cannot enter by passive diffusion. This size cut-off to free diffusion is thought to result from the NPC structure (see Figure 12–8). The channel nucleoporins with extensive unstructured regions form a disordered tangle (much like a kelp bed in the ocean) that restricts the diffusion of large macromolecules while allowing smaller molecules to pass.

Because many cell proteins are too large to diffuse passively through the NPCs, the nuclear compartment and the cytosol can maintain different protein compositions. Mature cytosolic ribosomes, for example, are about 30 nm in diameter and thus cannot diffuse through the NPC, confining protein synthesis to the cytosol. But how does the nucleus export newly made ribosomal subunits or import large molecules, such as DNA polymerases and RNA polymerases, which have subunit molecular masses of 100,000–200,000 daltons? As we discuss next, these and most other transported protein and RNA molecules bind to specific receptor proteins that actively ferry large molecules through NPCs. Even small proteins like histones frequently use receptor-mediated mechanisms to cross the NPC, thereby increasing transport efficiency.

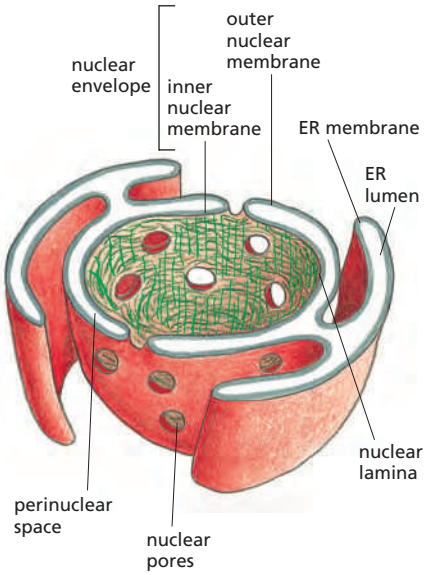


Figure 12–7 The nuclear envelope. The double-membrane envelope is penetrated by pores in which nuclear pore complexes (not shown) are positioned. The outer nuclear membrane is continuous with the endoplasmic reticulum (ER). The ribosomes that are normally bound to the cytosolic surface of the ER membrane and outer nuclear membrane are not shown. The nuclear lamina is a fibrous protein meshwork underlying the inner membrane.

Nuclear Localization Signals Direct Nuclear Proteins to the Nucleus

When proteins are experimentally extracted from the nucleus and reintroduced into the cytosol, even the very large ones reaccumulate efficiently in the nucleus. Sorting signals called **nuclear localization signals** (**NLSs**) are responsible for the selectivity of this active nuclear import process. The signals have been precisely defined by using recombinant DNA technology for numerous nuclear proteins, as well as for proteins that enter the nucleus only transiently (**Figure 12–9**). In many nuclear proteins, the signals consist of one or two short sequences that are rich in the positively charged amino acids lysine and arginine (see Table 12–3, p. 648), with the precise sequence varying for different proteins. Other nuclear proteins contain different signals, some of which are not yet characterized.

Nuclear localization signals can be located almost anywhere in the amino acid sequence and are thought to form loops or patches on the protein surface. Many function even when linked as short peptides to lysine side chains on the surface of a cytosolic protein, suggesting that the precise location of the signal within the amino acid sequence of a nuclear protein is not important. Moreover, as long as one of the protein subunits of a multicomponent complex displays a nuclear localization signal, the entire complex will be imported into the nucleus.

One can visualize the transport of nuclear proteins through NPCs by coating gold particles with a nuclear localization signal, injecting the particles into the cytosol, and then following their fate by electron microscopy (**Figure 12–10**). The particles bind to the tentaclelike fibrils that extend from the scaffold nucleoporins at the rim of the NPC into the cytosol, and then proceed through the center of the NPC. Presumably, the unstructured regions of the nucleoporins that form a diffusion barrier for large molecules (mentioned earlier) are pushed away to allow the coated gold particles to squeeze through.

Macromolecular transport across NPCs differs fundamentally from the transport of proteins across the membranes of other organelles, in that it occurs

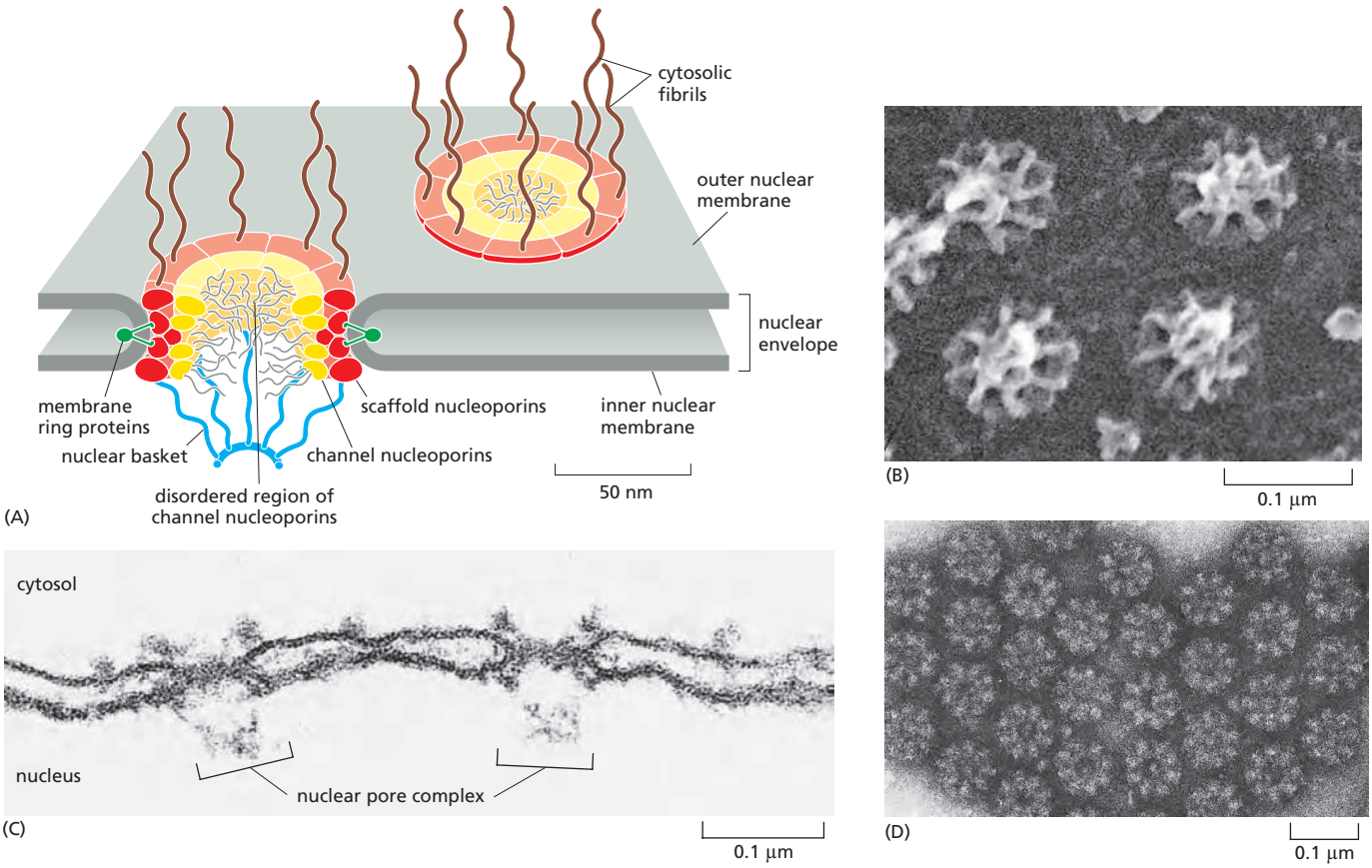


Figure 12–8 The arrangement of NPCs in the nuclear envelope. (A) In a vertebrate NPC, nucleoporins are arranged with striking eightfold rotational symmetry. In addition, immunoelectron microscopic studies show that the proteins that make up the central portion of the NPC are oriented symmetrically across the nuclear envelope, so that the nuclear and cytosolic sides look identical. The eightfold rotational and twofold transverse symmetry explains how such a huge structure can be formed from only about 30 different proteins: many of the nucleoporins are present in 8, 16, or 32 copies. Based on their approximate localization in the central portion of the NPC, nucleoporins can be classified into (1) transmembrane ring proteins that span the nuclear envelope and anchor the NPC to the envelope; (2) scaffold nucleoporins that form layered ring structures. Some scaffold nucleoporins are membrane-bending proteins that stabilize the sharp membrane curvature where the nuclear envelope is penetrated; and (3) channel nucleoporins that line a central pore. In addition to folded domains that anchor the proteins in specific places, many channel nucleoporins contain extensive unstructured regions, where the polypeptide chains are intrinsically disordered. The central pore is filled with a tangled mesh of these disordered domains that blocks the passive diffusion of large macromolecules. The disordered regions contain a large number of phenylalanine-glycine (FG) repeats. Fibrils protrude from both the cytosolic and the nuclear sides of the NPC. By contrast to the twofold transverse symmetry of the NPC core, the fibrils facing the cytosol and nucleus are different: on the nuclear side, the fibrils converge at their distal end to form a basketlike structure. The precise arrangement of individual nucleoporins in the assembled NPC is still a matter of intense debate, because atomic resolution analyses have been hindered by the sheer size and flexible nature of the NPC, and by difficulties in purifying sufficient amounts of homogeneous material. A combination of electron microscopy, computational analyses, and crystal structures of nucleoporin subcomplexes has been used to develop the current models of the NPC architecture. (B) A scanning electron micrograph of the nuclear side of the nuclear envelope of an oocyte (see also Figure 9–52). (C) An electron micrograph showing a side view of two NPCs *(brackets)*; note that the inner and outer nuclear membranes are continuous at the edges of the pore. (D) An electron micrograph showing face-on views of negatively stained NPCs. The membrane has been removed by detergent extraction. Note that some of the NPCs contain material in their center, which is thought to be trapped macromolecules in transit through these NPCs. (A, adapted from A. Hoelz, E.W. Debler and G. Blobel, *Annu. Rev. Biochem.* 80:613–643, 2011. With permission from Annual Reviews; B, from M.W. Goldberg and T.D. Allen, *J. Cell Biol.* 119:1429–1440, 1992. With permission from The Rockefeller University Press; C, courtesy of Werner Franke and Ulrich Scheer; D, courtesy of Ron Milligan.)

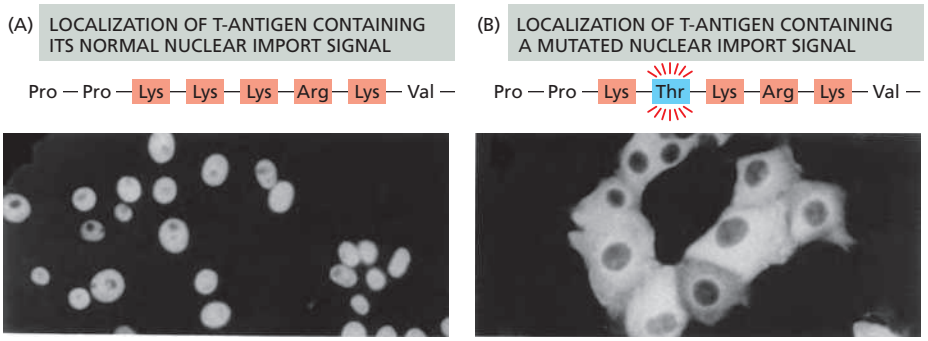


Figure 12–9 The function of a nuclear localization signal. Immunofluorescence micrographs showing the cell location of SV40 virus T-antigen containing or lacking a short sequence that serves as a nuclear localization signal. (A) The normal T-antigen protein contains the lysine-rich sequence indicated and is imported to its site of action in the nucleus, as indicated by immunofluorescence staining with antibodies against the T-antigen. (B) T-antigen with an altered nuclear localization signal (a threonine replacing a lysine) remains in the cytosol. (From D. Kalderon, B. Roberts, W. Richardson and A. Smith, *Cell* 39:499–509, 1984. With permission from Elsevier.)

through a large, expandable, aqueous pore, rather than through a protein transporter spanning one or more lipid bilayers. For this reason, fully folded nuclear proteins can be transported into the nucleus through an NPC, and newly formed ribosomal subunits are transported out of the nucleus as an assembled particle. By contrast, proteins have to be extensively unfolded to be transported into most other organelles, as we discuss later.

Nuclear Import Receptors Bind to Both Nuclear Localization Signals and NPC Proteins

To initiate nuclear import, most nuclear localization signals must be recognized by **nuclear import receptors**, sometimes called *importins*, most of which are encoded by a family of related genes. Each family member encodes a receptor protein that can bind and transport the subset of cargo proteins containing the appropriate nuclear localization signal (**Figure 12–11**A). Nuclear import receptors do not always bind to nuclear proteins directly. Additional adaptor proteins can form a bridge between the import receptors and the nuclear localization signals on the proteins to be transported (Figure 12–11B). Some adaptor proteins are structurally related to nuclear import receptors, suggesting a common evolutionary origin. By using a variety of import receptors and adaptors, cells are able to recognize the broad repertoire of nuclear localization signals that are displayed on nuclear proteins.

The import receptors are soluble cytosolic proteins that bind both to the nuclear localization signal on the cargo protein and to the phenylalanine-glycine (FG) repeats in the unstructured domains of the channel nucleoporins that line the central pore. FG-repeats are also found in the cytoplasmic and nuclear fibrils. FG-repeats in the unstructured tangle of the pore are thought to do double duty. They interact weakly, which gives the protein tangle gel-like properties that impose a permeability barrier to large macromolecules, and they serve as docking sites for nuclear import receptors. FG-repeats line the path through the NPCs taken by the import receptors and their bound cargo proteins. According to one model of nuclear transport, the receptor–cargo complexes move along the transport path by repeatedly binding, dissociating, and then re-binding to adjacent FG-repeat sequences. In this way, the complexes may hop from one nucleoporin to another to traverse the tangled interior of the NPC in a random walk. As import receptors bind to FG-repeats during this journey, they would disrupt interaction between the repeats and locally dissolve the gel phase of the protein tangle that fills the pore, allowing the passage of the receptor–cargo complex. Once inside the nucleus, the import receptors dissociate from their cargo and return to the cytosol. As we will see, this dissociation only occurs on the nuclear side of the NPC and thereby confers directionality to the import process.

Nuclear Export Works Like Nuclear Import, But in Reverse

The nuclear export of large molecules, such as new ribosomal subunits and RNA molecules, occurs through NPCs and also depends on a selective transport

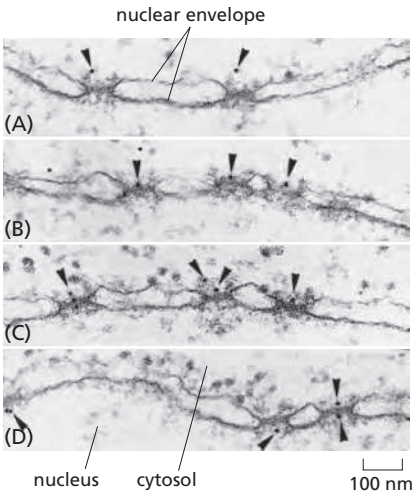


Figure 12–10 Visualizing active import through NPCs. This series of electron micrographs shows colloidal gold spheres *(arrowheads)* coated with peptides containing nuclear localization signals entering the nucleus through NPCs. The gold particles were injected into the cytosol of living cells, which then were fixed and prepared for electron microscopy at various times after injection. (A) Gold particles are first seen in proximity to the cytosolic fibrils of the NPCs. (B, C) They are then seen at the center of the NPCs, exclusively on the cytosolic face. (D) They then appear on the nuclear face. These gold particles have much larger diameters than the diffusion channels in the NPC and are imported by active transport. (From N. Panté and U. Aebi, *Science* 273:1729–1732, 1996. With permission from AAAS.)

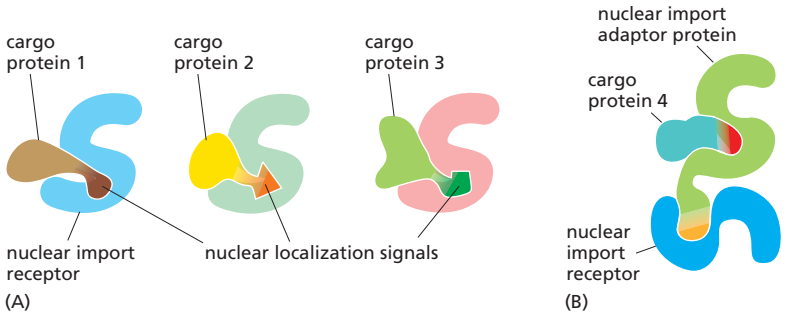


Figure 12–11 Nuclear import receptors (importins). (A) Different nuclear import receptors bind different nuclear localization signals and thereby different cargo proteins. (B) Cargo protein 4 requires an adaptor protein to bind to its nuclear import receptor. The adaptors are structurally related to nuclear import receptors and recognize nuclear localization signals on cargo proteins. They also contain a nuclear localization signal that binds them to an import receptor, but this signal only becomes exposed when they are loaded with a cargo protein.

system. The transport system relies on **nuclear export signals** on the macromolecules to be exported, as well as on complementary **nuclear export receptors**, or *exportins*. These receptors bind to both the export signal and NPC proteins to guide their cargo through the NPC to the cytosol.

Many nuclear export receptors are structurally related to nuclear import receptors, and they are encoded by the same gene family of **nuclear transport receptors**, or *karyopherins*. In yeast, there are 14 genes encoding karyopherins; in animal cells, the number is significantly larger. It is often not possible to tell from their amino acid sequence alone whether a particular family member works as a nuclear import or nuclear export receptor. As might be expected, therefore, the import and export transport systems work in similar ways but in opposite directions: the import receptors bind their cargo molecules in the cytosol, release them in the nucleus, and are then exported to the cytosol for reuse, while the export receptors function in the opposite fashion.

The Ran GTPase Imposes Directionality on Transport Through NPCs

The import of nuclear proteins through NPCs concentrates specific proteins in the nucleus and thereby increases order in the cell. The cell fuels this ordering process by harnessing energy stored in concentration gradients of the GTP-bound form of the monomeric GTPase **Ran**, which is required for both nuclear import and export.

Like other GTPases, Ran is a molecular switch that can exist in two conformational states, depending on whether GDP or GTP is bound (discussed in Chapter 3). Two Ran-specific regulatory proteins trigger the conversion between the two states: a cytosolic *GTPase-activating protein* (*GAP*) triggers GTP hydrolysis and thus converts Ran-GTP to Ran-GDP, and a nuclear *guanine exchange factor* (*GEF*) promotes the exchange of GDP for GTP and thus converts Ran-GDP to Ran-GTP. Because *Ran-GAP* is located in the cytosol and *Ran-GEF* is located in the nucleus where it is anchored to chromatin, the cytosol contains mainly Ran-GDP, and the nucleus contains mainly Ran-GTP (**Figure 12–12**).

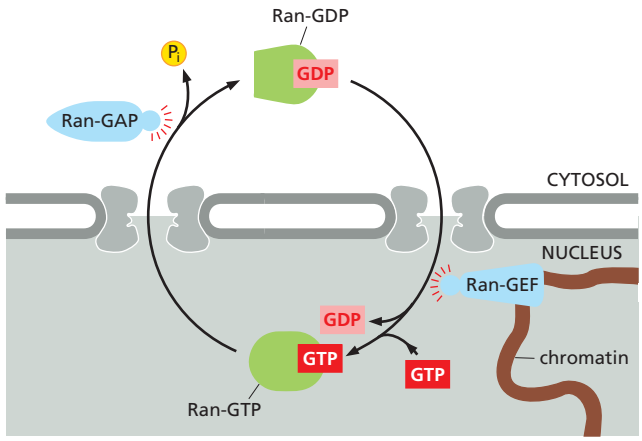


Figure 12–12 The compartmentalization of Ran-GDP and Ran-GTP. Localization of Ran-GDP in the cytosol and Ran-GTP in the nucleus results from the localization of two Ran regulatory proteins: Ran GTPase-activating protein (Ran-GAP) is located in the cytosol, and Ran guanine nucleotide exchange factor (Ran-GEF) binds to chromatin and is therefore located in the nucleus.

Ran-GDP is imported into the nucleus by its own import receptor, which is specific for the GDP-bound conformation of Ran. The Ran-GDP receptor is structurally unrelated to the main family of nuclear transport receptors. However, it also binds to FG-repeats in NPC channel nucleoporins.

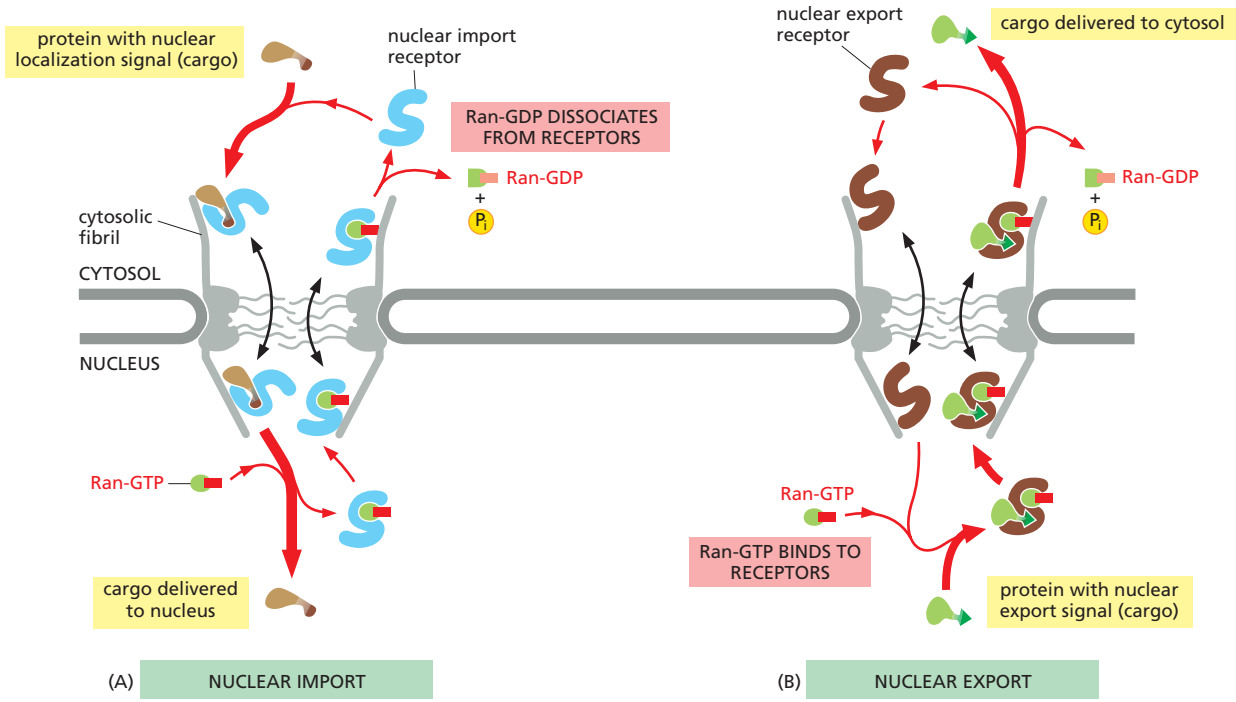


Figure 12–13 How GTP hydrolysis by Ran in the cytosol provides directionality to nuclear transport. Movement through the NPC of loaded nuclear transport receptors occurs along the FG-repeats displayed by certain NPC proteins. The differential localization of Ran-GTP in the nucleus and Ran-GDP in the cytosol provides directionality *(red arrows)* to both nuclear import (A) and nuclear export (B). Ran-GAP stimulates the hydrolysis of GTP to produce Ran-GDP on the cytosolic side of the NPC (see Figure 12–12).

This gradient of the two conformational forms of Ran drives nuclear transport in the appropriate direction. Docking of nuclear import receptors to FG-repeats on the cytosolic side of the NPC, for example, occurs whether or not these receptors are loaded with appropriate cargo. Import receptors, facilitated by FG-repeat binding, then enter the channel. If they reach the nuclear side of the pore complex, Ran-GTP binds to them, and, if the receptors arrive loaded with cargo molecules, the Ran-GTP binding causes the receptors to release their cargo (**Figure 12–13**A). Because the Ran-GDP in the cytosol does not bind to import (or export) receptors, unloading occurs only on the nuclear side of the NPC. In this way, the nuclear localization of Ran-GTP creates the directionality of the import process.

Having discharged its cargo in the nucleus, the empty import receptor with Ran-GTP bound is transported back through the pore complex to the cytosol. There, Ran-GAP triggers Ran-GTP to hydrolyze its bound GTP, thereby converting it to Ran-GDP, which dissociates from the receptor. The receptor is then ready for another cycle of nuclear import.

Nuclear export occurs by a similar mechanism, except that Ran-GTP in the nucleus promotes cargo binding to the export receptor, rather than promoting cargo dissociation. Once the export receptor moves through the pore to the cytosol, it encounters Ran-GAP, which induces the receptor to hydrolyze its GTP to GDP. As a result, the export receptor releases both its cargo and Ran-GDP in the cytosol. Free export receptors are then returned to the nucleus to complete the cycle (Figure 12–13B).

Transport Through NPCs Can Be Regulated by Controlling Access to the Transport Machinery

Some proteins contain both nuclear localization signals and nuclear export signals. These proteins continually shuttle back and forth between the nucleus and the cytosol. The relative rates of their import and export determine the steady-state localization of such *shuttling proteins*: if the rate of import exceeds the rate of export, a protein will be located mainly in the nucleus; conversely, if the rate of export exceeds the rate of import, a protein will be located mainly in the cytosol. Thus, changing the rate of import, export, or both, can change the location of a protein.

Some shuttling proteins move continuously into and out of the nucleus. In other cases, however, the transport is stringently controlled. As discussed in Chapter 7, cells control the activity of some transcription regulators by keeping them out of the nucleus until they are needed there (Figure 12–14). In many cases, cells control transport by regulating nuclear localization and export signals—turning them on or off, often by phosphorylation of amino acids close to the signal sequences (Figure 12–15).

Other transcription regulators are bound to inhibitory cytosolic proteins that either anchor them in the cytosol (through interactions with the cytoskeleton or specific organelles) or mask their nuclear localization signals so that they cannot interact with nuclear import receptors. An appropriate stimulus releases the gene regulatory protein from its cytosolic anchor or mask, and it is then transported into the nucleus. One important example is the latent gene regulatory protein that controls the expression of proteins involved in cholesterol metabolism. The protein is made and stored in an inactive form as a transmembrane protein in the ER. When a cell is deprived of cholesterol, the protein is transported from the ER to the Golgi apparatus where it encounters specific proteases that cleave off the cytosolic domain, releasing it into the cytosol. This domain is then imported into the nucleus, where it activates the transcription of genes required for both cholesterol uptake and synthesis (Figure 12–16).

As we discuss in detail in Chapter 6, cells control the export of RNAs from the nucleus in a similar way. snRNAs, miRNAs, and tRNAs bind to the same family of nuclear export receptors just discussed, and they use the same Ran-GTP gradient to fuel the transport process. By contrast, the export of mRNAs out of the nucleus uses a different mechanism. mRNAs are exported as large assemblies, which can be as large as 100 million daltons (see Figure 6–37) and can contain hundreds of proteins of a few dozen different types. These mRNA ribonucleoprotein complexes (mRNPs) first dock at the nuclear side of the NPC, where they are extensively remodeled. Although Ran-GTP is indirectly involved in the export (because it imports the proteins that bind to the mRNA molecules), the translocation across the NPC is thought to be driven by ATP hydrolysis. How export directionality is assured is unclear. It is likely that the many accessory proteins tethered to the NPC's nuclear and cytoplasmic fibrils have important roles in remodeling the mRNPs as they pass through the pores, in particular stripping away nuclear proteins as the mRNPs exit on the cytosolic side of the NPC, thereby ensuring that transport is unidirectional. Upon entry into the cytosol, these nuclear mRNP proteins are rapidly returned to the nucleus.

Figure 12–14 The control of nuclear transport in the early *Drosophila* embryo. The embryo at this stage is a syncytium, shown here in cross section, with many nuclei in a common cytoplasm, arranged around the periphery, just beneath the plasma membrane. The transcription regulatory protein Dorsal is produced uniformly throughout the peripheral cytoplasm, but it can act only when inside the nuclei. The Dorsal protein has been stained with an enzyme-coupled antibody that yields a brown product, revealing that Dorsal is excluded from the nuclei at the dorsal side *(top)* of the embryo but is concentrated in the nuclei toward the ventral side *(bottom)* of the embryo. The regulated traffic of Dorsal into the nuclei controls the differential development between the back and belly of the animal. (Courtesy of Siegfried Roth.)

