

MOLECULAR BIOLOGY OF THE CELL

THIRD EDITION

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Julian Lewis • Martin Raff • Keith Roberts
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Front cover: The photograph shows a rat nerve cell in culture. It is labeled (*yellow*) with a fluorescent antibody that stains its cell body and dendritic processes. Nerve terminals (*green*) from other neurons (not visible), which have made synapses on the cell, are labeled with a different antibody. (Courtesy of Olaf Mundigl and Pietro de Camilli.)

Dedication page: Gavin Borden, late president of Garland Publishing, weathered in during his mid-1980s climb near Mount McKinley with MBoC author Bruce Alberts and famous mountaineer guide Mugs Stump (1940–1992).

Back cover: The authors, in alphabetical order, crossing Abbey Road in London on their way to lunch. Much of this third edition was written in a house just around the corner. (Photograph by Richard Olivier.)

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Part III

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Cancer

Chapter 24

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Intracellular Compartments and Protein Sorting

12

- The Compartmentalization of Higher Cells
- The Transport of Molecules into and out of the Nucleus
- The Transport of Proteins into Mitochondria and Chloroplasts
- Peroxisomes
- The Endoplasmic Reticulum

Unlike a bacterium, which generally consists of a single intracellular compartment surrounded by a plasma membrane, a eucaryotic cell is elaborately subdivided into functionally distinct, membrane-bounded 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 eucaryotic cell, it is essential to know what occurs in each of these compartments, how molecules move between them, and how the compartments themselves are created and maintained.

Proteins play a central part in the compartmentalization of a eucaryotic cell. They catalyze the reactions that occur in each organelle and selectively transport small molecules into and out of its interior, or *lumen*. Proteins also serve as organelle-specific surface markers that direct new deliveries of proteins and lipids to the appropriate organelle. A mammalian 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. Each newly synthesized protein is then delivered specifically to the cell compartment that requires it. We shall make the intracellular transport of proteins the central theme of this chapter as well as of 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 Higher Cells

In this introductory section we give a brief overview of the compartments of the cell and of the relationships between them. In doing so, we organize the organelles conceptually into a small number of discrete families, discussing how proteins are directed to specific organelles and how they cross organelle membranes.

All Eucaryotic Cells Have the Same Basic Set of Membrane-bounded Organelles ¹

Many vital biochemical processes take place in or on membrane surfaces. Lipid metabolism, for example, is catalyzed mostly by membrane-bound enzymes, and oxidative phosphorylation and photosynthesis both require a membrane in or-

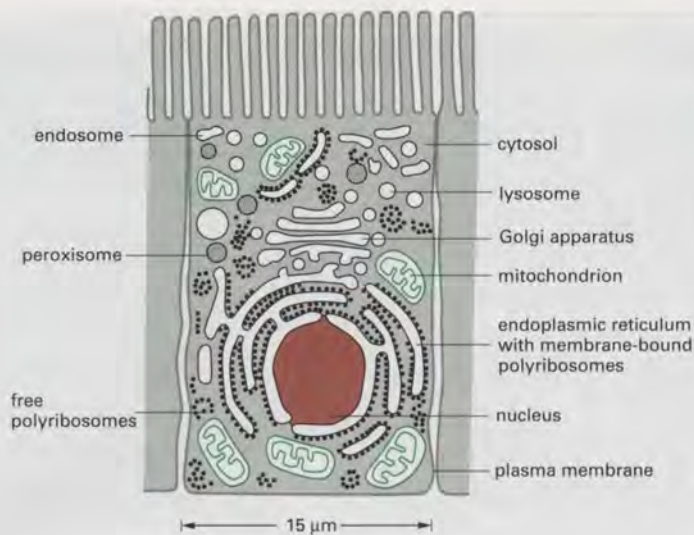


Figure 12-1 The major intracellular compartments of an animal cell. The cytosol (gray), endoplasmic reticulum, Golgi apparatus, nucleus, mitochondrion, endosome, lysosome, and peroxisome are distinct compartments isolated from the rest of the cell by at least one selectively permeable membrane.

der to couple the transport of H^+ to the synthesis of ATP. Intracellular membrane systems, however, do more for the cell than just provide increased membrane area: they create enclosed compartments that are separate from the cytosol, thus providing the cell with functionally specialized aqueous spaces. Because the lipid bilayer of organelle membranes is impermeable to most hydrophilic molecules, the membrane of each organelle must contain transport proteins that are responsible for the import and export of 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.

The major intracellular compartments common to eucaryotic cells are illustrated in Figure 12-1. The *nucleus* contains the main genome and 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 is the site of protein synthesis and of most of the cell's intermediary metabolism—that is, the many reactions by which some small molecules are degraded and others are synthesized to provide the building blocks of macromolecules (discussed in Chapter 2).

About half the total area of membrane in a cell encloses the labyrinthine spaces of the *endoplasmic reticulum (ER)*. The ER has many ribosomes bound to its cytosolic surface; these are engaged in the synthesis of integral membrane proteins and soluble proteins, most of which are destined for secretion or for other organelles. We shall see that this reflects an important difference between how proteins are directed to the ER and how they are directed to other cytoplasmic organelles: whereas proteins are translocated into other organelles only after their synthesis is complete, they are translocated into the ER during their synthesis, and hence the ribosomes on which they are made are tethered to the ER membrane. The ER also produces the lipid for the rest of the cell and functions as a store for Ca^{2+} ions. The *Golgi apparatus* consists of organized stacks of disclike compartments called *Golgi cisternae*; it receives lipids and proteins from the ER and dispatches them to a variety of destinations, usually covalently modifying them en route.

Mitochondria and (in plants) *chloroplasts* generate most of the ATP used to drive cellular reactions that require an input of free energy. *Lysosomes* contain digestive enzymes that degrade defunct intracellular organelles, as well as macromolecules and particles taken in from outside the cell by endocytosis. On their way to lysosomes, endocytosed material must first pass through a series of compartments called *endosomes*. *Peroxisomes* (also known as *microbodies*) are small vesicular compartments that contain enzymes utilized in a variety of oxidative

Table 12-1 The Relative Volumes Occupied by the Major Intracellular Compartments in a Liver Cell (Hepatocyte)

Intracellular Compartment	Percent of Total Cell Volume	Approximate Number per Cell*
Cytosol	54	1
Mitochondria	22	1700
Rough ER cisternae	9	1
Smooth ER cisternae plus Golgi cisternae	6	
Nucleus	6	1
Peroxisomes	1	400
Lysosomes	1	300
Endosomes	1	200

*All the cisternae of the rough and smooth endoplasmic reticulum are thought to be joined to form a single large compartment. The Golgi apparatus, in contrast, is organized into a number of discrete sets of stacked cisternae in each cell, and the extent of interconnection between these sets has not been clearly established.

reactions. In general, each membrane-bounded organelle carries out the same set of basic functions in all cell types but varies in abundance and can have additional properties that differ from cell type to cell type according to the specialized functions of differentiated cells.

On average, the membrane-bounded 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 all. In the two mammalian cells analyzed in Table 12-2, for example, the endoplasmic reticulum has a total membrane surface area that is, respectively, 25 times and 12 times that of the plasma membrane. In terms of its area and mass the plasma membrane is only a minor membrane in most eucaryotic cells (Figure 12-2).

Table 12-2 Relative Amounts of Membrane Types in Two Types of Eucaryotic Cells

Membrane Type	Percent 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	7	4
Inner membrane	32	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, since the average hepatocyte has a volume of about $5000 \mu\text{m}^3$ compared with about $1000 \mu\text{m}^3$ for the pancreatic exocrine cell. Total cell membrane areas are estimated at about $110,000 \mu\text{m}^2$ and $13,000 \mu\text{m}^2$, respectively.

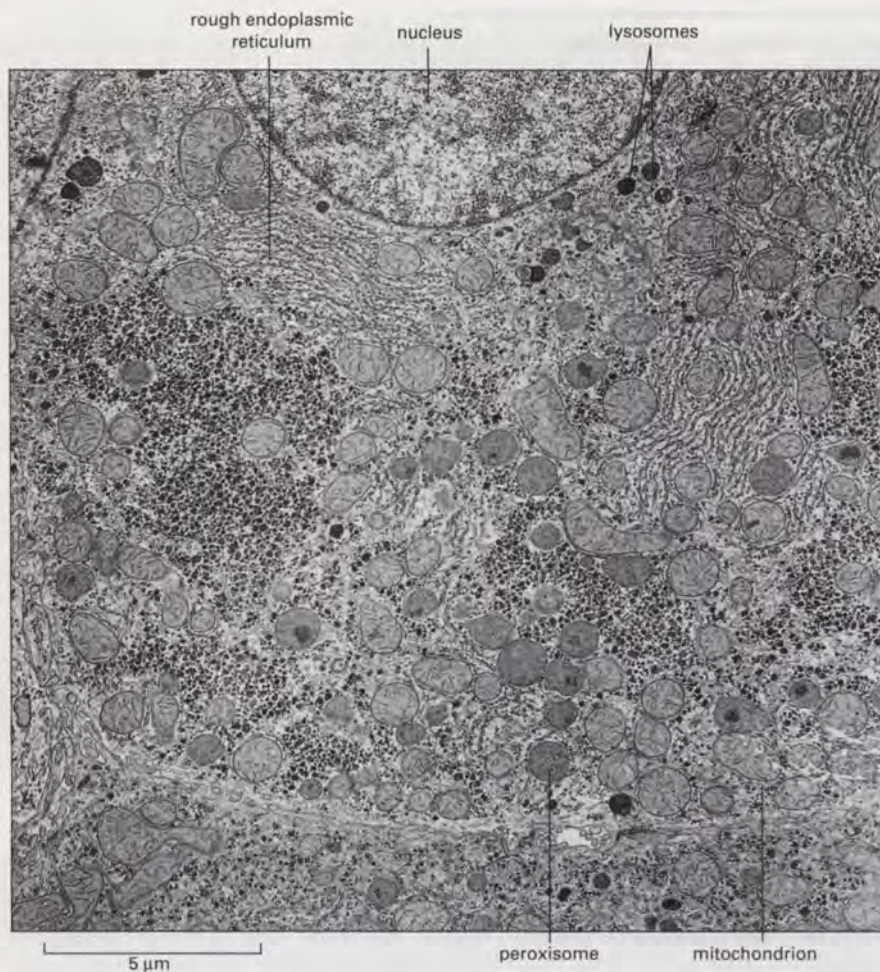


Figure 12-2 Electron micrograph of part of a liver cell seen in cross-section. Examples of most of the major intracellular compartments are indicated. (Courtesy of Daniel S. Friend.)

Membrane-bounded organelles are not randomly distributed in the cytosol; instead they often have characteristic positions. 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 seem to depend on interactions of the organelles with the cytoskeleton: the localization of both the ER and the Golgi apparatus, for example, is dependent 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, or *centrosome*, from which the microtubule array emanates (discussed in Chapter 16).

The Topological Relationships of Membrane-bounded Organelles Can Be Interpreted in Terms of Their Evolutionary Origins ²

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 eucaryotic cells are thought to have been simple organisms that resembled bacteria, which generally have a plasma membrane but no internal membranes. The plasma membrane in such cells therefore provides all membrane-dependent functions, including the pumping of ions, ATP synthesis, protein secretion, and lipid synthesis. Typical present-day eucaryotic cells are 10 to 30 times larger in linear dimension and 1000 to 10,000 times greater in volume than a typical bacterium such as *E. coli*. The profusion of internal membranes can be seen in part as an

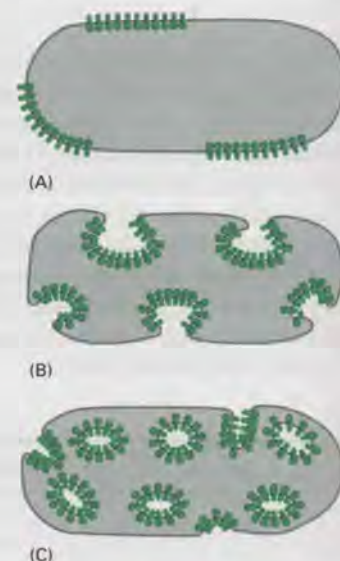


Figure 12-3 Organization of specialized membranes in bacteria. (A) Membrane patches on the cell surface consisting of clusters of specialized membrane proteins. (B) Invaginated patches of plasma membrane that increase the amount of membrane available for a specialized function such as photosynthesis. (C) Internalization of the specialized invaginated membrane to form vesicles, whose interior surface is topologically equivalent to the exterior surface of the cell. Membrane-bounded vesicles of this type are present in some types of photosynthetic bacteria; their topological relationship to the cell surface is similar to that of the ER, Golgi apparatus, endosomes, and lysosomes in eucaryotic cells.

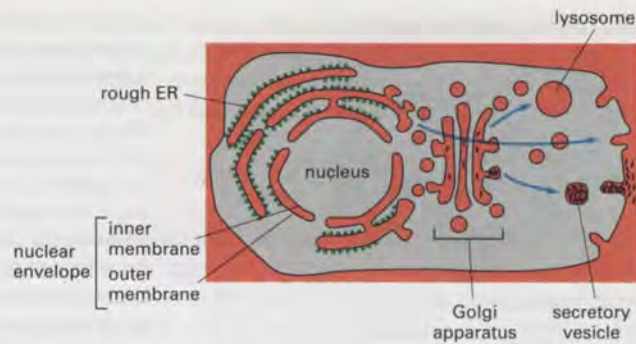


Figure 12-4 Topological relationships between compartments in a eucaryotic cell. Topologically equivalent spaces are shown in red. In principle, cycles of vesicle budding and fusion permit any lumen to communicate with any other and with the cell exterior. The blue arrows indicate the outward direction of vesicle traffic from the ER to Golgi apparatus to plasma membrane (or lysosomes), and the black dots represent protein molecules that are secreted by the cell. Some organelles, most notably mitochondria and (in plant cells) chloroplasts, however, do not take part in this vesicular communication and so are isolated from the traffic between organelles shown here.

adaptation to this increase in size: the eucaryotic cell has a much smaller ratio of surface to volume, and its area of plasma membrane is presumably too small to sustain the many vital functions for which membranes are required.

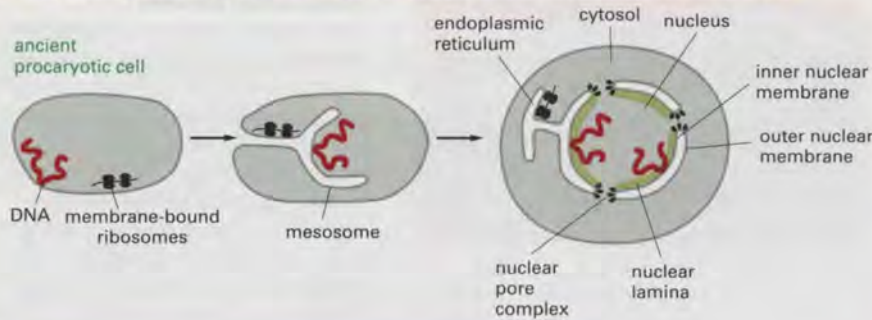
The evolution of internal membranes evidently has gone hand in hand with specialization of membrane function. Some present-day bacteria have specialized patches of plasma membrane in which a selected set of membrane proteins coalesce to carry out a group of related functions (Figure 12-3A). These specialized membrane patches, such as the "purple membrane" containing bacteriorhodopsin in *Halobacterium* (discussed in Chapter 10) and the chromatophores in photosynthetic bacteria (discussed in Chapter 14), represent primitive organelles. In some photosynthetic bacteria the patches have become elaborated into extensive invaginations of the plasma membrane (Figure 12-3B); in others the invaginations seem to have pinched off completely, forming sealed membrane-bounded vesicles specialized for photosynthesis (Figure 12-3C).

A eucaryotic organelle that originated by the type of pathway illustrated in Figure 12-3 might be expected to have an interior that is topologically equivalent to the exterior of the cell. We shall see that this is the case for the ER, Golgi apparatus, endosome, and lysosome—as well as for the many vesicular intermediates (*transport vesicles*) in the secretory and endocytic pathways. We can therefore think of all of these organelles as members of the same family, and, 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 that bud off from one organelle and fuse with another (Figure 12-4). Ribosomes are found attached to the cytosolic side of the plasma membrane in bacteria, and so the evolutionary origin of the ER membrane from the plasma membrane may explain why ribosomes are attached to the ER membrane in eucaryotic cells.

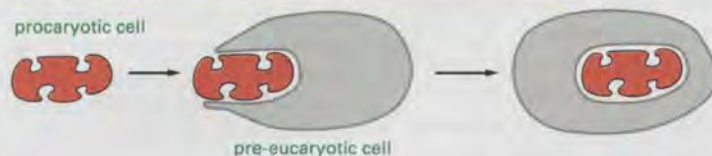
This evolutionary scheme also offers a reasonable explanation for the architecture of the cell nucleus with its double membrane. In bacteria the single chromosome is attached at special sites to the inside of the plasma membrane. It is possible therefore that the double-layered nuclear envelope originated as a deep invagination of the plasma membrane, as shown in Figure 12-5A. This scheme would explain why the nuclear compartment is topologically equivalent to the cytosol. In fact, in higher eucaryotic cells the nuclear envelope breaks down during mitosis, allowing the nuclear contents to disperse in the cytosol, a situation that never occurs for the contents of any other membrane-bounded organelle. As the scheme in Figure 12-5A also predicts, the space between the two nuclear membranes is topologically equivalent to the exterior of the cell and is continuous with the lumen of the ER.

As discussed in Chapter 14, mitochondria and plastids (of which chloroplasts are one form) differ from the other membrane-bounded organelles in that 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 (discussed in Chapters 1 and 14). According to the hypothetical scheme shown in Figure 12-5B, the inner membrane of mitochondria and plastids corresponds to the original

(A) PROPOSED EVOLUTIONARY PATHWAY FOR NUCLEUS AND ENDOPLASMIC RETICULUM



(B) PROPOSED EVOLUTIONARY PATHWAY FOR MITOCHONDRIA



plasma membrane of the bacterium, while the lumen of these organelles evolved from the bacterial cytosol. As might be expected from such origins, these two organelles remain isolated from the extensive vesicular traffic that connects the interiors of most of the other membrane-bounded organelles to one another and to the outside of the cell.

This evolutionary scheme groups the intracellular compartments in eucaryotic cells into five distinct families: (1) the nucleus and the cytosol, which communicate through the nuclear pores 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, lysosomes, and numerous classes of transport vesicles; (3) the mitochondria; (4) the plastids (in plants only); and (5) the peroxisomes (whose evolutionary origins are discussed later).

Proteins Can Move Between Compartments in Different Ways³

All proteins begin being synthesized 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. Most 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 (in plants), or peroxisomes; sorting signals can also direct the transport of proteins from the ER to other destinations in the cell.

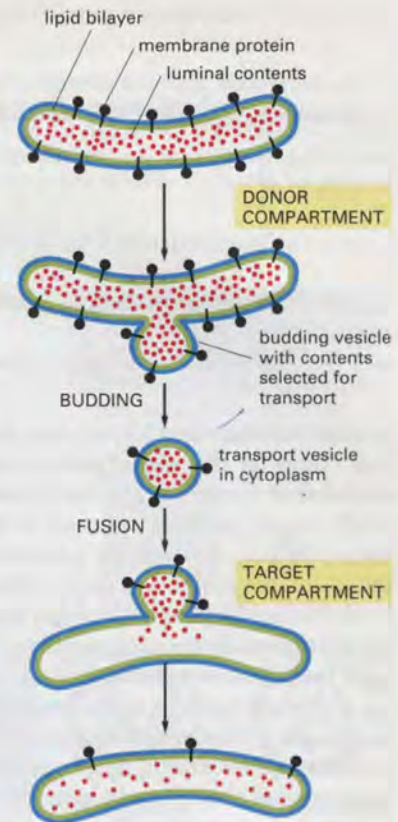
To understand the general principles by which sorting signals operate, it is important to distinguish three fundamentally different ways by which proteins move from one compartment to another. (1) The protein traffic between the cytosol and nucleus occurs between topologically equivalent spaces, which are in continuity through the nuclear pore complexes. This process is called **gated transport** because the nuclear pore complexes function as selective gates that can actively transport specific macromolecules and macromolecular assemblies, although they also allow free diffusion of smaller molecules. (2) In **transmembrane transport** membrane-bound *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 in order to snake

Figure 12-5 Hypotheses for the evolutionary origins of some membrane-bounded organelles. The origins of mitochondria, chloroplasts, ER, and the cell nucleus could explain the topological relationships of these intracellular compartments in eucaryotic cells. (A) A possible pathway for the evolution of the cell nucleus and the ER. In some bacteria the single DNA molecule is attached to an invagination of the plasma membrane, called a *mesosome*. Such an invagination in a very ancient procaryotic cell could have spread to form an envelope around the DNA while still allowing access of the DNA to the cell cytosol (as is required for DNA to direct protein synthesis). This envelope is presumed to have eventually pinched off completely from the plasma membrane, producing a nuclear compartment surrounded by a double membrane. As illustrated, the nuclear envelope is organized by a fibrous shell called the *nuclear lamina* and is penetrated by communicating channels called *nuclear pore complexes*. Because it is surrounded by two membranes that are in continuity where they are penetrated by these pores, the nuclear compartment is topologically equivalent to the cytosol. The lumen of the ER is continuous with the space between the inner and outer nuclear membranes and topologically equivalent to the extracellular space. (B) Mitochondria (and chloroplasts) are thought to have originated when a bacterium was engulfed by a larger pre-eucaryotic cell. They retain their autonomy. This may explain why the lumens of these organelles remain isolated from the vesicular traffic that interconnects the lumens of many other intracellular compartments.

Figure 12-6 The “sidedness” of membranes is preserved during vesicular transport. Note that the original orientation of both proteins and lipids in the donor-compartment membrane is preserved in the target-compartment membrane and that soluble molecules are transferred from lumen to lumen.

through the membrane. The initial transport of selected proteins from the cytosol into the ER lumen or into mitochondria, for example, occurs in this way. (3) In **vesicular transport**, *transport vesicles* ferry proteins from one compartment to another. The vesicles become loaded with a cargo of molecules derived from the lumen of one compartment as they pinch off from its membrane; they discharge their cargo into a second compartment by fusing with its membrane. The transfer of soluble proteins from the ER to the Golgi apparatus, for example, occurs in this way. Because the transported proteins do not cross a membrane, they move only between compartments that are topologically equivalent (Figure 12-6). We discuss vesicular transport in more detail in Chapter 13. The three ways in which proteins are transported between different compartments are summarized in Figure 12-7.

