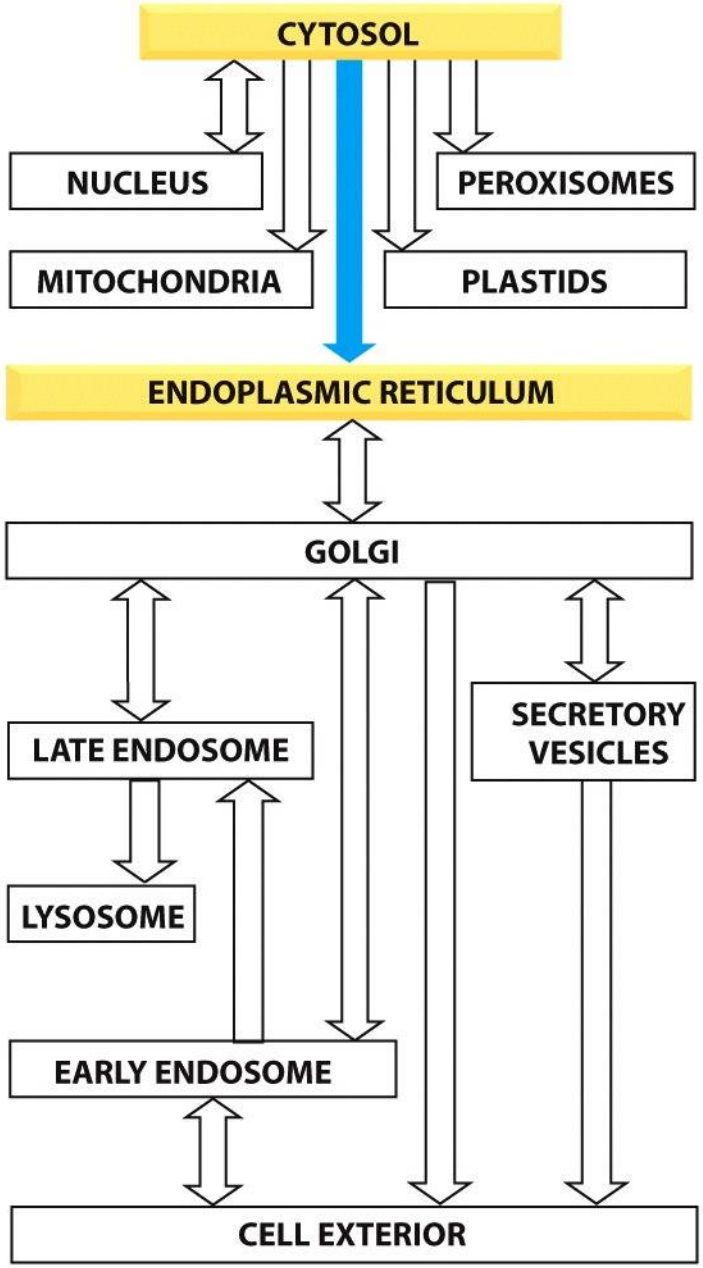
Molecular Biology of the Cell

Chapter 12

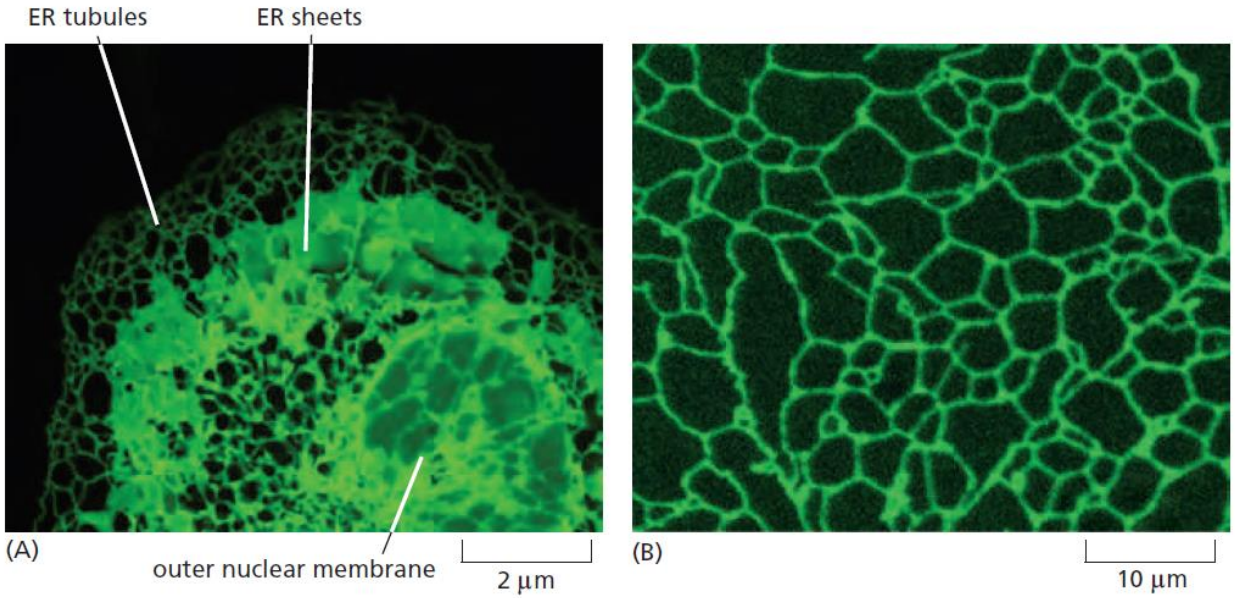
Intracellular Compartments and Protein Sorting 2


CYTOSOL
NUCLEUS
PEROXISOMES
MITOCHONDRIA
PLASTIDS
ENDOPLASMIC RETICULUM
GOLGI
LATE ENDOSOME
SECRETORY VESICLES
LYSOSOME
EARLY ENDOSOME
CELL EXTERIOR

The Endoplasmic Reticulum

- All eukaryotic cells have an endoplasmic reticulum (ER).
- Its membrane typically constitutes more than half of the total membrane of an average animal cell.
- The ER is organized into a **netlike labyrinth of branching tubules and flattened sacs** that extends throughout the cytosol.
- The tubules and sacs interconnect, and their membrane is continuous with the outer nuclear membrane; the compartment that they enclose therefore is also continuous with the space between the inner and outer nuclear membranes.
- The ER has a central role in both lipid and protein biosynthesis, and it also serves as an intracellular Ca2+ store that is used in many cell signaling responses.

- The ER membrane is the site of production of **all the transmembrane proteins and lipids** for most of the cell's organelles, including the ER itself, the Golgi apparatus, lysosomes, endosomes, secretory vesicles, and the plasma membrane.
- The ER membrane is also the site at which **most of the lipids** for mitochondrial and peroxisomal membranes are made.
- In addition, almost **all of the proteins** that will be secreted to the cell exterior—plus those destined for the lumen of the ER, Golgi apparatus, or lysosomes—are initially delivered to the ER lumen.