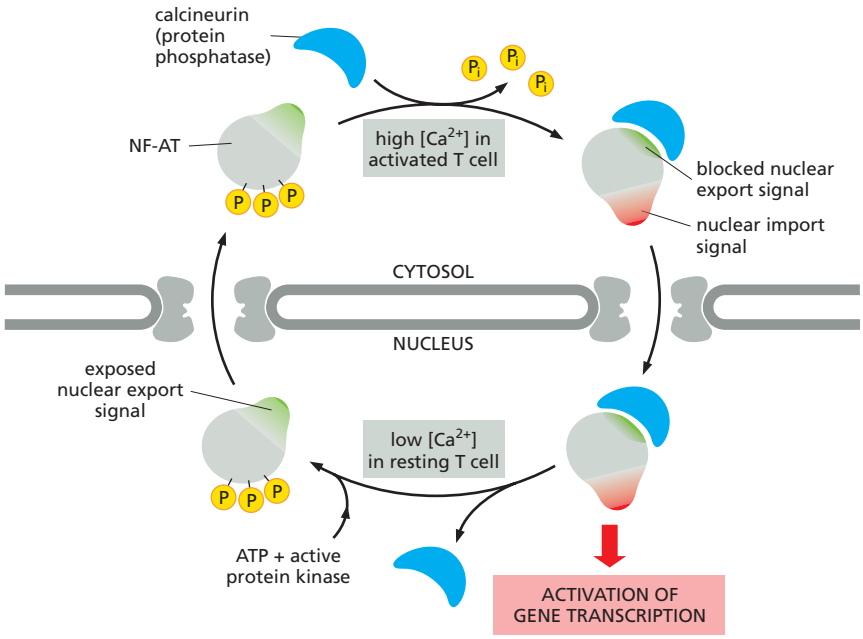


Figure 12–15 The control of nuclear import during T cell activation. The nuclear factor of activated T cells (NF-AT) is a transcription regulatory protein that, in the resting T cell, is found in the cytosol in a phosphorylated state. When T cells are activated by foreign antigen (discussed in Chapter 24), the intracellular Ca^{2+} concentration increases. In high Ca^{2+}, the protein phosphatase calcineurin binds to NF-AT and dephosphorylates it. The dephosphorylation exposes nuclear import signals and blocks a nuclear export signal. The complex of NF-AT and calcineurin is therefore imported into the nucleus, where NF-AT activates the transcription of numerous genes required for T cell activation.

The response shuts off when Ca^{2+} levels decrease, releasing NF-AT from calcineurin. Rephosphorylation of NF-AT inactivates the nuclear import signals and re-exposes the nuclear export signal, causing NF-AT to relocate to the cytosol. Some of the most potent immunosuppressive drugs, including cyclosporin A and FK506, inhibit the ability of calcineurin to dephosphorylate NF-AT and thereby block the nuclear accumulation of NF-AT and T cell activation (Movie 12.1).

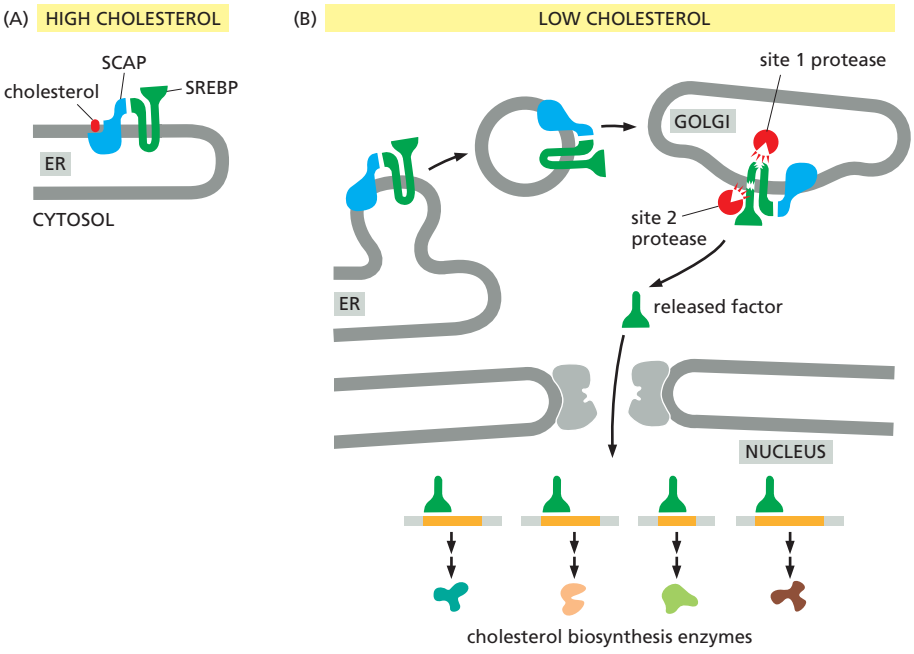


Figure 12–16 Feedback regulation of cholesterol biosynthesis. SREBP (sterol response element binding protein), a latent transcription regulator that controls expression of cholesterol biosynthetic enzymes, is initially synthesized as an ER membrane protein. It is anchored in the ER if there is sufficient cholesterol in the membrane by interaction with another ER membrane protein, called SCAP (SREBP cleavage activation protein), which binds cholesterol. If the cholesterol binding site on SCAP is empty (at low cholesterol concentrations), SCAP changes conformation and is packaged together with SREBP into transport vesicles, which deliver their cargo to the Golgi apparatus, where two Golgi-resident proteases cleave SREBP to free its cytosolic domain from the membrane. The cytosolic domain then moves into the nucleus, where it binds to the promoters of genes that encode proteins involved in cholesterol biosynthesis and activates their transcription. In this way, more cholesterol is made when its concentration falls below a threshold.

During Mitosis the Nuclear Envelope Disassembles

The **nuclear lamina**, located on the nuclear side of the inner nuclear membrane, is a meshwork of interconnected protein subunits called **nuclear lamins**. The lamins are a special class of intermediate filament proteins (discussed in Chapter 16) that polymerize into a two-dimensional lattice (**Figure 12–17**). The nuclear lamina gives shape and stability to the nuclear envelope, to which it is anchored by attachment to both the NPCs and transmembrane proteins of the inner nuclear membrane. The lamina also interacts directly with chromatin, which itself interacts with transmembrane proteins of the inner nuclear membrane. Together with the lamina, these inner membrane proteins provide structural links between the DNA and the nuclear envelope.

When a nucleus is dismantled during mitosis, the NPCs and nuclear lamina disassemble and the nuclear envelope fragments. The dismantling process is at least partly a consequence of direct phosphorylation of nucleoporins and lamins by the cyclin-dependent protein kinase (Cdk) that is activated at the onset of mitosis (discussed in Chapter 17). During this process, some NPC proteins become bound to nuclear import receptors, which play an important part in the reassembly of NPCs at the end of mitosis. Nuclear envelope membrane proteins—no longer tethered to the pore complexes, lamina, or chromatin—disperse throughout the ER membrane. The dynein motor protein, which moves along microtubules (discussed in Chapter 16), actively participates in tearing the nuclear envelope off the chromatin. Together, these processes break down the barriers that normally separate the nucleus and cytosol, and the nuclear proteins that are not bound to membranes or chromosomes intermix completely with the proteins of the cytosol (**Figure 12–18**).

Later in mitosis, the nuclear envelope reassembles on the surface of the daughter chromosomes. In addition to its crucial role in nuclear transport, the Ran GTPase also acts as a positional marker for chromatin during cell division, when the nuclear and cytosolic components intermix. Because Ran-GEF remains bound to chromatin when the nuclear envelope breaks down, Ran molecules close to chromatin are mainly in their GTP-bound conformation. By contrast, Ran molecules further away have a high likelihood of encountering Ran-GAP, which is distributed throughout the cytosol; these Ran molecules are mainly in their GDP-bound conformation. As a result, the chromosomes in mitotic cells are surrounded by a cloud of Ran-GTP. Ran-GTP releases the NPC proteins in proximity to the chromosomes from nuclear import receptors. The free NPC proteins attach

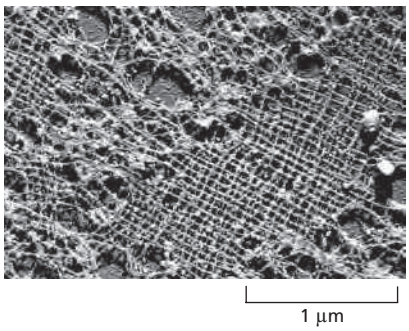


Figure 12–17 The nuclear lamina. An electron micrograph of a portion of the nuclear lamina in a *Xenopus* oocyte prepared by freeze-drying and metal shadowing. The lamina is formed by a regular lattice of specialized intermediate filaments. Lamins are only present in metazoan cells. Other, yet-unknown proteins may serve similar functions in species that lack lamins. (Courtesy of Ueli Aebi.)

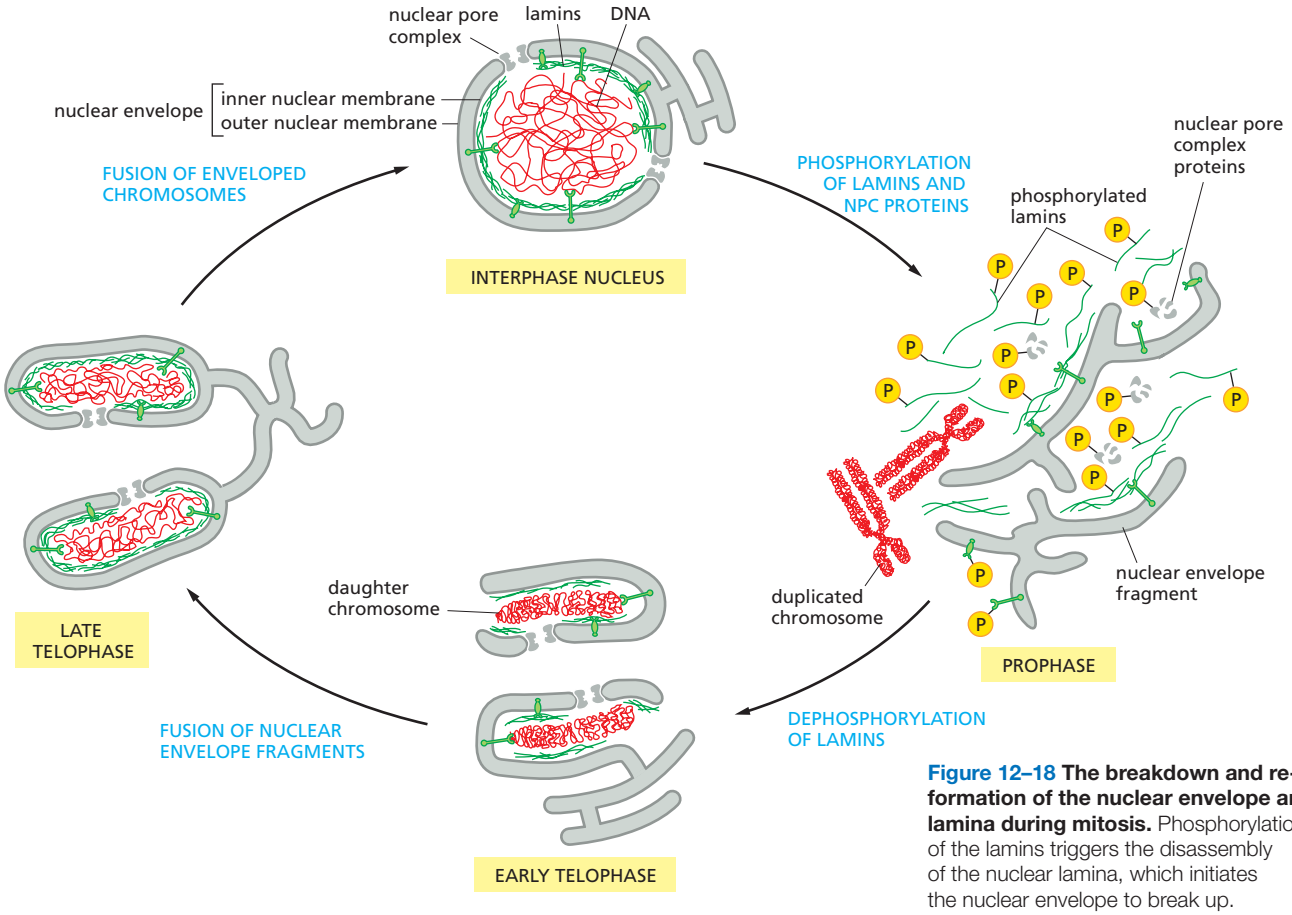


Figure 12–18 The breakdown and re-formation of the nuclear envelope and lamina during mitosis. Phosphorylation of the lamins triggers the disassembly of the nuclear lamina, which initiates the nuclear envelope to break up. Dephosphorylation of the lamins reverses the process. An analogous phosphorylation and dephosphorylation cycle occurs for some nucleoporins and proteins of the inner nuclear membrane, and some of these dephosphorylations are also involved in the reassembly process. As indicated, the nuclear envelope initially re-forms around individual decondensing daughter chromosomes. Eventually, as decondensation progresses, these structures fuse to form a single complete nucleus.

Mitotic breakdown of the nuclear envelope occurs in all metazoan cells. However, in many other species, such as yeasts, the nuclear envelope remains intact during mitosis, and the nucleus divides by fission.

to the chromosome surface, where they assemble into new NPCs. At the same time, inner nuclear membrane proteins and dephosphorylated lamins bind again to chromatin. ER membranes wrap around groups of chromosomes until they form a sealed nuclear envelope (**Movie 12.2**). During this process, the NPCs start actively re-importing proteins that contain nuclear localization signals. Because the nuclear envelope is initially closely applied to the surface of the chromosomes, the newly formed nucleus excludes all proteins except those initially bound to the mitotic chromosomes and those that are selectively imported through NPCs. In this way, all other large proteins, including ribosomes, are kept out of the newly assembled nucleus.

As we discuss in Chapter 17, the cloud of Ran-GTP surrounding chromatin is also important in assembling the mitotic spindle in a dividing cell.

Summary

The nuclear envelope consists of an inner and an outer nuclear membrane that are continuous with each other and with the ER membrane, and the space between the inner and outer nuclear membrane is continuous with the ER lumen. RNA molecules, which are made in the nucleus, and ribosomal subunits, which are assembled there, are exported to the cytosol; in contrast, all the proteins that function in the nucleus are synthesized in the cytosol and are then imported. The extensive traffic of materials between the nucleus and cytosol occurs through nuclear pore complexes (NPCs), which provide a direct passageway across the nuclear envelope. Small molecules diffuse passively through the NPCs, but large macromolecules have to be actively transported.

Proteins containing nuclear localization signals are actively transported into the nucleus through NPCs, while proteins containing nuclear export signals are transported out of the nucleus to the cytosol. Some proteins, including the nuclear

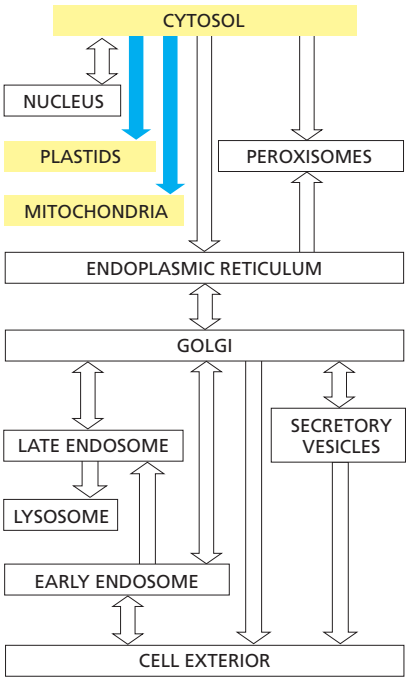
import and export receptors, continually shuttle between the cytosol and nucleus. The monomeric GTPase Ran provides both the free energy and the directionality for nuclear transport. Cells regulate the transport of nuclear proteins and RNA molecules through the NPCs by controlling the access of these molecules to the transport machinery. Newly transcribed messenger RNA and ribosomal RNA are exported from the nucleus as parts of large ribonucleoprotein complexes. Because nuclear localization signals are not removed, nuclear proteins can be imported repeatedly, as is required each time that the nucleus reassembles after mitosis.

THE TRANSPORT OF PROTEINS INTO MITOCHONDRIA AND CHLOROPLASTS

Mitochondria and chloroplasts (a specialized form of plastids in green algae and plant cells) are double-membrane-enclosed organelles. They specialize in ATP synthesis, using energy derived from electron transport and oxidative phosphorylation in mitochondria and from photosynthesis in chloroplasts (discussed in Chapter 14). Although both organelles contain their own DNA, ribosomes, and other components required for protein synthesis, most of their proteins are encoded in the cell nucleus and imported from the cytosol. Each imported protein must reach the particular organelle subcompartment in which it functions.

There are different subcompartments in mitochondria (**Figure 12–19**A): the internal **matrix space** and the **intermembrane space**, which is continuous with the cristae space. These compartments are formed by the two concentric mitochondrial membranes: the **inner membrane**, which encloses the matrix space and forms extensive invaginations called *cristae*, and the **outer membrane**, which is in contact with the cytosol. Protein complexes provide boundaries at the junctions where the cristae invaginate and divide the inner membrane into two domains: one inner membrane domain surrounds the cristae space, and the other domain abuts the outer membrane. Chloroplasts also have an outer and inner membrane, which enclose an intermembrane space, and the stroma, which is the chloroplast equivalent of the mitochondrial matrix space (Figure 12–19B). They have an additional subcompartment, the *thylakoid space*, which is surrounded by the *thylakoid membrane*. The thylakoid membrane derives from the inner membrane during plastid development and is pinched off to become discontinuous with it. Each of the subcompartments in mitochondria and chloroplasts contains a distinct set of proteins.

New mitochondria and chloroplasts are produced by the growth of preexisting organelles, followed by fission (discussed in Chapter 14). The growth depends mainly on the import of proteins from the cytosol. The imported proteins must be transported across a number of membranes in succession and end up in the appropriate place. The process of protein movement across membranes is called *protein translocation*. This section explains how it occurs.



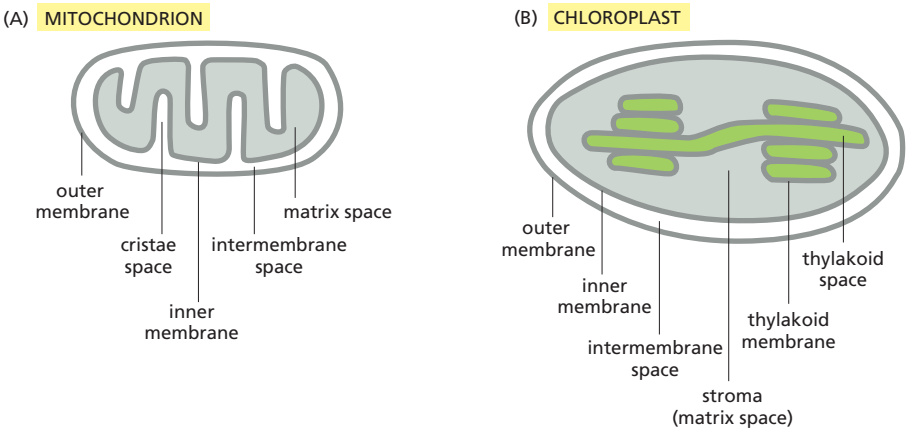


Figure 12–19 The subcompartments of mitochondria and chloroplasts. In contrast to the cristae of mitochondria (A), the thylakoids of chloroplasts (B) are not connected to the inner membrane and therefore form a sealed compartment with a separate internal space.

Translocation into Mitochondria Depends on Signal Sequences and Protein Translocators

Proteins imported into **mitochondria** are usually taken up from the cytosol within seconds or minutes of their release from ribosomes. Thus, in contrast to protein translocation into the ER, which often takes place simultaneously with translation by a ribosome docked on the rough ER membrane (described later), mitochondrial proteins are first fully synthesized as **mitochondrial precursor proteins** in the cytosol and then translocated into mitochondria by a *post-translational* mechanism. One or more signal sequences direct all mitochondrial precursor proteins to their appropriate mitochondrial subcompartment. Many proteins entering the matrix space contain a signal sequence at their N-terminus that a signal peptidase rapidly removes after import. Other imported proteins, including all outer membrane and many inner membrane and intermembrane space proteins, have internal signal sequences that are not removed. The signal sequences are both necessary and sufficient for the import and correct localization of the proteins: when genetic engineering techniques are used to link these signals to a cytosolic protein, the signals direct the protein to the correct mitochondrial subcompartment.

The signal sequences that direct precursor proteins into the mitochondrial matrix space are best understood. They all form an amphiphilic α helix, in which positively charged residues cluster on one side of the helix, while uncharged hydrophobic residues cluster on the opposite side. Specific receptor proteins that initiate protein translocation recognize this configuration rather than the precise amino acid sequence of the signal sequence (**Figure 12–20**).

Multisubunit protein complexes that function as **protein translocators** mediate protein movement across mitochondrial membranes. The **TOM complex** transfers proteins across the outer membrane, and two **TIM complexes** (TIM23 and TIM22) transfer proteins across the inner membrane (**Figure 12–21**). These complexes contain some components that act as receptors for mitochondrial precursor proteins, and other components that form the translocation channels.

The TOM complex is required for the import of all nucleus-encoded mitochondrial proteins. It initially transports their signal sequences into the intermembrane space and helps to insert transmembrane proteins into the outer membrane. β-barrel proteins, which are particularly abundant in the outer membrane, are then passed on to an additional translocator, the **SAM complex**, which helps them to fold properly in the outer membrane. The TIM23 complex transports some soluble proteins into the matrix space and helps to insert transmembrane proteins into the inner membrane. The TIM22 complex mediates the insertion of a subclass of inner membrane proteins, including the transporter that moves ADP, ATP, and phosphate in and out of mitochondria. Yet another protein translocator in the inner mitochondrial membrane, the **OXA complex**, mediates the insertion of

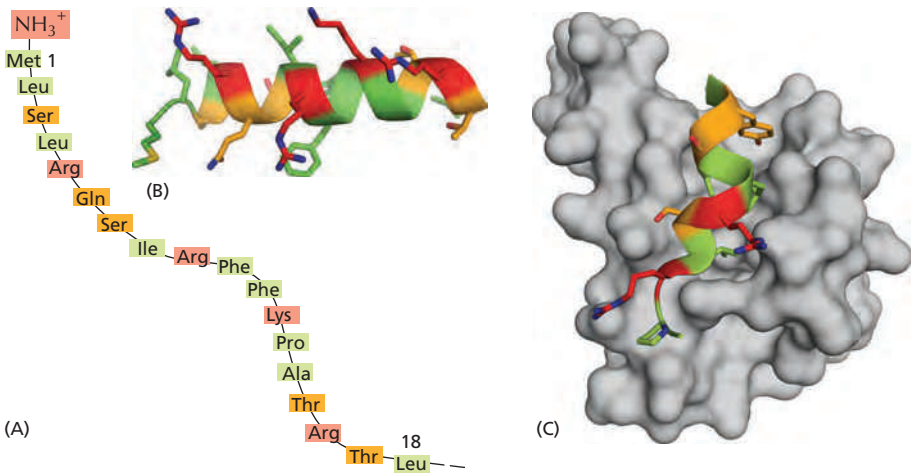


Figure 12–20 A signal sequence for mitochondrial protein import. Cytochrome oxidase is a large multiprotein complex located in the inner mitochondrial membrane, where it functions as the terminal enzyme in the electron-transport chain (discussed in Chapter 14). (A) The first 18 amino acids of the precursor to subunit IV of this enzyme serve as a signal sequence for import of the subunit into the mitochondrion. (B) When the signal sequence is folded as an α helix, the positively charged amino acids *(red)* are clustered on one face of the helix, while the nonpolar ones *(green)* are clustered primarily on the opposite face. Uncharged polar amino acids are shaded *orange*; nitrogen atoms on the side chains of Arg and Gln are colored *blue.* Signal sequences that direct proteins into the matrix space always have the potential to form such an amphiphilic α helix, which is recognized by specific receptor proteins on the mitochondrial surface. (C) The structure of a signal sequence (of alcohol dehydrogenase, another mitochondrial matrix enzyme), bound to an import receptor *(gray)*, as determined by nuclear magnetic resonance. The amphiphilic α helix binds with its hydrophobic face to a hydrophobic groove in the receptor (PDB code: 1OM2).

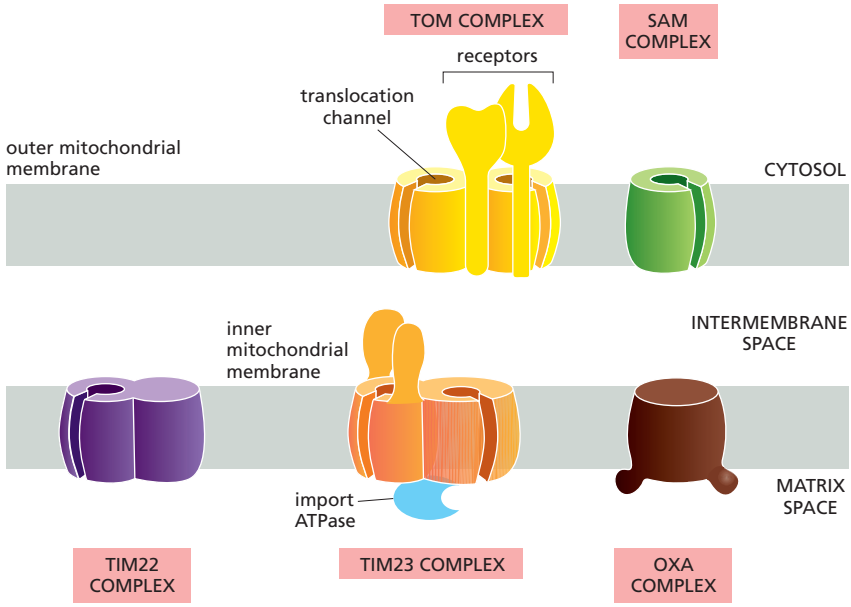


Figure 12–21 The protein translocators in the mitochondrial membranes. The TOM, TIM, SAM, and OXA complexes are multimeric membrane protein assemblies that catalyze protein translocation across mitochondrial membranes. The protein components of the TIM22 and TIM23 complexes that line the import channel are structurally related, suggesting a common evolutionary origin of both TIM complexes. On the matrix side, the TIM23 complex is bound to a multimeric protein complex containing mitochondrial hsp70, which acts as an import ATPase, using ATP hydrolysis to pull proteins through the pore. In animal cells, subtle variations exist in the subunit composition of the translocator complexes to adapt the mitochondrial import machinery to the particular needs of specialized cell types. SAM = Sorting and Assembly Machinery; OXA = cytochrome OXidase Activity; TIM = Translocator of the Inner Mitochondrial membrane; TOM = Translocator of the Outer Membrane.

those inner membrane proteins that are synthesized within mitochondria. It also helps to insert some imported inner membrane proteins that are initially transported into the matrix space by the other complexes.

Mitochondrial Precursor Proteins Are Imported as Unfolded Polypeptide Chains

We have learned almost everything we know about the molecular mechanism of protein import into mitochondria from analyses of cell-free, reconstituted translocation systems, in which purified mitochondria in a test tube import radiolabeled mitochondrial precursor proteins. By changing the conditions in the test tube, it is possible to establish the biochemical requirements for the import.

Mitochondrial precursor proteins do not fold into their native structures after they are synthesized; instead, they remain unfolded in the cytosol through interactions with other proteins. Some of these interacting proteins are general *chaperone proteins* of the *hsp70 family* (discussed in Chapter 6), whereas others are dedicated to mitochondrial precursor proteins and bind directly to their signal sequences. All the interacting proteins help to prevent the precursor proteins from aggregating or folding up spontaneously before they engage with the TOM complex in the outer mitochondrial membrane. As a first step in the import process, the import receptors of the TOM complex bind the signal sequence of the mitochondrial precursor protein. The interacting proteins are then stripped off, and the unfolded polypeptide chain is fed—signal sequence first—into the translocation channel.

In principle, a protein could reach the mitochondrial matrix space by either crossing the two membranes all at once or crossing one at a time. One can distinguish between these possibilities by cooling a cell-free mitochondrial import system to arrest the proteins at an intermediate step in the translocation process. The result is that the arrested proteins no longer contain their N-terminal signal sequence, indicating that the N-terminus must be in the matrix space where the signal peptidase is located, but the bulk of the protein can still be attacked from outside the mitochondria by externally added proteolytic enzymes. Clearly, the precursor proteins can pass through both mitochondrial membranes at once to enter the matrix space (**Figure 12–22**). The TOM complex first transports the signal sequence across the outer membrane to the intermembrane space, where it binds to a TIM complex, opening the channel in the complex. The polypeptide chain is then either translocated into the matrix space or inserted into the inner membrane.