Each of the three modes of protein transfer is usually selectively guided by sorting signals in the transported protein that are recognized by complementary receptor proteins in the target organelle. If a large protein is to be imported into the nucleus, for example, it must possess a sorting signal that is recognized by receptor proteins associated with the nuclear pore complex. If a protein is to be transferred directly across a membrane, it must possess a sorting signal that is recognized by the translocator in the membrane to be crossed. Likewise, if a protein is to be incorporated into certain types of transport vesicles or to be retained in certain organelles, its sorting signal must be recognized by a complementary receptor in the appropriate membrane.

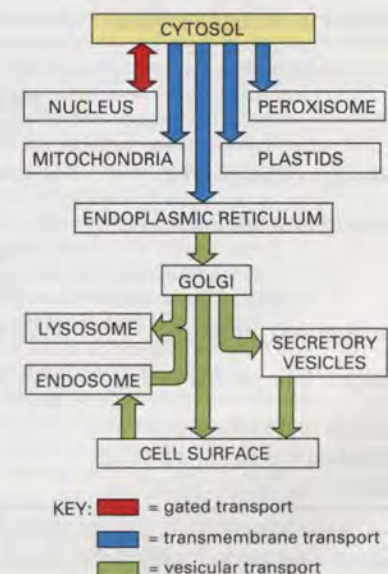


Signal Peptides and Signal Patches Direct Proteins to the Correct Cellular Address ⁴

There are at least two types of sorting signals on proteins. One type resides in a continuous stretch of amino acid sequence, typically 15 to 60 residues long. This **signal peptide** is often (but not always) removed from the finished protein by a specialized **signal peptidase** once the sorting process has been completed. The other type consists of a specific three-dimensional arrangement of atoms on the

Figure 12-7 A simplified “road map” of protein traffic. Proteins can move from one compartment to another by gated transport (red), transmembrane transport (blue), or vesicular transport (green). The signals that direct a given protein’s movement through the system, and thereby determine its eventual location in the cell, are contained in its amino acid sequence. The journey begins with the synthesis of a protein on a ribosome and terminates when the final destination is reached. At each intermediate station (boxes) a decision is made as to whether the protein is to be retained or transported further. In principle, a signal could be required either for retention in or for exit from each of the compartments shown, with the alternative fate being the *default pathway* (one that requires no signal). The vesicular transport of proteins from the ER through the Golgi apparatus to the cell surface, for example, appears not to require any specific sorting signals; specific sorting signals therefore are required to retain in the ER and the Golgi apparatus those specialized proteins that are resident there.

We shall use this figure repeatedly as a guide throughout this chapter and the next, highlighting the particular pathway being discussed.



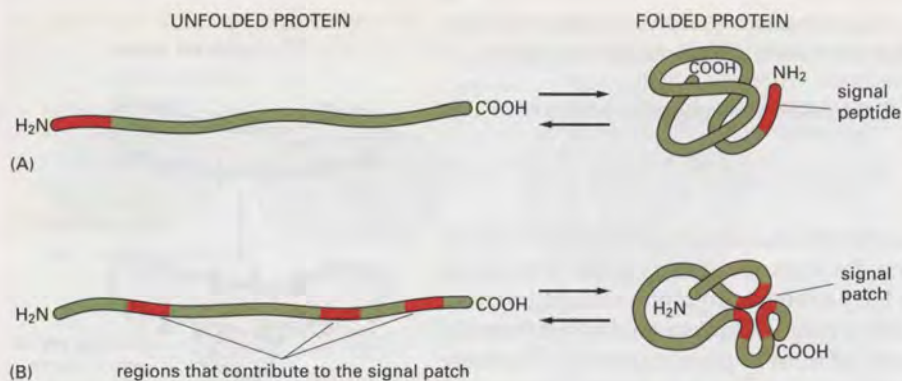


Figure 12-8 Two ways that a sorting signal can be built into a protein. (A) The signal resides in a single discrete stretch of amino acid sequence, called a *signal peptide*, that is exposed in the folded protein. Signal peptides often occur at the end of the polypeptide chain (as shown), but they can also be located elsewhere. (B) A *signal patch* can be formed by the juxtaposition of amino acids from regions that are physically separated before the protein folds (as shown); alternatively, separate patches on the surface of the folded protein that are spaced a fixed distance apart could form the signal. In either case the transport signal depends on the three-dimensional conformation of the protein, which makes it difficult to locate the signal precisely.

protein's surface that forms when the protein folds up. The amino acid residues that comprise this **signal patch** can be distant from one another in the linear amino acid sequence, and they generally remain in the finished protein (Figure 12-8). Signal peptides are used to direct proteins from the cytosol into the ER, mitochondria, chloroplasts, peroxisomes, and nucleus, and they are also used to retain soluble proteins in the ER. Signal patches identify certain enzymes that are to be marked with specific sugar residues that then direct them from the Golgi apparatus into lysosomes; signal patches are also used in other sorting steps that have been less well characterized.

Different types of signal peptides are used to specify different destinations in the cell. Proteins destined for initial transfer to the ER usually have a signal peptide at their amino terminus, which characteristically includes a sequence composed of about 5 to 10 hydrophobic amino acids. Most of these proteins will in turn pass from the ER to the Golgi apparatus, but those with a specific sequence of four amino acids at their carboxyl terminus are retained as permanent ER residents. Proteins destined for mitochondria have signal peptides of yet another type, in which positively charged amino acids alternate with hydrophobic ones. Proteins destined for peroxisomes usually have a specific signal sequence of three amino acids at their carboxyl terminus. Many proteins destined for the nucleus carry a signal peptide formed from a cluster of positively charged amino

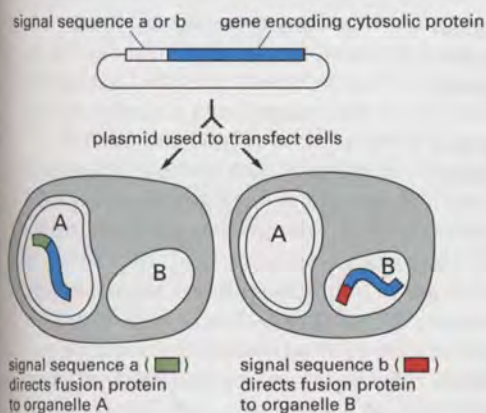
Table 12-3 Some Typical Signal Peptides

Function of Signal Peptide	Example of Signal Peptide
Import into ER	⁺ H ₃ N-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-
Retain in lumen of ER	-Lys-Asp-Glu-Leu-COO ⁻
Import into mitochondria	⁺ H ₃ N-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 nucleus	-Pro-Pro-Lys-Lys-Lys-Arg-Lys-Val-
Import into peroxisomes	-Ser-Lys-Leu-
Attach to membranes via the covalent linkage of a myristic acid to the amino terminus	⁺ H ₃ N-Gly-Ser-Ser-Lys-Ser-Lys-Pro-Lys-

Positively charged amino acids are shown in red and negatively charged amino acids in green. An extended block of hydrophobic amino acids is enclosed in a yellow box. H₃N⁺ indicates the amino terminus of a protein; COO⁻ indicates the carboxyl terminus.

A transfection approach for defining signal sequences

One way to show that a signal sequence is required and sufficient to target a protein to a specific intracellular compartment is to create a fusion protein in which the signal sequence is attached by genetic engineering techniques to a protein that is normally resident in the cytosol. After the cDNA encoding this protein is transfected into cells, the location of the fusion protein is determined by immunostaining or by cell fractionation.



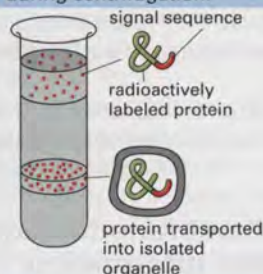
By altering the signal sequence using site-directed mutagenesis, one can determine which structural features are important for its function.

A biochemical approach for studying the mechanism of protein translocation

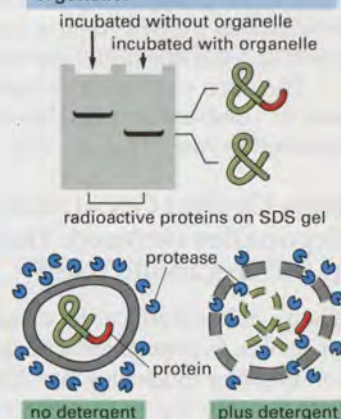
In this approach a labeled protein containing a specific signal sequence is transported into isolated organelles *in vitro*. The labeled protein is usually produced by cell-free translation of a purified mRNA encoding the protein; radioactive amino acids are used to label the newly synthesized protein so that it can be distinguished from the many other proteins that are present in the *in vitro* translation system.

Three methods are commonly used to test if the labeled protein has been translocated into the organelle:

1. The labeled protein co-fractionates with the organelle during centrifugation.



2. The signal sequence is removed by a specific protease that is present inside the organelle.

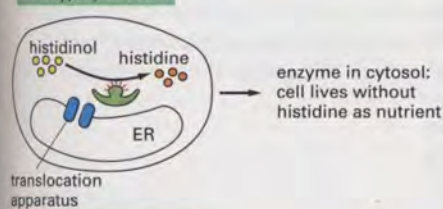


3. The protein is protected from digestion when proteases are added to the incubation medium but is susceptible if a detergent is first added to disrupt the organelle membrane.

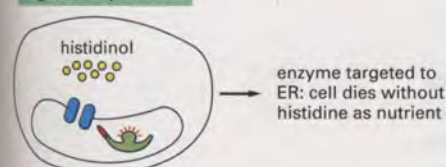
By exploiting such *in vitro* assays, one can determine what components (proteins, ATP, GTP, etc.) are required for the translocation process.

Genetic approaches for studying the mechanism of protein translocation

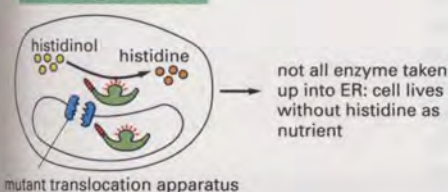
wild-type yeast cell



engineered yeast cell



mutant engineered cell



Yeast cells with mutations in genes that encode components of the translocation machinery have been useful for studying protein translocation. Because mutant cells that cannot translocate proteins across their membranes will die, the trick is to design a strategy that allows weak mutations that cause only a partial defect in protein translocation to be isolated.

One way uses genetic engineering to design special yeast cells. The enzyme histidinol dehydrogenase, for example, normally resides in the cytosol, where it is required to produce the essential amino acid histidine from its precursor histidinol. A yeast strain is constructed in which the histidinol dehydrogenase gene is replaced by a re-engineered gene encoding a fusion protein with an added signal sequence that misdirects the enzyme into the endoplasmic reticulum (ER). When such cells are grown without histidine, they die because all of the histidinol dehydrogenase is sequestered in the ER, where it is of no use. Cells with a mutation that partially inactivates the mechanism for translocating proteins from the cytosol to the ER, however, will survive because enough of the dehydrogenase will be retained in the cytosol to produce histidine. Often one obtains a cell in which the mutant protein still functions partially at normal temperature but is completely inactive at higher temperature. A cell carrying such a temperature-sensitive mutation dies at higher temperature, whether or not histidine is present, as it cannot transport any protein into the ER. This allows the normal gene that was disabled by the mutation to be identified by transfecting the mutant cells with a yeast plasmid vector into which random yeast genomic DNA fragments have been cloned: the specific DNA fragment that rescues the mutant cells when they are grown at high temperature should encode the wild-type version of the mutant gene.

acids, which is commonly found at internal sites of the polypeptide chain. Some typical signal peptides are listed in Table 12-3.

The importance of each of these signal peptides for protein targeting has been shown by experiments in which the peptide is transferred from one protein to another by genetic engineering techniques: placing the amino-terminal ER signal peptide at the beginning of a cytosolic protein, for example, redirects the protein to the ER. Even though their amino acid sequences can vary greatly, the signal peptides of all proteins having the same destination are functionally interchangeable: physical properties, such as hydrophobicity, often appear to be more important in the signal-recognition process than the exact amino acid sequence.

Signal patches are far more difficult to analyze than signal peptides, and so less is known about their structure. Because they result from a complex three-dimensional protein-folding pattern, they cannot be easily transferred experimentally from one protein to another.

The main ways of studying how proteins are directed from the cytosol to a specific compartment and how they are translocated across membranes are illustrated in Panel 12-1 (p. 559).

Cells Cannot Construct Their Membrane-bounded Organelles *de Novo*: They Require Information in the Organelle Itself⁵

When a cell reproduces by division, it has to duplicate its membrane-bounded organelles. In general, cells do this by enlarging the existing organelles by incorporating new molecules into them; the enlarged organelles then divide and are distributed to the two daughter cells. Thus each daughter cell inherits from its mother a complete set of specialized cell membranes. This inheritance is essential because a cell could not make such membranes *de novo*. If the ER were completely removed from a cell, for example, how could the cell reconstruct it? The membrane proteins that define the ER and carry out many of its functions are themselves products of the ER. A new ER could not be made without an existing ER or, at the very least, a membrane that contains the translocators required to import specific proteins into the ER (and lacks the translocators required to import the proteins that function in other organelles).

Thus it seems that the information required to construct a membrane-bounded organelle does not reside exclusively in the DNA that specifies the organelle's proteins. *Epigenetic* 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 progeny cell 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 its nucleotide and amino acid sequences.

Summary

Eucaryotic cells contain intracellular membranes that enclose nearly half the cell's total volume in separate intracellular compartments called organelles. The main types of membrane-bounded organelles that are present in all eucaryotic cells are the endoplasmic reticulum, Golgi apparatus, nucleus, mitochondria, lysosomes, endosomes, and peroxisomes; plant cells also contain plastids, such as chloroplasts. Each organelle contains a distinct set of proteins that mediates its unique functions.

Each newly synthesized organelle protein finds its way from the ribosome where it is made to the organelle where it functions by following a specific pathway, guided by signals in its amino acid sequence that function as signal peptides or signal patches. The signal peptides and patches are recognized by complementary receptor proteins in the target organelle. Proteins that function in the cytosol do not contain signal peptides or signal patches and therefore remain in the cytosol after they are synthesized.

The Transport of Molecules into and out of the Nucleus ⁶

The **nuclear envelope** encloses the DNA and defines the nuclear compartment. It is formed from two concentric membranes that, as we have seen, are continuous with the endoplasmic reticulum. Although the inner and outer nuclear membranes are continuous, the two membranes maintain distinct protein compositions. The **inner nuclear membrane** contains specific proteins that act as binding sites for the feltlike *nuclear lamina* that supports it. The inner membrane is surrounded by the **outer nuclear membrane**, which closely resembles the membrane of the rough endoplasmic reticulum (Figure 12-9). Like the membrane of the rough ER, 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 12-9).

Bidirectional traffic occurs continuously between the cytosol and the nucleus. The many proteins that function in the nucleus—including histones, DNA and RNA polymerases, gene regulatory proteins, and RNA-processing proteins—are selectively imported into the *nuclear compartment* from the cytosol where they are made. At the same time, tRNAs and mRNAs 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, imported into the nucleus—where they assemble with newly made ribosomal RNA into particles—and then exported again to the cytosol as part of a ribosomal subunit; each of these steps involves selective transport across the nuclear envelope.

Nuclear Pores Perforate the Nuclear Envelope ⁷

The nuclear envelope in all eucaryotes, from yeasts to humans, is perforated by *nuclear pores*. Each pore is formed by a large, elaborate structure known as the **nuclear pore complex**, which has an estimated molecular mass of about 125 million and is thought to be composed of more than 100 different proteins, arranged with a striking octagonal symmetry (Figures 12-10 and 12-11).

Each pore complex contains one or more open aqueous channels through which water-soluble molecules that are smaller than a certain size can passively

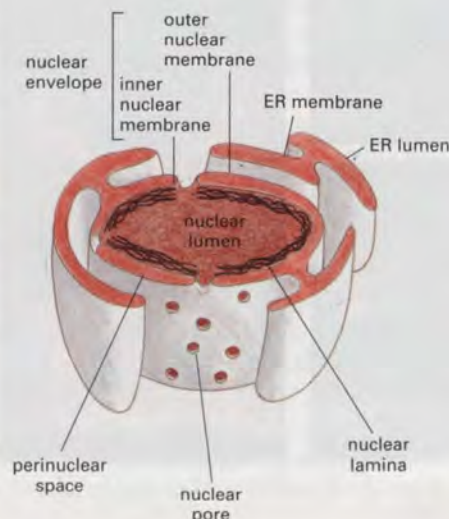
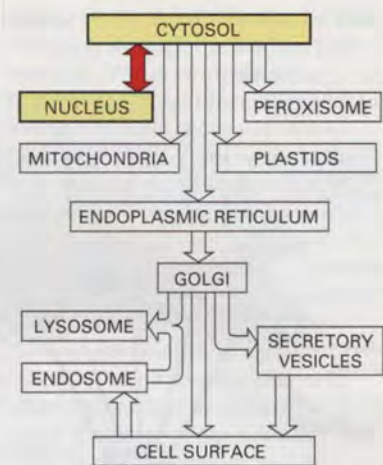


Figure 12-9 The nuclear envelope.

The double-membrane envelope is penetrated by nuclear pores and is continuous with the endoplasmic reticulum. The ribosomes that are bound to the cytosolic surface of the ER membrane and outer nuclear membrane are not shown.

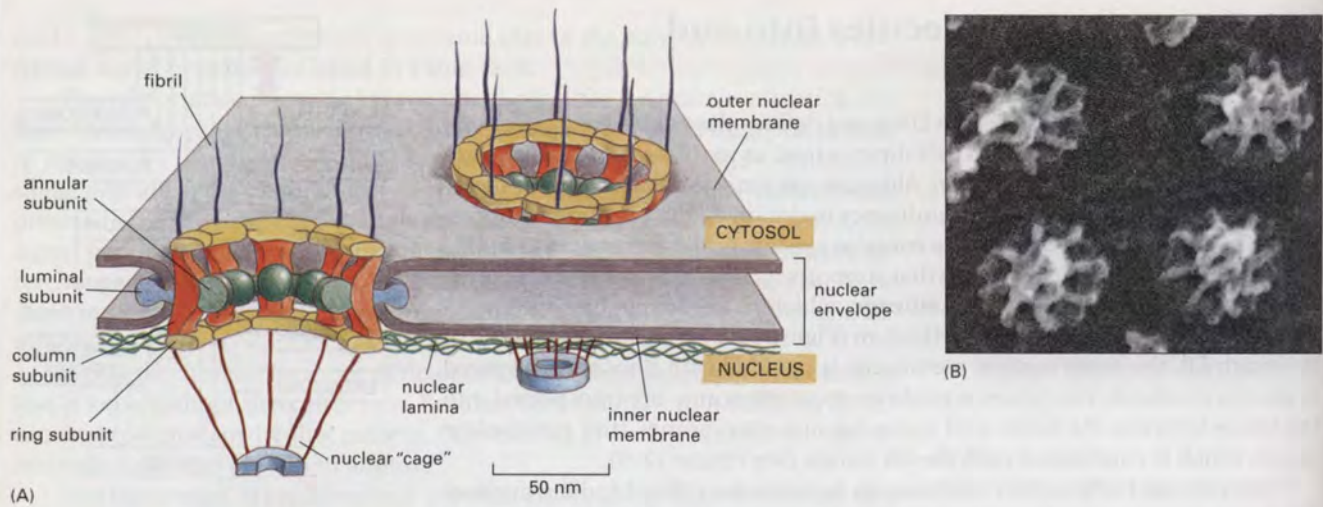


Figure 12-10 The arrangement of the nuclear pore complexes in the nuclear envelope. (A) A sketch showing a small region of the nuclear envelope. In cross-section the nuclear pore complex appears composed of three parts: (1) a column component which forms the bulk of the pore wall; (2) an annular component, which extends "spokes" toward the center of the pore; and (3) a luminal component, which is formed by a large transmembrane glycoprotein that is thought to help anchor the complex to the nuclear membrane. In addition, fibrils protrude from both the cytosolic and nuclear sides of the complex. On the nuclear side the fibrils converge to form cage-like structures, which are shown in a scanning electron micrograph of the nuclear side of the nuclear envelope of an oocyte in (B). (B, from M.W. Goldberg and T.D. Allen, *J. Cell Biol.* 119:1429-1440, 1992, by copyright permission of the Rockefeller University Press.)

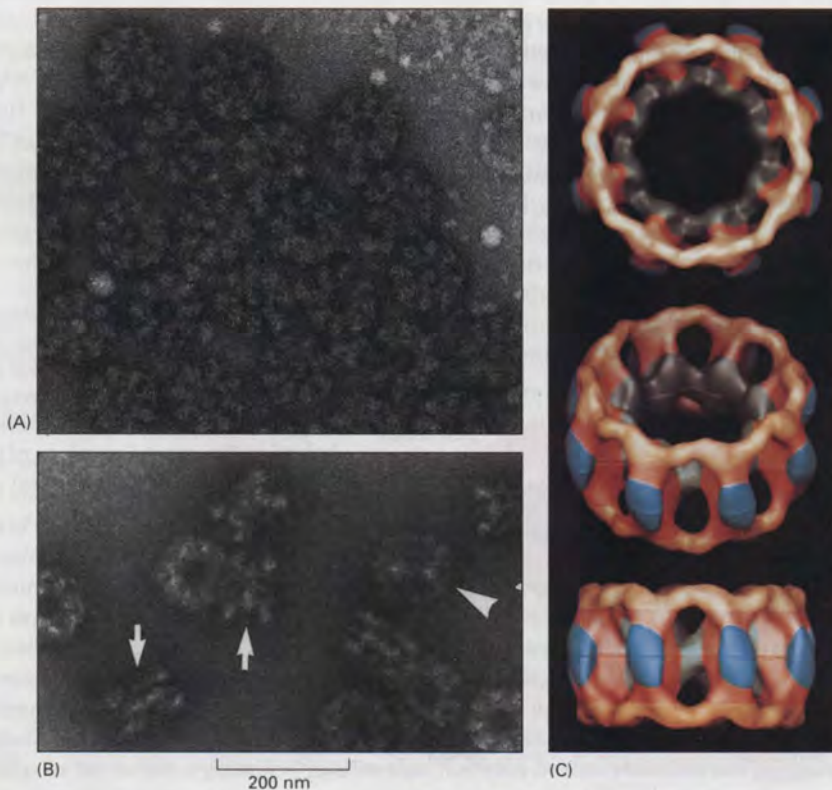


Figure 12-11 Electron micrograph and computer reconstruction of nuclear pore complexes. (A) and (B) Negatively stained views of nuclear pore complexes released from the envelope by detergent. In (B) some nuclear pore complexes can be seen on their side. (C) Three-dimensional computer reconstructions showing top, tilted, and side views of pore complexes. (From J.E. Hinshaw and R. Milligan, *Cell* 69:1133-1141, 1992. © Cell Press.)