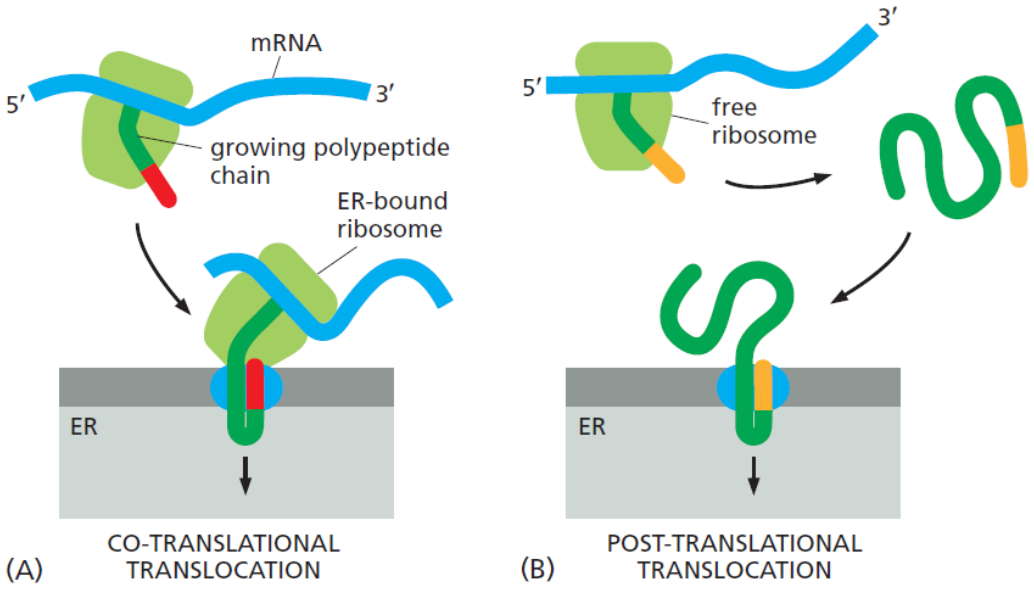
Fluorescent micrographs of the endoplasmic reticulum.

(A) An **animal cell in tissue culture** that was genetically engineered to express an ER membrane protein fused to a fluorescent protein. The ER extends as a network of tubules and sheets throughout the entire cytosol, so that all regions of the cytosol are close to some portion of the ER membrane. The outer nuclear membrane, which is continuous with the ER, is also stained.

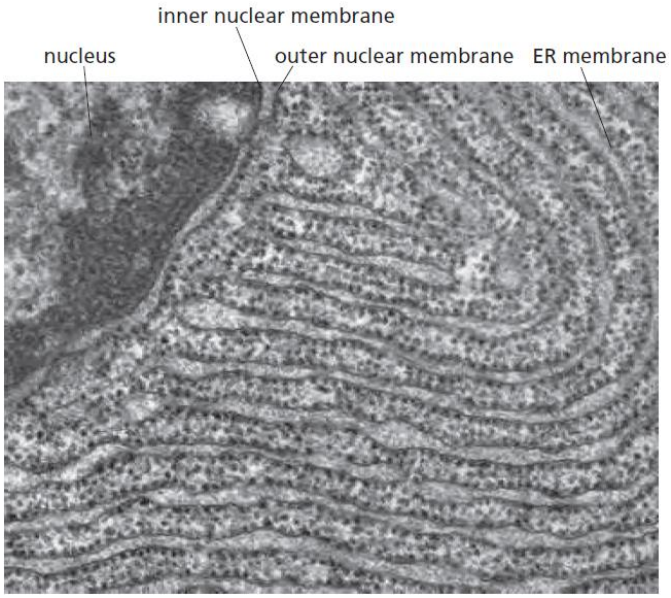
(B) Part of an ER network in a **living plant cell** that was genetically engineered to express a fluorescent protein in the ER.

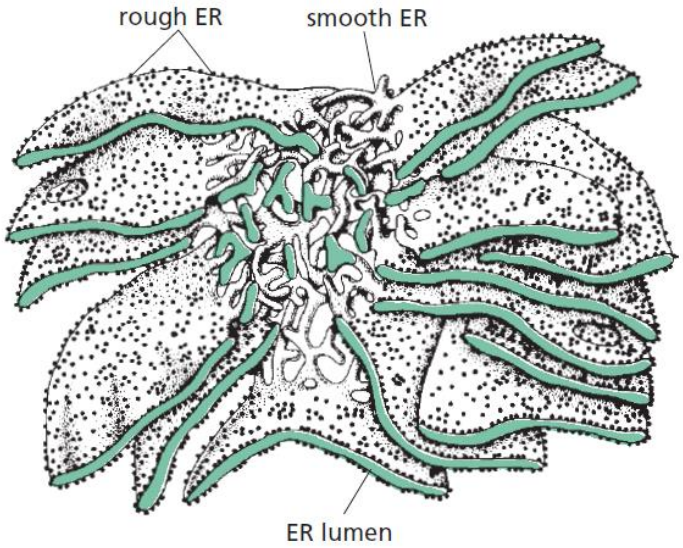
The ER Is Structurally and Functionally Diverse

- Various functions of the ER are essential to every cell.
- Their relative importance varies greatly between individual cell types.
- To meet different functional demands, **distinct regions** of the ER become highly specialized.
- We observe such **functional specialization** as dramatic changes in ER structure, and different cell types can therefore possess characteristically different types of ER membrane.
- One of the most remarkable ER specializations is the **rough ER**.
- Mammalian cells begin to import most proteins into the ER before complete synthesis of the polypeptide chain—that is, import is a **co-translational** process.
- In contrast, the import of proteins into mitochondria, chloroplasts, nuclei, and peroxisomes is a **post-translational** process.



- In co-translational transport, the ribosome that is synthesizing the protein is attached directly to the ER membrane, enabling one end of the protein to be translocated into the ER while the rest of the polypeptide chain is being synthesized.
- These membrane-bound ribosomes coat the surface of the ER, creating regions termed **rough endoplasmic reticulum**, or **rough ER**;
- regions of ER that lack bound ribosomes are called **smooth endoplasmic reticulum**, or **smooth ER**.
- Most cells have scanty regions of smooth ER, and the ER is often partly smooth and partly rough.
- Areas of smooth ER from which transport vesicles carrying newly synthesized proteins and lipids bud off for transport to the Golgi apparatus are called **transitional ER**.





- In certain specialized cells, the smooth ER is abundant and has additional functions.

It is prominent, for example, in cells that specialize in **lipid metabolism**, such as cells that synthesize steroid hormones from cholesterol;

the expanded smooth ER accommodates the enzymes that make cholesterol and modify it to form the hormones.