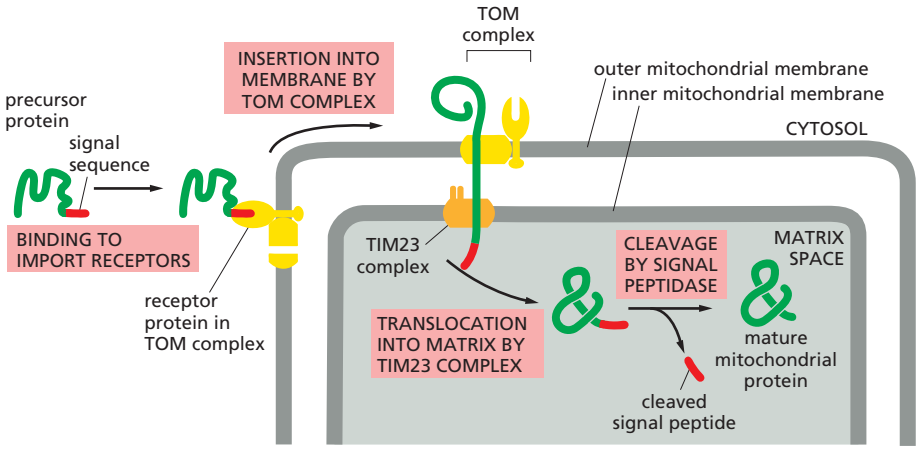


Figure 12–22 Protein import by mitochondria. The N-terminal signal sequence of the mitochondrial precursor protein is recognized by receptors of the TOM complex. The protein is then translocated through the TIM23 complex so that it transiently spans both mitochondrial membranes (Movie 12.3). The signal sequence is cleaved off by a signal peptidase in the matrix space to form the mature protein. The free signal sequence is then rapidly degraded (not shown).

Although the TOM and TIM complexes usually work together to translocate precursor proteins across both membranes at the same time, they can work independently. In isolated outer membranes, for example, the TOM complex can translocate the signal sequence of precursor proteins across the membrane. Similarly, if the outer membrane is experimentally disrupted in isolated mitochondria, the exposed TIM23 complex can efficiently import precursor proteins into the matrix space.

ATP Hydrolysis and a Membrane Potential Drive Protein Import Into the Matrix Space

Directional transport requires energy, which in most biological systems is supplied by ATP hydrolysis. ATP hydrolysis fuels mitochondrial protein import at two discrete sites, one outside the mitochondria and one in the matrix space. In addition, protein import requires another energy source, which is the membrane potential across the inner mitochondrial membrane (Figure 12–23).

The first requirement for energy occurs at the initial stage of the translocation process, when the unfolded precursor protein, associated with chaperone proteins, interacts with the import receptors of the TOM complex. As discussed in Chapter 6, the binding and release of newly synthesized polypeptides from the chaperone proteins requires ATP hydrolysis.

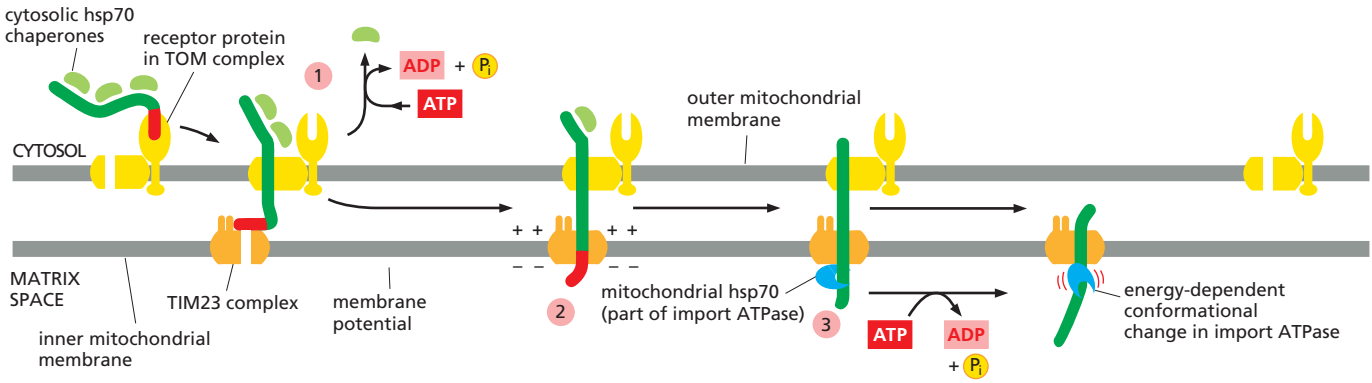


Figure 12–23 The role of energy in protein import into the mitochondrial matrix space. (1) Bound *cytosolic hsp70* chaperone is released from the precursor protein in a step that depends on ATP hydrolysis. After initial insertion of the signal sequence and of adjacent portions of the polypeptide chain into the TOM complex translocation channel, the signal sequence interacts with a TIM complex. (2) The signal sequence is then translocated into the matrix space in a process that requires the energy in the membrane potential across the inner membrane. (3) *Mitochondrial hsp70*, which is part of an import ATPase complex, binds to regions of the polypeptide chain as they become exposed in the matrix space, pulling the protein through the translocation channel, using the energy of ATP hydrolysis.

Once the signal sequence has passed through the TOM complex and is bound to a TIM complex, further translocation through the TIM translocation channel requires the membrane potential, which is the electrical component of the electrochemical H^+ gradient across the inner membrane (see Figure 11–4). Pumping of H^+ from the matrix space to the intermembrane space, driven by electron transport processes in the inner membrane (discussed in Chapter 14), maintains the electrochemical gradient. The energy in the electrochemical H^+ gradient across the inner membrane therefore not only powers most of the cell's ATP synthesis, but it also drives the translocation of the positively charged signal sequences through the TIM complexes by electrophoresis.

Mitochondrial hsp70 also plays a crucial part in the import process. Mitochondria containing mutant forms of the protein fail to import precursor proteins. The mitochondrial hsp70 is part of a multisubunit protein assembly that is bound to the matrix side of the TIM23 complex and acts as a motor to pull the precursor protein into the matrix space. Like its cytosolic cousin, mitochondrial hsp70 has a high affinity for unfolded polypeptide chains, and it binds tightly to an imported protein chain as soon as the chain emerges from the TIM translocator in the matrix space. The hsp70 then undergoes a conformational change and releases the protein chain in an ATP-dependent step, exerting a ratcheting/pulling force on the protein being imported. This energy-driven cycle of binding and subsequent release provides the final driving force needed to complete protein import after a protein has initially inserted into the TIM23 complex (see Figure 12–23).

After the initial interaction with mitochondrial hsp70, many imported matrix proteins are passed on to another chaperone protein, *mitochondrial hsp60*. As discussed in Chapter 6, hsp60 helps the unfolded polypeptide chain to fold by binding and releasing it through cycles of ATP hydrolysis.

Bacteria and Mitochondria Use Similar Mechanisms to Insert Porins into their Outer Membrane

The outer mitochondrial membrane, like the outer membrane of Gram-negative bacteria (see Figure 11–17), contains abundant pore-forming β-barrel proteins called **porins**, and it is thus freely permeable to inorganic ions and metabolites (but not to most proteins). In contrast to other outer membrane proteins, which are anchored in the membrane through transmembrane α-helical regions, the TOM complex cannot integrate porins into the lipid bilayer. Instead, porins are first transported unfolded into the intermembrane space, where they transiently bind specialized chaperone proteins, which keep the porins from aggregating (**Figure 12–24**A). They then bind to the SAM complex in the outer membrane, which both inserts them into the outer membrane and helps them fold properly.

One of the central subunits of the SAM complex is homologous to a bacterial outer membrane protein that helps insert β-barrel proteins into the bacterial outer

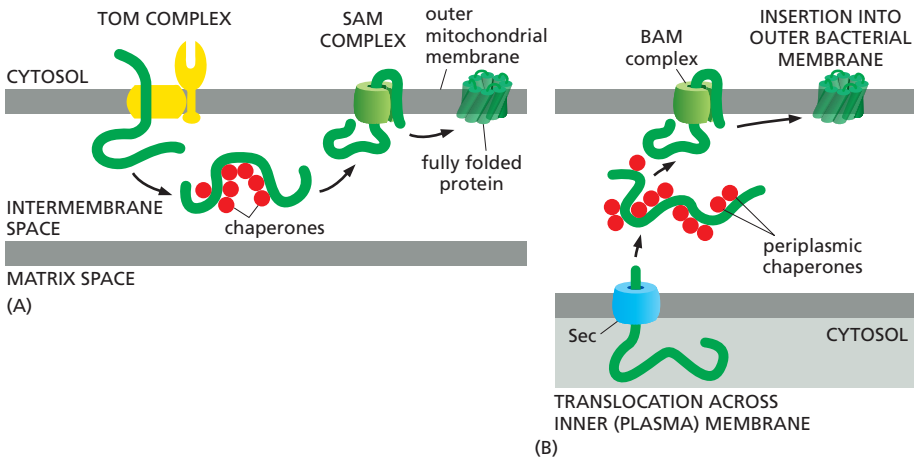


Figure 12–24 Integration of porins into the outer mitochondrial and bacterial membranes. (A) After translocation through the TOM complex in the outer mitochondrial membrane, β-barrel proteins bind to chaperones in the intermembrane space. The SAM complex then inserts the unfolded polypeptide chain into the outer membrane and helps the chain fold. (B) A structurally related BAM complex in the outer membrane of Gram-negative bacteria catalyzes β-barrel protein insertion and folding (see Figure 11–17).

membrane from the periplasmic space (the equivalent of the intermembrane space in mitochondria) (Figure 12–24B). This conserved pathway for inserting β-barrel proteins further underscores the endosymbiotic origin of mitochondria.

Transport Into the Inner Mitochondrial Membrane and Intermembrane Space Occurs Via Several Routes

The same mechanism that transports proteins into the matrix space using the TOM and TIM23 translocators (see Figure 12–22) also mediates the initial translocation of many proteins that are destined for the inner mitochondrial membrane or the intermembrane space. In the most common translocation route, only the N-terminal signal sequence of the transported protein actually enters the matrix space (**Figure 12–25**A). A hydrophobic amino acid sequence, strategically placed after the N-terminal signal sequence, acts as a *stop-transfer sequence*, preventing

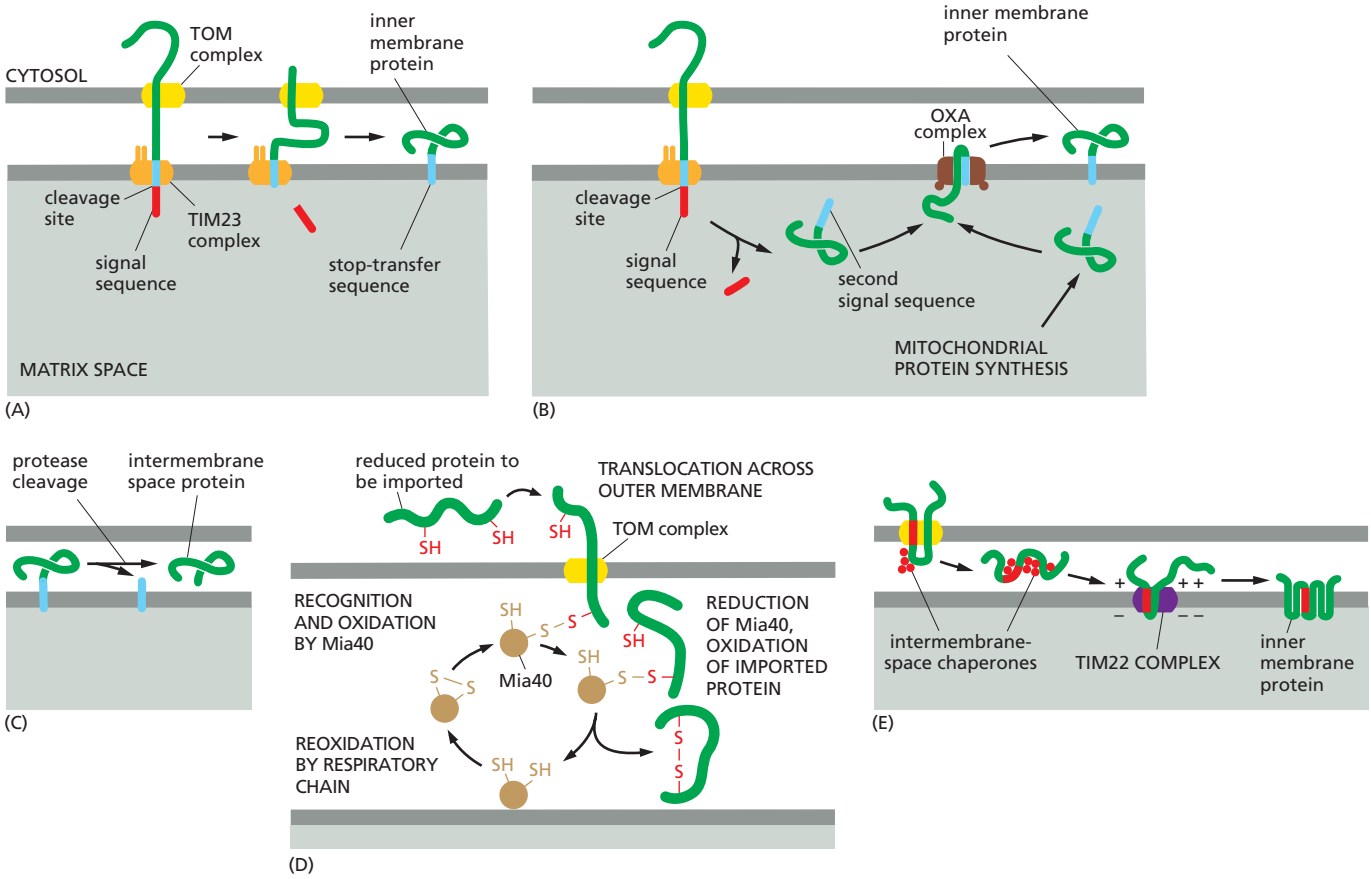


Figure 12–25 Protein import from the cytosol into the inner mitochondrial membrane and intermembrane space. (A) The N-terminal signal sequence *(red)* initiates import into the matrix space (see Figure 12–22). A hydrophobic sequence *(blue)* that follows the matrix-targeting signal sequence binds to the TIM23 translocator *(orange)* in the inner membrane and stops translocation. The remainder of the protein is then pulled into the intermembrane space through the TOM translocator in the outer membrane, and the hydrophobic sequence is released into the inner membrane anchoring the protein there. (B) A second route for protein integration into the inner membrane first delivers the protein completely into the matrix space. Cleavage of the signal sequence *(red)* used for the initial translocation unmasks an adjacent hydrophobic signal sequence *(blue)* at the new N-terminus. This signal then directs the protein into the inner membrane, using the same OXA-dependent pathway that inserts proteins that are encoded by the mitochondrial genome and translated in the matrix space. (C) Some soluble proteins of the intermembrane space also use the pathways shown in (A) and (B) before they are released into the intermembrane space by a second signal peptidase, which has its active site in the intermembrane space and removes the hydrophobic signal sequence. (D) Some soluble intermembrane-space proteins become oxidized by the Mia40 protein (Mia = mitochondrial intermembrane space assembly) during import. Mia40 forms a covalent intermediate through an intermolecular disulfide bond, which helps pull the transported protein through the TOM complex. Mia40 becomes reduced in the process, and then is reoxidized by the electron transport chain, so that it can catalyze the next round of import. (E) Multipass inner membrane proteins that function as metabolite transporters contain internal signal sequences and snake through the TOM complex as a loop. They then bind to the chaperones in the intermembrane space, which guide the proteins to the TIM22 complex. The TIM22 complex is specialized for the insertion of multipass inner membrane proteins.

further translocation across the inner membrane. The remainder of the protein then crosses the outer membrane through the TOM complex into the intermembrane space; the signal sequence is cleaved off in the matrix, and the hydrophobic sequence, released from TIM23, remains anchored in the inner membrane.

In another transport route to the inner membrane or intermembrane space, the TIM23 complex initially translocates the entire protein into the matrix space (Figure 12–25B). A matrix signal peptidase then removes the N-terminal signal sequence, exposing a hydrophobic sequence at the new N-terminus. This signal sequence guides the protein to the OXA complex, which inserts the protein into the inner membrane. As mentioned earlier, the OXA complex is primarily used to insert proteins that are encoded and translated in the mitochondrion into the inner membrane, and only a few imported proteins use this pathway. Translocators that are closely related to the OXA complex are found in the plasma membrane of bacteria and in the thylakoid membrane of chloroplasts, where they insert membrane proteins by a similar mechanism.

Many proteins that use these pathways to the inner membrane remain anchored there through their hydrophobic signal sequence (see Figure 12–25A,B). Others, however, are released into the intermembrane space by a protease that removes the membrane anchor (Figure 12–25C). Many of these cleaved proteins remain attached to the outer surface of the inner membrane as peripheral subunits of protein complexes that also contain transmembrane proteins.

Certain intermembrane-space proteins that contain cysteine motifs are imported by a yet different route. These proteins form a transient covalent disulfide bond to the Mia40 protein (Figure 12–25D). The imported proteins are then released in an oxidized form containing intrachain disulfide bonds. Mia40 becomes reduced in the process, and is then reoxidized by passing electrons to the electron transport chain in the inner mitochondrial membrane. In this way, the energy stored in the redox potential in the mitochondrial electron transport chain is tapped to drive protein import.

Mitochondria are the principal sites of ATP synthesis in the cell, but they also contain many metabolic enzymes, such as those of the citric acid cycle. Thus, in addition to proteins, mitochondria must also transport small metabolites across their membranes. While the outer membrane contains porins, which make the membrane freely permeable to such small molecules, the inner membrane does not. Instead, a family of metabolite-specific transporters transfers a vast number of small molecules across the inner membrane. In yeast cells, these transporters comprise a family of 35 different proteins, the most abundant of which transport ATP, ADP, and phosphate. These are multipass transmembrane proteins, which do not have cleavable signal sequences at their N-termini but instead contain internal signal sequences. They cross the TOM complex in the outer membrane, and intermembrane-space chaperones guide them to the TIM22 complex, which inserts them into the inner membrane by a process that requires the membrane potential, but not mitochondrial hsp70 or ATP (Figure 12–25E). An energetically favorable partitioning of the hydrophobic transmembrane regions into the inner membrane is likely to drive this process.

Two Signal Sequences Direct Proteins to the Thylakoid Membrane in Chloroplasts

Protein transport into **chloroplasts** resembles transport into mitochondria. Both processes occur post-translationally, use separate translocation complexes in each membrane, require energy, and use amphiphilic N-terminal signal sequences that are removed after use. With the exception of some of the chaperone molecules, however, the protein components that form the translocation complexes differ. Moreover, whereas mitochondria harness the electrochemical H^+ gradient across their inner membrane to drive transport, chloroplasts, which have an electrochemical H^+ gradient across their thylakoid membrane but not their inner membrane, use GTP and ATP hydrolysis to power import across their

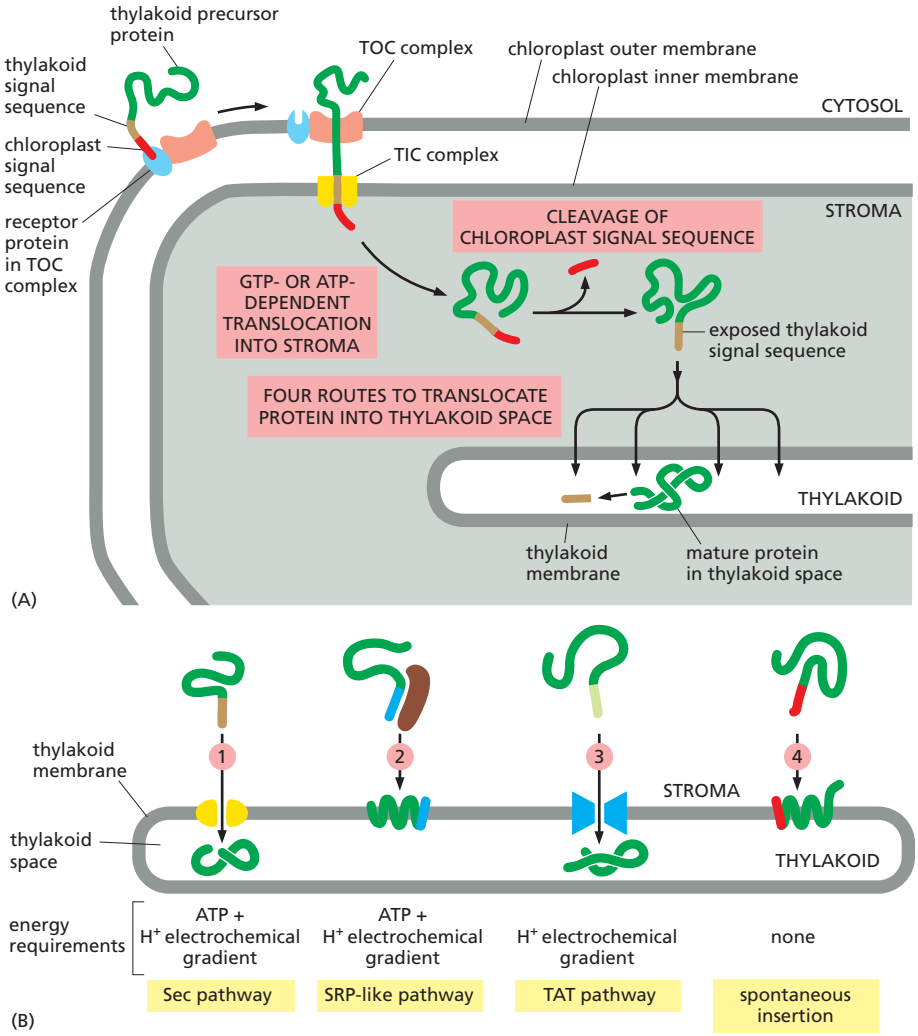


Figure 12–26 Translocation of chloroplast precursor proteins into the thylakoid space. (A) The precursor protein contains an N-terminal chloroplast signal sequence *(red)*, followed immediately by a thylakoid signal sequence *(brown)*. The chloroplast signal sequence initiates translocation into the stroma by a mechanism similar to that used for the translocation of mitochondrial precursor proteins into the matrix space, although the translocator complexes, TOC and TIC, are different. The signal sequence is then cleaved off, unmasking the thylakoid signal sequence, which initiates translocation across the thylakoid membrane. (B) Translocation into the thylakoid space or thylakoid membrane can occur by any one of at least four routes: (1) a *Sec pathway*, so called because it uses components that are homologs of Sec proteins, which mediate protein translocation across the bacterial plasma membrane (discussed later); (2) an *SRP-like pathway*, so called because it uses a chloroplast homolog of the signal-recognition particle, or SRP (discussed later); (3) a *TAT* (twin arginine translocation) *pathway*, so called because two arginines are critical in the signal sequences that direct proteins into this pathway, which depends on the H^+ gradient across the thylakoid membrane; and (4) a *spontaneous insertion pathway* that seems not to require any protein translocator.

double membrane. The functional similarities may thus result from convergent evolution, reflecting the common requirements for translocation across a double membrane.

Although the signal sequences for import into chloroplasts superficially resemble those for import into mitochondria, the same plant cells have both mitochondria and chloroplasts, so proteins must partition appropriately between the two organelles. In plants, for example, a bacterial enzyme can be directed specifically to mitochondria if it is experimentally joined to an N-terminal signal sequence of a mitochondrial protein; the same enzyme joined to an N-terminal signal sequence of a chloroplast protein ends up in chloroplasts. Thus, the import receptors on each organelle distinguish between the different signal sequences.

Chloroplasts have an extra membrane-enclosed compartment, the **thylakoid**. Many chloroplast proteins, including the protein subunits of the photosynthetic system and of the ATP synthase (discussed in Chapter 14), are located in the thylakoid membrane. Like the precursors of some mitochondrial proteins, the precursors of these proteins are translocated from the cytosol to their final destination in two steps. First, they pass across the double membrane into the matrix space (called the **stroma** in chloroplasts), and then they either integrate into the thylakoid membrane or translocate into the thylakoid space (Figure 12–26A). The precursors of these proteins have a hydrophobic thylakoid signal sequence following the N-terminal chloroplast signal sequence. After the N-terminal signal sequence has been used to import the protein into the stroma, a stromal signal peptidase removes it, unmasking the thylakoid signal sequence that initiates transport

across the thylakoid membrane. There are at least four routes by which proteins cross or become integrated into the thylakoid membrane, distinguished by their need for different stromal chaperones and energy sources (Figure 12–26B).

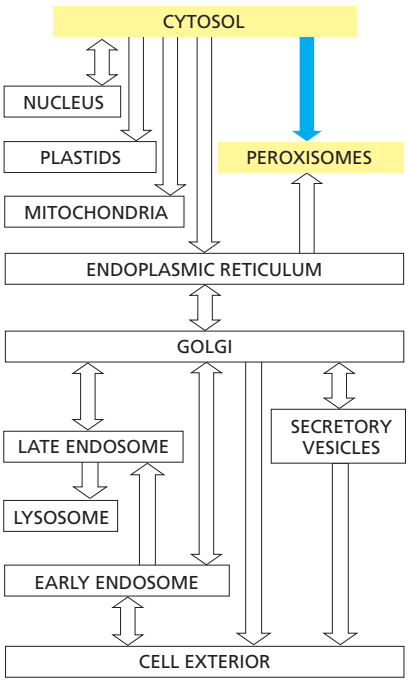
Summary

Although mitochondria and chloroplasts have their own genetic systems, they produce only a small proportion of their own proteins. Instead, the two organelles import most of their proteins from the cytosol, using similar mechanisms. In both cases, proteins are transported in an unfolded state across both outer and inner membranes simultaneously into the matrix space or stroma. Both ATP hydrolysis and a membrane potential across the inner membrane drive translocation into mitochondria, whereas GTP and ATP hydrolysis drive translocation into chloroplasts. Chaperone proteins of the cytosolic hsp70 family maintain the precursor proteins in an unfolded state, and a second set of hsp70 proteins in the matrix space or stroma pulls the polypeptide chain into the organelle. Only proteins that contain a specific signal sequence are translocated. The signal sequence can either be located at the N-terminus and cleaved off after import or be internal and retained. Transport into the inner membrane sometimes uses a second, hydrophobic signal sequence that is unmasked when the first signal sequence is removed. In chloroplasts, import from the stroma into the thylakoid can occur by several routes, distinguished by the chaperones and energy source used.

PEROXISOMES

Peroxisomes differ from mitochondria and chloroplasts in many ways. Most notably, they are surrounded by only a single membrane, and they do not contain DNA or ribosomes. Thus, because peroxisomes lack a genome, all of their proteins are encoded in the nucleus. Peroxisomes acquire most of these proteins by selective import from the cytosol, although some of them enter the peroxisome membrane via the ER.

Because we do not discuss peroxisomes elsewhere, we shall digress to consider some of the functions of this diverse family of organelles, before discussing their biosynthesis. Virtually all eukaryotic cells have peroxisomes. They contain oxidative enzymes, such as *catalase* and *urate oxidase*, at such high concentrations that, in some cells, the peroxisomes stand out in electron micrographs because of the presence of a crystalloid protein core (**Figure 12–27**).



Like mitochondria, peroxisomes are major sites of oxygen utilization. One hypothesis is that peroxisomes are a vestige of an ancient organelle that performed all the oxygen metabolism in the primitive ancestors of eukaryotic cells. When the oxygen produced by photosynthetic bacteria first accumulated in the atmosphere, it would have been highly toxic to most cells. Peroxisomes might have lowered the intracellular concentration of oxygen, while also exploiting its chemical reactivity to perform useful oxidation reactions. According to this view, the later development of mitochondria rendered peroxisomes largely obsolete because many of the same biochemical reactions—which had formerly been carried out in peroxisomes without producing energy—were now coupled to ATP formation by means of oxidative phosphorylation. The oxidation reactions performed by peroxisomes in present-day cells could therefore partly be those whose functions were not taken over by mitochondria.

Peroxisomes Use Molecular Oxygen and Hydrogen Peroxide to Perform Oxidation Reactions

Peroxisomes are so named because they usually contain one or more enzymes that use molecular oxygen to remove hydrogen atoms from specific organic substrates (designated here as R) in an oxidation reaction that produces *hydrogen peroxide* (H_2O_2):

$$RH_2 + O_2 \rightarrow R + H_2O_2$$

Catalase uses the H_2O_2 generated by other enzymes in the organelle to oxidize a variety of other substrates—including formic acid, formaldehyde, and alcohol—by the "peroxidation" reaction: $H_2O_2 + R'H_2 \rightarrow R' + 2H_2O$. This type of oxidation reaction is particularly important in liver and kidney cells, where the peroxisomes detoxify various harmful molecules that enter the bloodstream. About 25% of the ethanol we drink is oxidized to acetaldehyde in this way. In addition, when excess H_2O_2 accumulates in the cell, catalase converts it to H_2O through the reaction

$$2H_2O_2 \rightarrow 2H_2O + O_2$$

A major function of the oxidation reactions performed in peroxisomes is the breakdown of fatty acid molecules. The process, called *β oxidation*, shortens the alkyl chains of fatty acids sequentially in blocks of two carbon atoms at a time, thereby converting the fatty acids to acetyl CoA. The peroxisomes then export the acetyl CoA to the cytosol for use in biosynthetic reactions. In mammalian cells, β oxidation occurs in both mitochondria and peroxisomes; in yeast and plant cells, however, this essential reaction occurs exclusively in peroxisomes.

An essential biosynthetic function of animal peroxisomes is to catalyze the first reactions in the formation of *plasmalogens*, which are the most abundant class of phospholipids in myelin (**Figure 12–28**). Plasmalogen deficiencies cause profound abnormalities in the myelination of nerve-cell axons, which is one reason why many peroxisomal disorders lead to neurological disease.