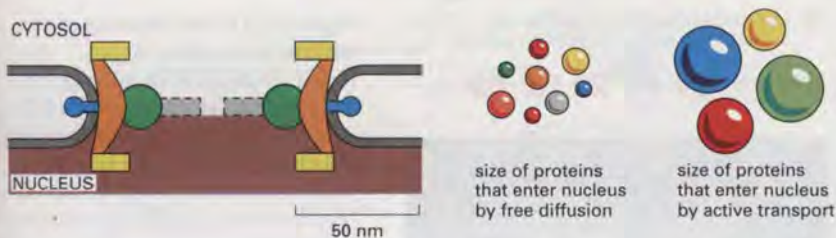


Figure 12-12 Possible paths for free diffusion through the nuclear pore complex. The drawing shows a hypothetical diaphragm inserted into the pore to restrict the size of the open channel to 9 nm, which is the pore size estimated from diffusion measurements. Nine nanometers is a much smaller diameter than that of the central opening apparent on the images of the nuclear pore complex derived from electron micrographs (or from that measured during active transport when the pore dilates to allow transport of particles of up to 26 nm in diameter). Thus it is likely that some pore components are lost during the preparation of specimens for electron microscopy and that these normally restrict free diffusion through the central opening. Such components may form a diaphragm (or plug) that opens and closes to allow passage of large objects during active transport, which is mediated by a sorting signal (discussed below). Although plugs can be seen in some preparations, it is not clear whether they are components of the pore complex or material that is being transported through it. Three-dimensional computer reconstructions suggest that the channels permitting free diffusion might not be located at the center of the pore complex but near its rim between the column components (see Figure 12-10A); this would mean that passive diffusion and active transport take place through different parts of the pore complex.

diffuse. The effective size of these channels has been determined by injecting labeled molecules (that are not nuclear components) into the cytosol and then measuring their rate of diffusion into the nucleus. Small molecules (5000 daltons or less) diffuse in so fast that the nuclear envelope can be considered to be freely permeable to them. A protein of 17,000 daltons takes 2 minutes to equilibrate between the cytosol and nucleus, while a protein of 44,000 daltons takes 30 minutes. A globular protein larger than about 60,000 daltons seems hardly able to enter the nucleus at all. A quantitative analysis of such data suggests that the nuclear pore complex contains a pathway for free diffusion equivalent to a water-filled cylindrical channel about 9 nm in diameter and 15 nm long; such a channel would occupy only a small fraction of the total pore volume (Figure 12-12).

Because many cellular proteins are too large to pass by diffusion through the nuclear pores, the nuclear envelope allows the nuclear compartment and the cytosol to maintain different complements of proteins. Mature cytosolic ribosomes, for example, are about 30 nm in diameter and thus cannot diffuse through the 9-nm channels; their exclusion from the nucleus ensures that all protein synthesis is confined to the cytosol. But how does the nucleus export newly made ribosomal subunits or import large molecules, such as DNA and RNA polymerases, which have subunit molecular weights of 100,000 to 200,000? As we discuss next, these and many other protein and RNA molecules bind to specific receptor proteins located in the pore complexes and are then actively transported across the nuclear envelope through the complexes.

Nuclear Localization Signals Direct Nuclear Proteins to the Nucleus⁸

In general, the more active the nucleus is in transcription, the greater the number of pore complexes its envelope contains. The nuclear envelope of a typical mammalian cell contains 3000 to 4000 pore complexes. If the cell is synthesizing DNA, it needs to import about 10^6 histone molecules from the cytosol every 3 minutes in order to package newly made DNA into chromatin, which means that, on average, each pore complex needs to transport about 100 histone molecules per minute. If the cell is growing rapidly, each pore complex also needs to transport about 6 newly assembled large and small ribosomal subunits per minute from the nucleus, where they are produced, to the cytosol, where they are used. And that is only a very small part of the total traffic that passes through the nuclear pores.

When proteins are experimentally extracted from the nucleus and micro-injected back into the cytosol, even the very large ones efficiently reaccumulate in the nucleus. The selectivity of this nuclear protein import resides in **nuclear localization signals**, which are present only in nuclear proteins. The signals have been precisely defined in many nuclear proteins using recombinant DNA technology. They can be located almost anywhere in the amino acid sequence and generally consist of a short sequence (typically from four to eight amino acids) that varies for different nuclear proteins but is rich in the positively charged amino acids lysine and arginine and usually contains proline. In many nuclear proteins this sequence is split into two blocks of two to four amino acids each,

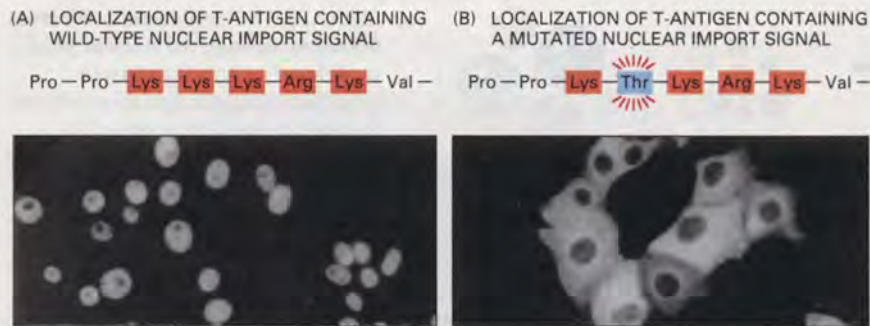


Figure 12-13 The function of a nuclear localization signal.

Immunofluorescence micrographs showing the cellular location of SV40 virus T-antigen containing or lacking a short peptide that serves as a nuclear localization signal. The wild-type 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 antibody against the T-antigen (A). T-antigen with an altered nuclear localization signal (a threonine replacing a lysine) remains in the cytosol (B). (From D. Kalderon, B. Roberts, W. Richardson, and A. Smith, *Cell* 39:499–509, 1984. © Cell Press.)

with the blocks separated from each other by about ten amino acids. The signals are thought to form loops on the protein surface.

Nuclear localization signals were first identified in the large viral protein called *T-antigen*, which is encoded by the SV40 virus and is needed for viral DNA replication in the host cell nucleus. The T-antigen normally accumulates in the nucleus shortly after being synthesized in the cytosol. A mutation in a single amino acid, however, prevents nuclear import (Figure 12-13). On the assumption that this mutation is in a nuclear localization signal sequence, short lengths of the DNA encoding this region of the normal T-antigen were fused to a gene coding for a cytosolic protein. The shortest sequence that caused the resulting fusion protein to be imported into the nucleus encoded a stretch of eight contiguous amino acids, which is normally located in an internal region of the T-antigen polypeptide chain (see Figure 12-13). Further experiments showed that the signal sequence could function even when it was linked as a short peptide to selected lysine side chains on the surface of a cytosolic protein, suggesting that the precise location of a nuclear localization signal within the amino acid sequence of a nuclear protein is not important. In fact, many nuclear proteins contain more than one nuclear localization signal.

Macromolecules Are Actively Transported into and out of the Nucleus Through Nuclear Pores⁹

The active transport of nuclear proteins through nuclear pore complexes can be directly visualized by coating gold particles with a nuclear protein, injecting the particles into the cytosol, and then following their fate by electron microscopy (Figures 12-14 and 12-15).

The initial interaction of a nuclear protein with the nuclear pore complex requires one or more cytosolic proteins that bind to the nuclear localization signals and help direct the nuclear protein to the pore complex, where it appears to bind to the fibrils that project from the rim of the complex. The nuclear protein then moves to the center of the pore complex, where it is actively transported across the nuclear envelope by a process that requires ATP hydrolysis (Figure 12-16). Studies with various sizes of gold beads indicate that the opening can dilate up to about 26 nm in diameter during the transport process: a poorly defined structure in the center of the nuclear pore complex appears to function like a close-fitting diaphragm that opens just the right amount when activated by a signal on an appropriate large protein (see Figure 12-12). The molecular basis of this mechanism, and how it operates to pump macromolecules both into and out of the nucleus, is a mystery.

It seems likely that the export of new ribosomal subunits and messenger RNA molecules through the nuclear pores also depends on a selective transport system. If 20-nm-diameter gold spheres, similar to those used in the experiments shown in Figure 12-15, are coated with small RNA molecules (tRNA or 5S RNA) and then injected into the nucleus of a frog oocyte, they are rapidly transported through the nuclear pores into the cytosol. If they are injected into the cytosol

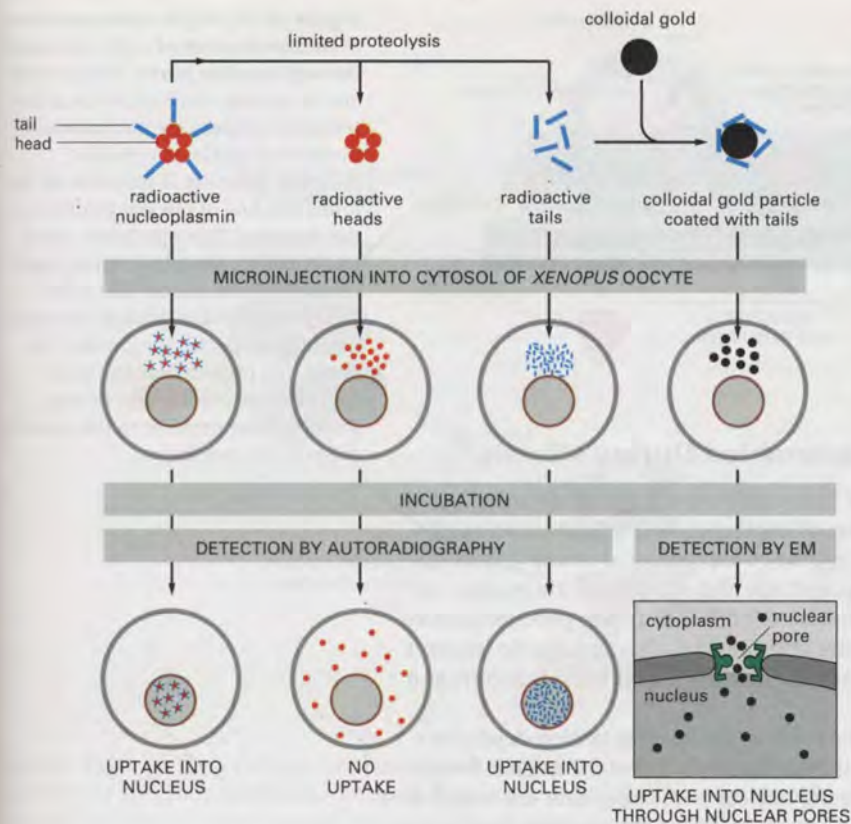


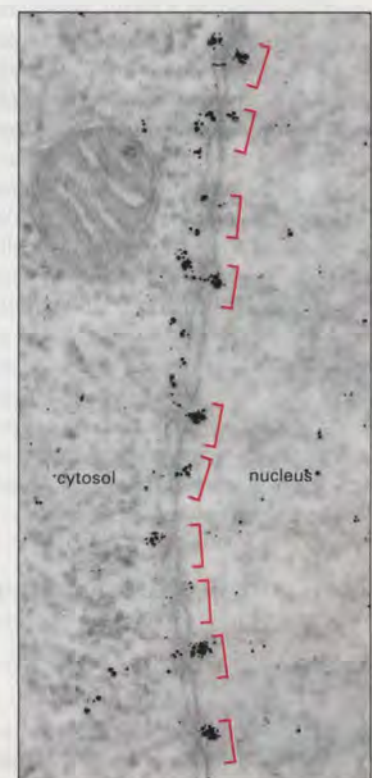
Figure 12-14 The uptake of nuclear proteins via nuclear pores.

Nucleoplasmin is a large nuclear protein with distinct head and tail domains. The heads can be cleaved from the tails by limited proteolysis. When injected into the cytosol of a frog oocyte, intact nucleoplasmin molecules rapidly accumulate in the nucleus even though they are too large to diffuse passively through the pore complex. The signal for this nuclear import resides in the tail domains, since injected tails are taken up by the nucleus but heads are not. The role of nuclear pores in this signal-directed import is demonstrated by electron microscopy using nucleoplasmin tails coupled to spheres of colloidal gold, which are easily visualized because of their high electron density. The attached nucleoplasmin tails direct the entry of the gold particles into the nucleus via nuclear pores (see Figure 12-15).

of the oocyte, on the other hand, they remain there. Thus it seems that, in addition to receptors that recognize nuclear protein import signals, the pore contains one or more receptors that recognize RNA molecules (or the proteins bound to them) destined for the cytosol. Using differently sized gold particles, one set coated with RNA and injected into the nucleus and the other set coated with nuclear protein import signals, it can be shown that a single pore complex allows traffic in both directions.

The mechanism of macromolecular transport across nuclear pores is fundamentally different from the transport mechanisms involved in the transfer of proteins across the membranes of other organelles in that it occurs through a large, regulated aqueous pore rather than through a protein transporter that spans one or more lipid bilayers. It is thought that a nuclear protein is transported through the pores while it is in a fully folded conformation, just as a newly formed ribosomal subunit is transported as an assembled particle; by contrast, proteins have to be unfolded during their transport into other organelles, as we discuss later.

Figure 12-15 Visualizing the specific import of a protein through nuclear pores. This electron micrograph shows colloidal gold spheres coated with nucleoplasmin (see Figure 12-14) entering the nucleus by means of nuclear pores (indicated by red brackets). The same result is obtained when the gold spheres are coated with the tail regions of nucleoplasmin molecules. These gold particles are much larger in diameter than the diffusion channel in the pore complex, implying that a pore has been induced to widen to permit their passage. Because the gold particles line up in the cytosol before they contact and enter the pore complex, it has been suggested that the fibrils that extend into the cytosol from the pore complex (see Figure 12-10A) guide the particles to their destination (From C. Feldherr, E. Kallenbach, and N. Schultz, *J. Cell Biol.* 99:2216-2222, 1984, by copyright permission of the Rockefeller University Press.)



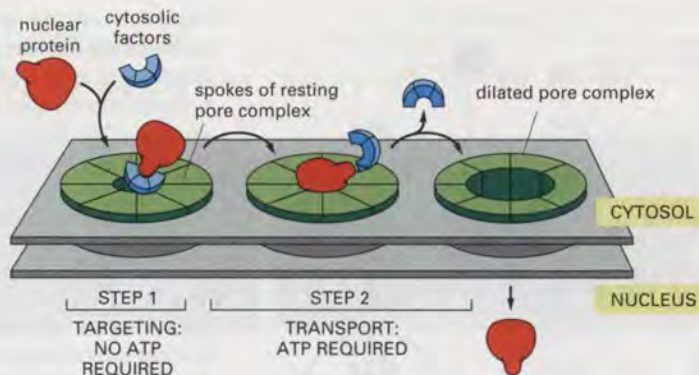


Figure 12-16 Highly schematic view of the mechanism of active transport through nuclear pores. The proteins and structures involved in the active transport process are not known. A diverse set of related cytosolic proteins, however, is required for the initial binding of nuclear proteins to the complex. These proteins, called *nucleoporins*, contain a simple sugar (N-acetylglucosamine) that aided their identification through the use of lectins and specific antibodies. The fibrils that project from the pore complex and are thought to help guide nuclear proteins to the center of the pore are not shown.

The Nuclear Envelope Is Disassembled During Mitosis¹⁰

The **nuclear lamina** is a meshwork of interconnected protein subunits called **nuclear lamins**. These 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 is thought to give shape and stability to the nuclear envelope, to which it is anchored by attachment to both the nuclear pore complexes and the inner nuclear membrane. As the chromatin is also thought to interact directly with the nuclear lamina, the lamina provides a structural link between the DNA and the nuclear envelope.

When a nucleus disassembles during mitosis, the nuclear lamina depolymerizes, at least partly as a consequence of the phosphorylation of the nuclear lamins at the onset of mitosis. At the same time the nuclear pore complexes disassemble into their various components. Depolymerization of the nuclear lamina is probably a prerequisite for the nuclear envelope to break up into membrane vesicles, which, together with the nuclear contents, disperse throughout the cytosol. Reassembly of the lamina occurs when the nuclear lamins are dephosphorylated and, as a result, repolymerize on the surface of the chromosomes; the re-assembled lamina then binds the vesicles of nuclear envelope membrane, which fuse with one another to re-form an envelope around each chromosome or group of chromosomes. During this process the nuclear pore complexes also re-assemble. The enveloped chromosomes then come together, and their membranes fuse to form a single nuclear envelope, which actively reimports all those proteins that contain nuclear localization signals (Figure 12-18). Because the new nuclear envelope is so closely applied to the surface of the chromosomes, it excludes all of the proteins in the cell except those bound to the mitotic chromosomes. Thus large proteins are kept out of the interphase nucleus unless they contain nuclear localization signals.

Nuclear localization signals are not cleaved off after transport into the nucleus. This is presumably because nuclear proteins need to be imported repeatedly, once after every cell division. In contrast, once a protein molecule has been imported into any of the other membrane-bounded organelles, it is passed on from generation to generation within that compartment and need never be translocated again; the signal peptide on these molecules is often removed following protein translocation.

Transport Between Nucleus and Cytosol Can Be Regulated by Preventing Access to the Transport Machinery¹¹

As discussed in Chapter 9, the activity of some gene regulatory proteins is controlled by keeping them out of the nuclear compartment until they are needed there. The nuclear localization signal of some of these proteins can be inactivated by phosphorylation. Others are bound to inhibitory cytosolic proteins that either

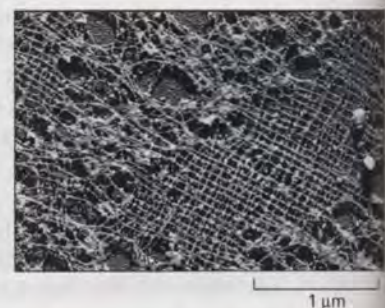


Figure 12-17 The nuclear lamina. Electron micrograph of a portion of the nuclear lamina in a *Xenopus* oocyte prepared by freeze drying and metal shadowing. The lamina is formed by a regular lattice of specialized intermediate filaments. (Courtesy of Ueli Aebi.)

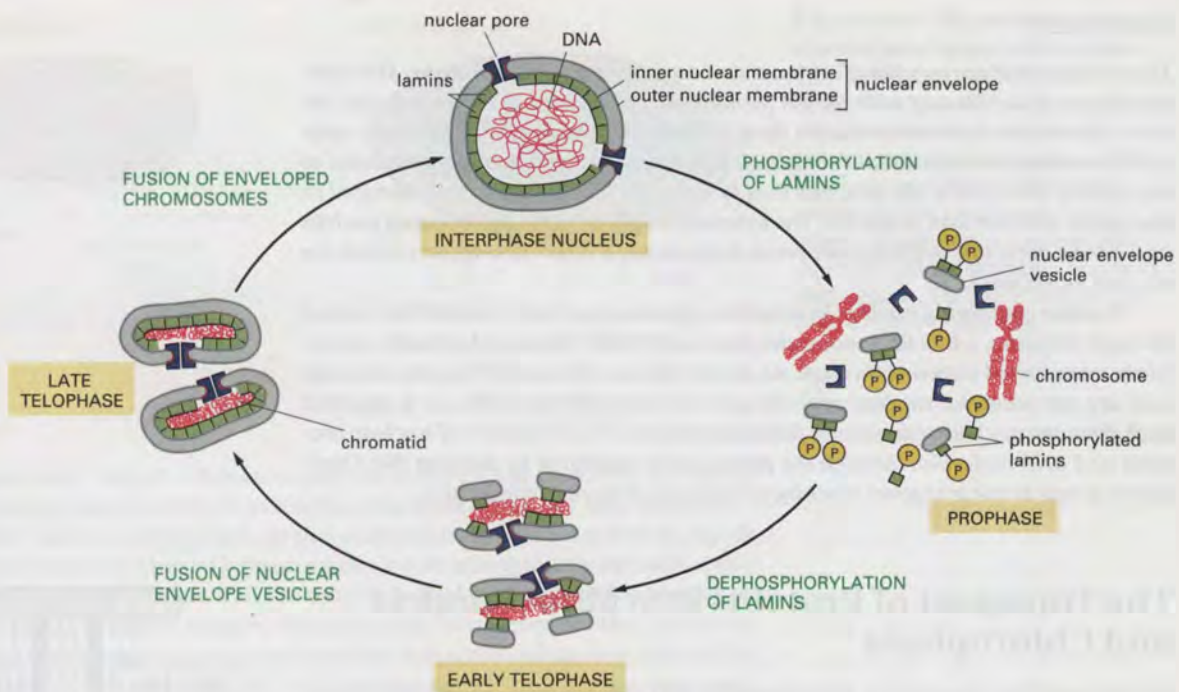


Figure 12-18 Breakdown and re-formation of the nuclear envelope during mitosis. The phosphorylation of the lamins is thought to help trigger the disassembly of the nuclear lamina, which in turn causes the nuclear envelope to break up into vesicles. Dephosphorylation of the lamins is thought to help reverse the process.

anchor them in the cytosol—presumably through interactions with the cytoskeleton or specific organelles—or mask their nuclear localization signals. When the cell receives an appropriate stimulus, the protein is released from its cytosolic anchor or mask and is transported into the nucleus (Figure 12-19).

Export of RNA from the nucleus may be controlled in a similar way. Like active import into the nucleus, export also requires a signal: in the case of most messenger RNA molecules, this is provided by a unique modification, the cap structure, at the 5' end of the RNA (discussed in Chapter 8). Incompletely processed pre-messenger RNAs include this cap but are anchored to the nuclear transcription and splicing machinery, which releases an RNA molecule only after its processing is completed: genetic studies in yeast have shown that mutations that prevent the pre-messenger RNA from properly engaging with the splicing machinery lead to the export of the unspliced RNA. Other RNAs, like transfer RNA or ribosomal RNA, which lack a 5' cap, must first be assembled with proteins and are then exported as part of these complexes. Presumably, nuclear export signals are contained in the protein subunits of these complexes, and these signals become activated after proper assembly with the RNA components, but the nature of these signals is not known.

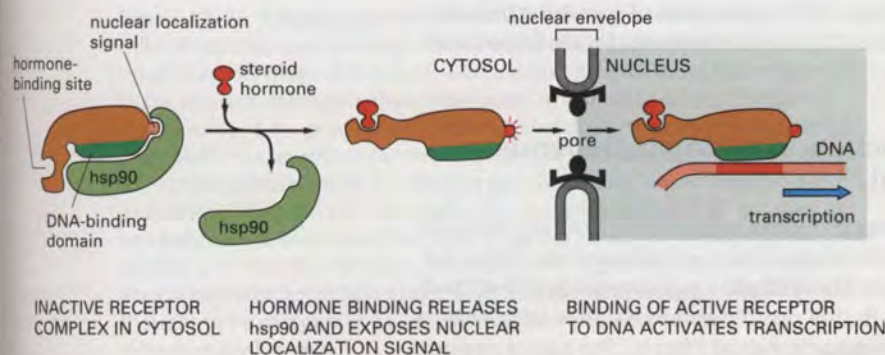


Figure 12-19 The nuclear import of the glucocorticoid receptor. The glucocorticoid receptor is a gene regulatory protein that, in the non-hormone-treated cell, is bound in the cytosol to the chaperone protein hsp90. When activated by the binding of the appropriate steroid hormone, it is released from hsp90 and is directed into the nucleus by a nuclear localization signal; once in the nucleus, it binds to specific DNA sequences and regulates the transcription of a discrete set of genes (discussed in Chapters 9 and 15).