- The main cell type in the liver, **the hepatocyte**, also has a substantial amount of smooth ER.

- It is the principal site of production of **lipoprotein particles**, which carry lipids via the bloodstream to other parts of the body.
- The enzymes that synthesize the lipid components of the particles are located in the membrane of the smooth ER,
- which also contains enzymes that catalyze a series of reactions to detoxify both lipid-soluble drugs and various harmful compounds produced by metabolism.

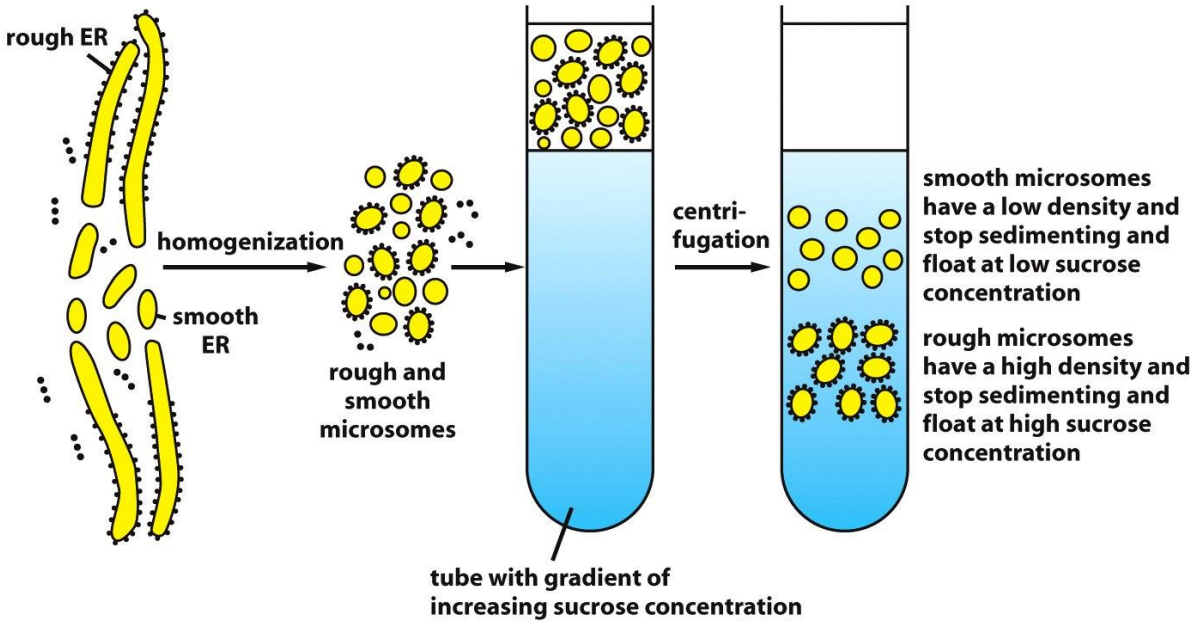
The most extensively studied of these **detoxification reactions** are carried out by the cytochrome P450 family of enzymes, which catalyze a series of reactions in which water-insoluble drugs or metabolites that would otherwise accumulate to toxic levels in cell membranes are rendered sufficiently **water-soluble** to leave the cell and be excreted in the urine.

Because the rough ER alone cannot house enough of these and other necessary enzymes, a substantial portion of the membrane in a hepatocyte normally consists of smooth ER.

- Another crucially important function of the ER in most eukaryotic cells is to sequester Ca2+ from the cytosol.
- The release of Ca2+ into the cytosol from the ER, and its subsequent reuptake, occurs in many rapid responses to extracellular signals.
- A **Ca2+ pump** transports Ca2+ from the cytosol into the ER lumen.
- A high concentration of **Ca2+-binding proteins** in the ER facilitates Ca2+ storage.
- In some cell types, and perhaps in most, specific regions of the ER are specialized for Ca2+ storage.
- Muscle cells have an abundant, modified smooth ER called the **sarcoplasmic reticulum**. The release and reuptake of Ca2+ by the sarcoplasmic reticulum trigger myofibril contraction and relaxation, respectively, during each round of muscle contraction.

- To study the functions and biochemistry of the ER, it is necessary to **isolate** it.

- When tissues or cells are disrupted by **homogenization**, the ER breaks into fragments, which **reseal** to form small (~100–200 nm in diameter) closed vesicles called **microsomes.**
- To the biochemist, microsomes represent small authentic versions of the ER, still capable of protein translocation, protein glycosylation, Ca2+ uptake and release, and lipid synthesis.
- Microsomes derived from rough ER are studded with ribosomes and are called **rough microsomes.**
- The ribosomes are always found on the outside surface, so the interior of the microsome is biochemically equivalent to the lumen of the ER.



- Many vesicles similar in size to rough microsomes, but lacking attached ribosomes, are also found in cell homogenates.
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- Such smooth microsomes are derived in part from smooth portions of the ER and in part from vesiculated fragments of the plasma membrane, Golgi apparatus, endosomes, and mitochondria (the ratio depending on the tissue).

- Thus, whereas rough microsomes are clearly derived from rough portions of ER, it is not easy to separate smooth microsomes derived from different organelles.
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- The smooth microsomes prepared from **liver or muscle cells** are an exception. Because of the unusually large quantities of smooth ER or sarcoplasmic reticulum, respectively, most of the smooth microsomes in homogenates of these tissues are derived from the smooth ER or sarcoplasmic reticulum.

- The ribosomes attached to rough microsomes make them more dense than smooth microsomes. As a result, we can use **equilibrium centrifugation** to separate the rough and smooth microsomes.

- **Microsomes have been invaluable in elucidating the molecular aspects of ER function**

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

- The ER signal sequence is guided to the ER membrane by at least two components:
- a **signal-recognition particle (SRP),** which cycles between the ER membrane and the cytosol and binds to the signal sequence,
- and an **SRP receptor** in the ER membrane.