Peroxisomes are unusually diverse organelles, and even in the various cell types of a single organism they may contain different sets of enzymes. They also adapt remarkably to changing conditions. Yeasts grown on sugar, for example, have few small peroxisomes. But when some yeasts are grown on methanol, numerous large peroxisomes are formed that oxidize methanol; and when grown on fatty acids, they develop numerous large peroxisomes that break down fatty acids to acetyl CoA by β oxidation.

Peroxisomes are also important in plants. Two types of plant peroxisomes have been studied extensively. One is present in leaves, where it participates in *photorespiration* (discussed in Chapter 14) (**Figure 12–29**A). The other type of peroxisome is present in germinating seeds, where it converts the fatty acids stored in seed lipids into the sugars needed for the growth of the young plant. Because this conversion of fats to sugars is accomplished by a series of reactions known as the *glyoxylate cycle*, these peroxisomes are also called *glyoxysomes* (Figure 12–29B). In the glyoxylate cycle, two molecules of acetyl CoA produced by fatty acid breakdown in the peroxisome are used to make succinic acid, which then leaves the peroxisome and is converted into glucose in the cytosol. The glyoxylate cycle does not occur in animal cells, and animals are therefore unable to convert the fatty acids in fats into carbohydrates.

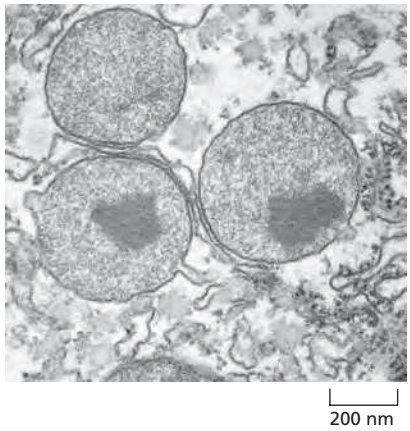


Figure 12–27 An electron micrograph of three peroxisomes in a rat liver cell. The paracrystalline, electron-dense inclusions are composed primarily of the enzyme urate oxidase. (Courtesy of Daniel S. Friend.)

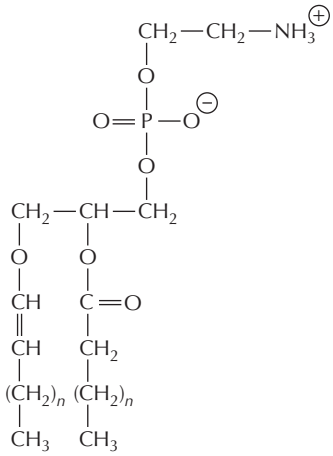


Figure 12–28 The structure of a plasmalogen. Plasmalogens are very abundant in the myelin sheaths that insulate the axons of nerve cells. They make up some 80–90% of the myelin membrane phospholipids. In addition to an ethanolamine head group and a long-chain fatty acid attached to the same glycerol phosphate backbone used for phospholipids, plasmalogens contain an unusual fatty alcohol that is attached through an ether linkage *(bottom left)*.

A Short Signal Sequence Directs the Import of Proteins into Peroxisomes

A specific sequence of three amino acids (Ser–Lys–Leu) located at the C-terminus of many peroxisomal proteins functions as an import signal (see Table 12–3, p. 648). Other peroxisomal proteins contain a signal sequence near the N-terminus. If either sequence is attached to a cytosolic protein, the protein is imported into peroxisomes. The import signals are first recognized by soluble receptor proteins in the cytosol. Numerous distinct proteins, called **peroxins**, participate in the import process, which is driven by ATP hydrolysis. A complex of at least six different peroxins forms a protein translocator in the peroxisome membrane. Even oligomeric proteins do not have to unfold to be imported. To allow the passage of such compactly folded cargo molecules, the pore formed by the transporter is thought to be dynamic in its dimensions, adapting in size to the particular cargo molecules to be transported. In this respect, the mechanism differs from that used by mitochondria and chloroplasts. One soluble import receptor, the peroxin Pex5 recognizes the C-terminal peroxisomal import signal. It accompanies its cargo all the way into peroxisomes and, after cargo release, cycles back to the cytosol. After

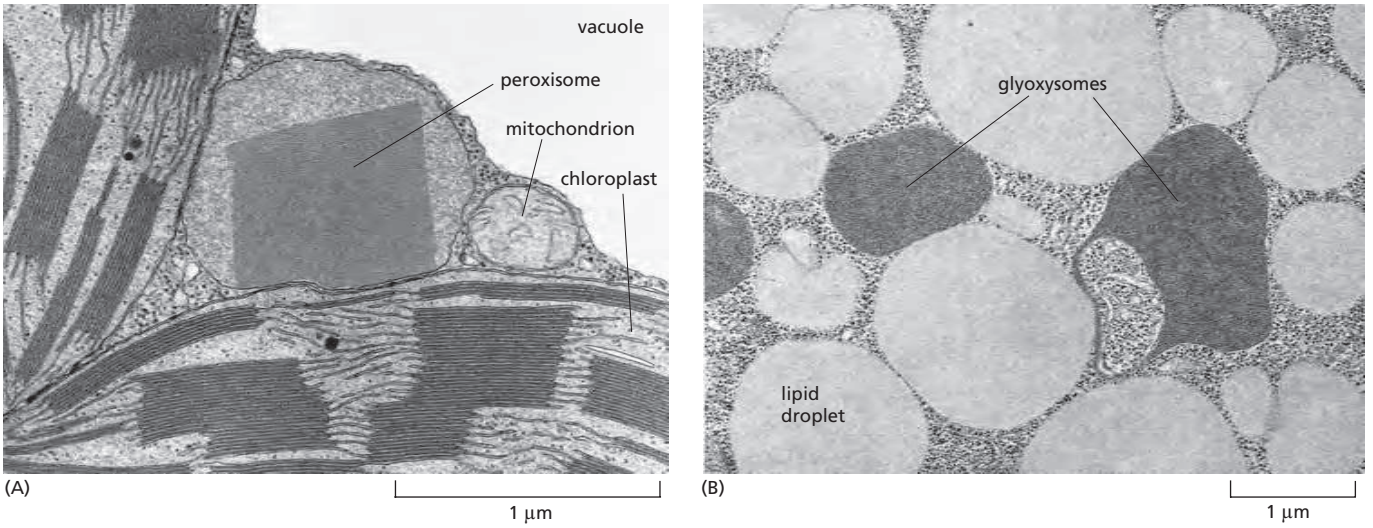


Figure 12–29 Electron micrographs of two types of peroxisomes found in plant cells. (A) A peroxisome with a paracrystalline core in a tobacco leaf mesophyll cell. Its close association with chloroplasts is thought to facilitate the exchange of materials between these organelles during photorespiration. The vacuole in plant cells is equivalent to the lysosome in animal cells. (B) Peroxisomes in a fat-storing cotyledon cell of a tomato seed 4 days after germination. Here the peroxisomes (glyoxysomes) are associated with the lipid droplets that store fat, reflecting their central role in fat mobilization and gluconeogenesis during seed germination. (A, from S.E. Frederick and E.H. Newcomb, *J. Cell Biol.* 43:343–353, 1969. With permission from The Rockefeller Press; B, from W.P. Wergin, P.J. Gruber and E.H. Newcomb, *J. Ultrastruct. Res.* 30:533–557, 1970. With permission from Academic Press.)

delivering its cargo to the peroxisome lumen, Pex5 undergoes ubiquitylation. This modification is required to release Pex5 back into the cytosol, where the ubiquitin is removed. An ATPase composed of Pex1 and Pex6 harnesses the energy of ATP hydrolysis to help release Pex5 from peroxisomes.

The importance of this import process and of peroxisomes is demonstrated by the inherited human disease *Zellweger syndrome,* in which a defect in importing proteins into peroxisomes leads to a profound peroxisomal deficiency. These individuals, whose cells contain "empty" peroxisomes, have severe abnormalities in their brain, liver, and kidneys, and they die soon after birth. A mutation in the gene encoding peroxin Pex5 causes one form of the disease. A defect in Pex7, the receptor for the N-terminal import signal, causes a milder peroxisomal disease.

It has long been debated whether new peroxisomes arise from preexisting ones by organelle growth and fission—as mentioned earlier for mitochondria and plastids—or whether they derive as a specialized compartment from the endoplasmic reticulum (ER). Aspects of both views are true (**Figure 12–30**). Most peroxisomal membrane proteins are made in the cytosol and insert into the membrane of

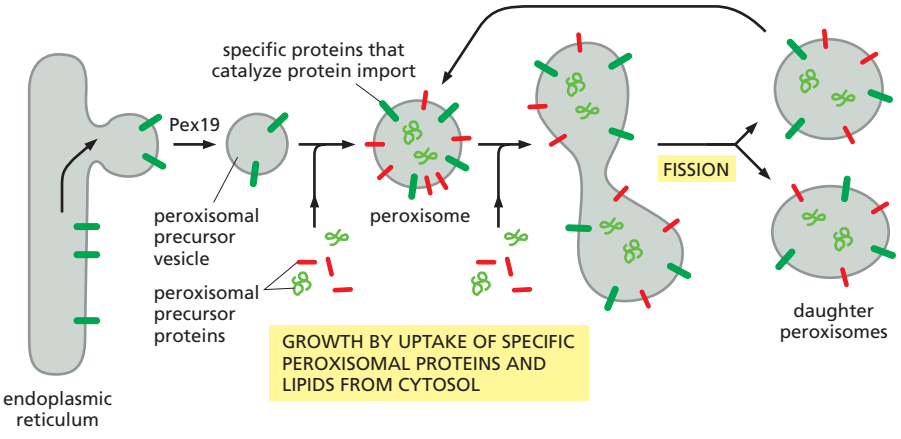


Figure 12–30 A model that explains how peroxisomes proliferate and how new peroxisomes arise. Peroxisomal precursor vesicles bud from the ER. At least two peroxisomal membrane proteins, Pex3 and Pex15, follow this route. The machinery that drives the budding reaction and that selects only peroxisomal proteins for packaging into these vesicles depends on Pex19 and other cytosolic proteins that are still unknown. Peroxisomal precursor vesicles may then fuse with one another or with preexisting peroxisomes. The peroxisome membrane contains import receptors and protein translocators that are required for the import of peroxisomal proteins made on cytosolic ribosomes, including new copies of the import receptors and translocator components. Presumably, the lipids required for growth are also imported, although some may derive directly from the ER in the membrane of peroxisomal precursor vesicles.

preexisting peroxisomes, but others are first integrated into the ER membrane, where they are packaged into specialized peroxisomal precursor vesicles. New precursor vesicles may then fuse with one another and begin importing additional peroxisomal proteins, using their own protein import machinery to grow into mature peroxisomes, which can undergo cycles of growth and fission.

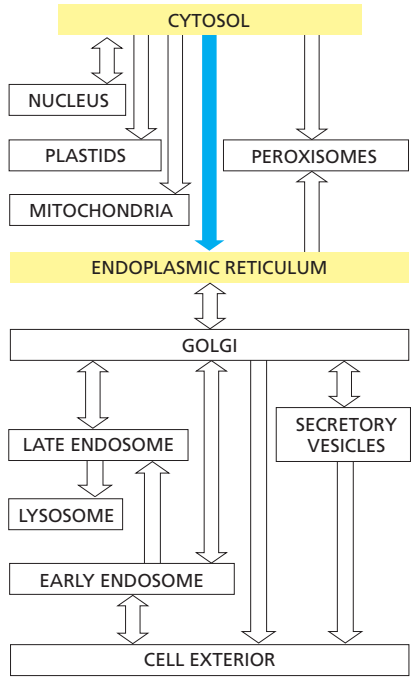
Summary

Peroxisomes are specialized for carrying out oxidation reactions using molecular oxygen. They generate hydrogen peroxide, which they employ for oxidative purposes—and contain catalase to destroy the excess. Like mitochondria and plastids, peroxisomes are self-replicating organelles. Because they do not contain DNA or ribosomes, however, all of their proteins are encoded in the cell nucleus. Some of these proteins are conveyed to peroxisomes via peroxisomal precursor vesicles that bud from the ER, but most are synthesized in the cytosol and directly imported. A specific sequence of three amino acids near the C-terminus of many of the latter proteins functions as a peroxisomal import signal. The mechanism of protein import differs from that of mitochondria and chloroplasts, in that even oligomeric proteins are imported from the cytosol without unfolding.

THE ENDOPLASMIC RETICULUM

All eukaryotic cells have an **endoplasmic reticulum** (**ER**). Its membrane typically constitutes more than half of the total membrane of an average animal cell (see Table 12–2, p. 643). The ER is organized into a netlike labyrinth of branching tubules and flattened sacs that extends throughout the cytosol (**Figure 12–31** and **Movie 12.4**). The tubules and sacs interconnect, and their membrane is continuous with the outer nuclear membrane; the compartment that they enclose therefore is also continuous with the space between the inner and outer nuclear membranes. Thus, the ER and nuclear membranes form a continuous sheet enclosing a single internal space, called the **ER lumen** or the *ER cisternal space*, which often occupies more than 10% of the total cell volume (see Table 12–1, p. 643).

As mentioned at the beginning of this chapter, the ER has a central role in both lipid and protein biosynthesis, and it also serves as an intracellular Ca^{2+} store that is used in many cell signaling responses (discussed in Chapter 15). The ER membrane is the site of production of all the transmembrane proteins and lipids for most of the cell's organelles, including the ER itself, the Golgi apparatus, lysosomes, endosomes, secretory vesicles, and the plasma membrane. The ER membrane is also the site at which most of the lipids for mitochondrial and peroxisomal membranes are made. In addition, almost all of the proteins that will be secreted to the cell exterior—plus those destined for the lumen of the ER, Golgi apparatus, or lysosomes—are initially delivered to the ER lumen.



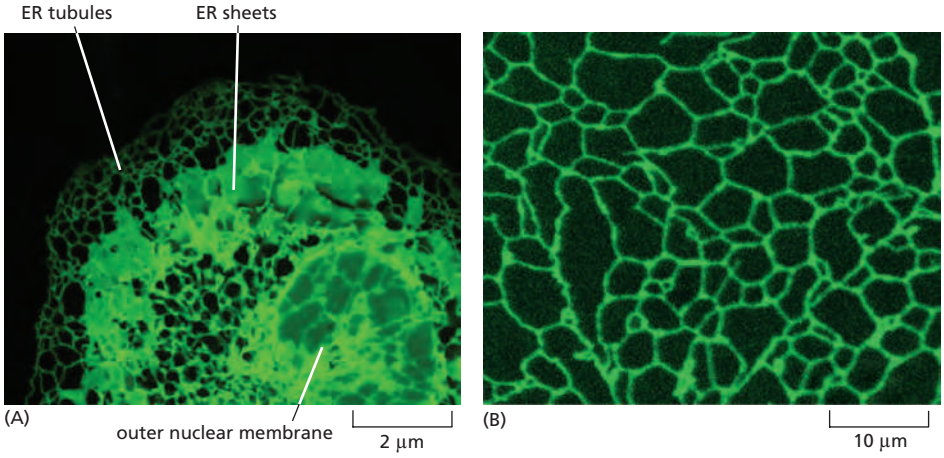


Figure 12–31 Fluorescent micrographs of the endoplasmic reticulum. (A) An animal cell in tissue culture that was genetically engineered to express an ER membrane protein fused to a fluorescent protein. The ER extends as a network of tubules and sheets throughout the entire cytosol, so that all regions of the cytosol are close to some portion of the ER membrane. The outer nuclear membrane, which is continuous with the ER, is also stained. (B) Part of an ER network in a living plant cell that was genetically engineered to express a fluorescent protein in the ER. (A, courtesy of Patrick Chitwood and Gia Voeltz; B, courtesy of Petra Boevink and Chris Hawes.)

The ER Is Structurally and Functionally Diverse

While the various functions of the ER are essential to every cell, their relative importance varies greatly between individual cell types. To meet different functional demands, distinct regions of the ER become highly specialized. We observe such functional specialization as dramatic changes in ER structure, and different cell types can therefore possess characteristically different types of ER membrane. One of the most remarkable ER specializations is the *rough ER*.

Mammalian cells begin to import most proteins into the ER before complete synthesis of the polypeptide chain—that is, import is a **co-translational** process (**Figure 12–32**A). In contrast, the import of proteins into mitochondria, chloroplasts, nuclei, and peroxisomes is a **post-translational** process (Figure 12–32B). In co-translational transport, the ribosome that is synthesizing the protein is attached directly to the ER membrane, enabling one end of the protein to be translocated into the ER while the rest of the polypeptide chain is being synthesized. These membrane-bound ribosomes coat the surface of the ER, creating regions termed **rough endoplasmic reticulum**, or **rough ER**; regions of ER that lack bound ribosomes are called **smooth endoplasmic reticulum**, or **smooth ER** (**Figure 12–33**).

Most cells have scanty regions of smooth ER, and the ER is often partly smooth and partly rough. Areas of smooth ER from which transport vesicles carrying newly synthesized proteins and lipids bud off for transport to the Golgi apparatus are called *transitional ER*. In certain specialized cells, the smooth ER is abundant and has additional functions. It is prominent, for example, in cells that specialize in lipid metabolism, such as cells that synthesize steroid hormones from cholesterol; the expanded smooth ER accommodates the enzymes that make cholesterol and modify it to form the hormones (see Figure 12–33B).

The main cell type in the liver, the *hepatocyte*, also has a substantial amount of smooth ER. It is the principal site of production of *lipoprotein particles*, which carry lipids via the bloodstream to other parts of the body. The enzymes that synthesize the lipid components of the particles are located in the membrane of the smooth ER, which also contains enzymes that catalyze a series of reactions to detoxify both lipid-soluble drugs and various harmful compounds produced by metabolism. The most extensively studied of these *detoxification reactions* are carried out by the *cytochrome P450* family of enzymes, which catalyze a series of reactions in which water-insoluble drugs or metabolites that would otherwise accumulate to toxic levels in cell membranes are rendered sufficiently water-soluble to leave the cell and be excreted in the urine. Because the rough ER alone cannot house enough of these and other necessary enzymes, a substantial portion of the membrane in a hepatocyte normally consists of smooth ER (see Table 12–2).

Another crucially important function of the ER in most eukaryotic cells is to sequester Ca^{2+} from the cytosol. The release of Ca^{2+} into the cytosol from the ER, and its subsequent reuptake, occurs in many rapid responses to extracellular

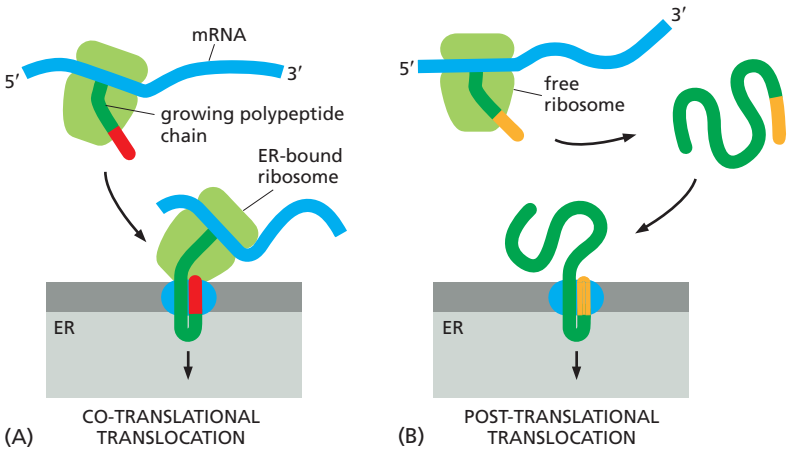


Figure 12–32 Co-translational and post-translational protein translocation. (A) Ribosomes bind to the ER membrane during co-translational translocation. (B) By contrast, cytosolic ribosomes complete the synthesis of a protein and release it prior to post-translational translocation. In both cases, the protein is directed to the ER by an ER signal sequence (*red* and *orange*).

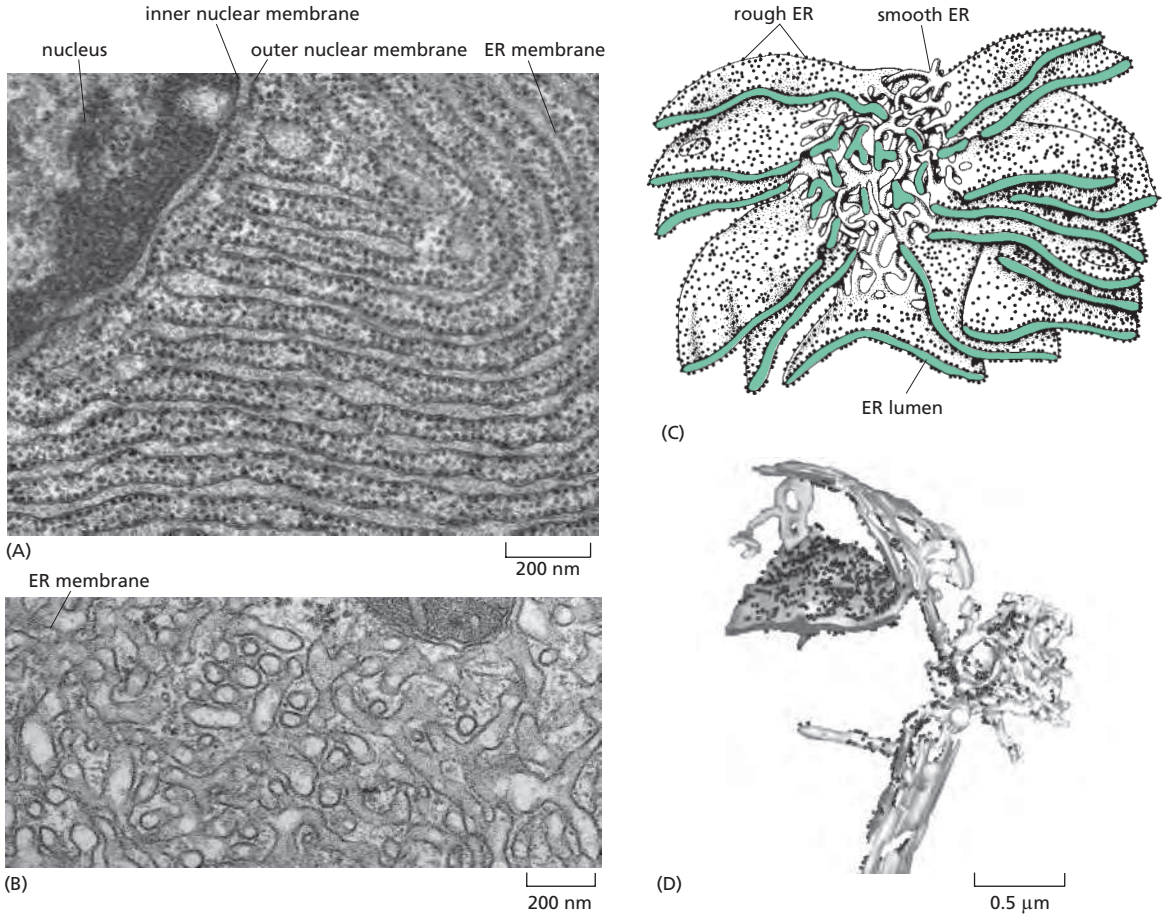


Figure 12–33 The rough and smooth ER. (A) An electron micrograph of the rough ER in a pancreatic exocrine cell that makes and secretes large amounts of digestive enzymes every day. The cytosol is filled with closely packed sheets of ER membrane that is studded with ribosomes. At the top left is a portion of the nucleus and its nuclear envelope; note that the outer nuclear membrane, which is continuous with the ER, is also studded with ribosomes. (B) Abundant smooth ER in a steroid-hormone-secreting cell. This electron micrograph is of a testosterone-secreting Leydig cell in the human testis. (C) A three-dimensional reconstruction of a region of smooth ER and rough ER in a liver cell. The rough ER forms oriented stacks of flattened cisternae, each having a lumenal space 20–30 nm wide. The smooth ER membrane is connected to these cisternae and forms a fine network of tubules 30–60 nm in diameter. The ER lumen is colored *green*. (D) A tomographic reconstruction of a portion of the ER network in a yeast cell. Membrane-bound ribosomes *(tiny dark spheres)* are seen in both flat sheets and tubular regions of irregular diameter, demonstrating that the ribosomes bind to ER membranes of different curvature in these cells. (A, courtesy of Lelio Orci; B, courtesy of Daniel S. Friend; C, after R.V. Krstić, Ultrastructure of the Mammalian Cell. New York: Springer-Verlag, 1979; D, from M. West et al., *J. Cell Biol.* 193:333–346, 2011. With permission from Rockefeller University Press.)

signals, as discussed in Chapter 15. A Ca^{2+} pump transports Ca^{2+} from the cytosol into the ER lumen. A high concentration of Ca^{2+}-binding proteins in the ER facilitates Ca^{2+} storage. In some cell types, and perhaps in most, specific regions of the ER are specialized for Ca^{2+} storage. Muscle cells have an abundant, modified smooth ER called the *sarcoplasmic reticulum*. The release and reuptake of Ca^{2+} by the sarcoplasmic reticulum trigger myofibril contraction and relaxation, respectively, during each round of muscle contraction (discussed in Chapter 16).

To study the functions and biochemistry of the ER, it is necessary to isolate it. This may seem to be a hopeless task because the ER is intricately interleaved with other components of the cytoplasm. Fortunately, when tissues or cells are disrupted by homogenization, the ER breaks into fragments, which reseal to form small (~100–200 nm in diameter) closed vesicles called **microsomes**. Microsomes are relatively easy to purify. To the biochemist, microsomes represent small authentic versions of the ER, still capable of protein translocation, protein glycosylation (discussed later), Ca^{2+} uptake and release, and lipid synthesis. Microsomes derived from rough ER are studded with ribosomes and are called *rough*

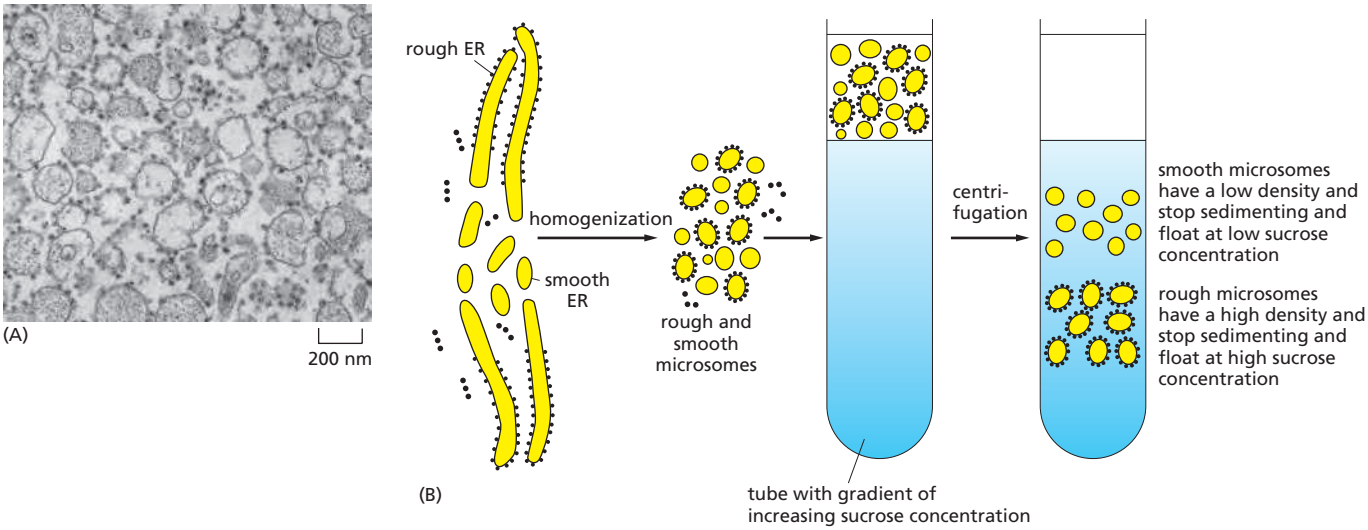


Figure 12–34 The isolation of purified rough and smooth microsomes from the ER. (A) A thin section electron micrograph of the purified rough ER fraction shows an abundance of ribosome-studded vesicles. (B) When sedimented to equilibrium through a gradient of sucrose, the two types of microsomes separate from each other on the basis of their different densities. Note that the smooth fraction will also contain non-ER-derived material. (A, courtesy of George Palade.)

microsomes. The ribosomes are always found on the outside surface, so the interior of the microsome is biochemically equivalent to the lumen of the ER (**Figure 12–34**A).

Many vesicles similar in size to rough microsomes, but lacking attached ribosomes, are also found in cell homogenates. Such *smooth microsomes* are derived in part from smooth portions of the ER and in part from vesiculated fragments of the plasma membrane, Golgi apparatus, endosomes, and mitochondria (the ratio depending on the tissue). Thus, whereas rough microsomes are clearly derived from rough portions of ER, it is not easy to separate smooth microsomes derived from different organelles. The smooth microsomes prepared from liver or muscle cells are an exception. Because of the unusually large quantities of smooth ER or sarcoplasmic reticulum, respectively, most of the smooth microsomes in homogenates of these tissues are derived from the smooth ER or sarcoplasmic reticulum. The ribosomes attached to rough microsomes make them more dense than smooth microsomes. As a result, we can use equilibrium centrifugation to separate the rough and smooth microsomes (Figure 12–34B). Microsomes have been invaluable in elucidating the molecular aspects of ER function, as we discuss next.