Summary

The nuclear envelope consists of an inner and an outer nuclear membrane. The outer membrane is continuous with the ER membrane, and the space between it and the inner 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, while all of the proteins that function in the nucleus are synthesized in the cytosol and are then imported. The extensive traffic of materials between nucleus and cytosol occurs through nuclear pores that provide a direct passageway across the nuclear envelope.

Proteins containing nuclear localization signals are actively transported inward through the pores, while RNA molecules and newly made ribosomal subunits are actively transported outward through the pores. Because the nuclear localization signals are not removed, nuclear proteins can be imported repeatedly, as is required each time the nucleus reassembles following mitosis. The transport of nuclear proteins and RNA molecules through the pores can be regulated by denying these molecules access to the transport machinery in the nuclear pore complexes.

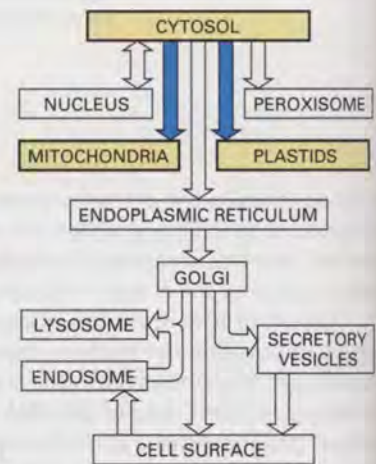
The Transport of Proteins into Mitochondria and Chloroplasts¹²

As discussed in Chapter 14, mitochondria and chloroplasts are double-membrane-bounded organelles that specialize in the synthesis of ATP, using energy derived from electron transport and oxidative phosphorylation in mitochondria and from photosynthetic phosphorylation in chloroplasts. Although both organelles contain their own DNA, ribosomes, and other machinery for protein synthesis, most of their proteins are encoded in the cell nucleus and imported from the cytosol. Moreover, each imported protein must reach the particular organelle subcompartment in which it functions. For mitochondria there are two subcompartments: the internal **matrix space** and the **intermembrane space**. These compartments are formed by the two distinct mitochondrial membranes: the **inner membrane**, which encloses the matrix space, and the **outer membrane**, which is in contact with the cytosol (Figure 12-20A). Chloroplasts have the same two subcompartments plus an additional subcompartment, the *thylakoid space*, which is surrounded by the *thylakoid membrane* (Figure 12-20B). Each of the subcompartments contains a distinct set of proteins. The growth of mitochondria and chloroplasts by the import of proteins from the cytosol is therefore a major feat, requiring that proteins be translocated across a number of membranes in succession and end up in the appropriate place.

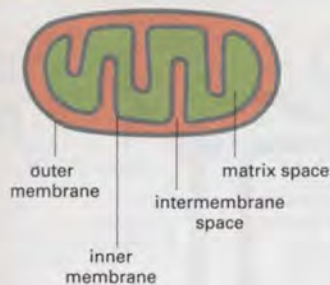
The relatively few proteins encoded by the genomes of these organelles are located mostly in the inner membrane in mitochondria and in the thylakoid membrane in chloroplasts. These organelle-encoded polypeptides generally form subunits of protein complexes whose other subunits are encoded by nuclear genes and are imported from the cytosol. The formation of such hybrid protein complexes requires a balanced synthesis of the two types of subunits; how protein synthesis is coordinated on different types of ribosomes located two membranes apart is still largely a mystery.

Translocation into the Mitochondrial Matrix Depends on a Matrix Targeting Signal¹³

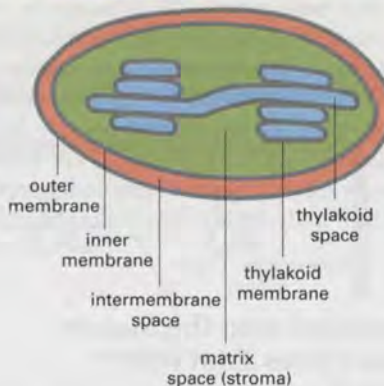
Proteins imported into the mitochondrial matrix are usually taken up from the cytosol within a minute or two of their release from polyribosomes. These **mitochondrial precursor proteins** almost always have a signal peptide (20–80 residues long) at their amino terminus that is rapidly removed after import by a protease (the *signal peptidase*) in the mitochondrial matrix. The signal peptide



(A) MITOCHONDRION



(B) CHLOROPLAST

**Figure 12-20 The subcompartments of mitochondria and chloroplasts.**

The topology of the chloroplast can be derived from that of the mitochondrion in a simple way: pinching off the invaginations of the inner mitochondrial membrane to create vesicles would generate a compartment that is topologically equivalent to the thylakoid vesicle in chloroplasts. Thylakoid vesicles may have evolved in this way.

can be remarkably simple. Molecular genetic experiments in which the signal peptide is progressively reduced in length have shown that, for one mitochondrial protein, only 12 amino acids at the amino terminus are needed to signal mitochondrial import. These 12 residues can be attached to any cytosolic protein and will direct the protein into the mitochondrial matrix. Physical studies of full-length signal peptides suggest that they can form amphipathic α -helical structures in which positively charged residues are clustered on one side of the helix while uncharged hydrophobic residues are clustered on the opposite side (Figure 12-21). This configuration is thought to be recognized by specific receptor proteins on the mitochondrial surface.

Translocation into the Mitochondrial Matrix Requires Both the Electrochemical Gradient Across the Inner Membrane and ATP Hydrolysis¹⁴

Almost everything we know about the molecular mechanism of protein import into mitochondria has been learned from analysis of cell-free, reconstituted transport systems. Mitochondria are first purified by differential centrifugation of homogenized cells and are then incubated with radiolabeled mitochondrial precursor proteins. The precursor proteins are generally taken up rapidly and efficiently into such mitochondria during a brief *in vitro* incubation. By changing the conditions in these experiments *in vitro*, it is possible to establish the biochemical requirements for protein transport into the mitochondria.

Vectorial movement and transport require energy. In most biological systems the energy is supplied by ATP hydrolysis. In the case of mitochondrial import, however, an electrochemical gradient across the inner mitochondrial membrane is required in addition to ATP hydrolysis. This gradient is maintained by the pumping of H^+ from the matrix to the intermembrane space, driven by electron transport processes in the inner membrane. The mitochondrial outer membrane,

Figure 12-21 A signal peptide for mitochondrial protein import.

Cytochrome oxidase is a large multiprotein complex located in the mitochondrial inner membrane, where it functions as the terminal enzyme in the electron-transport chain (discussed in Chapter 14). (A) The first 12 amino acids of the precursor to subunit IV of this enzyme serve as a signal peptide for the import of the subunit into the mitochondrion. (B) When the full-length signal peptide is folded as an α helix with 3.6 residues per turn and viewed from the top, the positively charged residues (red) are seen to be clustered on one face of the helix while the nonpolar residues (green) are clustered on the opposite face. Mitochondrial signal peptide sequences always have the potential to form such an amphipathic helix, which is thought to be recognized by specific receptor proteins on the mitochondrial surface.



like that of gram-negative bacteria (see Figure 11–14), contains large amounts of a pore-forming protein called *porin* and is thus freely permeable to inorganic ions and metabolites (but not to most proteins), so that no gradient can be maintained across it. The energy in the electrochemical gradient across the inner membrane is tapped to help drive most of the cell's ATP synthesis, but it is also used to drive the translocation of proteins bearing a mitochondrial import signal peptide: when ionophores that collapse the mitochondrial membrane potential are added, import is blocked. It is still uncertain how the electrochemical gradient contributes to protein translocation. The role of ATP hydrolysis is much better understood, as we see below.

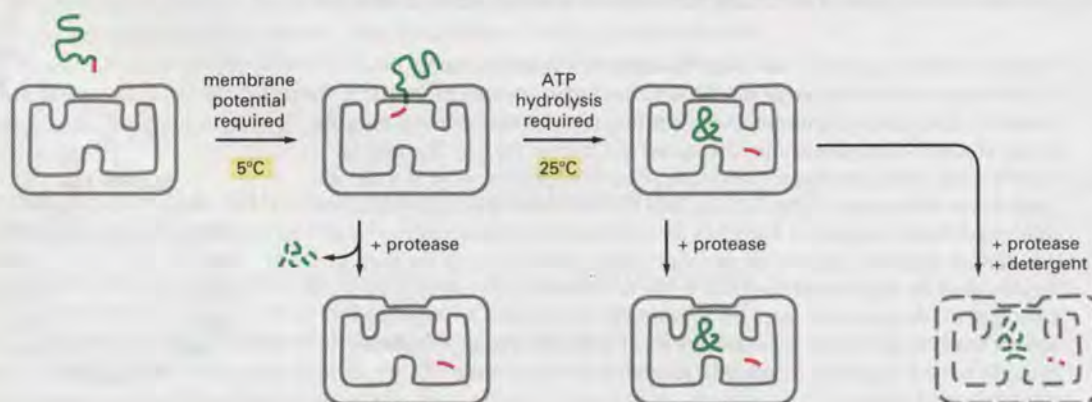
Mitochondrial Proteins Are Imported into the Matrix in a Two-Stage Process at Contact Sites That Join the Inner and Outer Membranes¹⁵

As a first step in mitochondrial import, the mitochondrial precursor proteins have to bind to receptor proteins that reside in the mitochondrial outer membrane and recognize the mitochondrial signal peptides. The next step is the translocation process itself.

A protein could reach the mitochondrial matrix by crossing the two membranes one at a time, or it could pass through both at once. To distinguish these possibilities, a cell-free import system is cooled to a low temperature, arresting the proteins at an intermediate step in the translocation process. The proteins that accumulate at this step have already had their amino-terminal signal peptide removed by the matrix signal peptidase, indicating that their amino terminus must be in the matrix space; yet the bulk of the protein can still be attacked from outside the mitochondria by externally added proteolytic enzymes (Figure 12–22). This result demonstrates that the precursor proteins can pass through both mitochondrial membranes at once to enter the matrix. It is thought that there are two protein translocators, one in the outer membrane and one in the inner membrane, whose functions are usually coupled to allow translocation across both membranes at the same time. Electron microscopists have noted numerous **contact sites** at which the inner and outer mitochondrial membranes appear to be joined, and it seems likely that translocation occurs at or near these sites.

Although precursor proteins are transported through both membranes at once, it is clear from the experiments described in Figure 12–22 that the import process occurs in two distinct stages, only the second of which is arrested at a low temperature. Of these, it is only the initial penetration, which is not affected by low temperatures, that requires the membrane potential: when cooled mitochondria containing partly translocated intermediates are warmed up, import is rapidly completed (see Figure 12–22) even if the potential across the inner membrane is collapsed. This second stage of the transport process, however, requires

Figure 12–22 Proteins transiently span both the inner and outer mitochondrial membranes during their translocation into the matrix. When isolated mitochondria are incubated with a precursor protein at 5°C, the precursor is only partially translocated. The amino-terminal signal peptide (red) is cleaved off in the matrix; most of the polypeptide chain remains outside the mitochondria, where it is accessible to proteolytic enzymes. Upon warming to 25°C, the translocation is completed. Once inside the mitochondrion, the polypeptide chain is protected from externally added proteolytic enzymes unless detergents are added to disrupt the mitochondrial membranes, which allows the imported proteins to be digested.



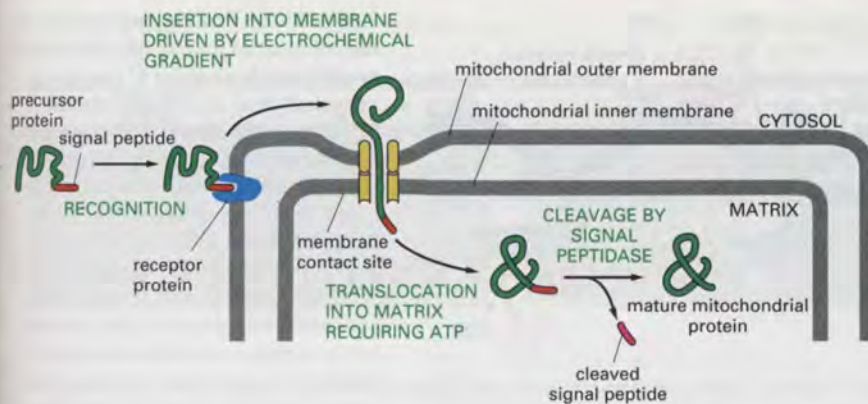


Figure 12-23 Protein import by mitochondria. The amino-terminal signal peptide of the precursor protein is recognized by receptors that reside in the outer membrane. The protein is thought to be translocated across both mitochondrial membranes at or near special contact sites, driven first by the electrochemical gradient across the inner membrane and then by ATP hydrolysis. The signal peptide is cleaved off by a signal peptidase in the matrix to form the mature protein; the free signal peptide is rapidly degraded.

ATP. Thus the first stage, which involves the insertion of the signal peptide and adjoining sequences into both mitochondrial membranes, is driven by the electrochemical gradient, and the second stage, in which the remainder of the polypeptide chain moves into the matrix, requires both ATP hydrolysis and a physiological temperature (Figure 12-23).

Proteins Are Imported into the Mitochondrial Matrix in an Unfolded State¹⁶

Transport of mitochondrial precursor proteins across the two mitochondrial membranes at a contact site is guided by members of the chaperone family of proteins, which are discussed in Chapter 5. It is difficult to envisage how a folded, water-soluble protein could straddle two (or even one) lipid bilayer while retaining its native three-dimensional conformation. It is now known that cytosolic chaperone proteins (called *chaperonins*) belonging to the hsp70 family, as well as helping to ensure the correct folding of cytosolic proteins, play an essential part in protein import into both mitochondria and the ER by binding the precursor in its unfolded state during translocation. As discussed in Chapter 5, the release of newly synthesized polypeptides from the hsp70 family of chaperone proteins requires ATP hydrolysis, and this partly accounts for the ATP dependence of the later stages of mitochondrial import.

The essential role of the chaperone proteins in translocation across internal cellular membranes was first indicated by genetic studies in yeasts. When the genes encoding certain members of the hsp70 family of chaperone proteins are inactivated, mitochondrial precursor proteins fail to be imported into mitochondria and accumulate in the cytosol instead. It is thought that newly synthesized precursor proteins, as they are released from polyribosomes in the cytosol, bind to hsp70 proteins, which prevent the precursor proteins from aggregating or folding up spontaneously before they bind to the protein translocator in the target membrane. The energy liberated by the hydrolysis of ATP is used to release the bound hsp70 proteins as the translocated protein is passed across the membrane. Experimentally, the requirement for hsp70 and ATP in the cytosol can be bypassed if the precursor proteins are artificially unfolded, for example, by a denaturation step in a concentrated solution of urea.

Sequential Binding of the Imported Protein to Mitochondrial hsp70 and hsp60 Drives Its Translocation and Assists Protein Folding¹⁷

Imported proteins are not only delivered to the mitochondrion by chaperone proteins: once they are extruded into the interior, they are received by closely related hsp70 proteins in the matrix space. *Mitochondrial hsp70* is crucial to the

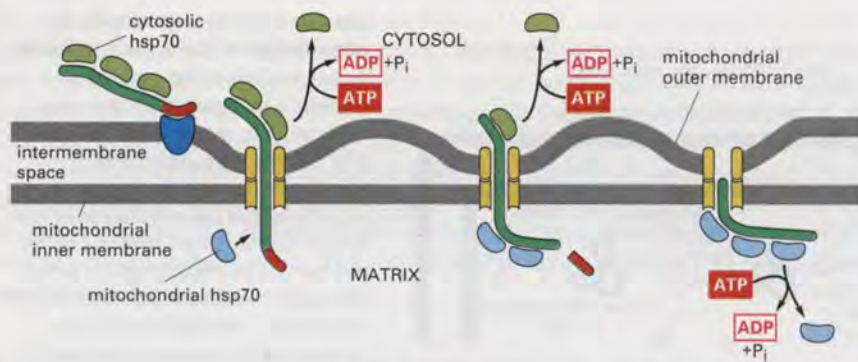


Figure 12-24 Protein import into the mitochondrial matrix requires hsp70 proteins on both sides of the mitochondrial double membrane.

After the initial insertion of the signal peptide and of adjacent portions of the polypeptide chain, the unfolded chain slides in a channel that spans both membranes. Bound *cytosolic hsp70* is released from the protein in a step that depends on ATP hydrolysis; concomitantly, *mitochondrial hsp70* binds to regions of the polypeptide chain as they become exposed in the matrix, thereby pulling the protein into the interior of the mitochondrion.

import process, as mitochondria containing mutant forms of the protein fail to import precursor proteins. Like its cytosolic cousin, mitochondrial hsp70 has a high affinity for unfolded polypeptide chains, and it binds tightly to an imported protein as soon as it emerges from the translocator. The hsp70 then releases the protein in an ATP-dependent step. This energy-driven cycle of binding and subsequent release could provide the driving force for protein import after the protein has initially inserted into the translocator (Figure 12-24): the sequential binding of multiple mitochondrial hsp70 proteins may pull the unfolded protein through a transmembrane channel into the matrix.

After the initial interaction with mitochondrial hsp70, many imported proteins are passed on to another chaperone protein, *mitochondrial hsp60*. As discussed in Chapter 5, hsp60 attaches to the unfolded polypeptide chain and facilitates its folding in an ATP-consuming reaction. Much of our current understanding of the function of hsp60 in facilitating protein folding is derived from studies on import of proteins into mitochondria.

Protein Transport into the Mitochondrial Inner Membrane and the Intermembrane Space Requires Two Signals¹⁸

Many of the functions of mitochondria require proteins that either are integrated into the mitochondrial inner membrane or operate in the intermembrane space. These proteins are transported from the cytosol by the same mechanism that transports proteins into the matrix, but they are then prevented by various means from ending their journey in the matrix. In many cases the precursor proteins are first transferred all the way into the matrix, as was illustrated in Figure 12-23. A very hydrophobic amino acid sequence, however, is strategically placed after the amino-terminal signal peptide that initiates import. Once the amino-terminal signal is cleaved by the matrix signal peptidase, the hydrophobic sequence can function as a new amino-terminal signal peptide to translocate the protein back again from the matrix into or across the inner membrane. Presumably, the final step in this pathway involves a mechanism that is also used to direct proteins encoded in the mitochondrion to the inner membrane (Figure 12-25A), and we see later that a similar mechanism is used for translocating proteins into or across the ER membrane and the procaryotic plasma membrane, where it has been more extensively investigated.

An alternative route to the inner membrane may avoid the excursion into the matrix space altogether. The translocator in the inner membrane binds to the hydrophobic sequence that follows the amino-terminal signal peptide that initiates import and prevents further translocation across the inner membrane. The two translocators in the outer and inner membranes become uncoupled, which causes the remainder of the protein to be pulled into the intermembrane space (Figure 12-25B). Different proteins may use one or the other of these two pathways to the inner membrane or intermembrane space. It is not clear which one is more commonly used.

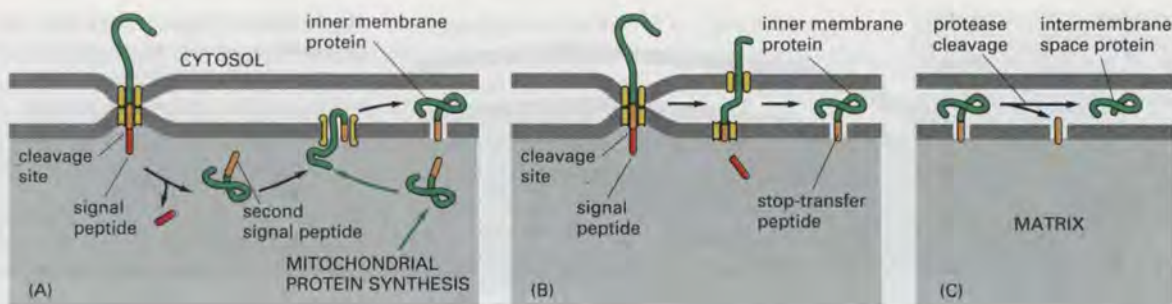


Figure 12-25 Import of proteins from the cytosol to the mitochondrial intermembrane space or inner membrane. (A) A pathway that requires two signal peptides and two translocation events is thought to be used to move some proteins from the cytosol to the inner membrane. The protein is first imported into the matrix space as in Figure 12-23. Cleavage of the signal peptide (red) used for the initial translocation, however, unmasks an adjacent hydrophobic signal peptide (orange) at the new amino terminus. This signal causes the protein to be integrated into the inner membrane by the same pathway that is used to insert proteins encoded by the mitochondrial genome into this membrane. (B) In an alternative mechanism, the hydrophobic sequence that follows the matrix targeting signal binds to the translocator and stops the translocation across the inner membrane. The remainder of the protein is then pulled into the intermembrane space and the hydrophobic sequence is released into the inner membrane. This mechanism is called the stop-transfer pathway and is discussed in detail later. (C) Some soluble proteins of the intermembrane space may also use the pathways shown in (A) and (B) before they are released into the intermembrane space by a second signal peptidase (with its active site in the intermembrane space), which removes the hydrophobic signal peptide.

After proteins destined for the intermembrane space have been inserted via their hydrophobic signal peptides into the inner membrane, some are cleaved by a signal peptidase in the intermembrane space to release the mature polypeptide chain as a soluble protein (Figure 12-25C). Many of these proteins ultimately become attached as peripheral membrane proteins to the outer surface of the inner membrane, where they form subunits of protein complexes that also contain transmembrane proteins.

Two Signal Peptides Are Required to Direct Proteins to the Thylakoid Membrane in Chloroplasts¹⁹

Protein transport into chloroplasts resembles transport into mitochondria in many respects: both occur posttranslationally, both require energy, and both utilize amphipathic amino-terminal signal peptides that are removed after use. There is at least one important difference, however: mitochondria exploit the electrochemical gradient across their inner membrane to help drive the transport, whereas chloroplasts, which have an electrochemical gradient across their thylakoid but not their inner membrane, appear to employ only ATP hydrolysis to power import across their double-membrane outer envelope.