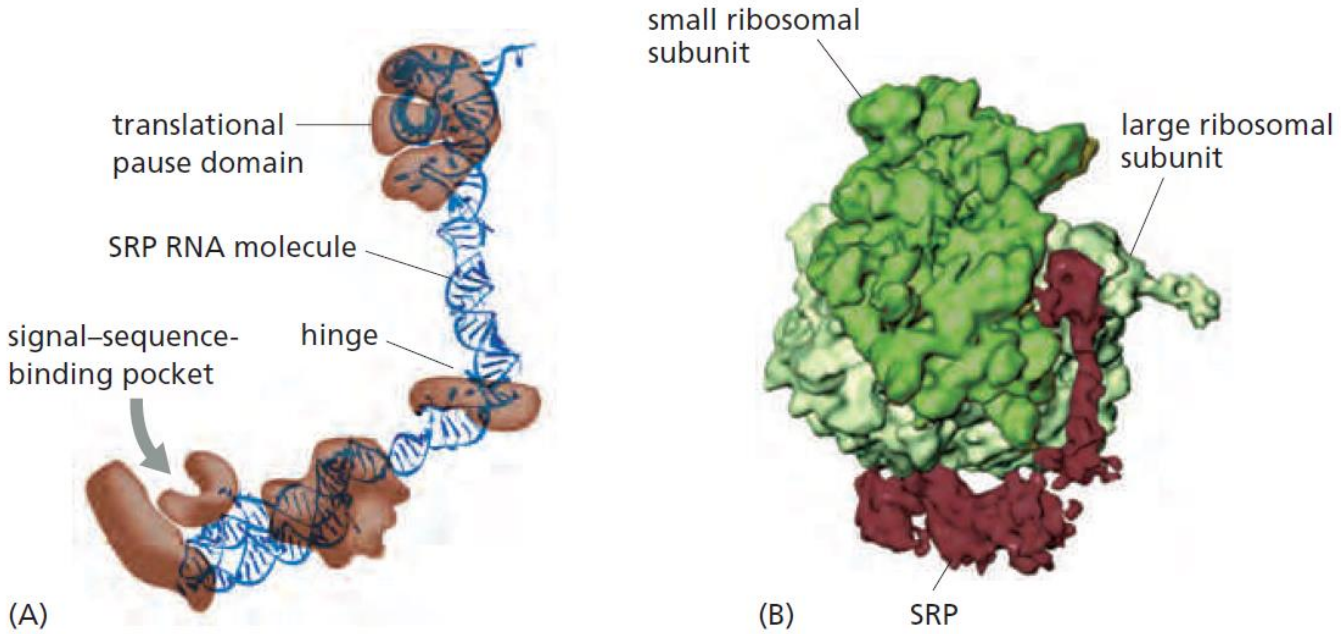
- The SRP is a large complex; in animal cells, it consists of six different polypeptide chains bound to a single small RNA molecule.

- ER signal sequences vary greatly in amino acid sequence, but each has **eight or more nonpolar amino acids at its center**.

- How can the SRP bind specifically to so many different sequences?

- The answer has come from the crystal structure of the SRP protein, which shows that the **signal-sequence-binding site is a large hydrophobic pocket lined by methionines**.

- Because methionines have unbranched, flexible side chains, the pocket is sufficiently plastic to accommodate **hydrophobic signal sequences** of different sequences, sizes, and shapes.

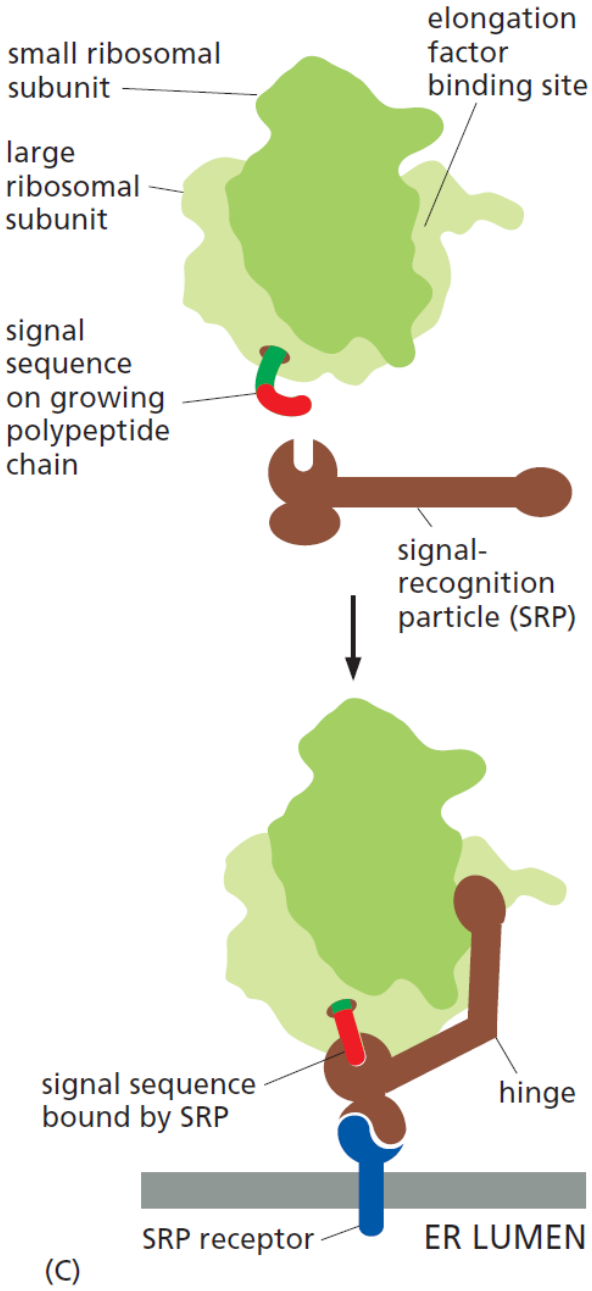


The signal-recognition particle (SRP).