Signal Sequences Were First Discovered in Proteins Imported into the Rough ER

The ER captures selected proteins from the cytosol as they are being synthesized. These proteins are of two types: *transmembrane proteins*, which are only partly translocated across the ER membrane and become embedded in it, and *water-soluble proteins*, which are fully translocated across the ER membrane and are released into the ER lumen. Some of the transmembrane proteins function in the ER, but many are destined to reside in the plasma membrane or the membrane of another organelle. The water-soluble proteins are destined either for secretion or for residence in the lumen of the ER or of another organelle. All of these proteins, regardless of their subsequent fate, are directed to the ER membrane by an **ER signal sequence**, which initiates their translocation by a common mechanism.

Signal sequences (and the signal sequence strategy of protein sorting) were first discovered in the early 1970s in secreted proteins that are translocated across the ER membrane as a first step toward their eventual discharge from the cell. In the key experiment, the mRNA encoding a secreted protein was translated by ribosomes *in vitro*. When microsomes were omitted from this cell-free system, the protein synthesized was slightly larger than the normal secreted protein. In the presence of microsomes derived from the rough ER, however, a protein of the correct size was produced. According to the *signal hypothesis*, the size difference reflects the initial presence of a signal sequence that directs the secreted protein

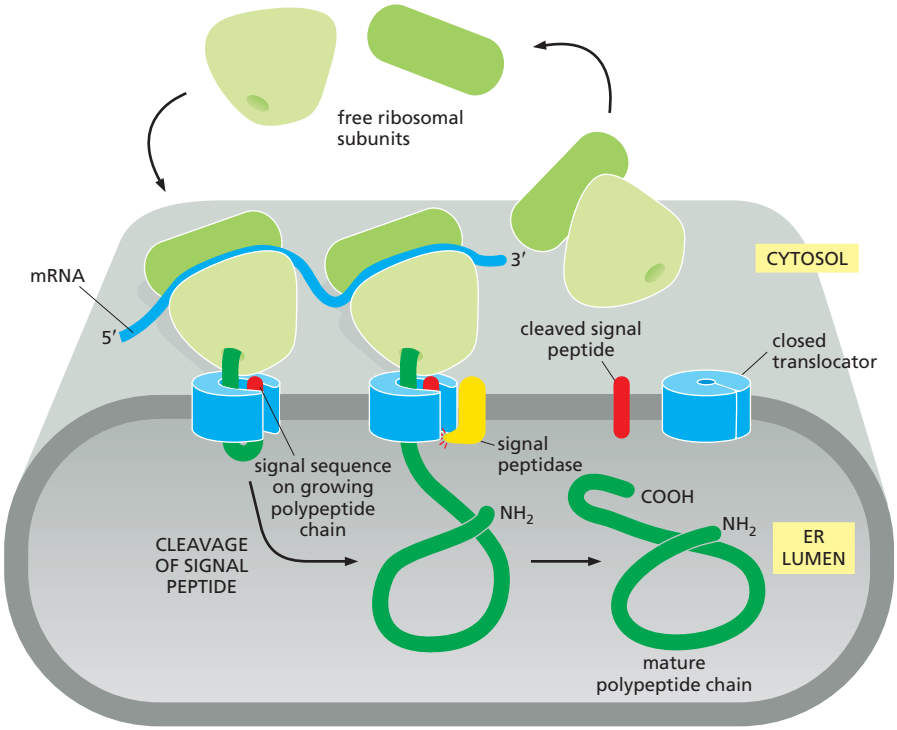


Figure 12–35 The signal hypothesis. A simplified view of protein translocation across the ER membrane, as originally proposed. When the ER signal sequence emerges from the ribosome, it directs the ribosome to a translocator on the ER membrane that forms a pore in the membrane through which the polypeptide is translocated. A signal peptidase is closely associated with the translocator and clips off the signal sequence during translation, and the mature protein is released into the lumen of the ER immediately after its synthesis is completed. The translocator is closed until the ribosome has bound, so that the permeability barrier of the ER membrane is maintained at all times.

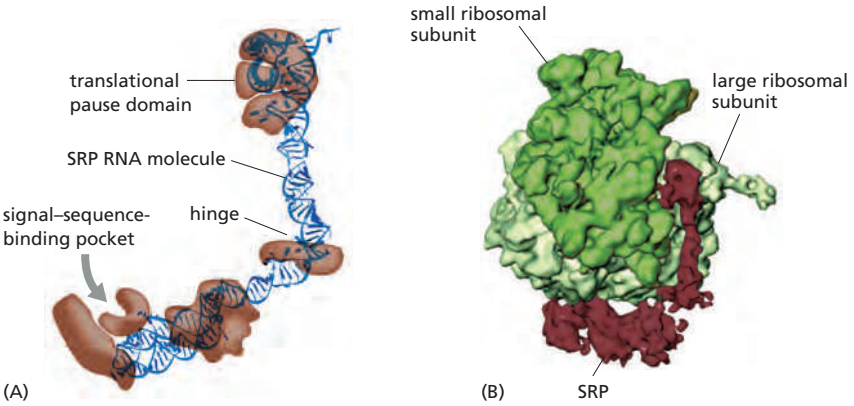
to the ER membrane and is then cleaved off by a *signal peptidase* in the ER membrane before the polypeptide chain has been completed (**Figure 12–35**). Cell-free systems in which proteins are imported into microsomes have provided powerful procedures for identifying, purifying, and studying the various components of the molecular machinery responsible for the ER import process.

A Signal-Recognition Particle (SRP) Directs the ER Signal Sequence to a Specific Receptor in the Rough ER Membrane

The ER signal sequence is guided to the ER membrane by at least two components: a **signal-recognition particle** (**SRP**), which cycles between the ER membrane and the cytosol and binds to the signal sequence, and an **SRP receptor** in the ER membrane. The SRP is a large complex; in animal cells, it consists of six different polypeptide chains bound to a single small RNA molecule. While the SRP and SRP receptor have fewer subunits in bacteria, homologs are present in all cells, indicating that this protein-targeting mechanism arose early in evolution and has been conserved.

ER signal sequences vary greatly in amino acid sequence, but each has eight or more nonpolar amino acids at its center (see Table 12–3, p. 648). How can the SRP bind specifically to so many different sequences? The answer has come from the crystal structure of the SRP protein, which shows that the signal-sequence-binding site is a large hydrophobic pocket lined by methionines. Because methionines have unbranched, flexible side chains, the pocket is sufficiently plastic to accommodate hydrophobic signal sequences of different sequences, sizes, and shapes.

The SRP is a rodlike structure, which wraps around the large ribosomal subunit, with one end binding to the ER signal sequence as it emerges from the ribosome as part of the newly made polypeptide chain; the other end blocks the elongation factor binding site at the interface between the large and small ribosomal subunits (**Figure 12–36**). This block halts protein synthesis as soon as the signal peptide has emerged from the ribosome. The transient pause presumably gives the ribosome enough time to bind to the ER membrane before completion of the polypeptide chain, thereby ensuring that the protein is not released into the cytosol. This safety



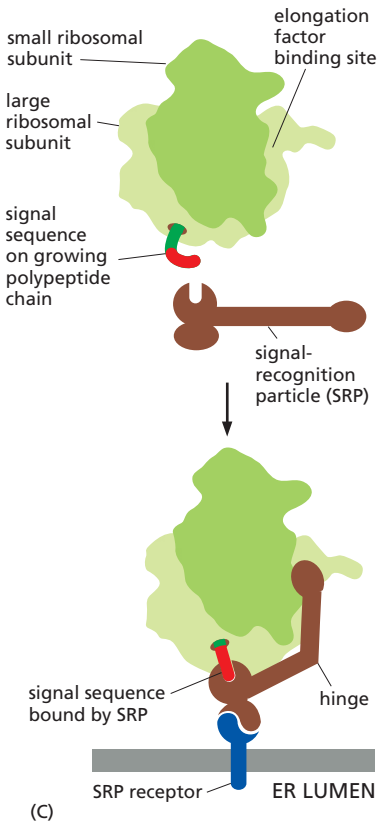


Figure 12–36 The signal-recognition particle (SRP). (A) A mammalian SRP is a rodlike ribonucleoprotein complex containing six protein subunits *(brown)* and one RNA molecule *(blue)*. The SRP RNA forms a backbone that links the protein domain containing the signal-sequence-binding pocket to the domain responsible for pausing translation. Crystal structures of various SRP pieces from different species are assembled here into a composite model to approximate the structure of a complete SRP. (B) The three-dimensional outline of the SRP bound to a ribosome was determined by cryoelectron microscopy. SRP binds to the large ribosomal subunit so that its signal-sequence-binding pocket is positioned near the growing polypeptide chain exit site, and its translational pause domain is positioned at the interface between the ribosomal subunits, where it interferes with elongation factor binding. (C) As a signal sequence emerges from the ribosome and binds to the SRP, a conformational change in the SRP exposes a binding site for the SRP receptor. (B, adapted from M. Halic et al., *Nature* 427:808–814, 2004. With permission from Macmillan Publishers Ltd.)

device may be especially important for secreted and lysosomal hydrolases, which could wreak havoc in the cytosol; cells that secrete large amounts of hydrolases, however, take the added precaution of having high concentrations of hydrolase inhibitors in their cytosol. The pause also ensures that large portions of a protein that could fold into a compact structure are not made before reaching the translocator in the ER membrane. Thus, in contrast to the post-translational import of proteins into mitochondria and chloroplasts, chaperone proteins are not required to keep the protein unfolded.

When a signal sequence binds, SRP exposes a binding site for the SRP receptor (see Figure 12–36B,C), which is a transmembrane protein complex in the rough ER membrane. The binding of the SRP to its receptor brings the SRP–ribosome complex to an unoccupied protein translocator in the same membrane. The SRP and SRP receptor are then released, and the translocator transfers the growing polypeptide chain across the membrane (**Figure 12–37**).

This co-translational transfer process creates two spatially separate populations of ribosomes in the cytosol. **Membrane-bound ribosomes**, attached to the

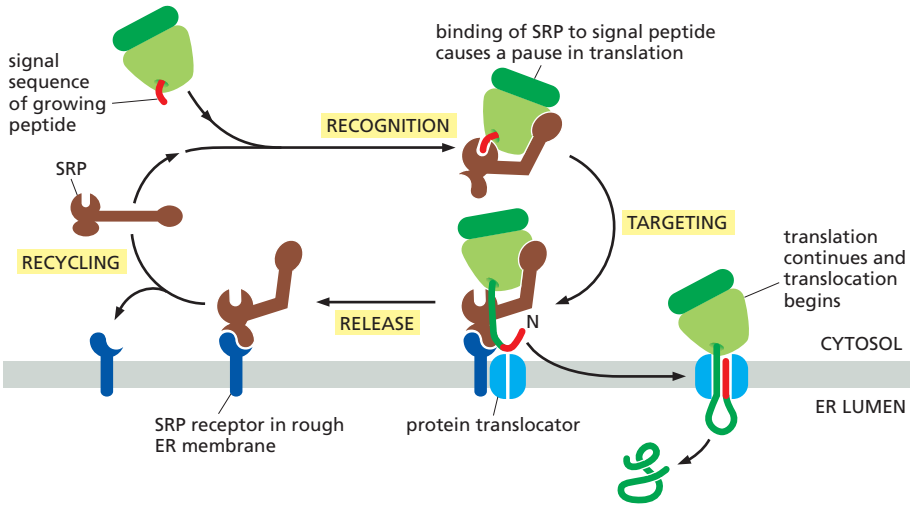


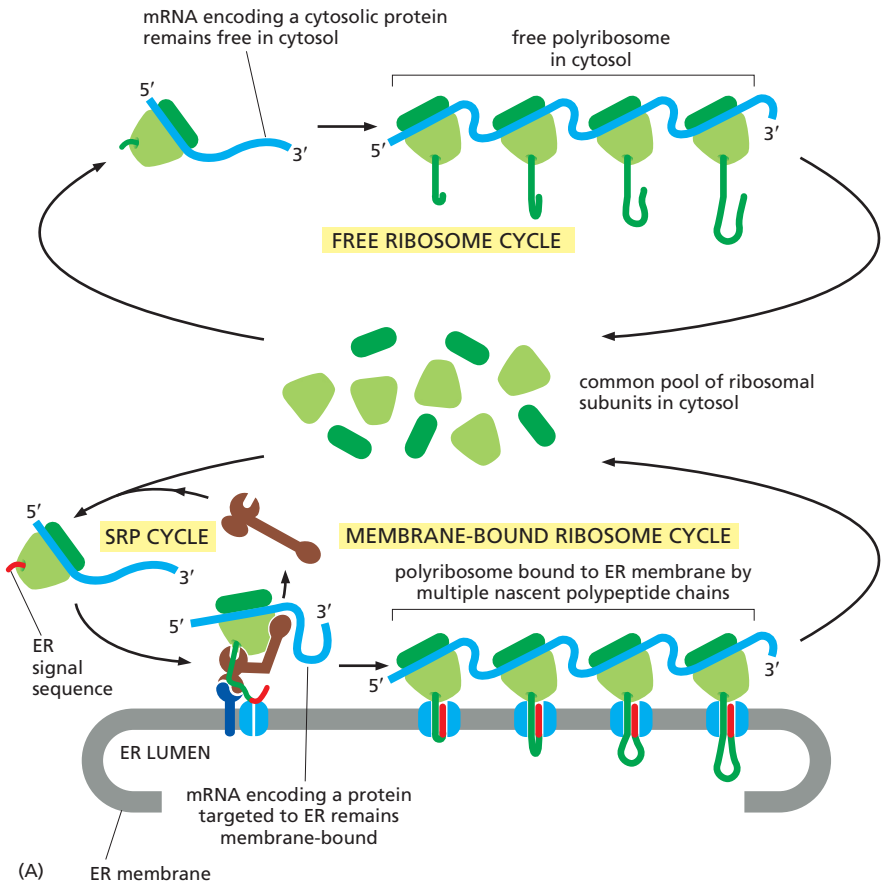
Figure 12–37 How ER signal sequences and SRP direct ribosomes to the ER membrane. The SRP and its receptor act in concert. The SRP binds to both the exposed ER signal sequence and the ribosome, thereby inducing a pause in translation. The SRP receptor in the ER membrane, which in animal cells is composed of two different polypeptide chains, binds the SRP–ribosome complex and directs it to the translocator. In a poorly understood reaction, the SRP and SRP receptor are then released, leaving the ribosome bound to the translocator in the ER membrane. The translocator then inserts the polypeptide chain into the membrane and transfers it across the lipid bilayer. Because one of the SRP proteins and both chains of the SRP receptor contain GTP-binding domains, it is thought that conformational changes that occur during cycles of GTP binding and hydrolysis (discussed in Chapter 15) ensure that SRP release occurs only after the ribosome has become properly engaged with the translocator in the ER membrane. The translocator is closed until the ribosome has bound, so that the permeability barrier of the ER membrane is maintained at all times.

cytosolic side of the ER membrane, are engaged in the synthesis of proteins that are being concurrently translocated into the ER. **Free ribosomes**, unattached to any membrane, synthesize all other proteins encoded by the nuclear genome. Membrane-bound and free ribosomes are structurally and functionally identical. They differ only in the proteins they are making at any given time.

Since many ribosomes can bind to a single mRNA molecule, a **polyribosome** is usually formed. If the mRNA encodes a protein with an ER signal sequence, the polyribosome becomes attached to the ER membrane, directed there by the signal sequences on multiple growing polypeptide chains. The individual ribosomes associated with such an mRNA molecule can return to the cytosol when they finish translation and intermix with the pool of free ribosomes. The mRNA itself, however, remains attached to the ER membrane by a changing population of ribosomes, each transiently held at the membrane by the translocator (**Figure 12–38**).

The Polypeptide Chain Passes Through an Aqueous Channel in the Translocator

It had long been debated whether polypeptide chains are transferred across the ER membrane in direct contact with the lipid bilayer or through a channel in a protein translocator. The debate ended with the identification of the translocator, which was shown to form a water-filled channel in the membrane through



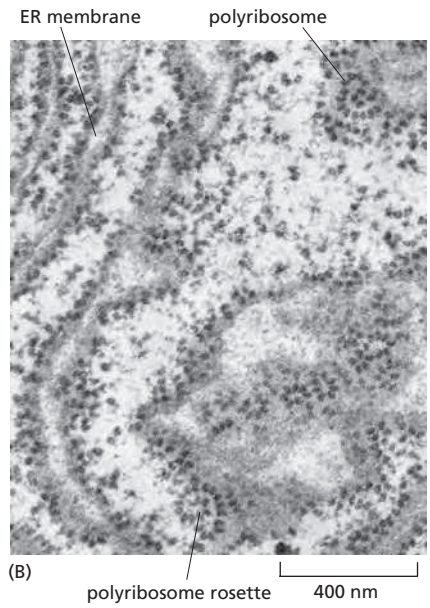


Figure 12–38 Free and membrane-bound polyribosomes. (A) A common pool of ribosomes synthesizes the proteins that stay in the cytosol and those that are transported into the ER. The ER signal sequence on a newly formed polypeptide chain binds to SRP, which directs the translating ribosome to the ER membrane. The mRNA molecule remains permanently bound to the ER as part of a polyribosome, while the ribosomes that move along it are recycled; at the end of each round of protein synthesis, the ribosomal subunits are released and rejoin the common pool in the cytosol. (B) A thin section electron micrograph of polyribosomes attached to the ER membrane. The plane of section in some places cuts through the ER roughly parallel to the membrane, giving a face-on view of the rosettelike pattern of the polyribosomes. (B, courtesy of George Palade.)

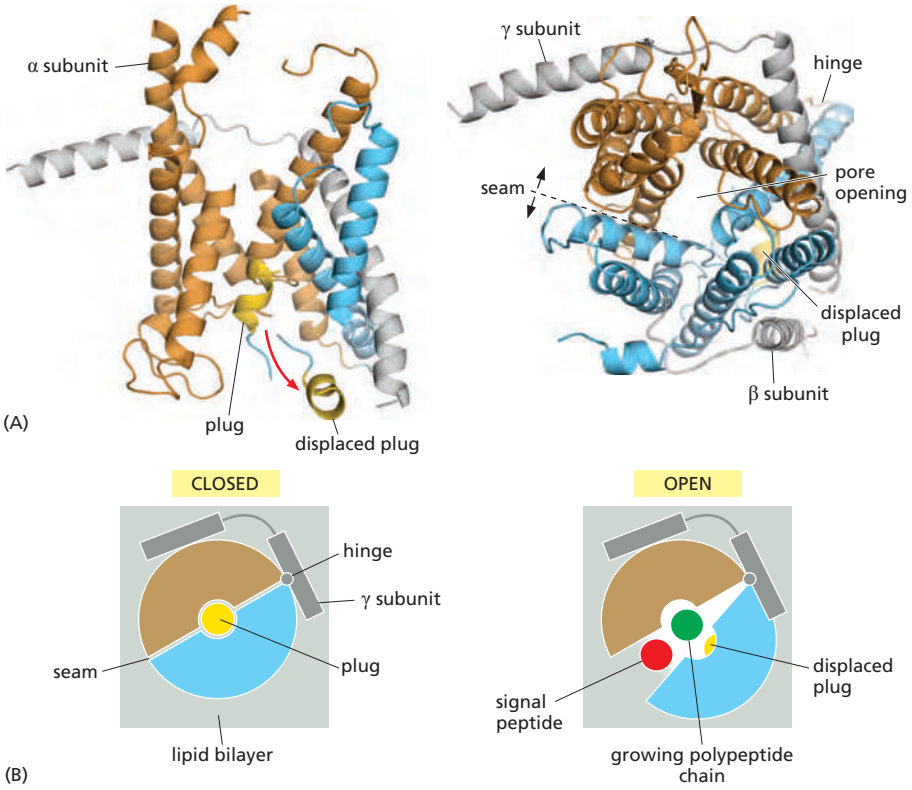


Figure 12–39 Structure of the Sec61 complex. (A) A side view *(left)* and a top view (*right*, seen from the cytosol) of the structure of the Sec61 complex of the archaeon *Methanococcus jannaschii*. The Sec61α subunit has an inverted repeat structure (see Figure 11–10) and is shown in *blue* and *beige* to indicate this pseudo-symmetry; the two smaller β and γ subunits are shown in *gray*. In the side view, some helices in front have been omitted to make the inside of the pore visible. The *yellow* short helix is thought to form a plug that seals the pore when the translocator is closed. To open, the complex rearranges itself to move the plug helix out of the way, as indicated by the *red* arrow. A ring of hydrophobic amino acid side chains is thought to form a tight-fitting diaphragm around translocating polypeptide chain to prevent leaks of other molecules across the membrane. The pore of the Sec61 complex can also open sideways at a lateral seam. (B) Models of the closed and open states of the translocator are shown in top view, illustrating how a signal sequence (or a transmembrane segment) could be released into the lipid bilayer after opening of the seam. (PDB codes: 1RH5 and 1RHZ.)

which the polypeptide chain passes. The core of the translocator, called the **Sec61 complex**, is built from three subunits that are highly conserved from bacteria to eukaryotic cells. The structure of the Sec61 complex suggests that α helices contributed by the largest subunit surround a central channel through which the polypeptide chain traverses the membrane (**Figure 12–39**). The channel is gated by a short α helix that is thought to keep the translocator closed when it is idle and to move aside when it is engaged in passing a polypeptide chain. According to this view, the pore is a dynamic gated channel that opens only transiently when a polypeptide chain traverses the membrane. In an idle translocator, it is important to keep the channel closed, so that the membrane remains impermeable to ions, such as Ca^{2+}, which otherwise would leak out of the ER. As a polypeptide chain is translocating, a ring of hydrophobic amino acid side chains is thought to provide a flexible seal to prevent ion leaks.

The structure of the Sec61 complex suggests that the pore can also open along a seam on its side. Indeed, some structures of the translocator show it locked in an open-seam conformation. This opening allows a translocating peptide chain lateral access into the hydrophobic core of the membrane, a process that is important both for the release of a cleaved signal peptide into the membrane (see Figure 12–35) and for the integration of transmembrane proteins into the bilayer, as we discuss later.

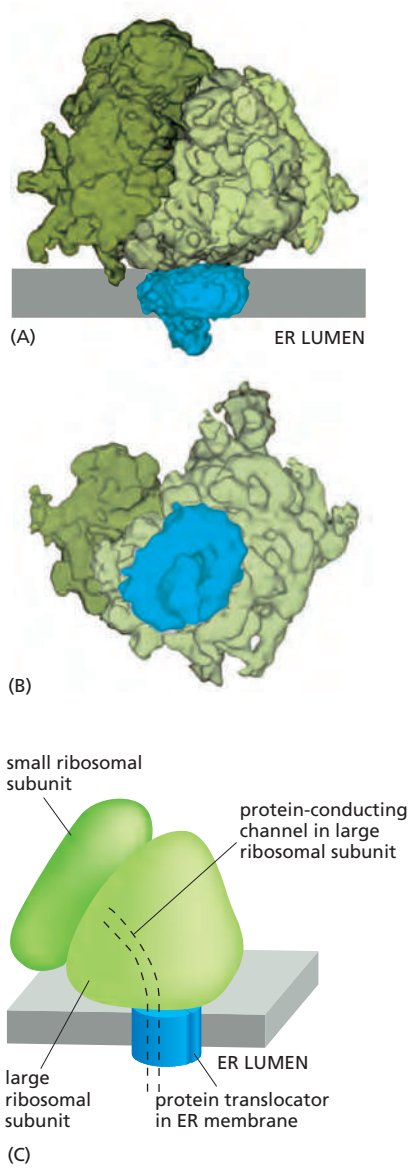


Figure 12–40 A ribosome *(green)* bound to the ER protein translocator *(blue)*. (A) A side-view reconstruction of the complex from electron microscopic images. (B) A view of the translocator seen from the ER lumen. The translocator contains Sec61, accessory proteins, and detergent used in the preparation. Domains of accessory proteins extend across the membrane and form the lumenal bulge. (C) A schematic drawing of a membrane-bound ribosome attached to the translocator, indicating the location of the tunnel in the large ribosomal subunit through which the growing polypeptide chain exits from the ribosome. The mRNA (not shown) would be located between the small and large ribosomal subunits. (Adapted from J.F. Ménétret et al., *J. Mol. Biol.* 348:445–457, 2005. With permission from Academic Press.)

In eukaryotic cells, four Sec61 complexes form a large translocator assembly that can be visualized on ER-bound ribosomes after detergent solubilization of the ER membrane (**Figure 12–40**). It is likely that this assembly includes other membrane complexes that associate with the translocator, such as enzymes that modify the growing polypeptide chain, including oligosaccharide transferase and the signal peptidase. The assembly of a translocator with these accessory components is called the **translocon**.

Translocation Across the ER Membrane Does Not Always Require Ongoing Polypeptide Chain Elongation

As we have seen, translocation of proteins into mitochondria, chloroplasts, and peroxisomes occurs post-translationally, after the protein has been made and released into the cytosol, whereas translocation across the ER membrane usually occurs during translation (co-translationally). This explains why ribosomes are bound to the ER but not to other organelles.

Some completely synthesized proteins, however, are imported into the ER, demonstrating that translocation does not always require ongoing translation. Post-translational protein translocation is especially common across the yeast ER membrane and the bacterial plasma membrane (which is thought to be evolutionarily related to the ER). To function in post-translational translocation, the ER translocator needs accessory proteins that feed the polypeptide chain into the pore and drive translocation (**Figure 12–41**). In bacteria, a translocation motor protein, the *SecA ATPase*, attaches to the cytosolic side of the translocator, where it undergoes cyclic conformational changes driven by ATP hydrolysis. Each time an ATP is hydrolyzed, a portion of the SecA protein inserts into the pore of the translocator, pushing a short segment of the passenger protein with it. As a result of this ratchet mechanism, the SecA ATPase progressively pushes the polypeptide chain of the transported protein across the membrane.

Eukaryotic cells use a different set of accessory proteins that associate with the Sec61 complex. These proteins span the ER membrane and use a small domain on the lumenal side of the ER membrane to deposit an hsp70-like chaperone protein (called *BiP*, for *bi*nding *p*rotein) onto the polypeptide chain as it emerges from the pore into the ER lumen. ATP-dependent cycles of BiP binding and release drive unidirectional translocation, as described earlier for the mitochondrial hsp70 proteins that pull proteins across mitochondrial membranes.

Proteins that are transported into the ER by a post-translational mechanism are first released into the cytosol, where they bind to chaperone proteins to prevent folding, as discussed earlier for proteins destined for mitochondria and chloroplasts.

In Single-Pass Transmembrane Proteins, a Single Internal ER Signal Sequence Remains in the Lipid Bilayer as a Membrane-spanning α Helix

The ER signal sequence in the growing polypeptide chain is thought to trigger the opening of the pore in the Sec61 protein translocator: after the signal sequence is released from the SRP and the growing chain has reached a sufficient length, the

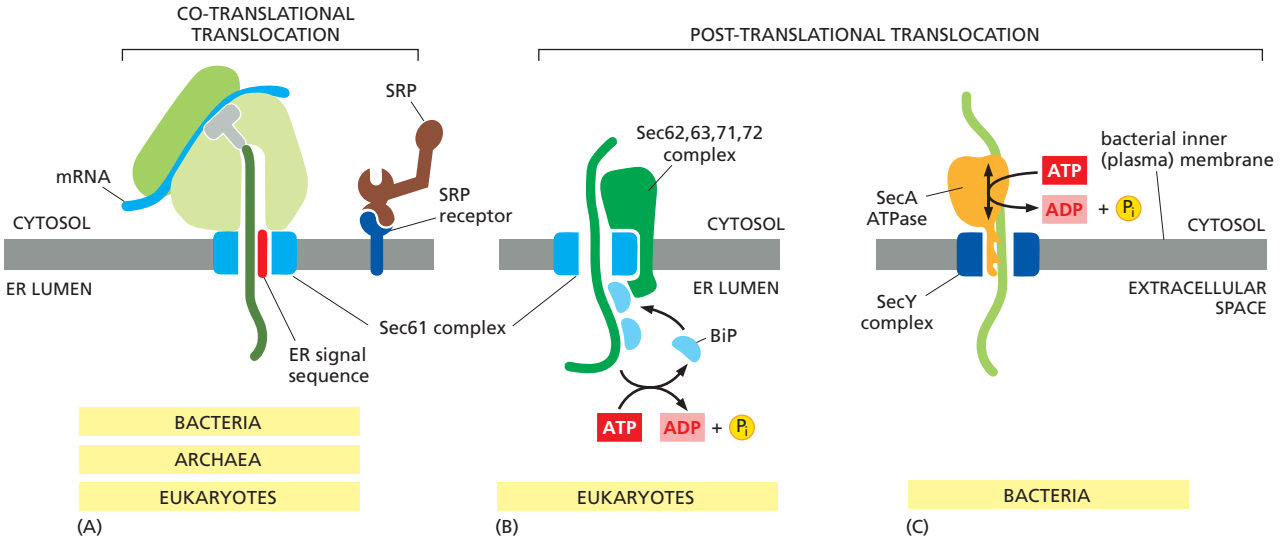


Figure 12–41 Three ways in which protein translocation can be driven through structurally similar translocators. (A) Co-translational translocation. The ribosome is brought to the membrane by the SRP and SRP receptor and then engages with the Sec61 protein translocator. The growing polypeptide chain is threaded across the membrane as it is made. No additional energy is needed, as the only path available to the growing chain is to cross the membrane. (B) Post-translational translocation in eukaryotic cells requires an additional complex composed of Sec62, Sec63, Sec71, and Sec72 proteins, which is attached to the Sec61 translocator and deposits BiP molecules onto the translocating chain as it emerges from the translocator in the lumen of the ER. ATP-driven cycles of BiP binding and release pull the protein into the lumen, a mechanism that closely resembles the mechanism of mitochondrial import in Figure 12–23. (C) Post-translational translocation in bacteria. The completed polypeptide chain is fed from the cytosolic side into the bacterial homolog of the Sec61 complex (called the SecY complex in bacteria) in the plasma membrane by the SecA ATPase. ATP hydrolysis-driven conformational changes drive a pistonlike motion in SecA, each cycle pushing about 20 amino acids of the protein chain through the pore of the translocator. The Sec pathway used for protein translocation across the thylakoid membrane in chloroplasts uses a similar mechanism (see Figure 12–26B).