Although the signal peptides for import into chloroplasts resemble those for import into mitochondria, mitochondria and chloroplasts are both present in the same plant cells, and proteins must choose appropriately between them. In plants, for example, a bacterial enzyme is directed specifically to mitochondria if it is experimentally joined to an amino-terminal signal sequence of a mitochondrial protein; the same enzyme joined to an amino-terminal signal sequence of a chloroplast protein ends up in chloroplasts. The different signal sequences, therefore, can be distinguished, presumably by the import receptors on each organelle.

Chloroplasts have an extra membrane-bounded compartment, the **thylakoid**. Many chloroplast proteins, including protein subunits of the photosynthetic system and of the ATP synthase, are embedded in the membrane of the thylakoid compartment. Like the precursors of some mitochondrial proteins, these proteins are transported from the cytosol to their final destination in two steps. First, they pass across the double membrane at contact sites into the matrix space of the chloroplast (termed the **stroma**), and then they are translocated into the thylakoid membrane (or across this membrane into the thylakoid space). The precursors of these proteins have a hydrophobic thylakoid signal peptide following the amino-terminal chloroplast signal peptide. After the amino-terminal signal peptide has been used to import the protein into the stroma, it is removed by a stromal signal peptidase (analogous to the matrix signal peptidase in mitochondria). This cleavage unmasks the thylakoid signal peptide, which then initiates transport across the thylakoid membrane (Figure 12-26). As with mitochondria, the second step is the pathway used to insert chloroplast-encoded proteins into the thylakoid membrane; the protein translocator required presumably originated in the chloroplast's bacterial ancestor.

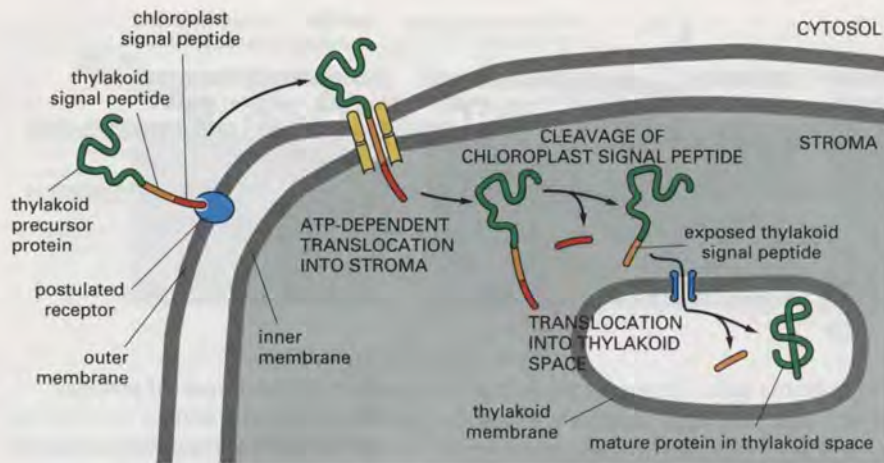


Figure 12-26 Translocation into the thylakoid space of chloroplasts. The precursor polypeptide contains an amino-terminal chloroplast signal peptide (red) followed immediately by a thylakoid signal peptide (orange). The chloroplast signal peptide initiates translocation into the stroma through a membrane contact site by a mechanism similar to that used for translocation into the mitochondrial matrix. The signal peptide is then cleaved off, unmasking the thylakoid signal peptide, which initiates translocation across the thylakoid membrane.

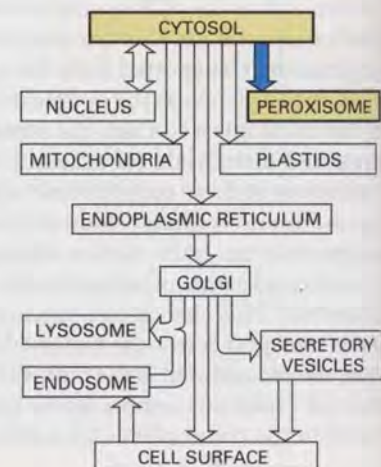
Summary

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. The transport processes involved have been most extensively studied in mitochondria, especially in yeasts. A protein is translocated into the mitochondrial matrix space by passing through sites of adhesion between the outer and inner membranes called contact sites. Translocation into mitochondria is driven by both ATP hydrolysis and the electrochemical gradient across the inner membrane, whereas translocation into chloroplasts is driven by ATP hydrolysis alone. The transported protein crosses the membranes of the mitochondrion or chloroplast in an unfolded state. Chaperone proteins of the cytosolic hsp70 family maintain the precursor proteins in an unfolded, translocation-competent state. Mitochondrial hsp70 in the matrix binds to the incoming polypeptide chain and is thought to pull the protein chain into the matrix. Once the protein is in the matrix, another stress protein, hsp60, helps the translocated protein fold up. Only proteins that contain a specific signal peptide are translocated into mitochondria or chloroplasts. The signal peptide is usually located at the amino terminus and is cleaved off after import. Transport across or into the inner membrane can occur as a second step if a hydrophobic signal peptide is also present in the imported protein; this second signal peptide is unmasked when the first signal peptide is removed. In the case of chloroplasts, import from the stroma into the thylakoid likewise requires a second signal peptide.

Peroxisomes²⁰

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. In spite of these differences, peroxisomes are thought to acquire their proteins by a similar process of selective import from the cytosol. Because peroxisomes have no genome, however, *all* of their proteins must be imported. Peroxisomes thus resemble the ER in being self-replicating membrane-bounded organelles that exist without genomes of their own.

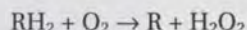
Because we do not discuss peroxisomes elsewhere, we shall digress to consider some of the functions of this diverse family of organelles before discussing their biosynthesis. Peroxisomes are found in all eucaryotic cells. They contain oxidative enzymes, such as *catalase* and *urate oxidase*, at such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



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

Peroxisomes Use Molecular Oxygen and Hydrogen Peroxide to Carry Out Oxidative Reactions ²¹

Peroxisomes are so called 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 oxidative reaction that produces *hydrogen peroxide* (H_2O_2):



Catalase utilizes the H_2O_2 generated by other enzymes in the organelle to oxidize a variety of other substrates—including phenols, formic acid, formaldehyde, and alcohol—by the “peroxidative” reaction: $H_2O_2 + R'H_2 \rightarrow R' + 2H_2O$. This type of oxidative reaction is particularly important in liver and kidney cells, whose peroxisomes detoxify various toxic molecules that enter the bloodstream. About a quarter 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 ($2H_2O_2 \rightarrow 2H_2O + O_2$).

A major function of the oxidative reactions carried out in peroxisomes is the breakdown of fatty acid molecules. In a process called β oxidation, the alkyl chains of fatty acids are shortened sequentially by blocks of two carbon atoms at a time that are converted to acetyl CoA and exported from the peroxisomes to the cytosol for reuse in biosynthetic reactions. β oxidation in mammalian cells occurs both in mitochondria and peroxisomes; in yeast and plant cells, however, this essential reaction is exclusively found in peroxisomes.

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

Peroxisomes also have very important roles in plants. Two very different types have been studied extensively. One type is present in leaves, where it catalyzes the oxidation of a side product of the crucial reaction that fixes CO_2 in carbohydrate (Figure 12–28A). This process is called *photorespiration* because it uses up O_2 and liberates CO_2 . The other type of peroxisome is present in germinating seeds, where it plays an essential role in converting 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 12–28B). In the glyoxylate cycle two molecules of acetyl CoA produced by fatty acid breakdown in the peroxisome are used to make succinic acid, which leaves the peroxisome and is converted into glucose. The glyoxylate cycle does not occur in animal cells, and animals are thus unable to convert the fatty acids in fats into carbohydrates.

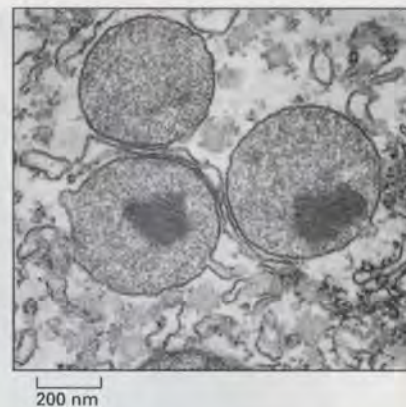


Figure 12–27 Peroxisomes. Electron micrograph of three peroxisomes in a rat liver cell. The paracrystalline electron-dense inclusions are the enzyme urate oxidase. (Courtesy of Daniel S. Friend.)

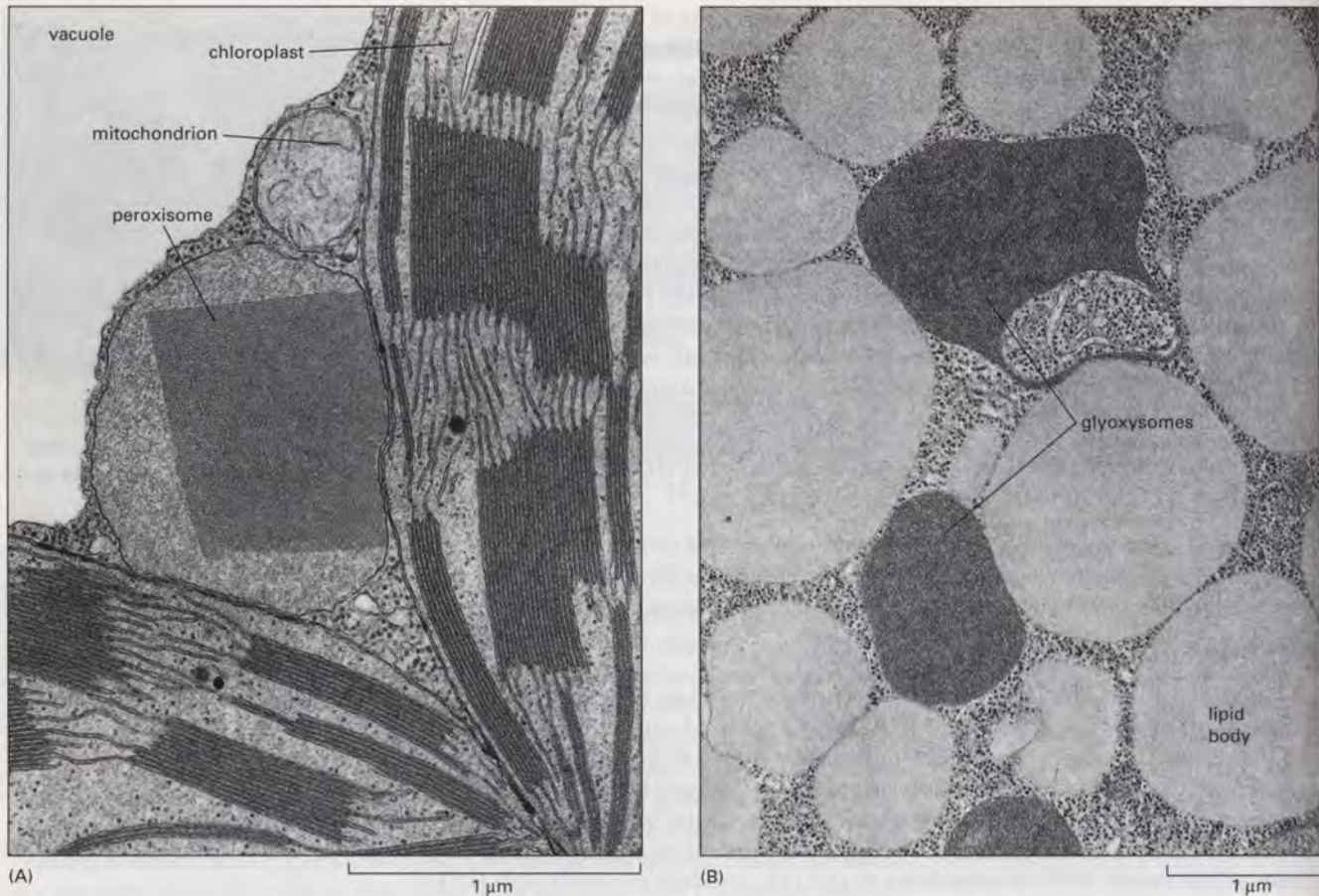
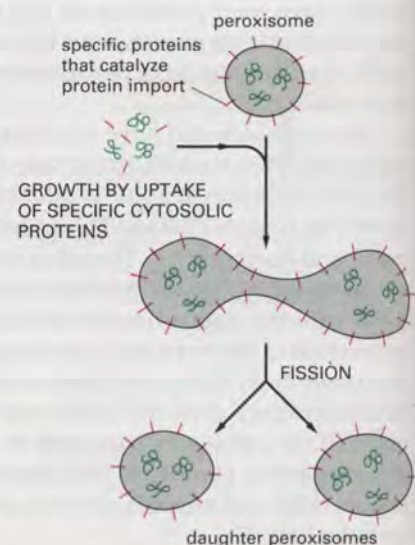


Figure 12-28 Electron micrographs of two types of peroxisomes found in plant cells. (A) A leaf peroxisome with a paracrystalline core in a tobacco leaf mesophyll cell. Its close association with chloroplasts is thought to facilitate the exchange of materials between these organelles during photorespiration. (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 bodies where fat is stored, reflecting their central role in fat mobilization and gluconeogenesis during seed germination. (A, courtesy of P.J. Gruber and E.H. Newcomb; B, courtesy of S.E. Frederick and E.H. Newcomb.)

A Short Signal Sequence Directs the Import of Proteins into Peroxisomes²²

A specific sequence of three amino acids located near the carboxyl terminus of many peroxisomal proteins functions as an import signal (see Table 12-3); if this sequence is experimentally attached to a cytosolic protein, the protein is imported into peroxisomes. The importance of this import process and of peroxisomes is dramatically demonstrated by the inherited human disease *Zellweger syndrome*, in which a defect in importing proteins into peroxisomes leads to a severe peroxisomal deficiency. These individuals, whose cells contain “empty” peroxisomes, have severe abnormalities in their brain, liver, and kidneys, and they die soon after birth. One form of this disease has been shown to be due to a mutation in the gene encoding a peroxisomal integral membrane protein called peroxisome assembly factor-1.

Figure 12-29 A model for how peroxisomes are assembled. The peroxisome membrane contains specific import receptor proteins. All peroxisomal proteins, including new copies of the import receptor, are synthesized by cytosolic ribosomes and then imported into the organelle. Thus peroxisomes form only from preexisting peroxisomes by a process of growth and fission. Presumably, the lipids required to make new peroxisome membrane are also imported. We discuss later how lipids made in the ER can be transported through the cytosol to other organelles.



Peroxisomes presumably have at least one unique protein exposed on their cytosolic surface to act as a receptor that recognizes the signal on the proteins to be imported. At one time it was thought that the membrane of the peroxisome forms by budding from the ER, while the content is imported from the cytosol. There is now evidence, however, suggesting that new peroxisomes arise only from preexisting ones, by organelle growth and fission, as described elsewhere for mitochondria and plastids and for the ER itself (Figure 12–29).

Summary

Peroxisomes are specialized for carrying out oxidative reactions using molecular oxygen. They generate hydrogen peroxide, which they also use for oxidative purposes—destroying the excess by means of the catalase they contain. Like mitochondria and plastids, peroxisomes are self-replicating organelles. Because they contain no DNA or ribosomes, they have to import all of their proteins from the cytosol. A specific three amino acid sequence near the carboxyl terminus of many of these proteins functions as a peroxisomal import signal.

The Endoplasmic Reticulum²³

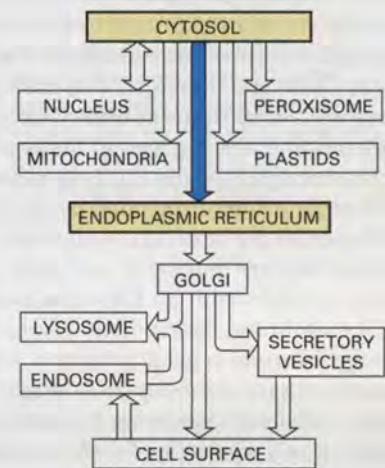
All eucaryotic cells have an **endoplasmic reticulum (ER)**. Its membrane typically constitutes more than half of the total membrane of an average animal cell (see Table 12–2). It is organized into a netlike labyrinth of branching tubules and flattened sacs extending throughout the cytosol (Figure 12–30). The tubules and sacs are all thought to interconnect, so that the ER membrane forms a continuous sheet enclosing a single internal space. This highly convoluted space is called the **ER lumen** or the *ER cisternal space*, and it often occupies more than 10% of the total cell volume (see Table 12–1). The ER membrane separates the ER lumen from the cytosol, and it mediates the selective transfer of molecules between these two compartments.

The ER plays a central part in lipid and protein biosynthesis. Its 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 also makes a major contribution to mitochondrial and peroxisomal membranes by producing most of their lipids. In addition, almost all of the proteins that will be secreted to the cell exterior—as well as those destined for the lumen of the ER, Golgi apparatus, or lysosomes—are initially delivered to the ER lumen.

Membrane-bound Ribosomes Define the Rough ER²⁴

The ER captures selected proteins from the cytosol as they are being synthesized. These proteins are of two types: (1) transmembrane proteins, which are only partly translocated across the ER membrane and become embedded in it, and (2) water-soluble proteins, which are fully translocated across the ER membrane and are released into the ER lumen. Some of the transmembrane proteins will remain 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 the lumen of an organelle or for secretion. All of these proteins, regardless of their subsequent fate, are directed to the ER membrane by the same kind of signal peptide and are translocated across it by the same mechanism.

In mammalian cells the import of proteins into the ER begins before the polypeptide chain is completely synthesized—that is, it occurs **co-translationally**. This distinguishes the process from the import of proteins into mitochondria, chloroplasts, nuclei, and peroxisomes, which is **posttranslational** and requires different signal peptides. Since one end of the protein is usually translocated into the ER as the rest of the polypeptide chain is being made, the protein is never released into the cytosol and therefore is never in danger of folding up before



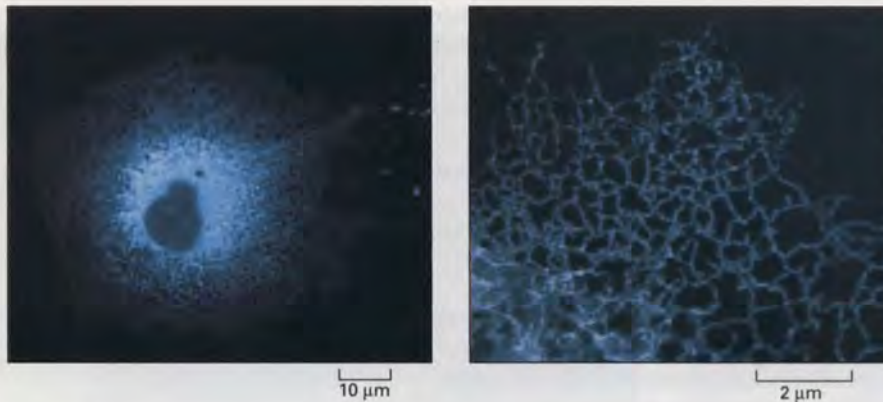


Figure 12-30 The endoplasmic reticulum. Fluorescence micrograph of a cultured mammalian cell stained with an antibody that binds to a protein retained in the ER. The ER extends as a network throughout the entire cytosol, so that all regions of the cytosol are close to some portion of the ER membrane. (Courtesy of Hugh Pelham.)

reaching the translocator in the membrane. In contrast to the posttranslational import of proteins into the mitochondria and chloroplasts, cytosolic chaperonins are therefore not required to keep the protein unfolded. The ribosome that is synthesizing the protein is directly attached to the ER membrane. These membrane-bound ribosomes coat the surface of the ER, creating regions termed **rough endoplasmic reticulum** (Figure 12-31).

There are, therefore, two spatially separate populations of ribosomes in the cytosol. **Membrane-bound ribosomes**, attached to the 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, make 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. When a ribosome happens to be making a protein with an **ER signal peptide**, the signal directs the ribosome to the ER membrane. Since many ribosomes can bind to a single mRNA molecule, a **polyribosome** is usually formed, which becomes attached to the ER membrane via the signal peptides on multiple growing polypeptide chains (Figure 12-32). The individual ribosomes associated with such an mRNA molecule can return to the cytosol when they finish translation near the 3' end of the mRNA molecule. The mRNA itself, however, tends to remain attached to the ER membrane by a changing population of ribosomes that are also held at the membrane by a ribosome receptor that helps to bind it there. In contrast, if an mRNA molecule encodes a protein that lacks an ER signal peptide, the polyribosome that forms remains free in the cytosol and its protein product is discharged there. Therefore, only those mRNA molecules that encode proteins with an ER signal peptide bind to rough ER membranes; those mRNA molecules that encode all other proteins remain free in the cytosol. The individual ribosomal subunits are thought to move



Figure 12-31 The rough ER. Electron micrograph of the rough ER, which receives its name from the many ribosomes on its cytosolic surface. (Courtesy of L. Orci.)

randomly between these two segregated populations of mRNA molecules (Figure 12–33).

Smooth ER Is Abundant in Some Specialized Cells²⁵

Regions of ER that lack bound ribosomes are called **smooth endoplasmic reticulum**, or **smooth ER**. In the great majority of cells such regions are scanty, and there is only a small region of the ER that is partly smooth and partly rough. This region is said to consist of **transitional elements** because it is from here that transport vesicles carrying newly synthesized proteins and lipids bud off for transport to the Golgi apparatus. In certain specialized cells, however, the smooth ER is abundant and has additional functions. In particular, it is usually prominent in cells that specialize in lipid metabolism: cells that synthesize steroid hormones from cholesterol, for example, have an expanded smooth ER compartment to accommodate the enzymes needed to make cholesterol and to modify it to form the hormones (Figure 12–34).

The main cell type in the liver, the *hepatocyte*, provides another example. It is the principal site of the production of lipoprotein particles; these particles carry lipids via the bloodstream to other sites in the body. The enzymes that synthesize the lipid components of lipoproteins 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 the *detoxification reactions* are catalyzed by the *cytochrome P450* family of enzymes, which catalyze a series of reactions whereby 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 major portion of the membrane in a hepatocyte normally consists of smooth ER (Figure 12–35 and see Table 12–2).

When large quantities of certain compounds, such as the drug phenobarbital, enter the circulation, detoxification enzymes are synthesized in the liver in unusually large amounts, and the smooth ER doubles in surface area within a few days. Once the drug disappears, the excess smooth ER membrane is specifically and rapidly removed by a lysosome-dependent process called *autophagocytosis* (discussed in Chapter 13). How these dramatic changes are regulated is not known.