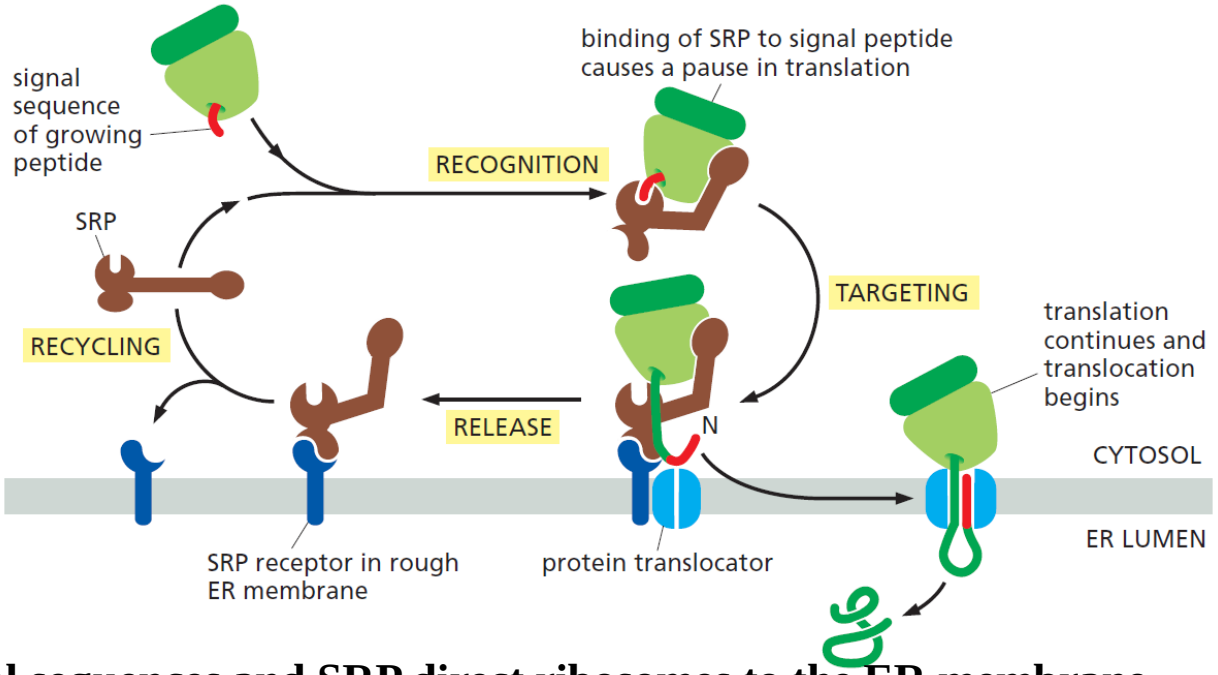
(A) A mammalian SRP is a rodlike ribonucleoprotein complex containing six protein subunits (brown) and one RNA molecule (blue). The SRP RNA forms a **backbone** that links the protein domain containing the **signal-sequencebinding pocket** to the domain responsible for . Crystal structures of various SRP pieces from different species are assembled here into a composite model to approximate the structure of a complete SRP. (B) The three-dimensional outline of the SRP bound to a ribosome was determined by cryoelectron microscopy. SRP binds to the large ribosomal subunit so that its signal-sequence-binding pocket is positioned near the growing polypeptide chain exit site, and its translational pause domain is positioned at the interface between the ribosomal subunits, where it interferes with **elongation factor binding**.

- The SRP is a **rodlike** structure, which wraps around the **large ribosomal subunit**, with one end binding to the ER signal sequence as it emerges from the ribosome as part of the newly made polypeptide chain;
- the other end blocks the **elongation factor binding site** at the interface between the large and small ribosomal subunits.
- This block **halts** protein synthesis as soon as the signal peptide has emerged from the ribosome.
- The transient pause presumably gives the ribosome enough time to bind to the ER membrane before completion of the polypeptide chain, thereby ensuring that the protein is not released into the cytosol.
- *This safety device may be especially important for secreted and lysosomal **hydrolases**, which could wreak havoc in the cytosol;
- cells that secrete large amounts of hydrolases, however, take the added precaution of having high concentrations of hydrolase inhibitors in their cytosol.
- * The pause also ensures that large portions of a protein that could **fold** into a compact structure are not made before reaching the translocator in the ER membrane.

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

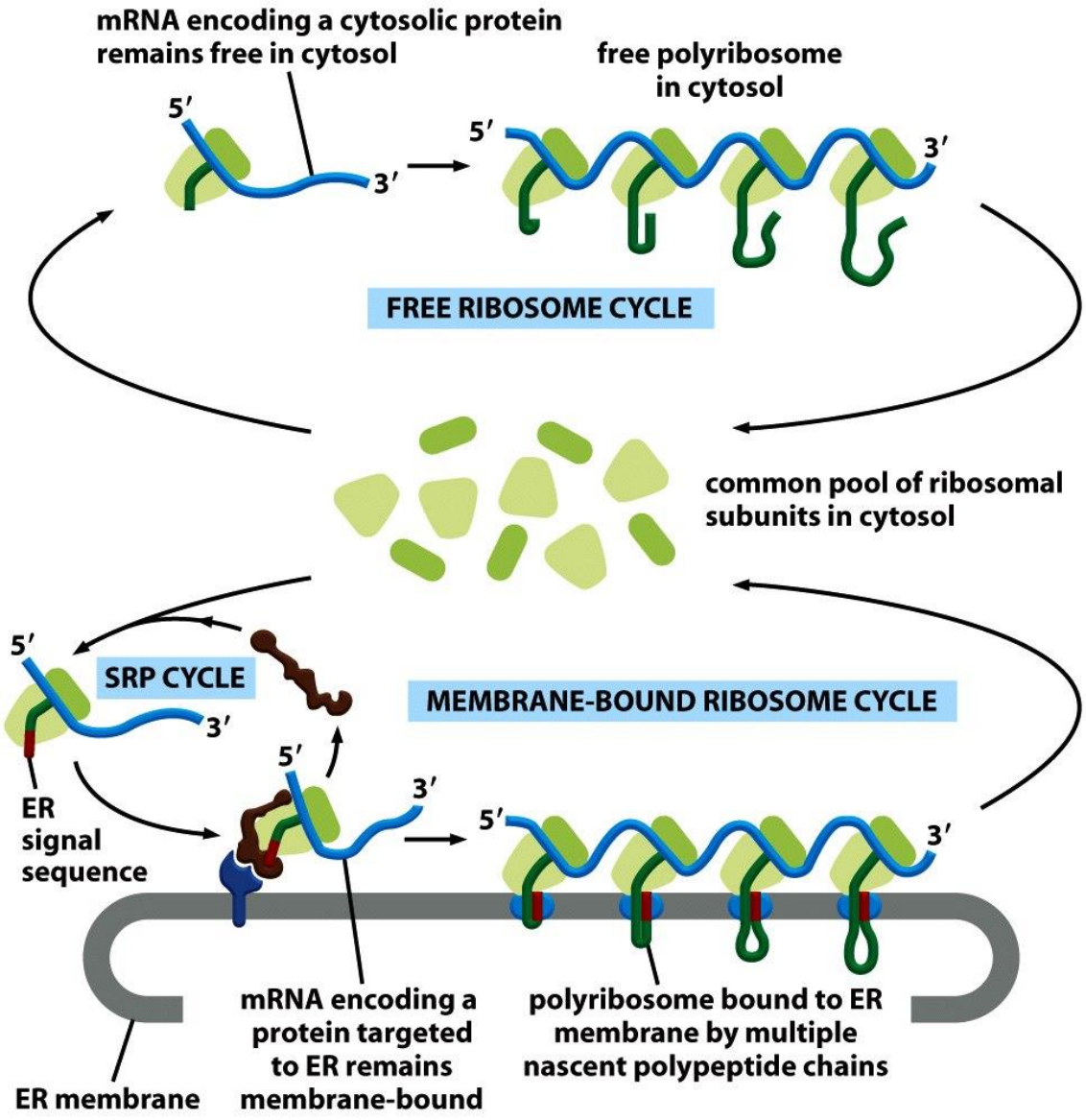


As a signal sequence emerges from the ribosome and binds to the SRP, a conformational change in the SRP exposes a binding site for the SRP receptor.