Whereas the Sec61 translocator, SRP, and SRP receptor are found in all organisms, SecA is found exclusively in bacteria, and the Sec62, 63, 71, 72 complex is found exclusively in eukaryotic cells. (Adapted from P. Walter and A.E. Johnson, *Annu. Rev. Cell Biol.* 10:87–119, 1994. With permission from Annual Reviews.)

signal sequence binds to a specific site inside the pore itself, thereby opening the pore. An ER signal sequence is therefore recognized twice: first by an SRP in the cytosol and then by a binding site in the pore of the protein translocator, where it serves as a **start-transfer signal** (or start-transfer peptide) that opens the pore (for example, see Figure 12–35 for how this works for a soluble protein). Dual recognition may help ensure that only appropriate proteins enter the lumen of the ER.

While bound in the translocation pore, a signal sequence is in contact not only with the Sec61 complex, which forms the walls of the pore, but also, along the lateral seam, with the hydrophobic core of the lipid bilayer. This was shown in chemical cross-linking experiments in which the signal sequence and the hydrocarbon chains of lipids were covalently linked together. When the nascent polypeptide chain grew long enough, the ER signal peptidase cleaved off the signal sequence and released it from the pore into the membrane, where it was rapidly degraded to amino acids by other proteases in the ER membrane. To release the signal sequence into the membrane, the translocator opens laterally along the seam (see Figures 12–35 and 12–39). The translocator is therefore gated in two directions: it opens to form a pore across the membrane to let the hydrophilic portions of proteins cross the lipid bilayer, and it opens laterally within the membrane to let hydrophobic portions of proteins partition into the lipid bilayer. Lateral gating of the pore is an essential step during the integration of transmembrane proteins.

The integration of membrane proteins requires that some parts of the polypeptide chain be translocated across the lipid bilayer whereas others are not. Despite this additional complexity, all modes of insertion of membrane proteins are simply variants of the sequence of events just described for transferring a soluble protein into the lumen of the ER. We begin by describing the three ways in

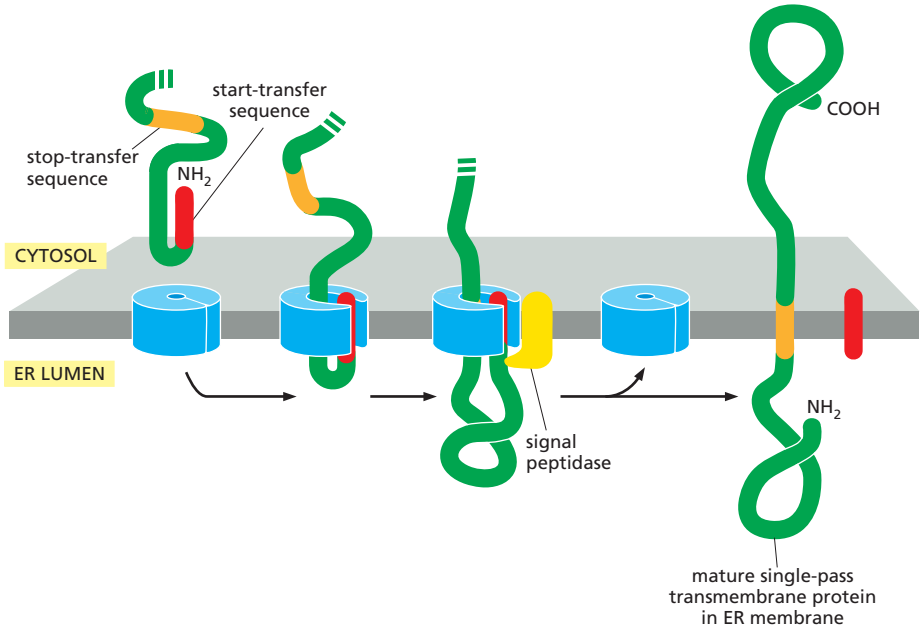


Figure 12–42 How a single-pass transmembrane protein with a cleaved ER signal sequence is integrated into the ER membrane. In this protein, the co-translational translocation process is initiated by an N-terminal ER signal sequence *(red)* that functions as a start-transfer signal, opening the translocator as in Figure 12–35. In addition to this start-transfer sequence, however, the protein also contains a stop-transfer sequence *(orange)*; when this sequence enters the translocator and interacts with a binding site within the pore, the translocator opens at the seam and discharges the protein laterally into the lipid bilayer, where the stop-transfer sequence remains to anchor the protein in the membrane. (In this figure and the two figures that follow, the ribosomes have been omitted for clarity.)

which **single-pass transmembrane proteins** (see Figure 10–17) become inserted into the ER membrane.

In the simplest case, an N-terminal signal sequence initiates translocation, just as for a soluble protein, but an additional hydrophobic segment in the polypeptide chain stops the transfer process before the entire polypeptide chain is translocated. This **stop-transfer signal** anchors the protein in the membrane after the ER signal sequence (the start-transfer signal) has been cleaved off and released from the translocator (**Figure 12–42**). The lateral gating mechanism transfers the stop-transfer sequence into the bilayer, where it remains as a single α-helical membrane-spanning segment, with the N-terminus of the protein on the lumenal side of the membrane and the C-terminus on the cytosolic side.

In the other two cases, the signal sequence is internal, rather than at the N-terminal end of the protein. As for an N-terminal ER signal sequence, the SRP binds to an internal signal sequence by recognizing its hydrophobic α-helical features. The SRP brings the ribosome making the protein to the ER membrane, and the ER signal sequence then serves as a start-transfer signal that initiates the protein's translocation. After release from the translocator, the internal start-transfer sequence remains in the lipid bilayer as a single membrane-spanning α helix.

Internal start-transfer sequences can bind to the translocation apparatus in either of two orientations; this in turn determines which protein segment (the one preceding or the one following the start-transfer sequence) is moved across the membrane into the ER lumen. In one case, the resulting membrane protein has its C-terminus on the lumenal side (pathway A in **Figure 12–43**), while in the other, it has its N-terminus on the lumenal side (pathway B in Figure 12–43). The orientation of the start-transfer sequence depends on the distribution of nearby charged amino acids, as described in the figure legend.

Combinations of Start-Transfer and Stop-Transfer Signals Determine the Topology of Multipass Transmembrane Proteins

In **multipass transmembrane proteins**, the polypeptide chain passes back and forth repeatedly across the lipid bilayer as hydrophobic α helices (see Figure 10–17). It is thought that an internal signal sequence serves as a start-transfer signal in these proteins to initiate translocation, which continues until the translocator encounters a stop-transfer sequence; in double-pass transmembrane proteins, for example, the polypeptide can then be released into the bilayer

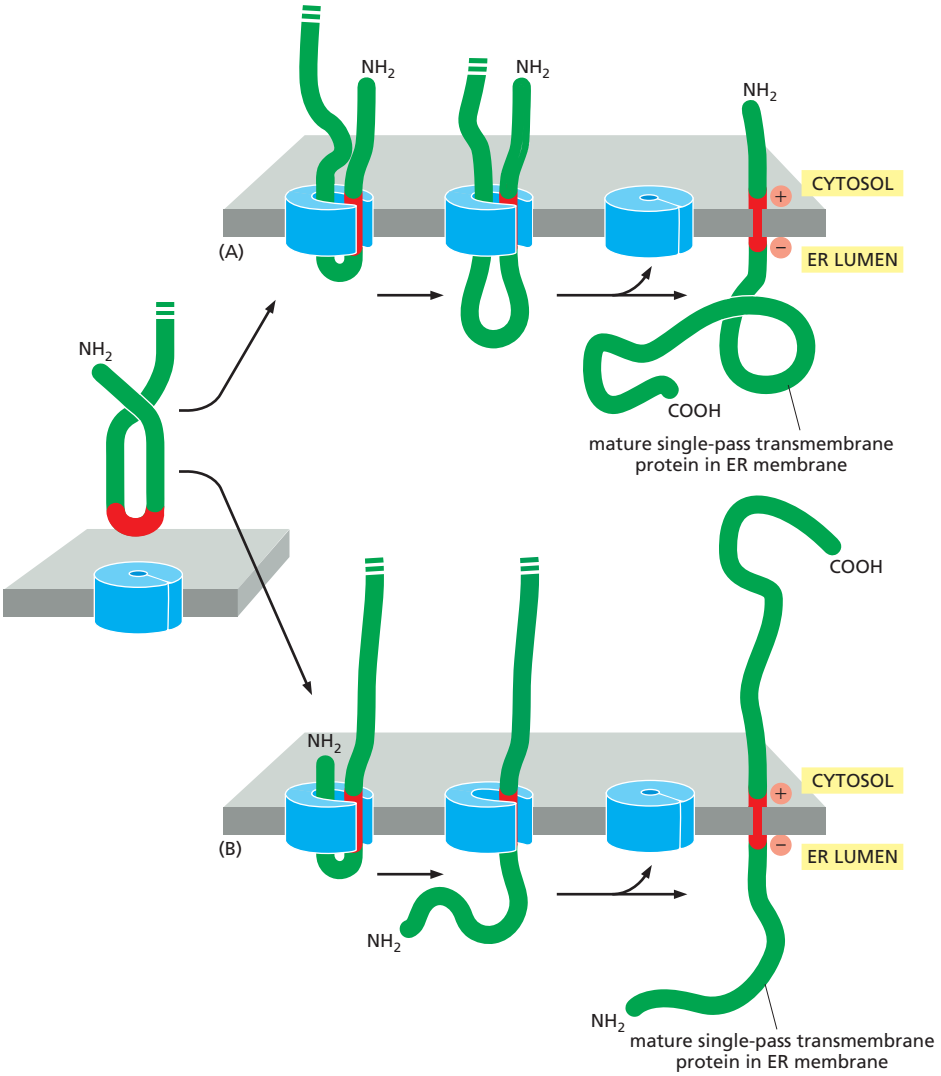


Figure 12–43 Integration of a single-pass transmembrane protein with an internal signal sequence into the ER membrane. An internal ER signal sequence that functions as a start-transfer signal can bind to the translocator in one of two ways, leading to a membrane protein that has either its C-terminus (pathway A) or its N-terminus (pathway B) in the ER lumen. Proteins are directed into either pathway by features in the polypeptide chain flanking the internal start-transfer sequence: if there are more positively charged amino acids immediately *preceding* the hydrophobic core of the start-transfer sequence than there are following it, the membrane protein is inserted into the translocator in the orientation shown in pathway A, whereas if there are more positively charged amino acids immediately *following* the hydrophobic core of the start-transfer sequence than there are preceding it, the membrane protein is inserted into the translocator in the orientation shown in pathway B. Because translocation cannot start before a start-transfer sequence appears outside the ribosome, translocation of the N-terminal portion of the protein shown in (B) can occur only after this portion has been fully synthesized.

Note that there are two ways to insert a single-pass membrane-spanning protein whose N-terminus is located in the ER lumen: that shown in Figure 12–42 and that shown here in (B).

(**Figure 12–44**). In more complex multipass proteins, in which many hydrophobic α helices span the bilayer, a second start-transfer sequence reinitiates translocation further down the polypeptide chain until the next stop-transfer sequence causes polypeptide release, and so on for subsequent start-transfer and stop-transfer sequences (**Figure 12–45** and **Movie 12.5**).

Hydrophobic start-transfer and stop-transfer signal sequences both act to fix the topology of the protein in the membrane by locking themselves into the membrane as membrane-spanning α helices; and they can do this in either orientation. Whether a given hydrophobic signal sequence functions as a start-transfer or stop-transfer sequence must depend on its location in a polypeptide chain, since its function can be switched by changing its location in the protein by using recombinant DNA techniques. Thus, the distinction between start-transfer and stop-transfer sequences results mostly from their relative order in the growing polypeptide chain. It seems that the SRP begins scanning an unfolded polypeptide chain for hydrophobic segments at its N-terminus and proceeds toward the C-terminus, in the direction that the protein is synthesized. By recognizing the first appropriate hydrophobic segment to emerge from the ribosome, the SRP sets the "reading frame" for membrane integration: after the SRP initiates translocation, the translocator recognizes the next appropriate hydrophobic segment in the direction of transfer as a stop-transfer sequence, causing the region of the polypeptide chain in between to be threaded across the membrane. A similar

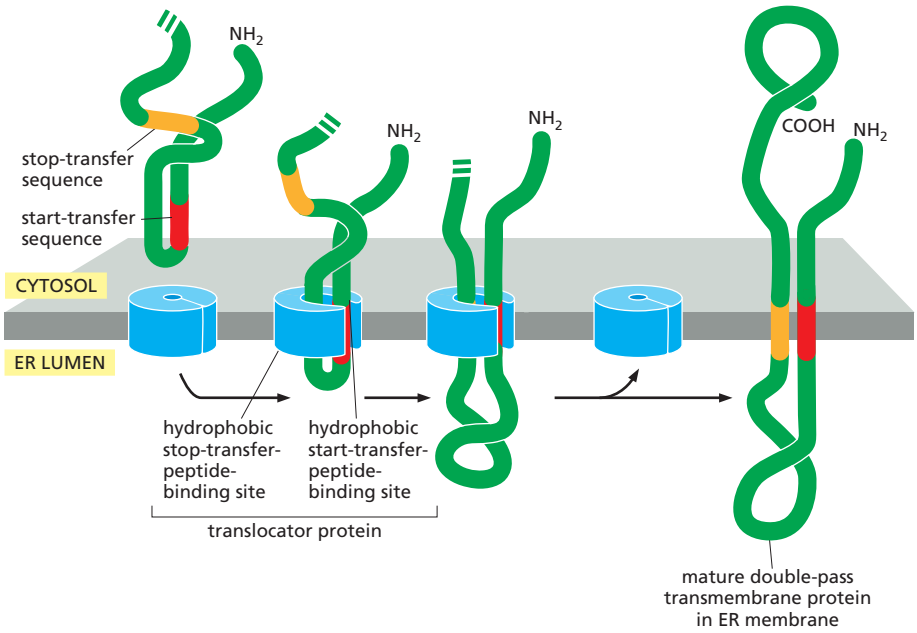


Figure 12–44 Integration of a double-pass transmembrane protein with an internal signal sequence into the ER membrane. In this protein, an internal ER signal sequence acts as a start-transfer signal (as in Figure 12–43) and initiates the transfer of the C-terminal part of the protein. At some point after a stop-transfer sequence has entered the translocator, the translocator discharges the sequence laterally into the membrane.

scanning process continues until all of the hydrophobic regions in the protein have been inserted into the membrane as transmembrane α helices.

Because membrane proteins are always inserted from the cytosolic side of the ER in this programmed manner, all copies of the same polypeptide chain will have the same orientation in the lipid bilayer. This generates an asymmetrical ER membrane in which the protein domains exposed on one side are different from those exposed on the other side. This asymmetry is maintained during the many membrane budding and fusion events that transport the proteins made in the ER to other cell membranes (discussed in Chapter 13). Thus, the way in which a newly synthesized protein is inserted into the ER membrane determines the orientation of the protein in all of the other membranes as well.

When proteins are extracted with detergent from a membrane and then reconstituted into artificial lipid vesicles, a random mixture of right-side-out and inside-out protein orientations usually results. Thus, the protein asymmetry observed in cell membranes seems not to be an inherent property of the proteins, but instead results solely from the process by which proteins are inserted into the ER membrane from the cytosol.

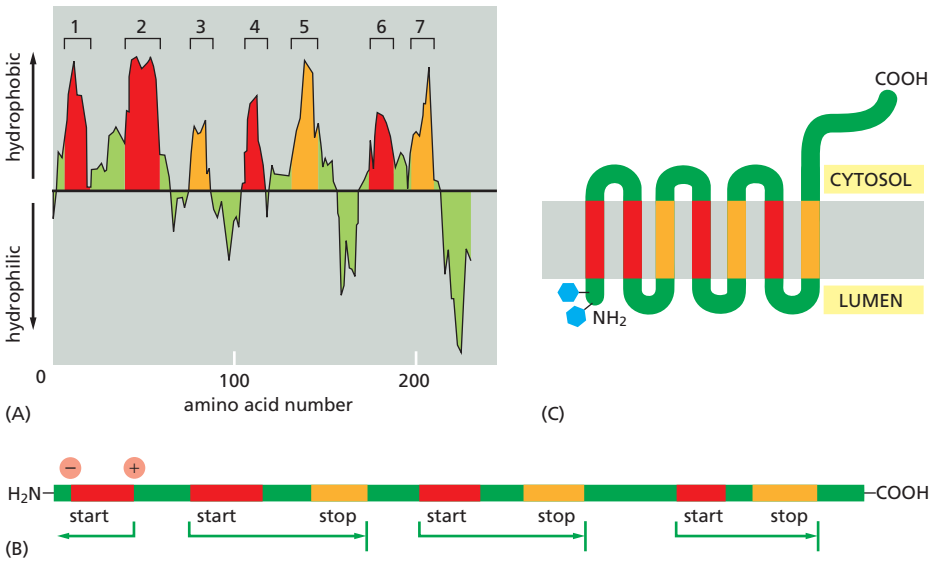


Figure 12–45 The insertion of the multipass membrane protein rhodopsin into the ER membrane. Rhodopsin is the light-sensitive protein in rod photoreceptor cells in the mammalian retina (discussed in Chapter 15). (A) A hydropathy plot (see Figure 10–20) identifies seven short hydrophobic regions in rhodopsin. (B) The hydrophobic region nearest the N-terminus serves as a start-transfer sequence that causes the preceding N-terminal portion of the protein to pass across the ER membrane. Subsequent hydrophobic sequences function in alternation as start-transfer and stop-transfer sequences. The *green arrows* indicate the paired start and stop signals inserted into the translocator. (C) The final integrated rhodopsin has its N-terminus located in the ER lumen and its C-terminus located in the cytosol. The *blue hexagons* represent covalently attached oligosaccharides.

ER Tail-anchored Proteins Are Integrated into the ER Membrane by a Special Mechanism

Many important membrane proteins are anchored in the membrane by a C-terminal transmembrane, hydrophobic α helix. These **ER tail-anchored proteins** include a large number of SNARE protein subunits that guide vesicular traffic (discussed in Chapter 13). When such a tail-anchored protein inserts into the ER membrane from the cytosol, only a few amino acids that follow the transmembrane α helix on its C-terminal side are translocated into the ER lumen, while most of the protein remains in the cytosol. Because of the unique position of the transmembrane α helix in the protein sequence, translation terminates while the C-terminal amino acids that will form the transmembrane α helix have not yet emerged from the ribosome exit tunnel. Recognition by SRP is therefore not possible. It was long thought that these proteins are released from the ribosome and the hydrophobic C-terminal tail spontaneously partitions into the ER membrane. Such a mechanism could not explain, however, why ER tail-anchored proteins insert into the ER membrane selectively and not also into all other membranes in the cell. It is now clear that a specialized targeting machinery is involved that is fueled by ATP hydrolysis (**Figure 12–46**). Although the components and details differ, this post-translational targeting mechanism is conceptually similar to SRP-dependent protein targeting (see Figure 12–37).

Not all tail-anchored proteins are inserted into the ER, however. Some proteins contain a C-terminal membrane anchor that contains additional sorting information that directs the protein to mitochondria or peroxisomes. How these proteins are sorted there remains unknown.

Translocated Polypeptide Chains Fold and Assemble in the Lumen of the Rough ER

Many of the proteins in the lumen of the ER are in transit, *en route* to other destinations; others, however, normally reside there and are present at high concentrations. These **ER resident proteins** contain an **ER retention signal** of four amino acids at their C-terminus that is responsible for retaining the protein in the ER (see Table 12–3. p. 648; discussed in Chapter 13). Some of these proteins function as catalysts that help the many proteins that are translocated into the ER lumen to fold and assemble correctly.

One important ER resident protein is *protein disulfide isomerase* (*PDI*), which catalyzes the oxidation of free sulfhydryl (SH) groups on cysteines to form disulfide (S–S) bonds. Almost all cysteines in protein domains exposed to either the extracellular space or the lumen of organelles in the secretory and endocytic pathways are disulfide-bonded. By contrast, disulfide bonds form only very rarely in domains exposed to the cytosol, because of the reducing environment there.

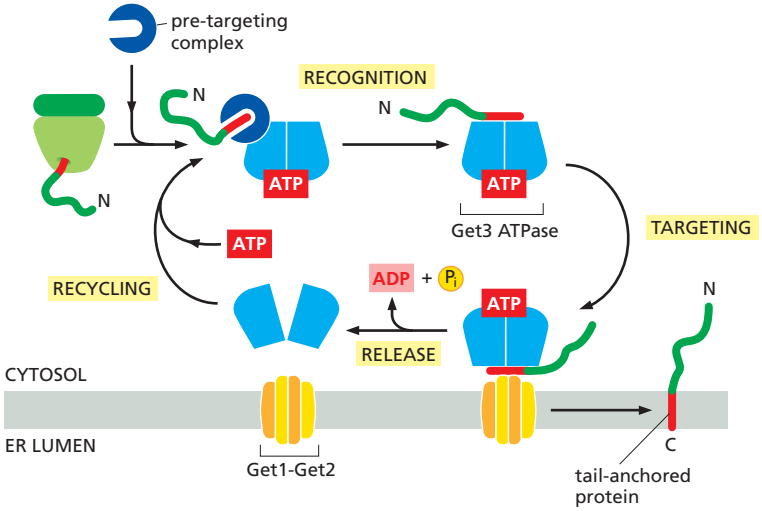


Figure 12–46 The insertion mechanism for tail-anchored proteins. In this post-translational pathway for the insertion of tail-anchored ER membrane proteins, a soluble pre-targeting complex captures the hydrophobic C-terminal α helix after it emerges from the ribosomal exit tunnel and loads it onto the Get3 ATPase. The resulting complex is targeted to the ER membrane by interaction with the Get1–Get2 receptor complex that functions as a membrane protein insertion machine. After Get3 hydrolyzes its bound ATP, the tail-anchored protein is released from the receptor and inserted into the ER membrane. ADP release and renewed ATP binding recycles Get3 back to the cytosol.

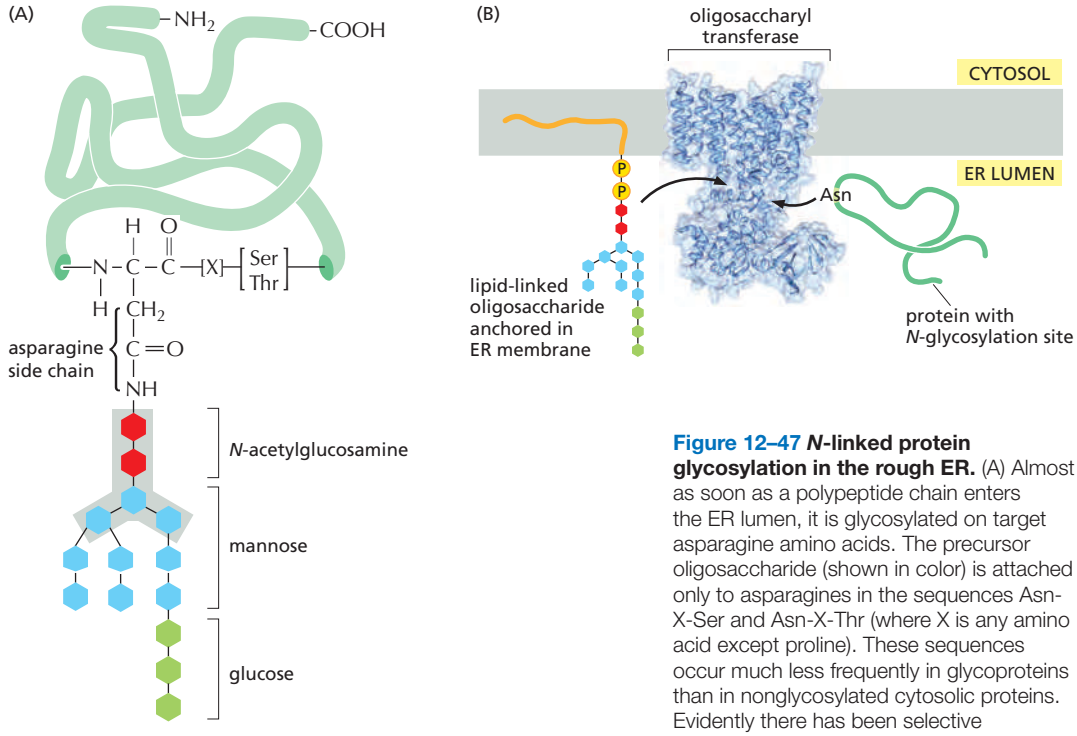


Figure 12–47 *N*-linked protein glycosylation in the rough ER. (A) Almost as soon as a polypeptide chain enters the ER lumen, it is glycosylated on target asparagine amino acids. The precursor oligosaccharide (shown in color) is attached only to asparagines in the sequences Asn-X-Ser and Asn-X-Thr (where X is any amino acid except proline). These sequences occur much less frequently in glycoproteins than in nonglycosylated cytosolic proteins. Evidently there has been selective pressure against these sequences during protein evolution, presumably because glycosylation at too many sites would interfere with protein folding. The five sugars in the *gray* box form the core region of this oligosaccharide. For many glycoproteins, only the core sugars survive the extensive oligosaccharide trimming that takes place in the Golgi apparatus. (B) The precursor oligosaccharide is transferred from a dolichol lipid anchor to the asparagine as an intact unit in a reaction catalyzed by a transmembrane *oligosaccharyl transferase* enzyme. One copy of this enzyme is associated with each protein translocator in the ER membrane. (The translocator is not shown.) Oligosaccharyl transferase contains 13 transmembrane α helices and a large ER lumenal domain that contains its substrate-binding sites. The asparagine binds a tunnel that penetrates the enzyme interior. There, the amino group of the asparagine is twisted out of the plane that stabilizes the otherwise poorly reactive amide bond, activating it for reaction with the dolichol–oligosaccharide. The structure shown is of a prokaryotic homolog that closely resembles the catalytic subunit of the eukaryotic oligosaccharyl transferase. (PDB code: 3RCE.)

Another ER resident protein is the chaperone protein **BiP**. We have already discussed how BiP pulls proteins post-translationally into the ER through the Sec61 ER translocator. Like other chaperones (discussed in Chapter 13), BiP recognizes incorrectly folded proteins, as well as protein subunits that have not yet assembled into their final oligomeric complexes. It does so by binding to exposed amino acid sequences that would normally be buried in the interior of correctly folded or assembled polypeptide chains. An example of a BiP-binding site is a stretch of alternating hydrophobic and hydrophilic amino acids that would normally be buried in a β sheet with its hydrophobic side oriented towards the hydrophobic core of the folded protein. The bound BiP both prevents the protein from aggregating and helps keep it in the ER (and thus out of the Golgi apparatus and later parts of the secretory pathway). Like some other members of the hsp70 family of chaperone proteins, which bind unfolded proteins and facilitate their import into mitochondria and chloroplasts, BiP hydrolyzes ATP to shuttle between high- and low-affinity binding states, which allow it to hold on to and let go of its substrate proteins in a dynamic cycle.

Most Proteins Synthesized in the Rough ER Are Glycosylated by the Addition of a Common *N*-Linked Oligosaccharide

The covalent addition of oligosaccharides to proteins is one of the major biosynthetic functions of the ER. About half of the soluble and membrane-bound proteins that are processed in the ER—including those destined for transport to the Golgi apparatus, lysosomes, plasma membrane, or extracellular space—are **glycoproteins** that are modified in this way. Many proteins in the cytosol and nucleus are also glycosylated, but not with oligosaccharides: they carry a much simpler sugar modification, in which a single *N*-acetylglucosamine group is added to a serine or threonine of the protein.