Another function of the ER in most eucaryotic cells is to sequester Ca^{2+} from the cytosol. The release of Ca^{2+} into the cytosol from the ER, and its subsequent reuptake, mediate many rapid responses to extracellular signals, as discussed in Chapter 15. The storage of Ca^{2+} in the ER lumen is facilitated by the high concen-

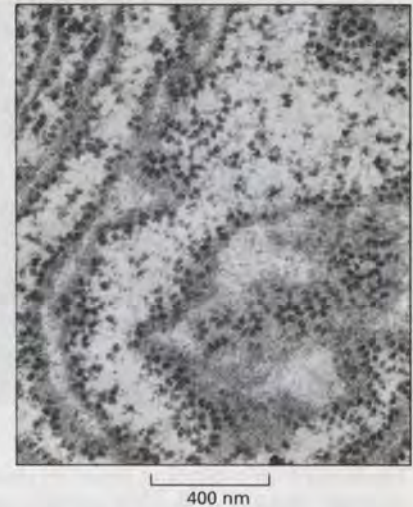


Figure 12–32 Polyribosomes. Thin-section electron micrograph of polyribosomes attached to the ER membrane. The plane of section in some places cuts through the ER roughly parallel to the membrane, giving a face-on view of the rosettelike pattern of the polyribosomes. (Courtesy of George Palade.)

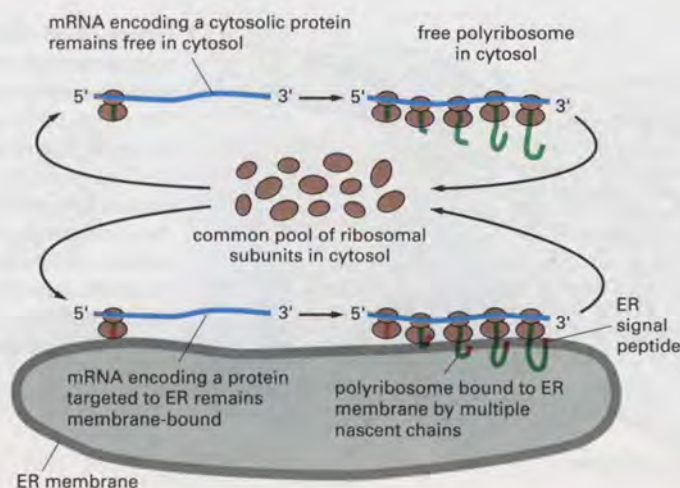


Figure 12–33 Free and membrane-bound ribosomes. A common pool of ribosomes is used to synthesize both the proteins that stay in the cytosol and those that are transported into the ER. It is the ER signal peptide on a newly formed polypeptide chain that directs the engaged ribosome to the ER membrane. The mRNA molecule may remain 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.

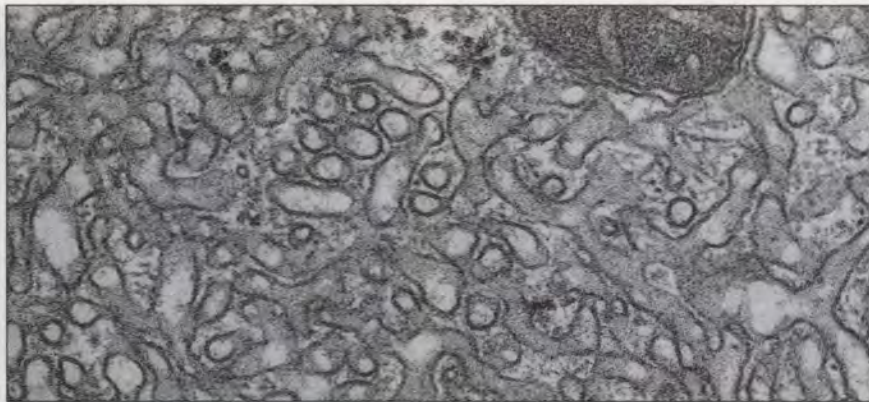


Figure 12-34 Abundant smooth ER in a steroid-hormone-secreting cell. This electron micrograph is of a testosterone-secreting Leydig cell in the human testis.

trations of Ca^{2+} -binding proteins there. In some cell types, and perhaps in most, specific regions of the ER are specialized for Ca^{2+} storage. Muscle cells, for example, have an abundant specialized smooth ER, called the *sarcoplasmic reticulum*, which sequesters Ca^{2+} from the cytosol by means of a Ca^{2+} -ATPase that pumps in Ca^{2+} ; the release and reuptake of Ca^{2+} by the sarcoplasmic reticulum mediates the contraction and relaxation of the myofibrils during each round of muscle contraction (discussed in Chapter 16).

We shall now return to the two major roles of the ER: the synthesis and modification of proteins and the synthesis of lipids.

Rough and Smooth Regions of ER Can Be Separated by Centrifugation²⁶

In order to study the functions and biochemistry of the ER, it is necessary to isolate the ER membrane. At first sight this seems a hopeless task since the ER is intricately interleaved with other components of the cytosol. Fortunately, when tissues or cells are disrupted by homogenization, the ER is fragmented and re-seals into many small (~100 nm in diameter) closed vesicles called **microsomes**, which are relatively easy to purify. Microsomes derived from rough ER are studded with ribosomes and are called *rough microsomes*. The ribosomes are always found on the *outside* surface, so that the interior of the microsome is biochemically equivalent to the luminal space of the ER (Figure 12-36). Because they can be readily purified in functional form, rough microsomes are especially useful for studying the many processes carried out by the rough ER. To the biochemist they represent small authentic versions of the rough ER, still capable of protein synthesis, protein glycosylation, and lipid synthesis.

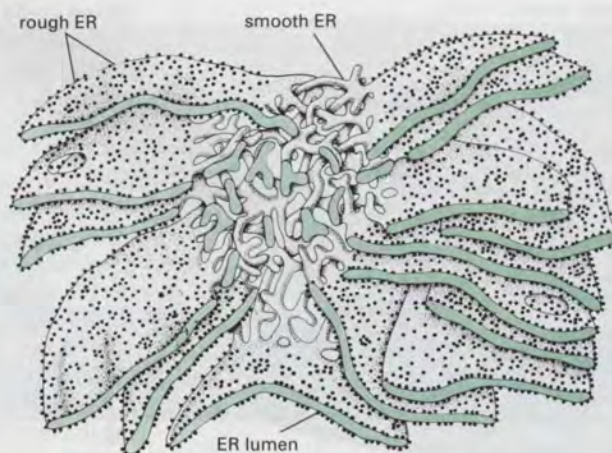
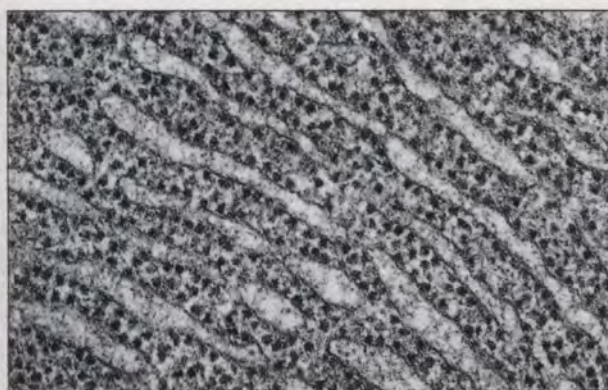
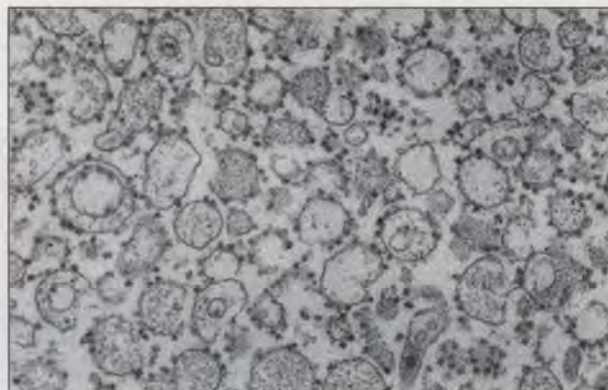


Figure 12-35 Three-dimensional reconstruction of a region of the smooth and rough ER in a liver cell. The rough ER forms oriented stacks of flattened cisternae, each having a luminal space 20 to 30 nm wide. The smooth ER membrane is connected to these cisternae and forms a fine network of tubules 30 to 60 nm in diameter. (After R.V. Krstić, *Ultrastructure of the Mammalian Cell*. New York: Springer-Verlag, 1979.)



(A)

200 nm



(B)

200 nm

Many vesicles of a size similar to that of rough microsomes but lacking attached ribosomes are also found in these homogenates. Such *smooth microsomes* are derived in part from smooth portions of the ER and in part from vesiculated fragments of plasma membrane, Golgi apparatus, endosomes, and mitochondria (the ratio depending on the tissue). Thus, whereas rough microsomes can be equated with rough portions of ER, the origins of smooth microsomes cannot be so easily assigned. The microsomes of the liver are an exception. Because of the unusually large quantities of smooth ER in the hepatocyte, most of the smooth microsomes in liver homogenates are derived from smooth ER.

The ribosomes attached to them make rough microsomes more dense than smooth microsomes. As a result, the rough and smooth microsomes can be separated from each other by equilibrium centrifugation (Figure 12-37). When the separated rough and smooth microsomes of liver are compared with respect to such properties as enzyme activity or polypeptide composition, they are very similar, although not identical: apparently most of the components of the ER membrane can diffuse freely between the rough and smooth regions, as would be expected for a continuous fluid membrane. The rough microsomes, however, contain more than 20 proteins that are not present in smooth microsomes, showing that some restraining mechanism must exist for a subset of ER membrane proteins. Some of the proteins in this subset help bind ribosomes to the rough ER, while others presumably produce the flattened shape of this part of the ER (see Figure 12-35). It is not clear whether these membrane proteins are retained by forming large two-dimensional aggregates in the lipid bilayer or whether they are instead held in place by interactions with a network of structural proteins on one or the other face of the ER membrane.

Figure 12-36 Electron micrographs of microsomes. When cells are disrupted by homogenization, the cisternae of rough ER (A) break up into small closed vesicles called *rough microsomes* (B). Similarly, the smooth ER breaks up into small vesicles that lack ribosomes and are called *smooth microsomes*. (A, courtesy of Daniel S. Friend; B, courtesy of George Palade.)

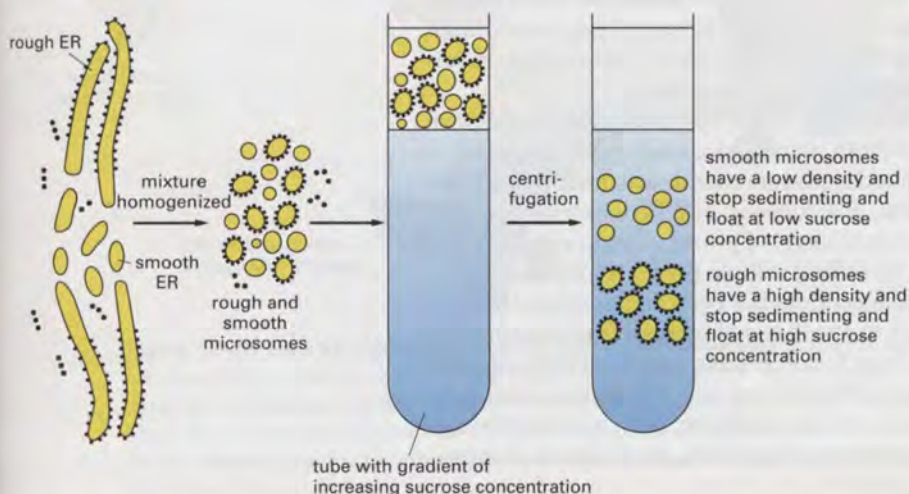


Figure 12-37 The isolation procedure used to purify rough and smooth microsomes from the ER. When sedimented to equilibrium through a gradient of sucrose, the two types of microsomes separate from each other on the basis of their different densities.

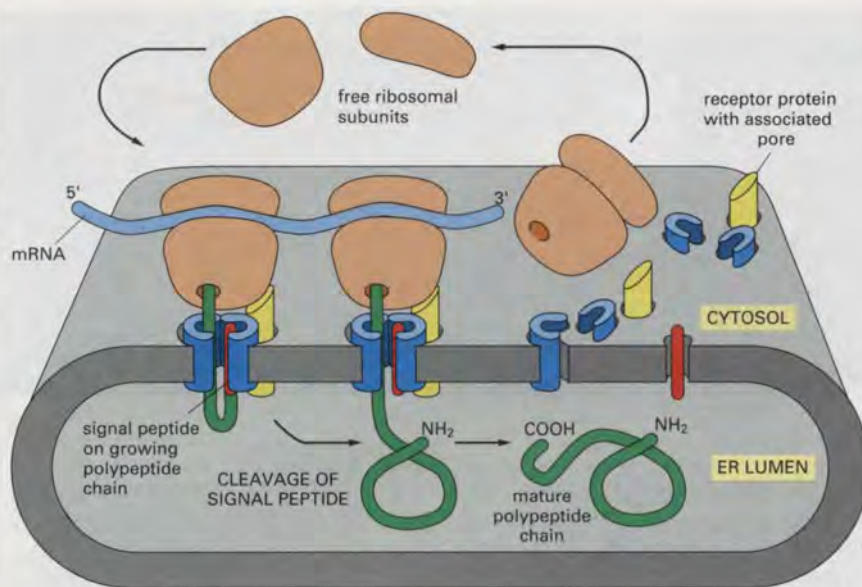


Figure 12-38 The original signal hypothesis. A simplified view of protein translocation across the ER membrane, as originally proposed. When the signal peptide emerges from the ribosome, it directs the ribosome to a receptor protein on the ER membrane. As it is synthesized, the polypeptide is postulated to be translocated across the ER membrane through a protein pore associated with the receptor. The signal peptide is clipped off during translation by a signal peptidase, and the mature protein is released into the lumen of the ER immediately after being synthesized. We now know that the hypothesis is correct in outline but that additional components besides those shown in this figure are required. The signal peptidase, for example, is a complex of five different membrane-bound polypeptide chains, with one complex apparently associated with every translocation pore.

Signal Peptides Were First Discovered in Proteins Imported into the Rough ER ²⁷

Signal peptides (and the signal peptide strategy of protein import) 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, the extra length being due to the presence of an amino-terminal *leader peptide*. In the presence of microsomes derived from the rough endoplasmic reticulum, however, a protein of the correct size was produced. These results were explained by the **signal hypothesis**, which postulated that the leader serves as a *signal peptide* that directs the secreted protein to the ER membrane and is then cleaved off by a signal peptidase in the ER membrane before the polypeptide chain is completed (Figure 12-38).

According to the signal hypothesis, the secreted protein should be extruded into the lumen of the microsome during its *in vitro* synthesis. This can be demonstrated with protease treatment: a newly synthesized protein made in the absence of microsomes is degraded when protease is added to the medium, whereas the same protein made in the presence of microsomes remains intact because it is protected by the microsomal membrane. When proteins without ER signal peptides are similarly synthesized *in vitro*, they are not imported into microsomes and therefore are degraded by protease treatment.

The signal hypothesis has been thoroughly tested by genetic and biochemical experiments and is found to apply to both plant and animal cells, as well as to protein translocation across the bacterial plasma membrane and, as we have seen, the membranes of mitochondria, chloroplasts, and peroxisomes. Amino-terminal ER signal peptides guide not only secreted proteins but also the precursors of all proteins made in the ER, including soluble proteins and membrane proteins. The signaling function of these peptides has been demonstrated directly by using recombinant DNA techniques to attach signal sequences to proteins that do not normally have them; the resulting fusion proteins are directed to the ER.

Cell-free systems in which proteins are imported into microsomes have provided powerful assay 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 ER Signal Peptides to a Specific Receptor in the Rough ER Membrane ²⁸

The ER signal peptide 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 peptide, and an *SRP receptor*, also known as a *docking protein*, in the ER membrane. The SRP was discovered when it was found that washing microsomes with salt eliminated their ability to import secreted proteins. Import could be restored by adding back the supernatant containing the salt extract. The "translocation factor" in the salt extract was then purified and found to be a complex particle consisting of six different polypeptide chains bound to a single small RNA molecule (Figure 12-39). SRP and SRP receptor are present in all eucaryotic cells and probably in procaryotic cells as well.

The SRP binds to the ER signal peptide as soon as the peptide emerges from the ribosome. This causes a pause in protein synthesis, which presumably gives the ribosome enough time to bind to the ER membrane before the synthesis of the polypeptide chain is completed, thereby ensuring that the protein is not released into the cytosol. This may provide a safety mechanism as many secreted proteins and lysosomal proteins are hydrolases that could wreak havoc in the cytosol. Cells that secrete large amounts of hydrolases take the added precaution of having high concentrations of hydrolase inhibitors in their cytosol.

Once formed, the SRP ribosome complex binds to the **SRP receptor**, which is an integral membrane protein exposed on the cytosolic surface of the rough ER membrane. The SRP is then released, and a poorly characterized translocation apparatus transfers the growing polypeptide chain across the membrane (Figure 12-40). 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 5) ensure that SRP release occurs only after the ribosome has become properly engaged with the translocation apparatus in the ER membrane.

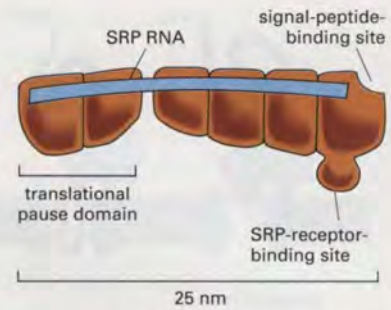


Figure 12-39 A highly schematic drawing of a signal-recognition particle (SRP). An SRP is an elongated complex containing six protein subunits and one RNA molecule (SRP RNA). One end of the SRP binds to an ER signal peptide on a growing polypeptide chain, while the other end binds to the ribosome itself and stops translation. The RNA in the particle may mediate an interaction with ribosomal RNA. (Adapted from V. Siegel and P. Walter, *Nature* 320:82-84, 1986.)

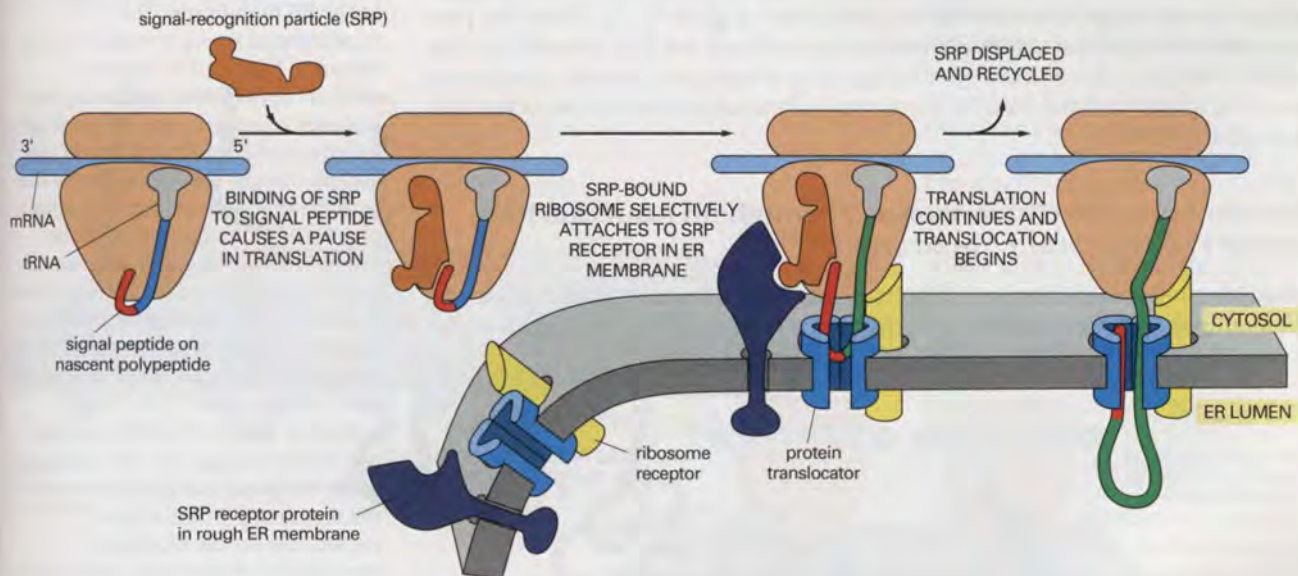


Figure 12-40 How ER signal peptides and SRP direct ribosomes to the ER membrane. The SRP and the SRP receptor are thought to act in concert. The SRP binds to the exposed ER signal peptide and to the ribosome, thereby inducing a pause in translation. The SRP receptor in the ER membrane, which is composed of two different polypeptide chains, binds the SRP ribosome complex. In a poorly understood reaction that involves multiple GTP-binding proteins, the SRP is released, leaving the ribosome on the ER membrane. A multisubunit protein translocation apparatus in the ER membrane then inserts the polypeptide chain into the membrane and transfers it across the lipid bilayer.

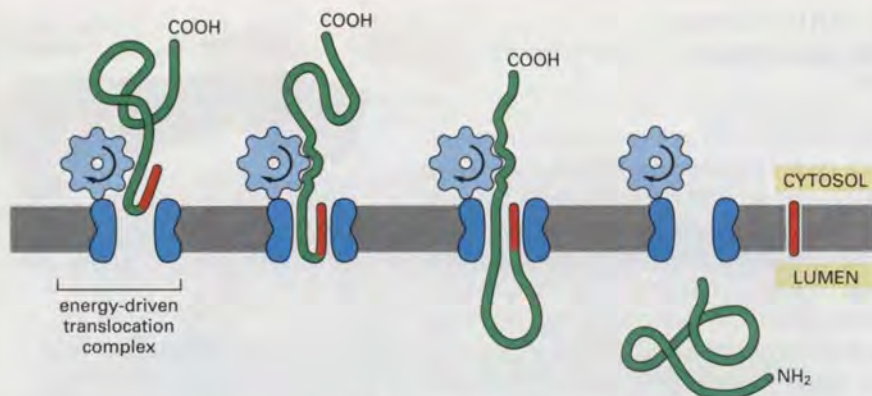


Figure 12-41 Translocation of a protein across the bacterial plasma membrane. In this schematic model, after a receptor in the translocation complex has bound the amino-terminal signal peptide, an energy-driven protein translocator threads the protein through the membrane, unfolding the polypeptide chain in the process. The energy is provided both by ATP hydrolysis and an electrochemical gradient across the membrane.