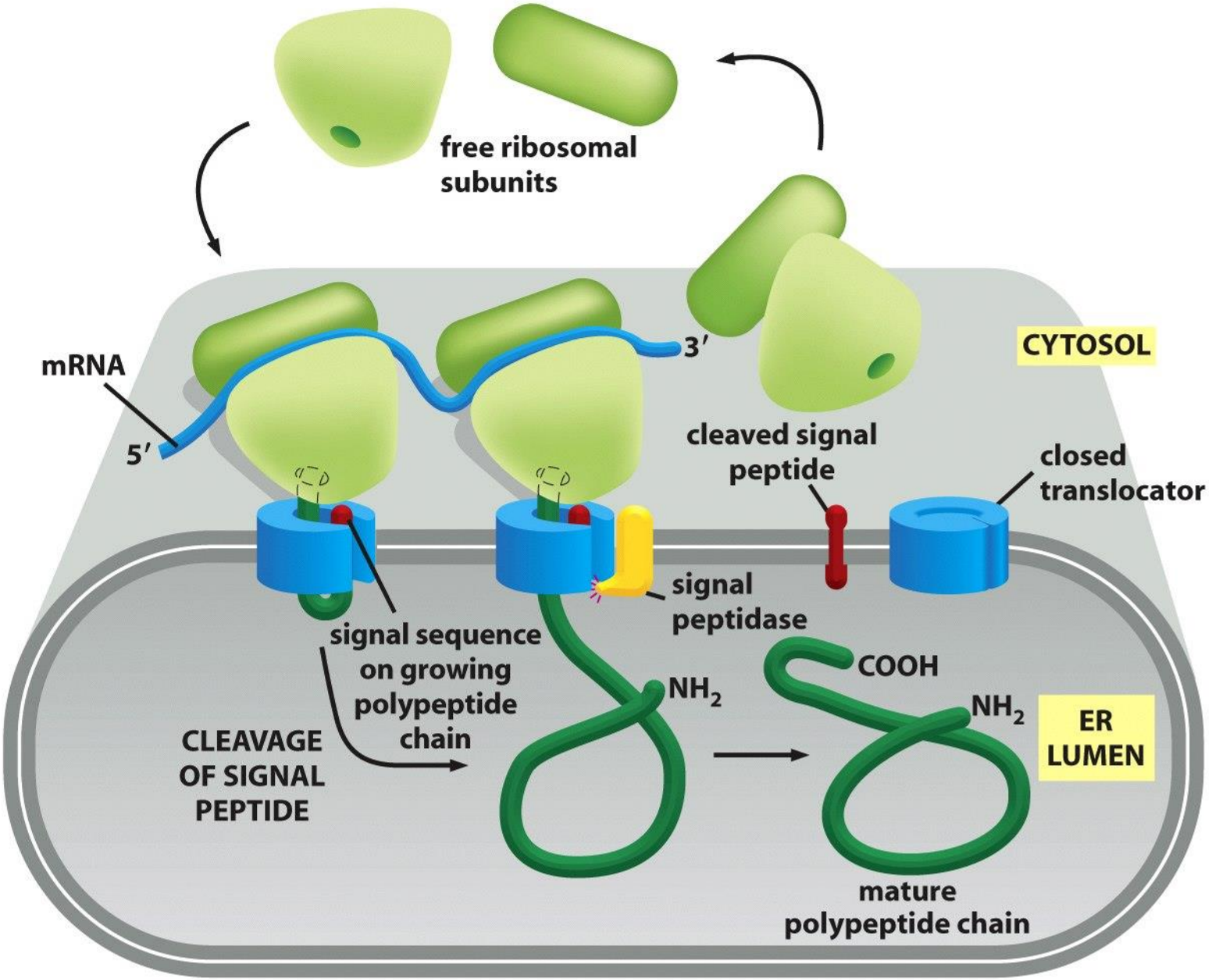
- **How ER signal sequences and SRP direct ribosomes to the ER membrane.**
- The SRP binds to both the exposed ER signal sequence and the ribosome, thereby inducing a pause in translation. The SRP receptor in the ER membrane, which in animal cells is composed of two different polypeptide chains, binds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