During the most common form of **protein glycosylation** in the ER, a preformed *precursor oligosaccharide* (composed of *N*-acetylglucosamine, mannose, and glucose, and containing a total of 14 sugars) is transferred *en bloc* to proteins. Because this oligosaccharide is transferred to the side-chain NH_2 group of an asparagine in the protein, it is said to be *N-linked* or *asparagine-linked* (**Figure 12–47**A). The transfer is catalyzed by a membrane-bound enzyme complex, an

oligosaccharyl transferase, which has its active site exposed on the lumenal side of the ER membrane; this explains why cytosolic proteins are not glycosylated in this way. A special lipid molecule called **dolichol** anchors the precursor oligosaccharide in the ER membrane. The precursor oligosaccharide is transferred to the target asparagine in a single enzymatic step immediately after that amino acid has reached the ER lumen during protein translocation. The precursor oligosaccharide is linked to the dolichol lipid by a high-energy pyrophosphate bond, which provides the activation energy that drives the glycosylation reaction (Figure 12–47B). One copy of oligosaccharyl transferase is associated with each protein translocator, allowing it to scan and glycosylate the incoming polypeptide chains efficiently.

The precursor oligosaccharide is built up sugar by sugar on the membrane-bound dolichol lipid and is then transferred to a protein. The sugars are first activated in the cytosol by the formation of *nucleotide (UDP or GDP)-sugar intermediates*, which then donate their sugar (directly or indirectly) to the lipid in an orderly sequence. Part way through this process, the lipid-linked oligosaccharide is flipped, with the help of a transporter, from the cytosolic to the lumenal side of the ER membrane (**Figure 12–48**).

All of the diversity of the *N*-linked oligosaccharide structures on mature glycoproteins results from the later modification of the original precursor oligosaccharide. While still in the ER, three glucoses (see Figure 12–47) and one mannose are quickly removed from the oligosaccharides of most glycoproteins. We shall return to the importance of glucose trimming shortly. This oligosaccharide "trimming," or "processing," continues in the Golgi apparatus, as we discuss in Chapter 13.

The *N*-linked oligosaccharides are by far the most common oligosaccharides, being found on 90% of all glycoproteins. Less frequently, oligosaccharides are linked to the hydroxyl group on the side chain of a serine, threonine, or hydroxylysine amino acid. A first sugar of these *O-linked oligosaccharides* is added in the ER and the oligosaccharide is then further extended in the Golgi apparatus (see Figure 13–32).

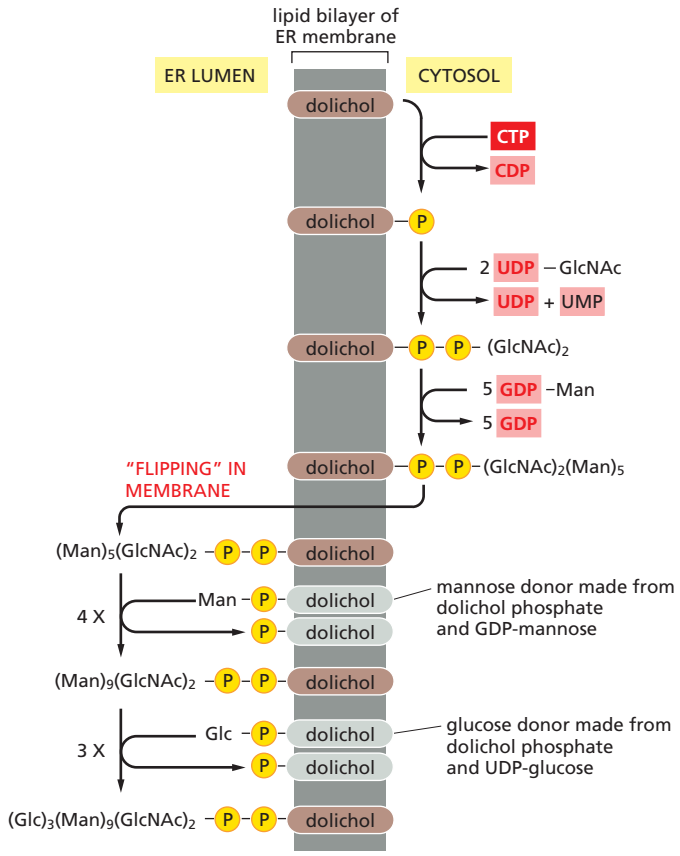


Figure 12–48 Synthesis of the lipid-linked precursor oligosaccharide in the rough ER membrane. The oligosaccharide is assembled sugar by sugar onto the carrier lipid dolichol (a polyisoprenoid; see Panel 2–5, pp. 98–99). Dolichol is long and very hydrophobic: its 22 five-carbon units can span the thickness of a lipid bilayer more than three times, so that the attached oligosaccharide is firmly anchored in the membrane. The first sugar is linked to dolichol by a pyrophosphate bridge. This high-energy bond activates the oligosaccharide for its eventual transfer from the lipid to an asparagine side chain of a growing polypeptide on the lumenal side of the rough ER. As indicated, the synthesis of the oligosaccharide starts on the cytosolic side of the ER membrane and continues on the lumenal face after the $(Man)_5(GlcNAc)_2$ lipid intermediate is flipped across the bilayer by a transporter (which is not shown). All the subsequent glycosyl transfer reactions on the lumenal side of the ER involve transfers from dolichol-P-glucose and dolichol-P-mannose; these activated, lipid-linked monosaccharides are synthesized from dolichol phosphate and UDP-glucose or GDP-mannose (as appropriate) on the cytosolic side of the ER and are then flipped across the ER membrane. GlcNAc = *N*-acetylglucosamine; Man = mannose; Glc = glucose.

Oligosaccharides Are Used as Tags to Mark the State of Protein Folding

It has long been debated why glycosylation is such a common modification of proteins that enter the ER. One particularly puzzling observation has been that some proteins require *N*-linked glycosylation for proper folding in the ER, yet the precise location of the oligosaccharides attached to the protein's surface does not seem to matter. A clue to the role of glycosylation in protein folding came from studies of two ER chaperone proteins, which are called **calnexin** and **calreticulin** because they require Ca^{2+} for their activities. These chaperones are carbohydrate-binding proteins, or *lectins*, which bind to oligosaccharides on incompletely folded proteins and retain them in the ER. Like other chaperones, they prevent incompletely folded proteins from irreversibly aggregating. Both calnexin and calreticulin also promote the association of incompletely folded proteins with another ER chaperone, which binds to cysteines that have not yet formed disulfide bonds.

Calnexin and calreticulin recognize *N*-linked oligosaccharides that contain a single terminal glucose, and they therefore bind proteins only after two of the three glucoses on the precursor oligosaccharide have been removed during glucose trimming by ER glucosidases. When the third glucose has been removed, the glycoprotein dissociates from its chaperone and can leave the ER.

How, then, do calnexin and calreticulin distinguish properly folded from incompletely folded proteins? The answer lies in yet another ER enzyme, a glucosyl transferase that keeps adding a glucose to those oligosaccharides that have lost their last glucose. It adds the glucose, however, only to oligosaccharides that are attached to unfolded proteins. Thus, an unfolded protein undergoes continuous cycles of glucose trimming (by glucosidase) and glucose addition (by glucosyl transferase), maintaining an affinity for calnexin and calreticulin until it has achieved its fully folded state (**Figure 12–49**).

Improperly Folded Proteins Are Exported from the ER and Degraded in the Cytosol

Despite all the help from chaperones, many protein molecules (more than 80% for some proteins) translocated into the ER fail to achieve their properly folded or oligomeric state. Such proteins are exported from the ER back into the cytosol, where they are degraded in proteasomes (discussed in Chapter 6). In many ways, the mechanism of retrotranslocation is similar to other post-translational

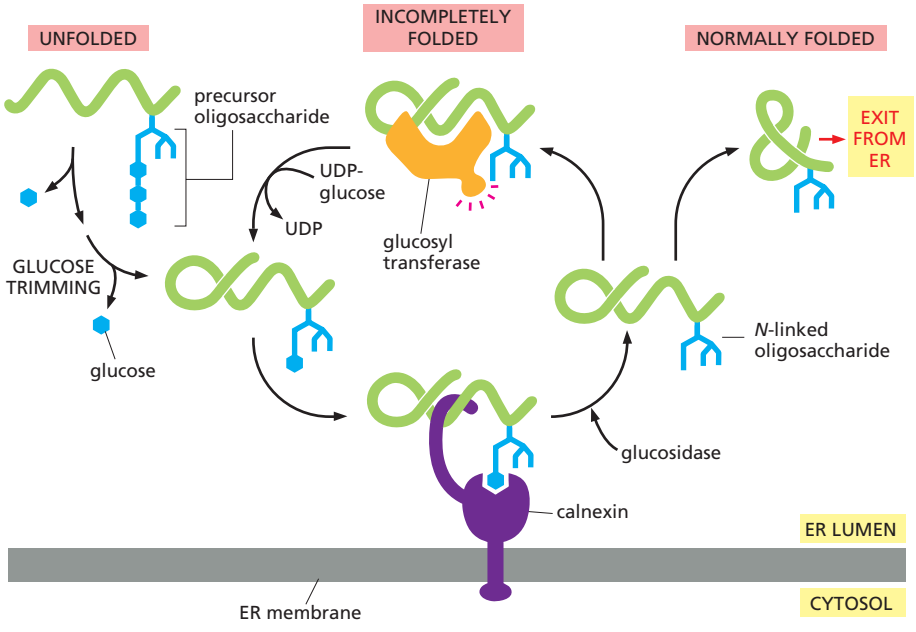


Figure 12–49 The role of *N*-linked glycosylation in ER protein folding. The ER-membrane-bound chaperone protein calnexin binds to incompletely folded proteins containing one terminal glucose on *N*-linked oligosaccharides, trapping the protein in the ER. Removal of the terminal glucose by a glucosidase releases the protein from calnexin. A glucosyl transferase is the crucial enzyme that determines whether the protein is folded properly or not: if the protein is still incompletely folded, the enzyme transfers a new glucose from UDP-glucose to the *N*-linked oligosaccharide, renewing the protein's affinity for calnexin and retaining it in the ER. The cycle repeats until the protein has folded completely. Calreticulin functions similarly, except that it is a soluble ER resident protein. Another ER chaperone, ERp57 (not shown), collaborates with calnexin and calreticulin in retaining an incompletely folded protein in the ER. ERp57 recognizes free sulfhydryl groups, which are a sign of incomplete disulfide bond formation.

modes of translocation. For example, like translocation into mitochondria or chloroplasts, chaperone proteins are necessary to keep the polypeptide chain in an unfolded state prior to and during translocation. Similarly, a source of energy is required to provide directionality to the transport and to pull the protein into the cytosol. Finally, a translocator is necessary.

Selecting proteins from the ER for degradation is a challenging process: misfolded proteins or unassembled protein subunits should be degraded, but folding intermediates of newly made proteins should not. Help in making this distinction comes from the *N*-linked oligosaccharides, which serve as timers that measure how long a protein has spent in the ER. The slow trimming of a particular mannose on the core oligosaccharide tree by an enzyme (a mannosidase) in the ER creates a new oligosaccharide structure that ER-lumenal lectins of the retrotranslocation apparatus recognize. Proteins that fold and exit from the ER faster than the mannosidase can remove its target mannose therefore escape degradation.

In addition to the lectins in the ER that recognize the oligosaccharides, chaperones and protein disulfide isomerases (enzymes mentioned earlier that catalyze the formation and breakage of S–S bonds) associate with the proteins that must be degraded. The chaperones prevent the unfolded proteins from aggregating, and the disulfide isomerases break disulfide bonds that may have formed incorrectly, so that a linear polypeptide chain can be translocated back into the cytosol.

Multiple translocator complexes move different proteins from the ER membrane or lumen into the cytosol. A common feature is that they each contain an E3 ubiquitin ligase enzyme, which attaches polyubiquitin tags to the unfolded proteins as they emerge into the cytosol, marking them for destruction. Fueled by the energy derived from ATP hydrolysis, a hexomeric ATPase of the family of AAA-ATPases (see Figure 6–85) pulls the unfolded protein through the translocator into the cytosol. An *N*-glycanase removes its oligosaccharide chains *en bloc*. Guided by its ubiquitin tag, the deglycosylated polypeptide is rapidly fed into proteasomes, where it is degraded (**Figure 12–50**).

Misfolded Proteins in the ER Activate an Unfolded Protein Response

Cells carefully monitor the amount of misfolded protein in various compartments. An accumulation of misfolded proteins in the cytosol, for example, triggers a *heat-shock response* (discussed in Chapter 6), which stimulates the transcription of genes encoding cytosolic chaperones that help to refold the proteins. Similarly, an accumulation of misfolded proteins in the ER triggers an **unfolded protein response**, which includes an increased transcription of genes encoding proteins involved in retrotranslocation and protein degradation in the cytosol, ER chaperones, and many other proteins that help to increase the protein-folding capacity of the ER.

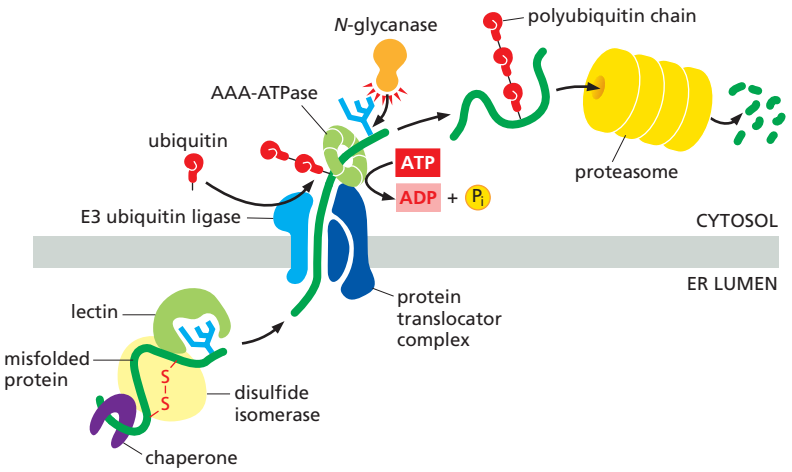
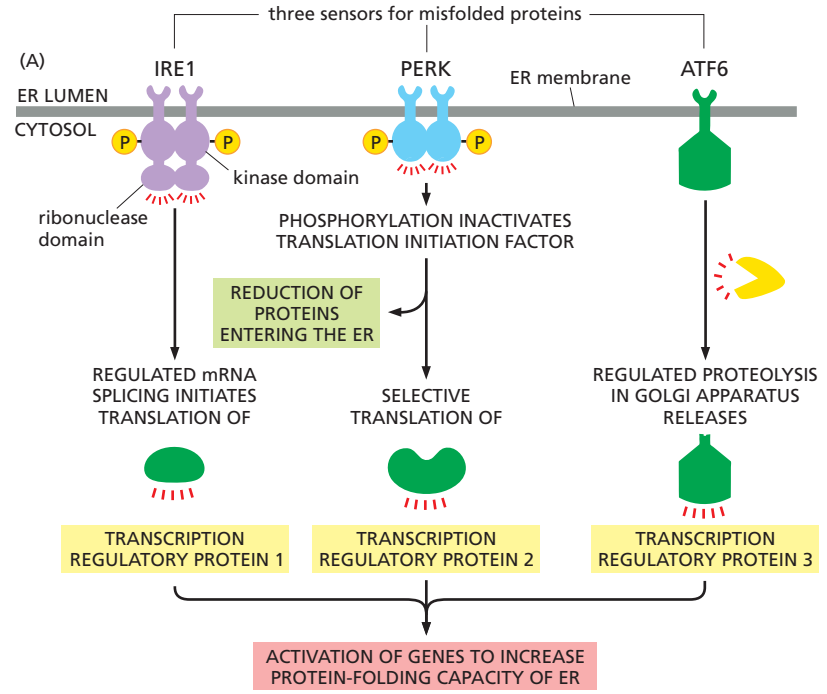


Figure 12–50 The export and degradation of misfolded ER proteins. Misfolded soluble proteins in the ER lumen are recognized and targeted to a translocator complex in the ER membrane. They first interact in the ER lumen with chaperones, disulfide isomerases, and lectins. They are then exported into the cytosol through the translocator. In the cytosol, they are ubiquitylated, deglycosylated, and degraded in proteasomes. Misfolded membrane proteins follow a similar pathway but use a different translocator.

How do misfolded proteins in the ER signal to the nucleus? There are three parallel pathways that execute the unfolded protein response (Figure 12–51A). The first pathway, which was initially discovered in yeast cells, is particularly remarkable. Misfolded proteins in the ER activate a transmembrane protein kinase in the ER, called IRE1, which causes the kinase to oligomerize and phosphorylate itself. (Some cell-surface receptor kinases in the plasma membrane are activated in a



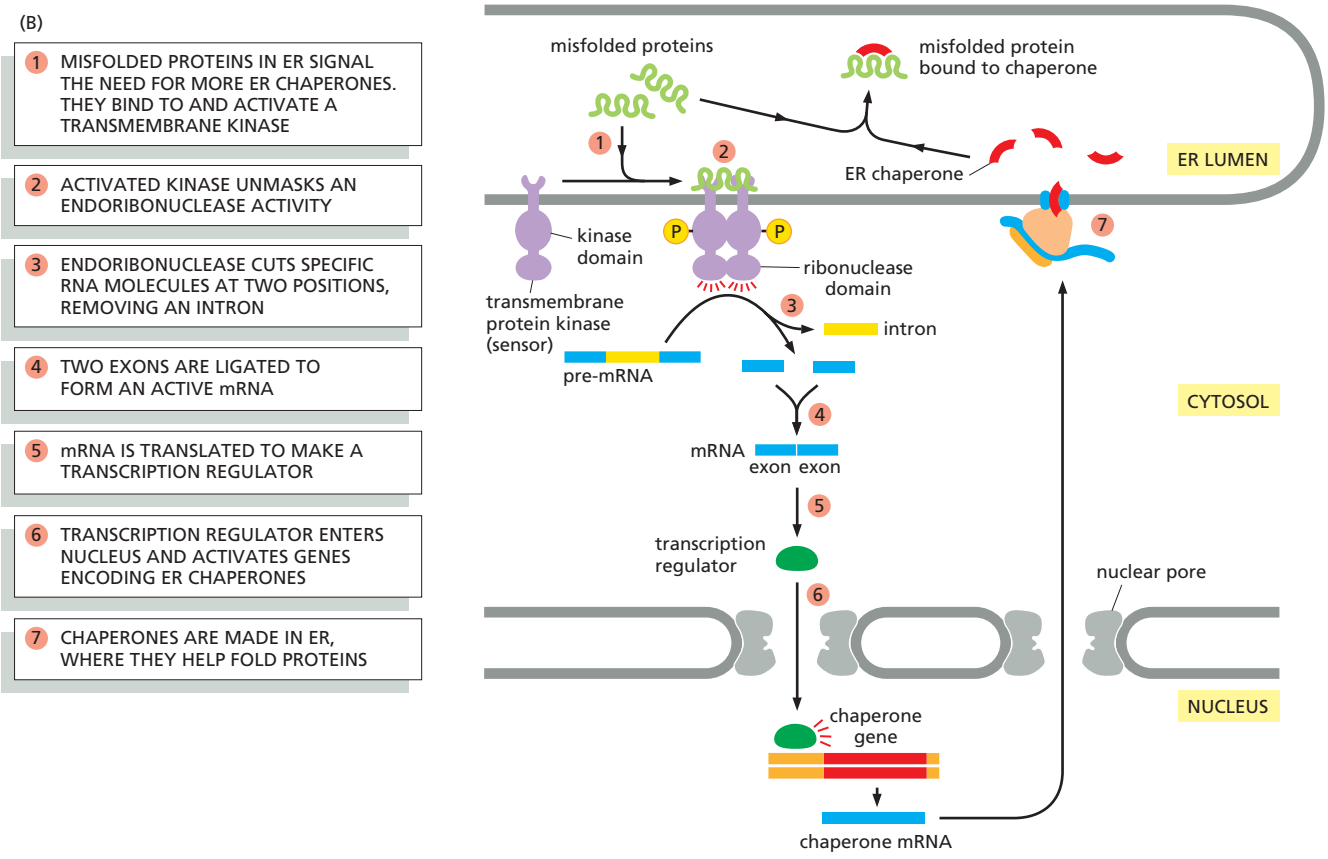


Figure 12–51 The unfolded protein response. (A) By three parallel intracellular signaling pathways, the accumulation of misfolded proteins in the ER lumen signals to the nucleus to activate the transcription of genes that encode proteins that help the cell cope with misfolded proteins in the ER. (B) Regulated RNA splicing is a key regulatory switch in pathway 1 of the unfolded protein response (Movie 12.6).

similar way, as discussed in Chapter 15.) The oligomerization and autophosphorylation of IRE1 activates an endoribonuclease domain in the cytosolic portion of the same molecule, which cleaves a specific cytosolic mRNA molecule at two positions, excising an intron. (This is a unique exception to the rule that introns are spliced out while the RNA is still in the nucleus.) The separated exons are then joined by an RNA ligase, generating a spliced mRNA, which is translated to produce an active transcription regulatory protein. This protein activates the transcription of genes encoding the proteins that help mediate the unfolded protein response (Figure 12–51B).

Misfolded proteins also activate a second transmembrane kinase in the ER, PERK, which inhibits a translation initiation factor by phosphorylating it, thereby reducing the production of new proteins throughout the cell. One consequence of the reduction in protein synthesis is to reduce the flux of proteins into the ER, thereby reducing the load of proteins that need to be folded there. Some proteins, however, are preferentially translated when translation initiation factors are scarce (discussed in Chapter 7, p. 424), and one of these is a transcription regulator that helps activate the transcription of the genes encoding proteins active in the unfolded protein response.

Finally, a third transcription regulator, ATF6, is initially synthesized as a transmembrane ER protein. Because it is embedded in the ER membrane, it cannot activate the transcription of genes in the nucleus. When misfolded proteins accumulate in the ER, however, the ATF6 protein is transported to the Golgi apparatus, where it encounters proteases that cleave off its cytosolic domain, which can now migrate to the nucleus and help activate the transcription of genes encoding proteins involved in the unfolded protein response. (This mechanism is similar to that described in Figure 12–16 for activation of the transcription regulator that controls cholesterol biosynthesis.) The relative importance of each of these three pathways in the unfolded protein response differs in different cell types, enabling each cell type to tailor the unfolded protein response to its particular needs.

Some Membrane Proteins Acquire a Covalently Attached Glycosylphosphatidylinositol (GPI) Anchor

As discussed in Chapter 10, several cytosolic enzymes catalyze the covalent addition of a single fatty acid chain or prenyl group to selected proteins. The attached lipids help direct and attach these proteins to cell membranes. A related process is catalyzed by ER enzymes that covalently attach a **glycosylphosphatidylinositol (GPI) anchor** to the C-terminus of some membrane proteins destined for the plasma membrane. This linkage forms in the lumen of the ER, where, at the same time, the transmembrane segment of the protein is cleaved off (**Figure 12–52**). A large number of plasma membrane proteins are modified in this way. Since they are attached to the exterior of the plasma membrane only by their GPI anchors,

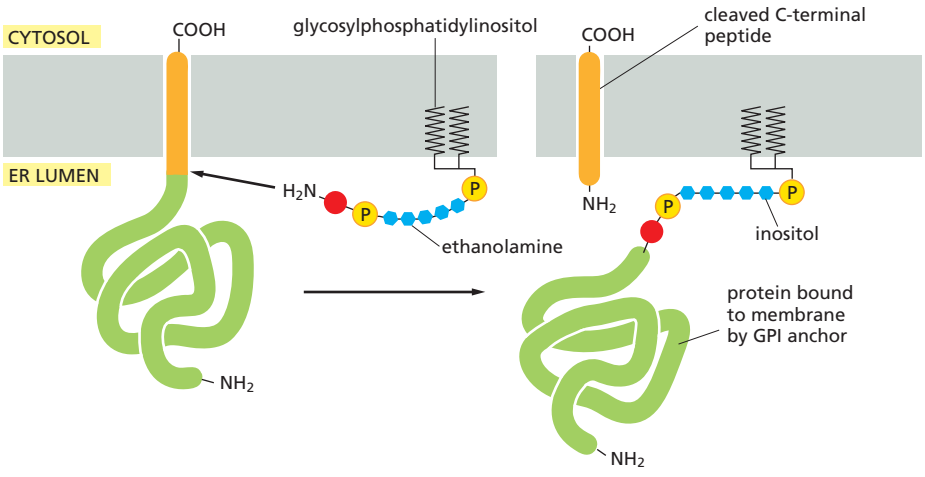


Figure 12–52 The attachment of a GPI anchor to a protein in the ER. GPI-anchored proteins are targeted to the ER membrane by an N-terminal signal sequence (not shown), which is removed (see Figure 12–42). Immediately after the completion of protein synthesis, the precursor protein remains anchored in the ER membrane by a hydrophobic C-terminal sequence of 15–20 amino acids; the rest of the protein is in the ER lumen. Within less than a minute, an enzyme in the ER cuts the protein free from its membrane-bound C-terminus and simultaneously attaches the new C-terminus to an amino group on a preassembled GPI intermediate. The sugar chain contains an inositol attached to the lipid from which the GPI anchor derives its name. It is followed by a glucosamine and three mannoses. The terminal mannose links to a phosphoethanolamine that provides the amino group to attach the protein. The signal that specifies this modification is contained within the hydrophobic C-terminal sequence and a few amino acids adjacent to it on the lumenal side of the ER membrane; if this signal is added to other proteins, they too become modified in this way. Because of the covalently linked lipid anchor, the protein remains membrane-bound, with all of its amino acids exposed initially on the lumenal side of the ER and eventually on the exterior of the plasma membrane.

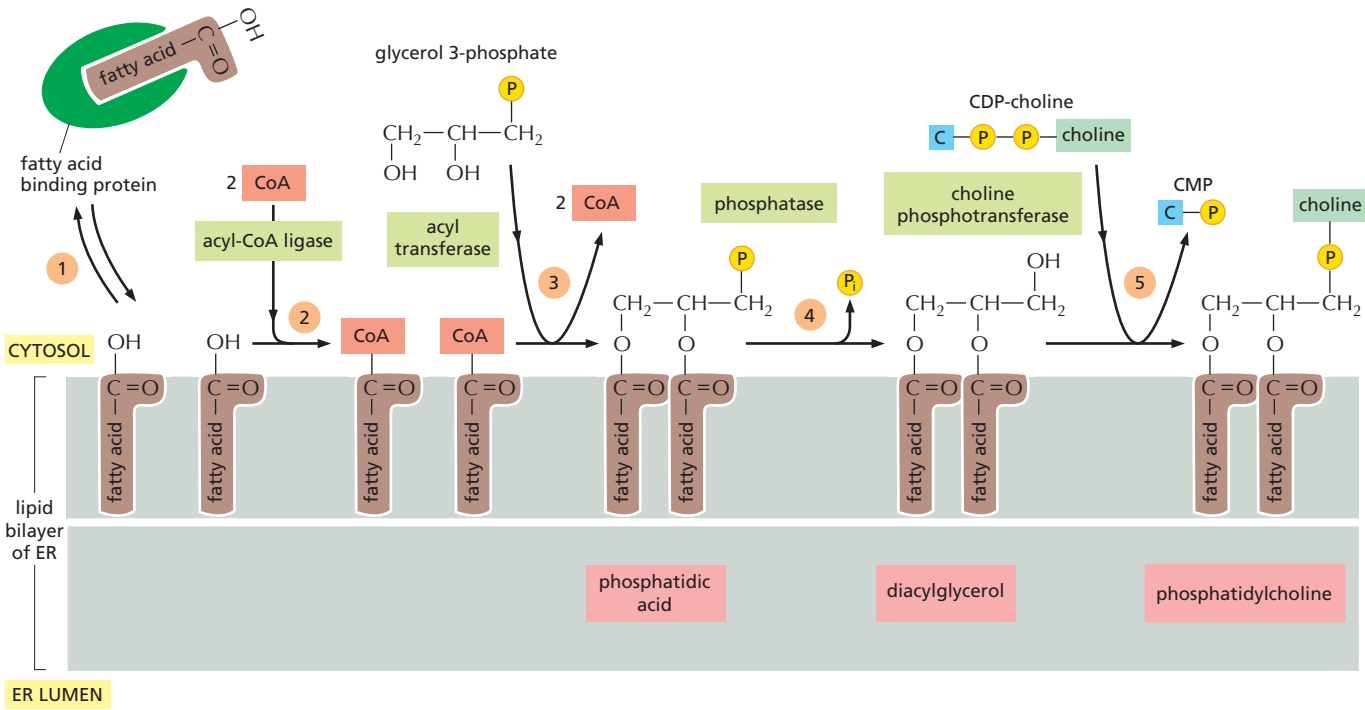


Figure 12–53 The synthesis of phosphatidylcholine. As illustrated, this phospholipid is synthesized from glycerol 3-phosphate, cytidine-diphosphocholine (CDP-choline), and fatty acids delivered to the ER by a cytosolic fatty acid binding protein.

they can in principle be released from cells in soluble form in response to signals that activate a specific phospholipase in the plasma membrane. Trypanosome parasites, for example, use this mechanism to shed their coat of GPI-anchored surface proteins when attacked by the immune system. GPI anchors may also be used to direct plasma membrane proteins into *lipid rafts* and thus segregate the proteins from other membrane proteins (see Figure 10–13).