Translocation Across the ER Membrane Does Not Always Require Ongoing Polypeptide Chain Elongation²⁹

As we have seen, translocation of proteins into mitochondria, chloroplasts, and peroxisomes occurs *posttranslationally*, after the protein is completed 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 usually not to other organelles. A ribosome attached to the rough ER may utilize the energy of protein synthesis to force its growing polypeptide chain through a channel formed by a translocator in the ER membrane. Recent studies *in vitro*, however, have shown that a small minority of protein precursors can be imported into the ER after their synthesis has been completed, thus demonstrating that translocation does not always require ongoing translation. Posttranslational protein translocation may occur even more commonly across the ER membrane in yeast cells and across the bacterial plasma membrane (which is thought to be evolutionarily related to the ER; see Figure 12-5). In both cases the translocation requires ATP hydrolysis, and in bacteria an electrochemical gradient is needed as well. A translocation apparatus has been reconstituted from purified bacterial components, one of which is an ATPase that is thought to help thread the protein through the membrane (Figure 12-41). Since the proteins that use a posttranslational translocation pathway are first released into the cytosol, they are prevented from folding up by binding to cytosolic chaperone proteins, just as we have seen for the posttranslational import into mitochondria and chloroplasts.

The Polypeptide Chain Passes Through an Aqueous Pore in the Translocation Apparatus³⁰

It has long been debated whether polypeptide chains are transferred across the ER membrane in direct contact with the lipid bilayer or through a pore in a pro-

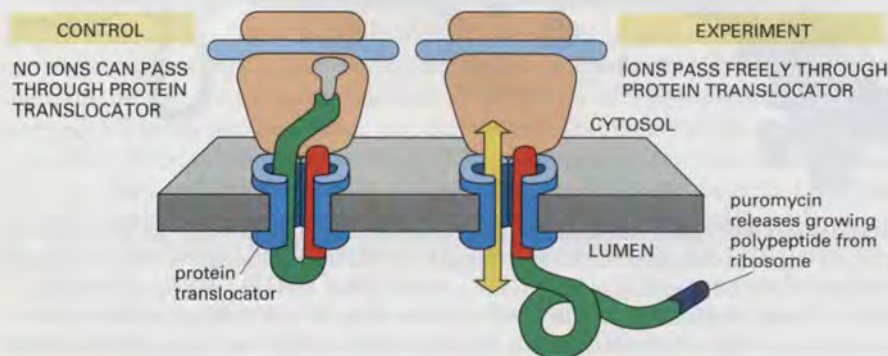


Figure 12-42 The demonstration of protein-translocating aqueous pores in the ER membrane. The experimental set-up is similar to that shown in Figure 10-5, with an artificial lipid bilayer separating two aqueous compartments. When rough microsomes are added to one of the compartments, they occasionally fuse with the lipid bilayer, incorporating a portion of ER membrane (with its bound ribosomes) into the bilayer. When the drug puromycin (dark blue) is added to the same compartment, it couples covalently to the carboxyl terminus of the growing polypeptide chain and releases it from the ribosome; pores of uniform size can now be detected as discrete increases in the electrical conductance across the membrane (the ion flow responsible for the increased electrical conductance is indicated by the yellow arrow). If the ribosomes are removed from the membrane with a high-salt wash, pores are no longer detected, indicating that ribosome binding is required to open (or assemble) the pore (not shown).

tein translocator. There is now strong evidence for a protein translocator. Normally, the pore in the translocator is plugged with the growing polypeptide chain that is in transit across the membrane; when the nascent chains are experimentally released from the ribosomes with the drug puromycin, however, the pores can be detected by the ion currents that flow through them. Although the pore is large enough to allow the passage of an unfolded polypeptide chain, it closes when the ribosome is removed from the membrane (Figure 12-42). Thus the pore seems to be a dynamic structure, opening when a ribosome with a growing polypeptide chain attaches to the membrane and closing when the ribosome detaches after the synthesis of the protein is completed.

The ER Signal Peptide Is Removed from Most Soluble Proteins After Translocation³¹

We have seen that in chloroplasts and mitochondria the signal peptides are cleaved from the precursor proteins once they have crossed the membrane. Similarly, amino-terminal ER signal peptides are removed by a signal peptidase on the luminal side of the ER membrane. The peptide by itself, however, is not sufficient to signal cleavage by the peptidase; this requires an adjacent cleavage site that is specifically recognized by the peptidase. We shall see below that ER signal peptides that are contained within the polypeptide chain rather than at the amino terminus do not have these recognition sites and are never cleaved; instead, they can serve to retain transmembrane proteins in the lipid bilayer after the translocation process has been completed.

The amino-terminal ER signal peptide of a soluble protein itself has two signaling functions: in addition to directing the protein to the ER membrane, it is thought to serve as a **start-transfer signal**, which remains bound to the translocation apparatus while the rest of the protein is threaded continuously through the membrane as a large loop. Once the carboxyl terminus of the protein has passed through the membrane, the signal peptide is released from the translocator pore, cleaved off by the signal peptidase, and rapidly degraded to amino acids by other proteases in the ER while the protein is released into the ER lumen (Figure 12-43).

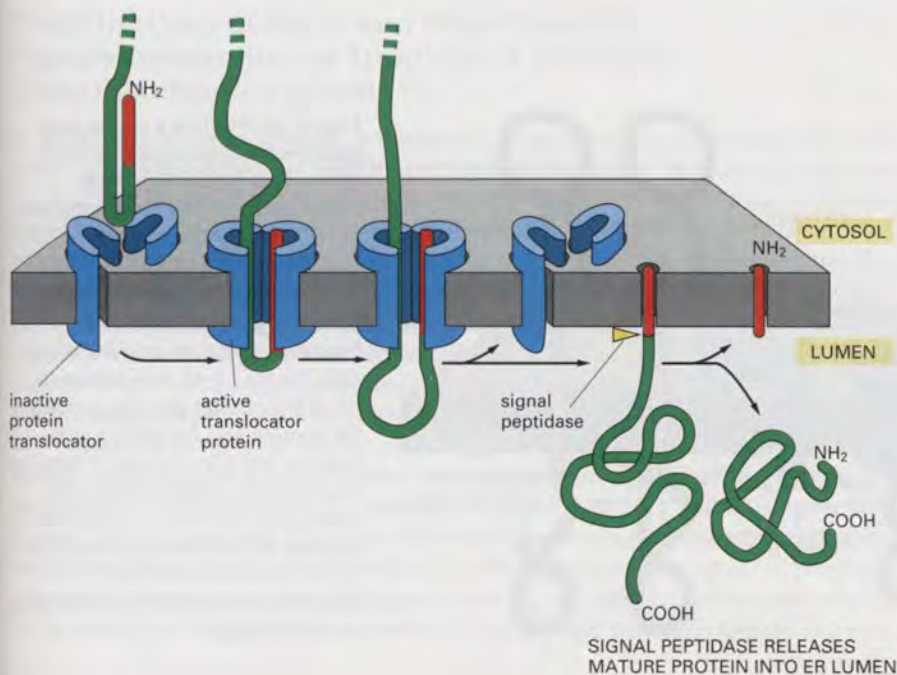


Figure 12-43 The translocation of a soluble protein across the ER membrane. In this hypothetical model the protein translocator in the membrane is postulated to exist in two alternative states—active or inactive. On binding an ER signal peptide (which acts as a start-transfer signal), the translocator adopts an active state and begins to transfer the polypeptide chain across the lipid bilayer as a loop. In this state it forms an aqueous pore across the membrane that can be detected electrophysiologically if the polypeptide chain is released (see Figure 12-42). After the protein has been completely translocated, the translocator reverts to an inactive conformation, which can no longer conduct ions across the membrane but is open to the lipid bilayer, allowing the hydrophobic signal peptide to diffuse out into the bilayer, where it is rapidly degraded. In this and the following two figures the ribosomes have been omitted for clarity.

In Single-Pass Transmembrane Proteins a Single Internal ER Signal Peptide Remains in the Lipid Bilayer as a Membrane-spanning α Helix³²

The translocation process for proteins destined to remain in the membrane is more complex than it is for soluble proteins, as some parts of the polypeptide chain are translocated across the lipid bilayer whereas others are not. Nevertheless, all modes of insertion of membrane proteins can be considered as 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 which **single-pass transmembrane proteins** (see Figure 10–13) become inserted into the ER.

In the simplest case an amino-terminal signal peptide 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 peptide** anchors the protein in the membrane after the ER signal (start-transfer) peptide is released from the translocator and is cleaved off (Figure 12–44). The stop-transfer peptide forms a single α -helical membrane-spanning segment, with the amino terminus of the protein on the luminal side of the membrane and the carboxyl terminus on the cytosolic side.

In the other two cases the signal peptide is internal, rather than at the amino-terminal end of the protein. Like the amino-terminal ER signal peptides, the internal signal peptide is recognized by SRP, which brings the ribosome making the protein to the ER membrane and serves as a start-transfer signal that initiates the translocation of the protein. After release from the translocator, the internal start-transfer peptide remains in the lipid bilayer as a single membrane-spanning α helix. Internal start-transfer peptides, however, can bind to the translocation apparatus in either of two orientations, and the orientation of the inserted start-transfer peptide, in turn, determines which protein segment (the one preceding or the one following the start-transfer peptide) is moved across the membrane into the ER lumen. In one case the resulting membrane protein has its carboxyl terminus on the luminal side (Figure 12–45A), while in the other it has its amino terminus on the luminal side (Figure 12–45B). The orientation of the start-transfer peptide depends on the distribution of nearby charged amino acids, as described in the figure legend.

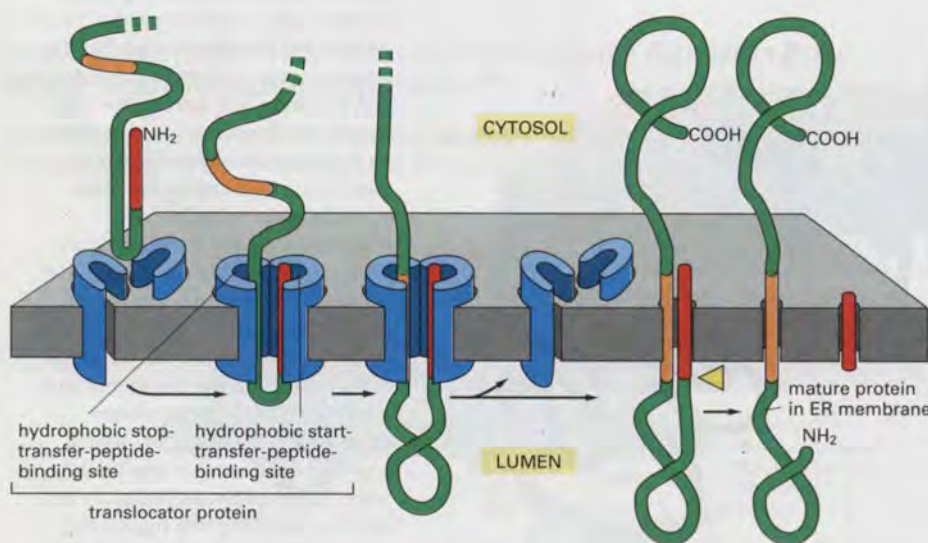


Figure 12–44 How a single-pass transmembrane protein with a cleaved ER signal peptide is integrated into the ER membrane. In this hypothetical model the co-translational translocation process is initiated by an amino-terminal ER signal peptide (red) that functions as a start-transfer signal as in Figure 12–43. In addition to the start-transfer peptide, however, the protein also contains a stop-transfer peptide (orange). When the stop-transfer peptide enters the translocator and interacts with a binding site, the translocator flips into its inactive state and discharges the protein laterally into the lipid bilayer.

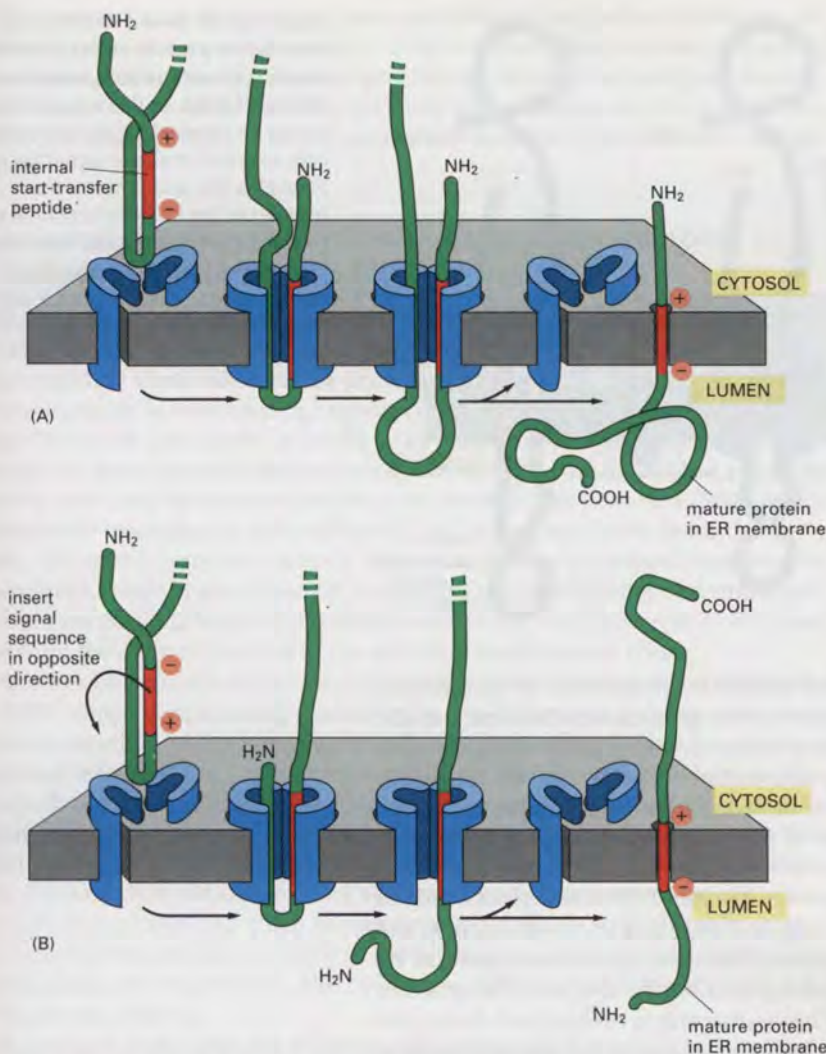


Figure 12-45 How a single-pass membrane protein with an internal signal peptide is integrated into the ER membrane. In this hypothetical model an internal ER signal peptide that functions as a start-transfer signal will bind to the translocator in such a way that its more positively charged end remains in the cytosol. If there are more positively charged amino acids immediately preceding the hydrophobic core of the start transfer peptide than there are following it on its carboxyl-terminal end, the start-transfer peptide will be inserted into the translocator in the orientation shown in (A), and the arm of the inserted loop carboxyl-terminal to the start-transfer sequence will be passed across the membrane. If, however, there are more positively charged amino acids immediately following the hydrophobic core of the start-transfer peptide than there are preceding it on its amino-terminal end, the start-transfer peptide will be inserted into the translocator in the orientation shown in (B), and the arm of the inserted loop amino-terminal to the start-transfer peptide will be passed across the membrane. Because translocation cannot start before a start-transfer sequence appears outside the ribosome, translocation of the amino-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 amino terminus is located in the ER lumen: that shown in Figure 12-44 and that shown in (B) here.

Combinations of Start- and Stop-Transfer Signals Determine the Topology of Multipass Transmembrane Proteins³³

In **multipass transmembrane proteins** the polypeptide chain passes back and forth repeatedly across the lipid bilayer (see Figure 10-13). It is thought that an internal signal peptide serves as a start-transfer signal in these proteins to initiate translocation, which continues until a stop-transfer peptide is reached. In double-pass transmembrane proteins, for example, the polypeptide is released into the bilayer at this point (Figure 12-46). In more complex multipass proteins, in which many hydrophobic α helices span the bilayer, a second start-transfer peptide reinitiates translocation further down the polypeptide chain until the next stop-transfer peptide causes polypeptide release, and so on for subsequent start-transfer and stop-transfer peptides (Figure 12-47).

Whether a given hydrophobic signal sequence will function as a start-transfer or stop-transfer peptide must depend on its location in a polypeptide chain, since its function can be switched by changing its location in the protein using recombinant DNA techniques. Thus the distinction between start-transfer and stop-transfer peptides 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 amino terminus and proceeds toward the car-

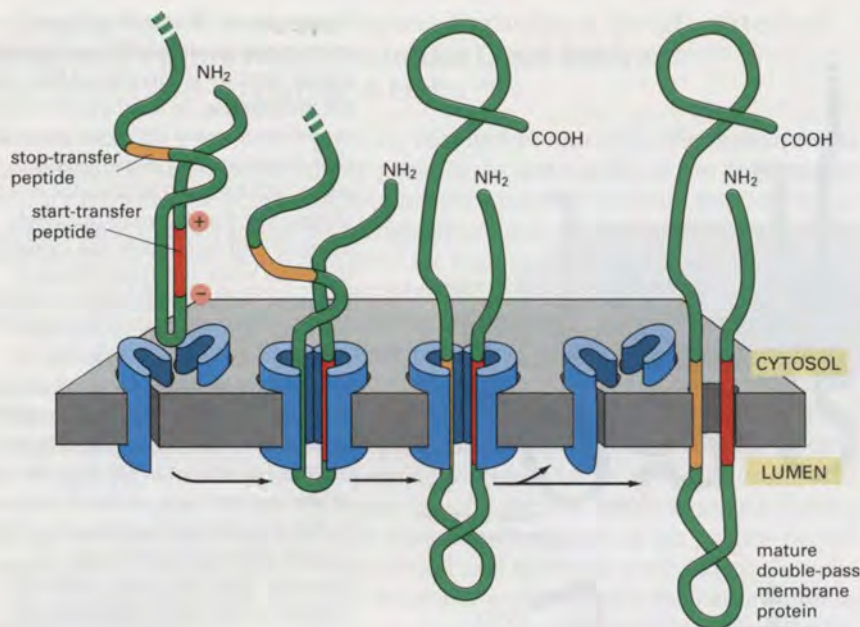


Figure 12-46 How a double-pass membrane protein with an internal signal sequence is integrated into the ER membrane. In this hypothetical model an internal ER signal peptide acts as a start-transfer signal (as in Figure 12-45) and initiates the transfer of the carboxyl terminal arm of the polypeptide chain. When a stop-transfer peptide enters the translocator, it discharges the protein laterally into the membrane.

boxyl 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": if translocation is initiated, the next appropriate hydrophobic segment will be recognized as a stop-transfer peptide, causing the region of the polypeptide chain in between to be threaded across the membrane. A similar scanning process continues until all of the hydrophobic regions in the protein have been inserted into the membrane.

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 domains exposed on the other. 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 the other membranes as well.

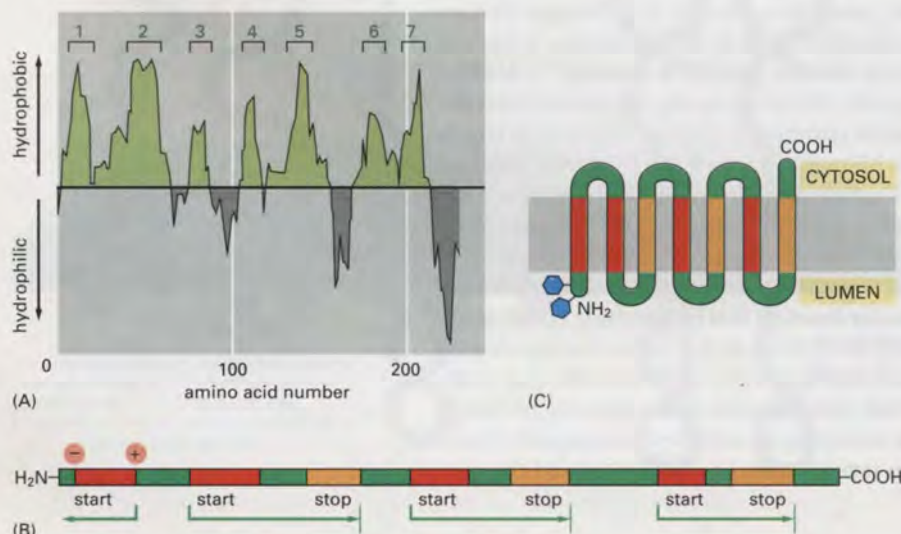


Figure 12-47 The insertion of the multipass membrane protein rhodopsin into the ER membrane. Rhodopsin is the light-sensitive protein in rod photoreceptor cells in the mammalian retina. (A) A hydrophobicity plot identifies seven short hydrophobic regions in rhodopsin. (B) The most amino-terminal region serves as a start-transfer peptide that causes the preceding amino-terminal portion of the protein to be passed across the ER membrane. Subsequent hydrophobic peptides will function in alternation as start-transfer and stop-transfer peptides. (C) The final integrated rhodopsin has its amino terminus located in the ER lumen and its carboxyl terminus located in the cytosol. The blue hexagons represent covalently attached oligosaccharides.

When proteins are dissociated from a membrane and reconstituted in 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 protein but to result solely from the process by which proteins are inserted into the ER membrane from the cytosol.

Translocated Polypeptide Chains Fold and Assemble in the Lumen of the Rough ER ³⁴

Many of the proteins in the lumen of the ER are in transit, *en route* to other destinations; others, however, are normally resident there and are present at high concentrations. These **ER resident proteins** contain an **ER retention signal** of four amino acids at their carboxyl terminus that is responsible for retaining the protein in the ER (see Table 12-3). Some of these proteins function as catalysts that help the many proteins that are translocated into the ER to fold and assemble correctly. One such ER resident protein is *protein disulfide isomerase (PDI)*, which catalyzes the oxidation of free sulfhydryl (SH) groups to form disulfide (S—S) bonds. Almost all cysteine residues in protein domains exposed to either the extracellular space or the lumen of organelles in the secretory and endocytic pathways are disulfide bonded; disulfide bonds do not form, however, in domains exposed to the cytosol because of the reducing environment there.

Another ER resident protein is a chaperone protein known as **binding protein (BiP)**, which is structurally related to the hsp70 proteins and, like them, recognizes incorrectly folded proteins, as well as protein subunits that have not yet assembled into their final oligomeric complexes. BiP, like other chaperone proteins, is thought to bind to exposed amino acid sequences that would normally be buried in the interior of correctly folded or assembled polypeptide chains. The bound BiP both prevents the proteins from aggregating and helps to keep them in the ER (and thus out of the Golgi apparatus and later parts of the secretory pathway); it may also help them to fold normally. Like the hsp70 family of proteins, which bind unfolded proteins in the cytosol and facilitate their import into mitochondria and chloroplasts, BiP hydrolyzes ATP to provide the energy for its role in protein folding.

As we have seen earlier for mitochondrial hsp70 (see Figure 12-24), the binding of BiP to an unfolded protein chain emerging in the ER lumen may help pull the protein into the ER. This pulling process may be particularly important for proteins that enter the ER posttranslationally because in this case there is no ribosome attached to the membrane to help push the nascent protein through the translocator during the protein's synthesis.