Free and membrane-bound polyribosomes

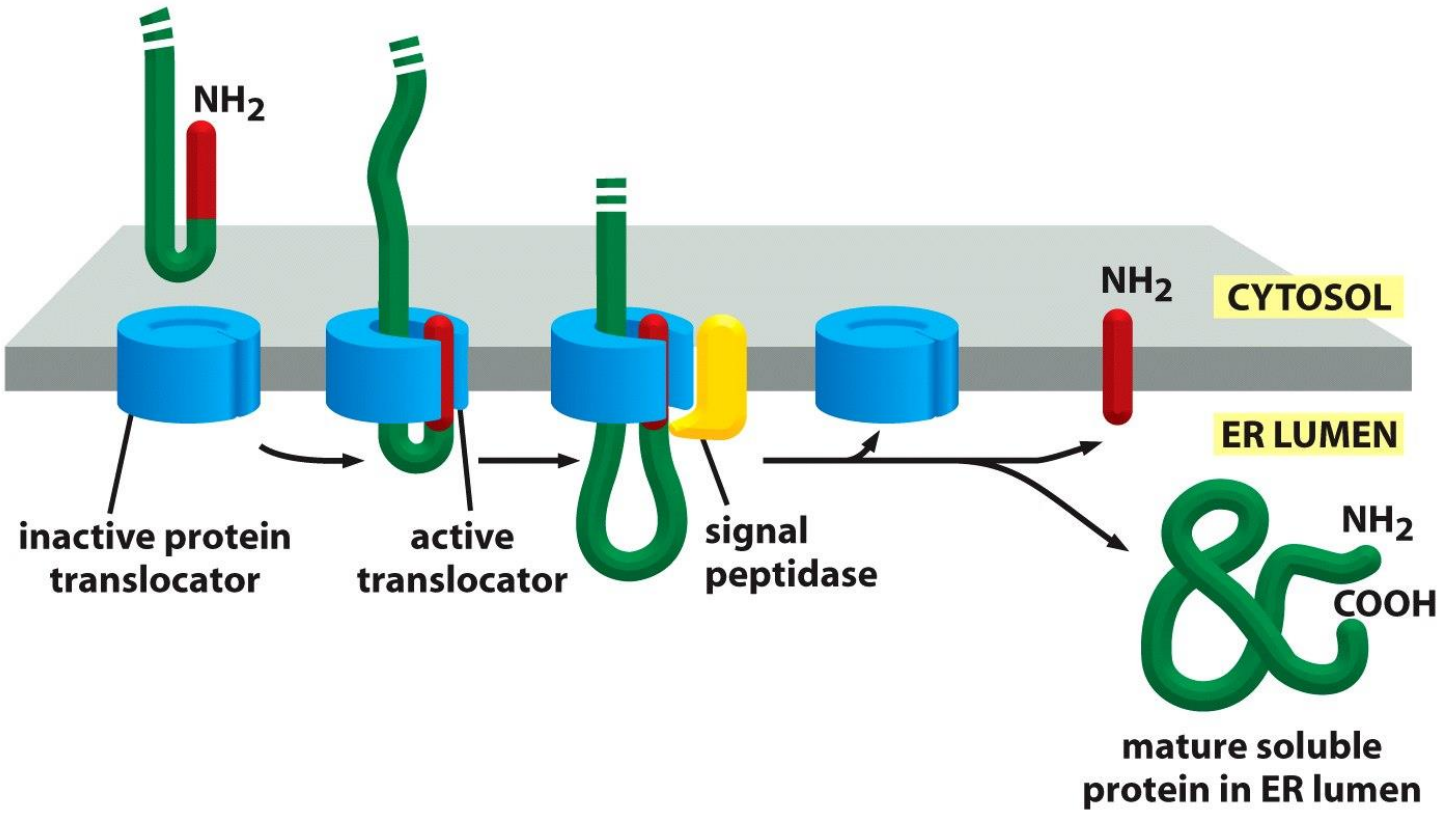


A common pool of ribosomes synthesizes the proteins that stay in the cytosol and those that are transported into the ER.

The signal hypothesis.



A model to explain how a soluble protein is translocated across the ER membrane



- On binding an ER signal sequence (which acts as a **start transfer signal**), the translocator **opens its pore**, allowing the transfer of the polypeptide chain across the lipid bilayer as a loop.
- After the protein has been completely translocated, the pore closes, but the translocator now opens laterally within the lipid bilayer, allowing the hydrophobic signal sequence to diffuse into the bilayer, where it is rapidly degraded.

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

- The ER signal sequence in the growing polypeptide chain is thought to trigger the opening of the pore in the **Sec61 protein translocator** (The core of the translocator, called the Sec61 complex):

after the signal sequence is released from the SRP and the growing chain has reached a sufficient length, the signal sequence binds to a **specific site** inside the pore itself, thereby **opening the pore**.

- An ER signal sequence is therefore recognized twice:

first by an **SRP** in the cytosol and then by a **binding site** in the pore of the protein translocator, where it serves as a **start-transfer signal** (or start-transfer peptide) that opens the pore.

- **Dual recognition** may help ensure that only appropriate proteins enter the lumen of the ER.

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

- While bound in the translocation pore, a signal sequence is in contact not only with the Sec61 complex, which forms the walls of the pore, but also, along the lateral seam, with the hydrophobic core of the lipid bilayer.

- When the nascent polypeptide chain grew long enough, the ER signal peptidase cleaved off the signal sequence and released it from the pore into the membrane, where it was rapidly degraded to amino acids by other proteases in the ER membrane.

- To release the signal sequence into the membrane, the **translocator opens laterally** along the seam.

- The translocator is therefore gated in two directions:

✓ it opens to form a pore across the membrane to let the **hydrophilic portions of proteins cross** the lipid bilayer, and

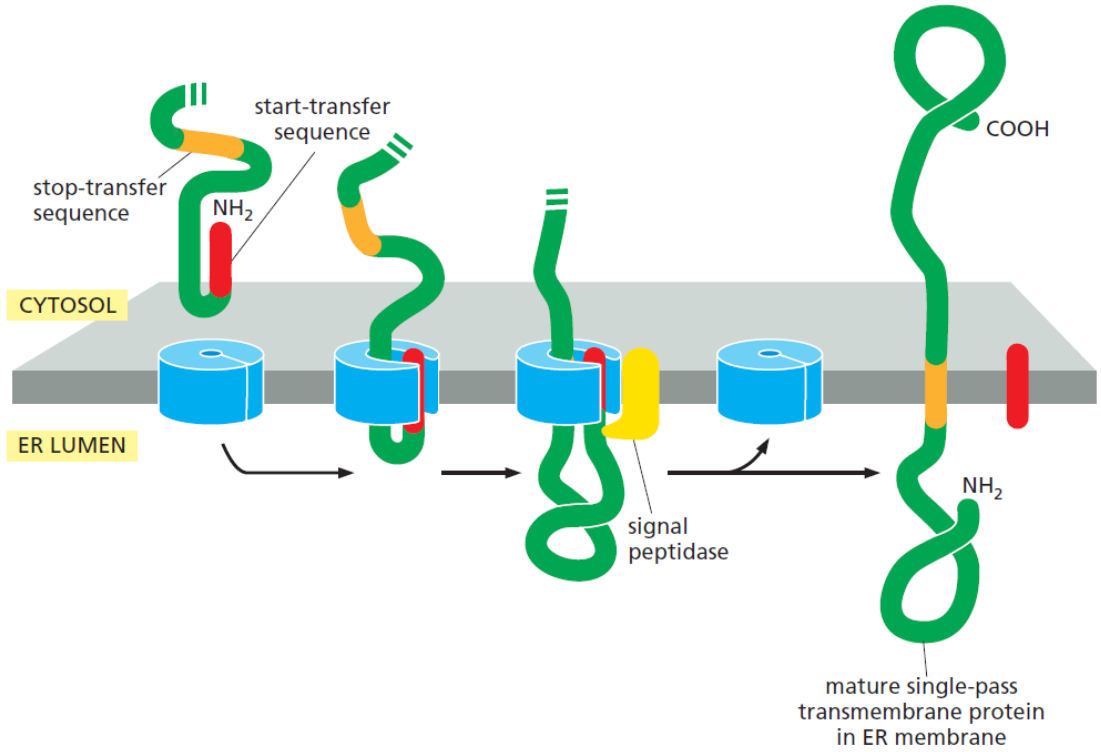
✓ it opens laterally within the membrane to let **hydrophobic portions of proteins partition** into the lipid bilayer.

- **Lateral gating of the pore is an essential step during the integration of transmembrane proteins.**