The ER Assembles Most Lipid Bilayers

The ER membrane is the site of synthesis of nearly all of the cell's major classes of lipids, including both phospholipids and cholesterol, required for the production of new cell membranes. The major phospholipid made is *phosphatidylcholine*, which can be formed in three steps from choline, two fatty acids, and glycerol phosphate (**Figure 12–53**). Each step is catalyzed by enzymes in the ER membrane, which have their active sites facing the cytosol, where all of the required metabolites are found. Thus, phospholipid synthesis occurs exclusively in the cytosolic leaflet of the ER membrane. Because fatty acids are not soluble in water, they are shepherded from their sites of synthesis to the ER by a fatty acid binding protein in the cytosol. After arrival in the ER membrane and activation with CoA, acyl transferases successively add two fatty acids to glycerol phosphate to produce phosphatidic acid. Phosphatidic acid is sufficiently water-insoluble to remain in the lipid bilayer; it cannot be extracted from the bilayer by the fatty acid binding proteins. It is therefore this first step that enlarges the ER lipid bilayer. The later steps determine the head group of a newly formed lipid molecule and therefore the chemical nature of the bilayer, but they do not result in net membrane growth. The two other major membrane phospholipids—phosphatidylethanolamine and phosphatidylserine (see Figure 10–3)—as well as the minor phospholipid phosphatidylinositol (PI), are all synthesized in this way.

Because phospholipid synthesis takes place in the cytosolic leaflet of the ER lipid bilayer, there needs to be a mechanism that transfers some of the newly formed phospholipid molecules to the lumenal leaflet of the bilayer. In synthetic lipid bilayers, lipids do not "flip-flop" in this way (see Figure 10–10). In the ER, however, phospholipids equilibrate across the membrane within minutes, which is almost 100,000 times faster than can be accounted for by spontaneous "flip-flop." This rapid trans-bilayer movement is mediated by a poorly characterized

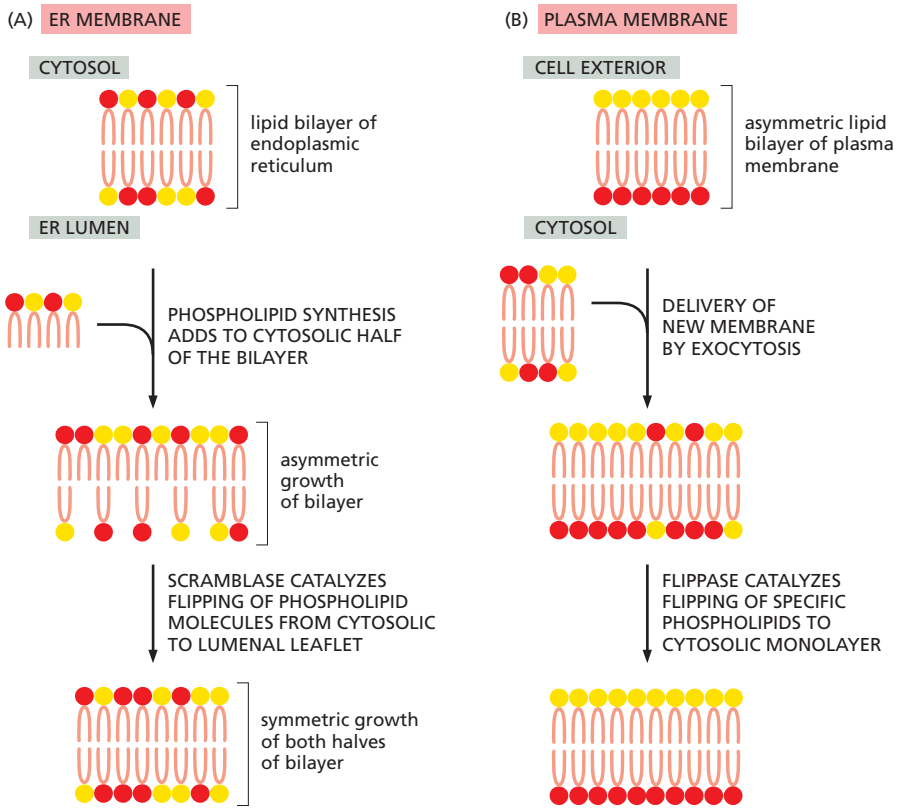


Figure 12–54 The role of phospholipid translocators in lipid bilayer synthesis. (A) Because new lipid molecules are added only to the cytosolic half of the ER membrane bilayer and lipid molecules do not flip spontaneously from one monolayer to the other, a transmembrane phospholipid translocator (called a scramblase) is required to transfer lipid molecules from the cytosolic half to the lumenal half so that the membrane grows as a bilayer. The scramblase is not specific for particular phospholipid head groups and therefore equilibrates the different phospholipids between the two monolayers. (B) Fueled by ATP hydrolysis, a head-group-specific flippase in the plasma membrane actively flips phosphatidylserine and phosphatidylethanolamine directionally from the extracellular to the cytosolic leaflet, creating the characteristically asymmetric lipid bilayer of the plasma membrane of animal cells (see Figure 10–15).

phospholipid translocator called a *scramblase*, which nonselectively equilibrates phospholipids between the two leaflets of the lipid bilayer (**Figure 12–54**). Thus, the different types of phospholipids are thought to be equally distributed between the two leaflets of the ER membrane.

The plasma membrane contains a different type of phospholipid translocator that belongs to the family of P-type pumps (discussed in Chapter 11). These *flippases* specifically recognize those phospholipids that contain free amino groups in their head groups (phosphatidylserine and phosphatidylethanolamine—see Figure 10–3) and transfers them from the extracellular to the cytosolic leaflet, using the energy of ATP hydrolysis. The plasma membrane therefore has a highly asymmetric phospholipid composition, which is actively maintained by the flippases (see Figure 10–15). The plasma membrane also contains a scramblase but, in contrast to the ER scramblase, which is always active, the plasma membrane enzyme is regulated and only activated in some situations, such as in apoptosis and in activated platelets, where it acts to abolish the lipid bilayer asymmetry; the resulting exposure of phosphatidylserine on the surface of apoptotic cells serves as a signal for phagocytic cells to ingest and degrade the dead cell.

The ER also produces cholesterol and ceramide (**Figure 12–55**). *Ceramide* is made by condensing the amino acid serine with a fatty acid to form the amino alcohol *sphingosine* (see Figure 10–3); a second fatty acid is then covalently added to form ceramide. The ceramide is exported to the Golgi apparatus, where it serves as a precursor for the synthesis of two types of lipids: oligosaccharide chains are added to form *glycosphingolipids* (glycolipids; see Figure 10–16), and phosphocholine head groups are transferred from phosphatidylcholine to other ceramide molecules to form *sphingomyelin* (discussed in Chapter 10). Thus, both glycolipids and sphingomyelin are produced relatively late in the process of membrane synthesis. Because they are produced by enzymes that have their active sites exposed to the Golgi lumen, they are found exclusively in the noncytosolic leaflet of the lipid bilayers that contain them.

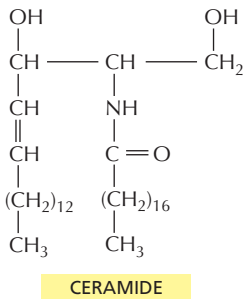


Figure 12–55 The structure of ceramide.

As discussed in Chapter 13, the plasma membrane and the membranes of the Golgi apparatus, lysosomes, and endosomes all form part of a membrane system that communicates with the ER by means of transport vesicles, which transfer both proteins and lipids. Mitochondria and plastids, however, do not belong to this system, and they therefore require different mechanisms to import proteins and lipids for growth. We have already seen that they import most of their proteins from the cytosol. Although mitochondria modify some of the lipids they import, they do not synthesize lipids *de novo*; instead, their lipids have to be imported from the ER, either directly or indirectly by way of other cell membranes. In either case, special mechanisms are required for the transfer.

The details of how lipid distribution between different membranes is catalyzed and regulated are not known. Water-soluble carrier proteins called *phospholipid exchange proteins* (or *phospholipid transfer proteins*) are thought to transfer individual phospholipid molecules between membranes, functioning much like fatty acid binding proteins that shepherd fatty acids through the cytosol (see Figure 12–54). In addition, mitochondria are often seen in close juxtaposition to ER membranes in electron micrographs, and specific junction complexes have been identified that hold the ER and outer mitochondrial membrane in close proximity. It is thought that these junction complexes provide specific contact-dependent lipid transfer mechanisms that operate between these adjacent membranes.

Summary

The extensive ER network serves as a factory for the production of almost all of the cell's lipids. In addition, a major portion of the cell's protein synthesis occurs on the cytosolic surface of the rough ER: virtually all proteins destined for secretion or for the ER itself, the Golgi apparatus, the lysosomes, the endosomes, and the plasma membrane are first imported into the ER from the cytosol. In the ER lumen, the proteins fold and oligomerize, disulfide bonds are formed, and N*-linked oligosaccharides are added. The pattern of* N*-linked glycosylation is used to indicate the extent of protein folding, so that proteins leave the ER only when they are properly folded. Proteins that do not fold or oligomerize correctly are translocated back into the cytosol, where they are deglycosylated, polyubiquitylated, and degraded in proteasomes. If misfolded proteins accumulate in excess in the ER, they trigger an unfolded protein response, which activates appropriate genes in the nucleus to help the ER cope.*

Only proteins that carry a special ER signal sequence are imported into the ER. The signal sequence is recognized by a signal-recognition particle (SRP), which binds both the growing polypeptide chain and the ribosome and directs them to a receptor protein on the cytosolic surface of the rough ER membrane. This binding to the ER membrane initiates the translocation process that threads a loop of polypeptide chain across the ER membrane through the hydrophilic pore of a protein translocator.

Soluble proteins—destined for the ER lumen, for secretion, or for transfer to the lumen of other organelles—pass completely into the ER lumen. Transmembrane proteins destined for the ER or for other cell membranes are translocated part way across the ER membrane and remain anchored there by one or more membrane-spanning α-helical segments in their polypeptide chains. These hydrophobic portions of the protein can act either as start-transfer or stop-transfer signals during the translocation process. When a polypeptide contains multiple, alternating start-transfer and stop-transfer signals, it will pass back and forth across the bilayer multiple times as a multipass transmembrane protein.

The asymmetry of protein insertion and glycosylation in the ER establishes the sidedness of the membranes of all the other organelles that the ER supplies with membrane proteins.

WHAT WE DON'T KNOW

- How do nuclear import receptors negotiate the tangled gel-like interior of a nuclear pore complex so efficiently?
- Is the nuclear pore complex a rigid structure or can it expand and contract, depending on the cargo transported?
- Sequence comparisons show that signal sequences for an individual protein such as insulin are quite conserved across species, much more so than would be expected from our current understanding that all that matters for their function are general structural features such as hydrophobicity. What other functions might signal sequences have that could account for their evolutionary sequence conservation?
- How are polyribosomes on the endoplasmic reticulum membrane arranged so that the next initiating ribosome will find an unoccupied translocator?
- Why does the signal-recognition particle have an indispensable RNA subunit?

PROBLEMS

Which statements are true? Explain why or why not.

12–1 Like the lumen of the ER, the interior of the nucleus is topologically equivalent to the outside of the cell.

12–2 ER-bound and free ribosomes, which are structurally and functionally identical, differ only in the proteins they happen to be making at a particular time.

12–3 To avoid the inevitable collisions that would occur if two-way traffic through a single pore were allowed, nuclear pore complexes are specialized so that some mediate import while others mediate export.

12–4 Peroxisomes are found in only a few specialized types of eukaryotic cell.

Discuss the following problems.

12–5 What is the fate of a protein with no sorting signal?

12–6 The rough ER is the site of synthesis of many classes of membrane proteins. Some of these proteins remain in the ER, whereas others are sorted to compartments such as the Golgi apparatus, lysosomes, and the plasma membrane. One measure of the difficulty of the sorting problem is the degree of "purification" that must be achieved during transport from the ER. Are proteins bound for the plasma membrane common or rare among all ER membrane proteins?

A few simple considerations allow one to answer this question. In a typical growing cell that is dividing once every 24 hours, the equivalent of one new plasma membrane must transit the ER every day. If the ER membrane is 20 times the area of a plasma membrane, what is the ratio of plasma membrane proteins to other membrane proteins in the ER? (Assume that all proteins on their way to the plasma membrane remain in the ER for 30 minutes on average before exiting, and that the ratio of proteins to lipids in the ER and plasma membranes is the same.)

12–7 Before nuclear pore complexes were well understood, it was unclear whether nuclear proteins diffused passively into the nucleus and accumulated there by binding to residents of the nucleus such as chromosomes, or whether they were actively imported and accumulated regardless of their affinity for nuclear components.

A classic experiment that addressed this problem used several forms of radioactive nucleoplasmin, which is a large pentameric protein involved in chromatin assembly. In this experiment, either the intact protein or the nucleoplasmin heads, tails, or heads with a single tail were injected into the cytoplasm of a frog oocyte or into the nucleus (**Figure Q12–1**). All forms of nucleoplasmin, except heads, accumulated in the nucleus when injected into the cytoplasm, and all forms were retained in the nucleus when injected there.

A. What portion of the nucleoplasmin molecule is responsible for localization in the nucleus?

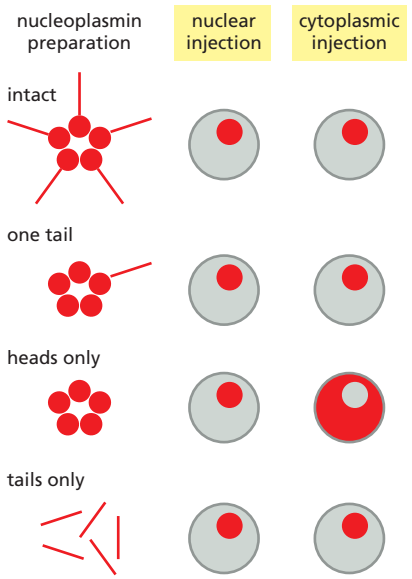


Figure Q12–1 Cellular location of injected nucleoplasmin and nucleoplasmin components (Problem 12–7). Schematic diagrams of autoradiographs show the cytoplasm and nucleus with the location of nucleoplasmin indicated by the *red* areas.

B. How do these experiments distinguish between active transport, in which a nuclear localization signal triggers transport by the nuclear pore complex, and passive diffusion, in which a binding site for a nuclear component allows accumulation in the nucleus?

12–8 Assuming that 32 million histone octamers are required to package the human genome, how many histone molecules must be transported per second per nuclear pore complex in cells whose nuclei contain 3000 nuclear pores and are dividing once per day?

12–9 The nuclear pore complex (NPC) creates a barrier to the free exchange of molecules between the nucleus and cytosol, but in a way that remains mysterious. In yeast, for example, the central pore of the NPC has a diameter of 35 nm and is 30 nm long, which is somewhat smaller than its vertebrate counterpart. Even so, it is large enough to accommodate virtually all components of the cytosol. Yet the pore allows passive diffusion of molecules only up to about 40 kd; entry of anything larger requires help from a nuclear import receptor. Selective permeability is controlled by protein components of the NPC that have unstructured, polar tails extending into the central pore. These tails are characterized by periodic repeats of the hydrophobic amino acids phenylalanine (F) and glycine (G).

At high enough concentration (~50 mM), the FG-repeat domains of these proteins can form a gel, with a meshwork of interactions between the hydrophobic FG repeats (**Figure Q12–2A**). These gels allow passive diffusion of small molecules, but they prevent entry of larger proteins such as the fluorescent protein mCherry fused to maltose binding protein (MBP) (**Figure Q12–2B**). (The fusion to MBP makes mCherry too large to enter the nucleus by passive diffusion.) However, if the nuclear import receptor, importin, is fused to a similar protein, MBP-GFP, the importin-MBP-GFP fusion readily enters the gel (Figure Q12–2B).

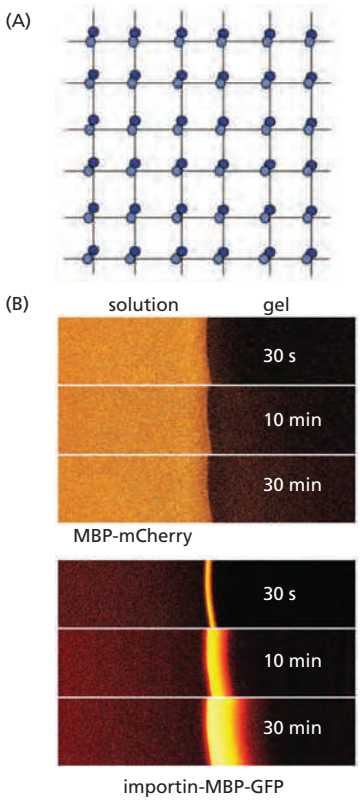


Figure Q12–2 FG-repeat gel and influx of proteins into the nucleus (Problem 12–9). (A) Cartoon of the meshwork (gel) formed by pairwise interactions between hydrophobic FG repeats. For FG-repeats separated by 17 amino acids, as is typical, the mesh formed by extended amino acid side chains would correspond to about 4 nm on a side, which would be large enough to account for the characteristic passive diffusion of proteins through nuclear pores. (B) Diffusion of MBP-mCherry and importin-MBP-GFP into a gel of FG-repeats. In each group, the solution is shown at left and the gel at right. The bright areas indicate regions that contain the fluorescent proteins.

A. FG-repeats only form gels *in vitro* at relatively high concentration (50 mM). Is this concentration reasonable for FG repeats in the NPC core? In yeast, there are about 5000 FG-repeats in each NPC. Given the dimensions of the yeast nuclear pore (35 nm diameter and 30 nm length), calculate the concentration of FG-repeats in the cylindrical volume of the pore. Is this concentration comparable to the one used *in vitro*?

B. A second question is whether the diffusion of importin-MBP-GFP through the FG-repeat gel is fast enough to account for the efficient flow of materials between the nucleus and cytosol. From experiments of the type shown in Figure Q12–2B, the diffusion coefficient (D) of importin-MBP-GFP through the FG-repeat gel was determined to be about 0.1 $\mu m^2/s$. The equation for diffusion is $t = x^2/2D$, where t is time and x is distance. Calculate the time it would take importin-MBP-GFP to diffuse through a yeast nuclear pore (30 nm) if the pore consisted of a gel of FG-repeats. Does this time seem fast enough for the needs of a eukaryotic cell?

12–10 Components of the TIM complexes, the multisubunit protein translocators in the mitochondrial inner membrane, are much less abundant than those of the TOM complex. They were initially identified using a genetic trick. The yeast *Ura3* gene, whose product is an enzyme that is normally located in the cytosol where it is essential for synthesis of uracil, was modified so that the protein carried an import signal for the mitochondrial matrix. A population of cells carrying the modified *Ura3* gene in place of the normal gene was then grown in the absence of uracil. Most cells died, but the rare cells that grew were shown to be defective for mitochondrial import. Explain how this selection identifies cells with defects in components required for import into the mitochondrial matrix. Why don't normal cells with the modified *Ura3* gene grow in the absence of uracil? Why do cells that are defective for mitochondrial import grow in the absence of uracil?

12–11 If the enzyme dihydrofolate reductase (DHFR), which is normally located in the cytosol, is engineered to carry a mitochondrial targeting sequence at its N-terminus, it is efficiently imported into mitochondria. If the modified DHFR is first incubated with methotrexate, which binds tightly to the active site, the enzyme remains in the cytosol. How do you suppose that the binding of methotrexate interferes with mitochondrial import?

12–12 Why do mitochondria need a special translocator to import proteins across the outer membrane, when the membrane already has large pores formed by porins?

12–13 Examine the multipass transmembrane protein shown in **Figure Q12–3**. What would you predict would be the effect of converting the first hydrophobic transmembrane segment to a hydrophilic segment? Sketch the arrangement of the modified protein in the ER membrane.

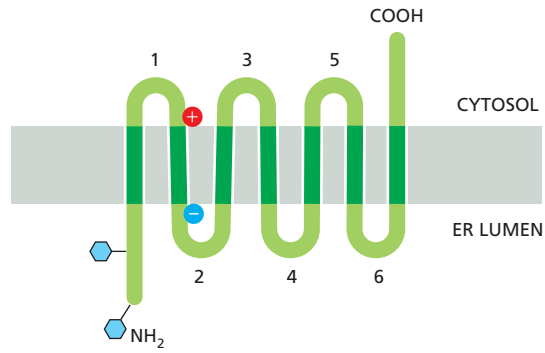


Figure Q12–3 Arrangement of a multipass transmembrane protein in the ER membrane (Problem 12–13). *Blue hexagons* represent covalently attached oligosaccharides. The positions of positively and negatively charged amino acids flanking the second transmembrane segment are shown.

12–14 All new phospholipids are added to the cytosolic leaflet of the ER membrane, yet the ER membrane has a symmetrical distribution of different phospholipids in its two leaflets. By contrast, the plasma membrane, which receives all its membrane components ultimately from the ER, has a very asymmetrical distribution of phospholipids in the two leaflets of its lipid bilayer. How is the symmetry generated in the ER membrane, and how is the asymmetry generated and maintained in the plasma membrane?

REFERENCES

General

Palade G (1975) Intracellular aspects of the process of protein synthesis. *Science* 189, 347–358.

The Compartmentalization of Cells

Blobel G (1980) Intracellular protein topogenesis. *Proc. Natl Acad. Sci. USA* 77, 1496–1500.

Devos DP, Gräf R & Field MC (2014) Evolution of the nucleus. *Curr. Opin. Cell Biol.* 28, 8–15.

Warren G & Wickner W (1996) Organelle inheritance. *Cell* 84, 395–400.

The Transport of Molecules Between the Nucleus and the Cytosol

Adam SA & Gerace L (1991) Cytosolic proteins that specifically bind nuclear location signals are receptors for nuclear import. *Cell* 66, 837–847.

Burke B & Stewart CL (2013) The nuclear lamins: flexibility in function. *Nat. Rev. Mol. Cell Biol.* 14, 13–24.

Cole CN & Scarcelli JJ (2006) Transport of messenger RNA from the nucleus to the cytoplasm. *Curr. Opin. Cell Biol.* 18, 299–306.

Güttinger S, Laurell E & Kutay U (2009) Orchestrating nuclear envelope disassembly and reassembly during mitosis. *Nat. Rev. Mol. Cell Biol.* 10, 178–191.

Hetzer MW & Wente SR (2009) Border control at the nucleus: biogenesis and organization of the nuclear membrane and pore complexes. *Dev. Cell* 17, 606–616.

Hoelz A, Debler EW & Blobel G (2011) The structure of the nuclear pore complex. *Annu. Rev. Biochem.* 80, 613–643.

Hülsmann BB, Labokha AA & Görlich D (2012) The permeability of reconstituted nuclear pores provides direct evidence for the selective phase model. *Cell* 150, 738–751.

Köhler A & Hurt E (2007) Exporting RNA from the nucleus to the cytoplasm. *Nat. Rev. Mol. Cell Biol.* 8, 761–773.

Rothballer A & Kutay U (2013) Poring over pores: nuclear pore complex insertion into the nuclear envelope. *Trends Biochem. Sci.* 38, 292–301.

Strambio-De-Castilla C, Niepel M & Rout MP (2010) The nuclear pore complex: bridging nuclear transport and gene regulation. *Nat. Rev. Mol. Cell Biol.* 11, 490–501.

Tran EJ & Wente SR (2006) Dynamic nuclear pore complexes: life on the edge. *Cell* 125, 1041–1053.

The Transport of Proteins Into Mitochondria and Chloroplasts

Chacinska A, Koehler CM, Milenkovic D et al. (2009) Importing mitochondrial proteins: machineries and mechanisms. *Cell* 138, 628–644.

Jarvis P & Robinson C (2004) Mechanisms of protein import and routing in chloroplasts. *Curr. Biol.* 14, R1064–R1077.

Kessler F & Schnell DJ (2009) Chloroplast biogenesis: diversity and regulation of the protein import apparatus. *Curr. Opin. Cell Biol.* 21, 494–500.

Prakash S & Matouschek A (2004) Protein unfolding in the cell. *Trends Biochem. Sci.* 29, 593–600.

Schleiff E & Becker T (2011) Common ground for protein translocation: access control for mitochondria and chloroplasts. *Nat. Rev. Mol. Cell Biol.* 12, 48–59.

Peroxisomes

Dimitrov L, Lam SK & Schekman R (2013) The role of the endoplasmic reticulum in peroxisome biogenesis. *Cold Spring Harb. Perspect. Biol.* 5, a013243.

Fujiki Y, Yagita Y & Matsuzaki T (2012) Peroxisome biogenesis disorders. *Biochim. Biophys. Acta* 1822, 1337–1342.

Schliebs W, Girzalsky W& Erdmann R (2010) Peroxisomal protein import and ERAD: variations on a common theme. *Nat. Rev. Mol. Cell Biol.* 11, 885–890.

Tabak HF, Braakman I & van der Zand A (2013) Peroxisome formation and maintenance are dependent on the endoplasmic reticulum. *Annu. Rev. Biochem.* 82, 723–744.

The Endoplasmic Reticulum

Akopian D, Shen K, Zhang X & Shan SO (2013) Signal recognition particle: an essential protein-targeting machine. *Annu. Rev. Biochem.* 82, 693–721.

Blobel G & Dobberstein B (1975) Transfer of proteins across membranes. I. Presence of proteolytically processed and unprocessed nascent immunoglobulin light chains on membrane-bound ribosomes of murine myeloma. *J. Cell Biol.* 67, 835–851.

Borgese N, Mok W, Kreibich G & Sabatini DD (1974) Ribosomal-membrane interaction: *in vitro* binding of ribosomes to microsomal membranes. *J. Mol. Biol.* 88, 559–580.

Braakman I & Bulleid NJ (2011) Protein folding and modification in the mammalian endoplasmic reticulum. *Annu. Rev. Biochem.* 80, 71–99.

Brodsky JL & Skach WR (2011) Protein folding and quality control in the endoplasmic reticulum: recent lessons from yeast and mammalian cell systems. *Curr. Opin. Cell Biol.* 23, 464–475.

Chen S, Novick P & Ferro-Novick S (2013) ER structure and function. *Curr. Opin. Cell Biol.* 25, 428–433.

Clark MR (2011) Flippin' lipids. *Nat. Immunol.* 12, 373–375.

Daleke DL (2003) Regulation of transbilayer plasma membrane phospholipid asymmetry. *J. Lipid Res.* 44, 233–242.

Deshaies RJ, Sanders SL, Feldheim DA & Schekman R (1991) Assembly of yeast Sec proteins involved in translocation into the endoplasmic reticulum into a membrane-bound multisubunit complex. *Nature* 349, 806–808.

Gething MJ (1999) Role and regulation of the ER chaperone BiP. *Semin. Cell Dev. Biol.* 10, 465–472.

Görlich D, Prehn S, Hartmann E et al. (1992) A mammalian homolog of SEC61p and SECYp is associated with ribosomes and nascent polypeptides during translocation. *Cell* 71, 489–503.

Hegde RS & Ploegh HL (2010) Quality and quantity control at the endoplasmic reticulum. *Curr. Opin. Cell Biol.* 22, 437–446.

Hegde RS & Keenan RJ (2011) Tail-anchored membrane protein insertion into the endoplasmic reticulum. *Nat. Rev. Mol. Cell Biol*. 12, 787–798.

Levine T & Loewen C (2006) Inter-organelle membrane contact sites: through a glass, darkly. *Curr. Opin. Cell Biol.* 18, 371–378.

López-Marqués RL, Holthuis JCM & Pomorski TG (2011) Pumping lipids with P4-ATPases. *Biol. Chem.* 392, 67–76.

Mamathambika BS & Bardwell JC (2008) Disulfide-linked protein folding pathways. *Annu. Rev. Cell Dev. Biol.* 24, 211–235.

Marciniak SJ & Ron D (2006) Endoplasmic reticulum stress signaling in disease. *Physiol. Rev.* 86, 1133–1149.

Milstein C, Brownlee GG, Harrison TM & Mathews MB (1972) A possible precursor of immunoglobulin light chains. *Nat. New Biol.* 239, 117–120.

Park E & Rapoport TA (2012) Mechanisms of Sec61/SecY-mediated protein translocation across membranes. *Annu. Rev. Biophys.* 41, 21–40.

Römisch K (2005) Endoplasmic reticulum-associated degradation. *Annu. Rev. Cell Dev. Biol.* 21, 435–456.

Rowland AA & Voeltz GK (2012) Endoplasmic reticulum-mitochondria contacts: function of the junction. *Nat. Rev. Mol. Cell Biol*. 13, 607–625.

Trombetta ES & Parodi AJ (2003) Quality control and protein folding in the secretory pathway. *Annu. Rev. Cell Dev. Biol.* 19, 649–676.

Tsai B, Ye Y & Rapoport TA (2002) Retro-translocation of proteins from the endoplasmic reticulum into the cytosol. *Nat. Rev. Mol. Cell Biol.* 3, 246–255.

Walter P & Ron D (2011) The unfolded protein response: from stress pathway to homeostatic regulation. *Science* 334, 1081–1086.

von Heijne G (2011) Introduction to theme "membrane protein folding and insertion". *Annu. Rev. Biochem.* 80, 157–160.