Most Proteins Synthesized in the Rough ER Are Glycosylated by the Addition of a Common N-linked Oligosaccharide ³⁵

The covalent addition of sugars to proteins is one of the major biosynthetic functions of the ER. Most of the soluble and membrane-bound proteins that are made in the ER, including those destined for transport to the Golgi apparatus, lysosomes, plasma membrane, or extracellular space are **glycoproteins**. In contrast, very few proteins in the cytosol are glycosylated, and those that are carry a much simpler sugar modification in which a single *N*-acetylglucosamine group is added to a serine or threonine residue of the protein.

An important advance in understanding the process of **protein glycosylation** was the discovery that a preformed oligosaccharide (composed of *N*-acetylglucosamine, mannose, and glucose and containing a total of 14 sugar residues) is transferred *en bloc* to proteins in the ER. Because this oligosaccharide is transferred to the side-chain NH₂ group of an asparagine amino acid in the protein, it is said to be *N-linked* or *asparagine-linked* (Figure 12-48). The transfer is cata-

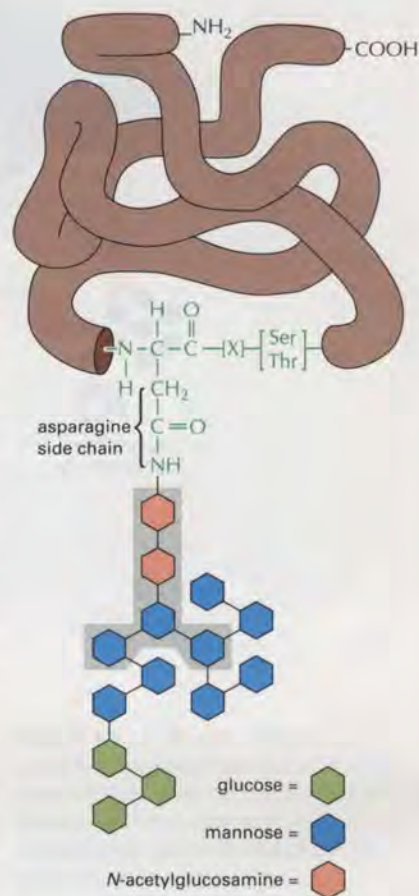


Figure 12-48 The asparagine-linked (N-linked) oligosaccharide that is added to most proteins in the rough ER membrane. The five sugar residues in the gray box form the "core region" of this oligosaccharide. For many glycoproteins only the core sugars survive the extensive oligosaccharide trimming process that takes place in the Golgi apparatus. Only asparagines in the sequences *Asn-X-Ser* or *Asn-X-Thr* (where X is any amino acid except proline) become glycosylated. These two 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.

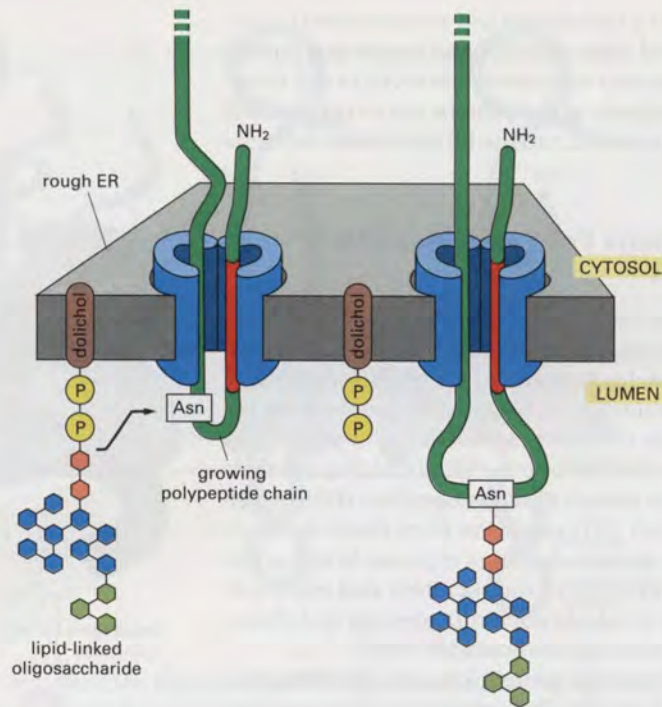


Figure 12-49 Protein glycosylation in the rough ER. Almost as soon as a polypeptide chain enters the ER lumen, it is glycosylated on target asparagine amino acids. The oligosaccharide shown in Figure 12-48 is transferred to the asparagine as an intact unit in a reaction catalyzed by a membrane-bound *oligosaccharyl transferase* enzyme. There is one copy of this enzyme associated with each protein translocator in the ER membrane.

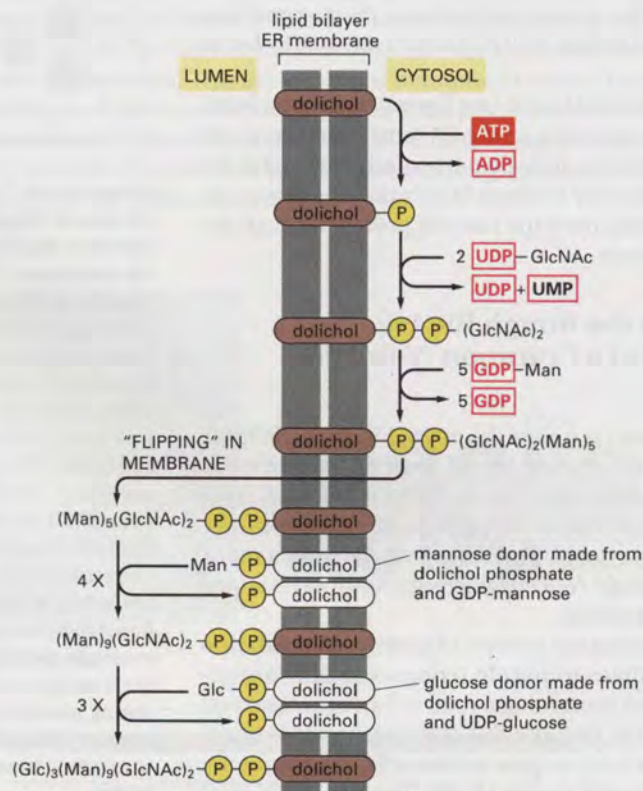


Figure 12-50 Synthesis of the lipid-linked precursor oligosaccharide in the rough ER membrane. The oligosaccharide is assembled sugar by sugar onto the carrier lipid dolichol (a polyisoprenoid—see Panel 2-4). Dolichol is long and very hydrophobic: its 22 five-carbon units can span the thickness of a lipid bilayer more than three times, so that the attached oligosaccharide is firmly anchored in the membrane. The first sugar group is linked to dolichol by a pyrophosphate bridge. This high-energy bond activates the oligosaccharide for its transfer from the lipid to an asparagine side chain of a nascent polypeptide on the luminal side of the rough ER. The synthesis of the oligosaccharide starts on the cytosolic side of the ER membrane and continues on the luminal face after the $(\text{Man})_5(\text{GlcNAc})_2$ lipid intermediate is flipped across the bilayer. All of the subsequent glycosyl transfer reactions on the luminal 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 thought to be flipped across the ER membrane. GlcNAc = *N*-acetylglucosamine; Man = mannose; Glc = glucose.

lyzed by a membrane-bound enzyme, an *oligosaccharyl transferase*, which has its active site exposed on the luminal side of the ER membrane; this explains why cytosolic proteins are not glycosylated in this way. The precursor oligosaccharide is held in the ER membrane by a special lipid molecule called **dolichol**, and it is transferred to the target asparagine in a single enzymatic step immediately after that amino acid emerges in the ER lumen during protein translocation (Figure 12–49). Since most proteins are co-translationally imported into the ER, *N*-linked oligosaccharides are almost always added during protein synthesis.

The lipid-linked precursor oligosaccharide is linked to the dolichol by a high-energy pyrophosphate bond, which provides the activation energy that drives the glycosylation reaction illustrated in Figure 12–49. The entire oligosaccharide is built up sugar by sugar on this membrane-bound lipid molecule prior to its transfer to a protein. The sugars are first activated in the cytosol by the formation of *nucleotide-sugar intermediates*, which then donate their sugar (directly or indirectly) to the lipid in an orderly sequence. Partway through this process, the lipid-linked oligosaccharide is flipped from the cytosolic to the luminal side of the ER membrane (Figure 12–50).

All of the diversity of the *N*-linked oligosaccharide structures on mature glycoproteins results from later modification of the original precursor structure. While still in the ER, three glucose residues (see Figure 12–48) and one mannose residue are quickly removed from the oligosaccharides of most glycoproteins. This oligosaccharide “trimming” or “processing” continues in the Golgi apparatus and is discussed in Chapter 13.

The *N*-linked oligosaccharides are by far the most common ones found on glycoproteins. Less frequently, oligosaccharides are linked to the hydroxyl group on the side chain of a serine, threonine, or hydroxylysine amino acid. These *O*-linked oligosaccharides are formed in the Golgi apparatus by pathways that are not yet fully understood (discussed in Chapter 13).

Some Membrane Proteins Exchange a Carboxyl-Terminal Transmembrane Tail for a Covalently Attached Glycosylphosphatidylinositol (GPI) Anchor After Entry into the ER³⁶

As discussed in Chapter 10, several cytosolic enzymes catalyze the covalent addition of a single fatty acid chain or prenyl group to selected proteins to help direct these proteins to cell membranes. A related process is catalyzed by enzymes in the rough ER: the carboxyl terminus of some membrane proteins destined for the plasma membrane is covalently attached to a sugar residue of a glycolipid. This linkage forms in the lumen of the ER by the mechanism illustrated in Figure 12–51, and it adds a **glycosylphosphatidylinositol (GPI) anchor**, which

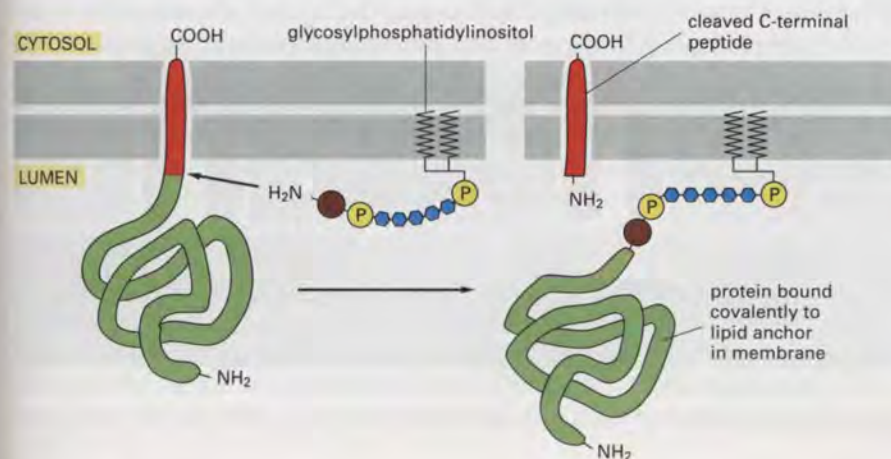


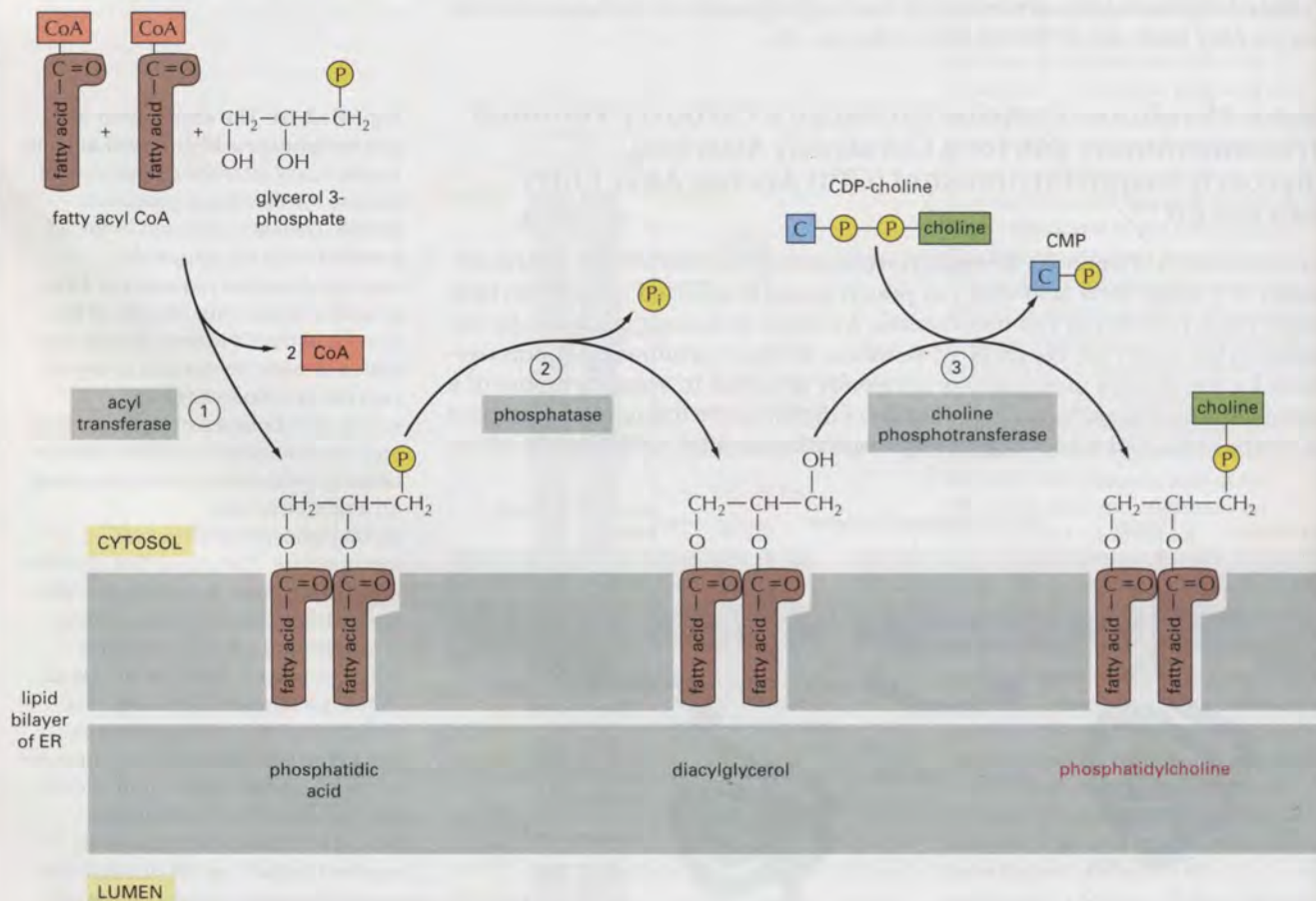
Figure 12–51 The attachment of a glycosylphosphatidylinositol anchor. Immediately after the completion of protein synthesis, the precursor protein remains anchored in the ER membrane by a hydrophobic carboxyl-terminal sequence of 15 to 20 amino acids, with the rest of the protein in the ER lumen. Within less than a minute, an enzyme in the ER cuts the protein free from its membrane-bound carboxyl terminus and simultaneously attaches the new carboxyl terminus to an amino group on a preassembled glycosylphosphatidylinositol intermediate. The signal that specifies this modification is contained within the hydrophobic carboxyl-terminal sequence and a few amino acids adjacent to it on the luminal 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 luminal side of the ER and eventually on the cell exterior.

contains two fatty acids, to the protein. At the same time the transmembrane segment of the protein is cleaved off. An increasing number of plasma membrane proteins have been shown to be modified in this way. Since these proteins are attached to the exterior of the plasma membrane only by their GPI anchors, in principle they can 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 if attacked by the immune system.

Most Membrane Lipid Bilayers Are Assembled in the ER ³⁷

The ER membrane produces nearly all of the lipids required for the elaboration of new cell membranes, including both phospholipids and cholesterol. The major phospholipid made is *phosphatidylcholine* (also called lecithin), which can be formed in three steps from choline, two fatty acids, and glycerol phosphate. Each step is catalyzed by enzymes in the ER membrane that have their active sites facing the cytosol, where all of the required metabolites are found. Thus phospholipid synthesis occurs exclusively in the cytosolic half of the ER bilayer. In the first step acyl transferases successively add two fatty acids to glycerol phosphate to produce phosphatidic acid, a compound sufficiently water-insoluble to remain in the lipid bilayer after it has been synthesized. It is this step that enlarges the lipid bilayer; the later steps determine the head group of a newly formed lipid molecule, and therefore the chemical nature of the bilayer, but do not result in net membrane growth (Figure 12–52). The two other major membrane phospholipids—phosphatidylethanolamine (PE) and phosphatidylserine (PS)—as well as the minor phospholipid phosphatidylinositol (PI), are all synthesized in this way.

Figure 12–52 The synthesis of phosphatidylcholine. This phospholipid is synthesized from fatty acyl-coenzyme A (fatty acyl CoA), glycerol 3-phosphate, and cytidine-bisphosphocholine (CDP-choline).



As phospholipid synthesis takes place in the cytosolic half of the ER bilayer, there needs to be a mechanism that transfers some of the newly formed phospholipid molecules to the other half of the bilayer. In synthetic lipid bilayers, lipids do not “flip-flop” in this way. 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 transbilayer movement is thought to be mediated by **phospholipid translocators** that are head-group-specific. In particular, the ER membrane seems to contain a translocator (a “*flippase*”) that transfers choline-containing phospholipids—but not ethanolamine-, serine-, or inositol-containing phospholipids—between cytosolic and luminal faces. This means that phosphatidylcholine reaches the luminal face much more readily than the other phospholipids. In this way the translocator is responsible for the asymmetric distribution of the lipids in the bilayer (Figure 12–53).

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

Phospholipid Exchange Proteins Help Transport Phospholipids from the ER to Mitochondria and Peroxisomes³⁸

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 that transfer both proteins and lipids. Mitochondria, plastids, and peroxisomes do not belong to this system, and they require different mechanisms for the import of proteins and lipids for growth. We have already seen that most (for mitochondria and plastids) or all (for peroxisomes) of the proteins in these organelles are imported 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 cellular membranes. In either case, special mechanisms are required for the transfer.

Water-soluble carrier proteins—called **phospholipid exchange proteins** (or *phospholipid transfer proteins*)—have been shown in *in vitro* experiments to have the ability to transfer individual phospholipid molecules between membranes. Each exchange protein recognizes only specific types of phospholipids. It functions by “extracting” a molecule of the appropriate phospholipid from a membrane and diffusing away with the lipid buried within its binding site. When it encounters another membrane, the exchange protein tends to discharge the bound phospholipid molecule into the new lipid bilayer (Figure 12–54). It has been proposed that phosphatidylserine is imported into mitochondria in this way and then decarboxylated to yield phosphatidylethanolamine, while phosphatidylcholine is imported intact.

Exchange proteins act to distribute phospholipids at random among all membranes present. In principle, such a random exchange process can result in a net transport of lipids from a lipid-rich to a lipid-poor membrane, allowing phosphatidylcholine and phosphatidylserine molecules, for example, to be transferred from the ER, where they are synthesized, to a mitochondrial or peroxisomal

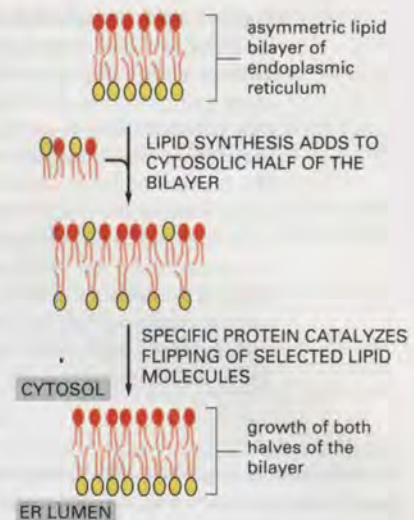
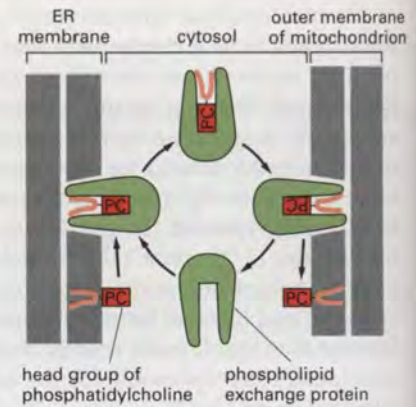


Figure 12–53 The role of phospholipid translocators in lipid bilayer synthesis. Since new lipid molecules are added only to the cytosolic half of the bilayer and lipid molecules do not flip spontaneously from one monolayer to the other, membrane-bound phospholipid translocator proteins (“flippases”) are required to transfer selected lipid molecules from the cytosolic half to the luminal half so that the membrane grows as a bilayer. Because the flippase in the ER membrane preferentially recognizes and transfers choline-containing head groups, an asymmetric bilayer is generated, with the luminal monolayer (which produces the outer half of the plasma membrane bilayer) highly enriched for phosphatidylcholine.

Figure 12-54 Phospholipid exchange proteins. Because phospholipids are insoluble in water, their passage between membranes requires a carrier protein. Phospholipid exchange proteins are water-soluble proteins that carry a single molecule of phospholipid at a time; they can pick up a lipid molecule from one membrane and release it at another and thereby redistribute phospholipids between membrane-bounded compartments. The transfer of phosphatidylcholine (PC) from ER to mitochondria can occur without the input of additional energy because the concentration of PC is high in the ER membrane (where it is made) and low in the mitochondrial outer membrane. One would predict that there must be a flippase in the outer mitochondrial membrane to equilibrate the lipids between the two leaflets of the bilayer, and there must be a mechanism to transfer lipids between the outer and inner mitochondrial membrane. These postulated pathways, however, remain to be discovered.



membrane. It might be that mitochondria and peroxisomes are the only “lipid-poor” organelles in the cytosol and that such an exchange process is sufficient, although other, more specific mechanisms probably also exist for transporting phospholipids to these organelles.

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 ER: all proteins destined for secretion and all proteins destined 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.

Only proteins that carry a special hydrophobic signal peptide are imported into the ER. The ER signal peptide 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 membrane initiates the translocation process that threads a loop of polypeptide chain across the ER membrane through a hydrophilic pore in 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 across the ER membrane but are not released into the lumen; instead, they remain anchored in the lipid bilayer by one or more membrane-spanning α -helical regions in their polypeptide chain. 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.

The asymmetry of lipid synthesis, protein insertion, and glycosylation in the ER establishes the polarity of the membranes of all of the other organelles that the ER supplies with lipids and membrane proteins.

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