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

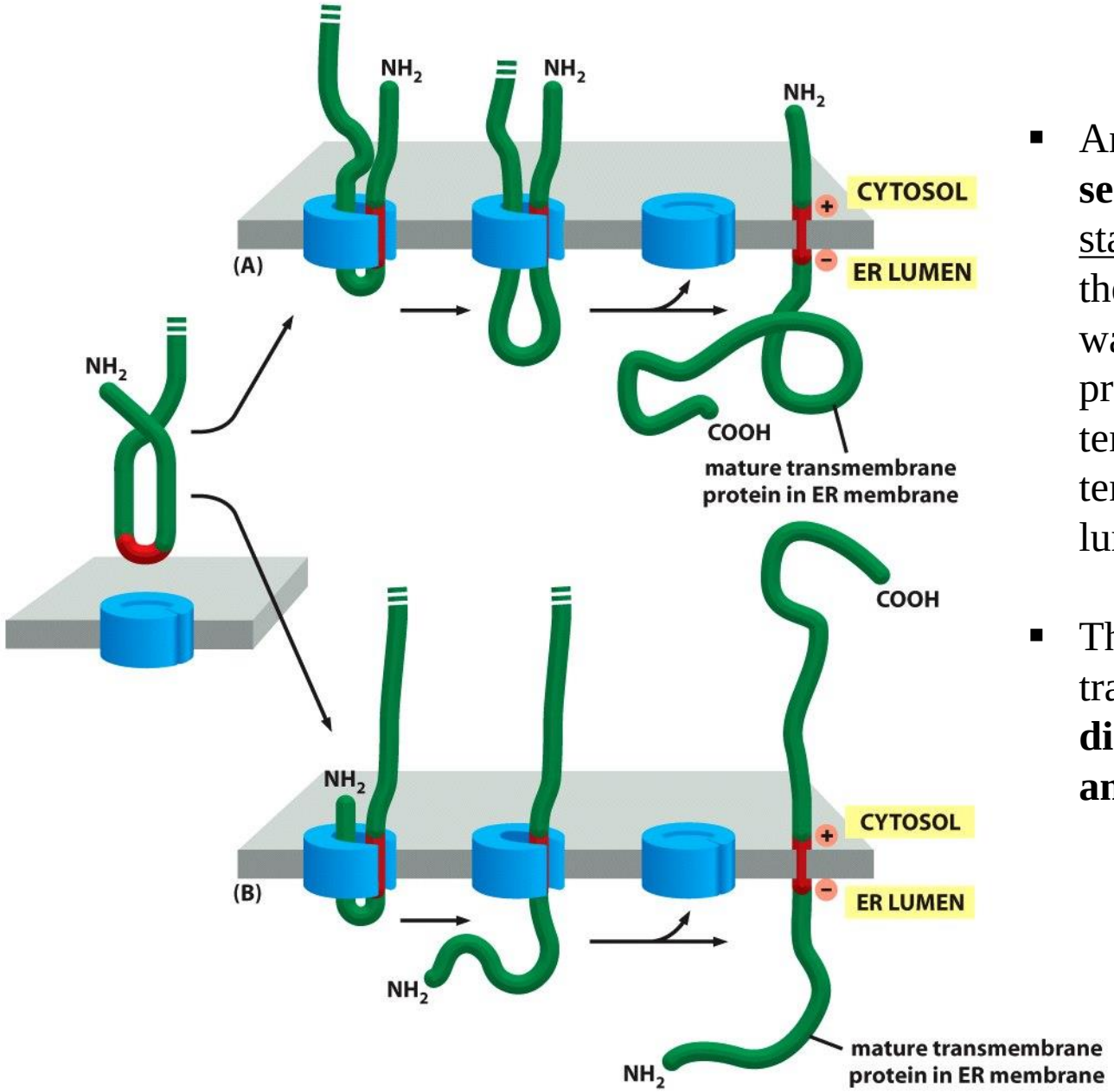
- The integration of membrane proteins requires that some parts of the polypeptide chain be translocated across the lipid bilayer whereas others are not.
- Despite this additional complexity, all modes of insertion of membrane proteins are simply variants of the sequence of events.
- There are the three ways in which single-pass transmembrane proteins become inserted into the ER membrane.

How a single-pass transmembrane protein with a cleaved ER signal sequence is integrated into the ER membrane



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

Integration of a single-pass transmembrane protein with an internal signal sequence into the ER membrane

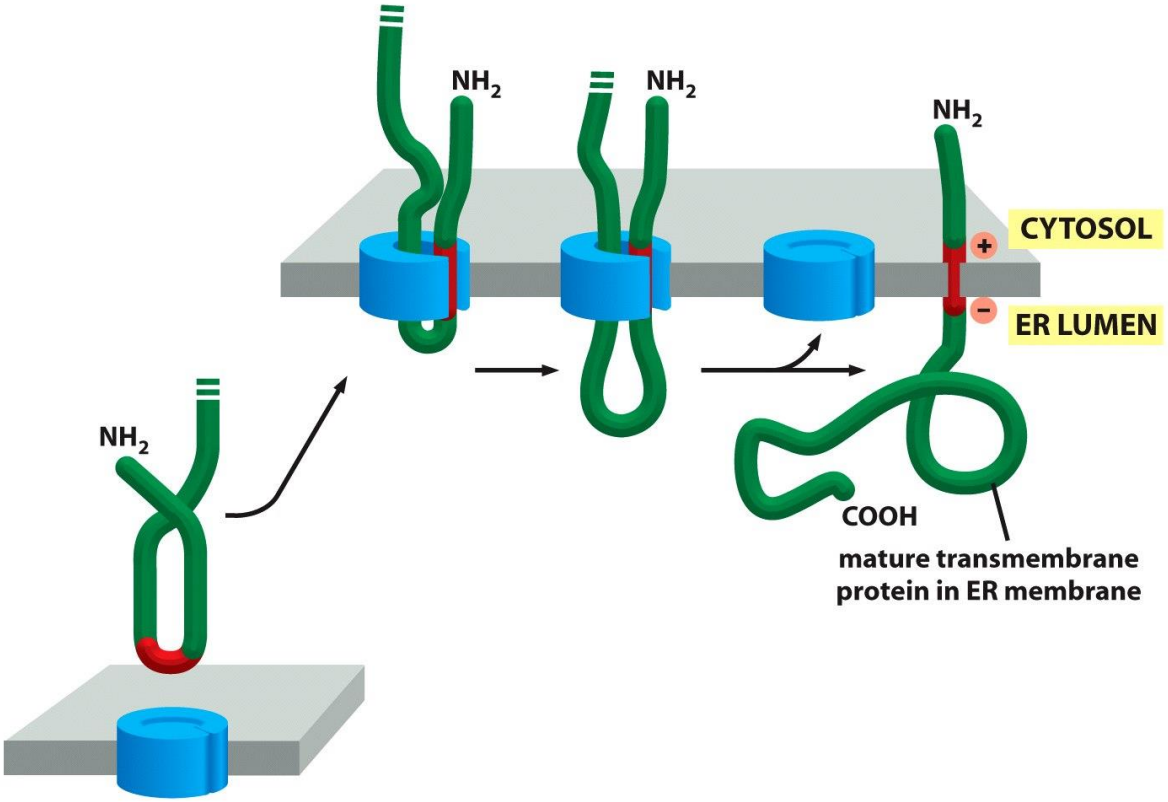


- An **internal ER signal sequence** that functions as a start-transfer signal can bind to the translocator in one of two ways, leading to a membrane protein that has either its C-terminus (pathway A) or its N-terminus (pathway B) in the ER lumen.
- The orientation of the start-transfer sequence depends on the **distribution of nearby charged amino acids.**

Integration of a singlepass transmembrane protein with an internal signal sequence into the ER membrane

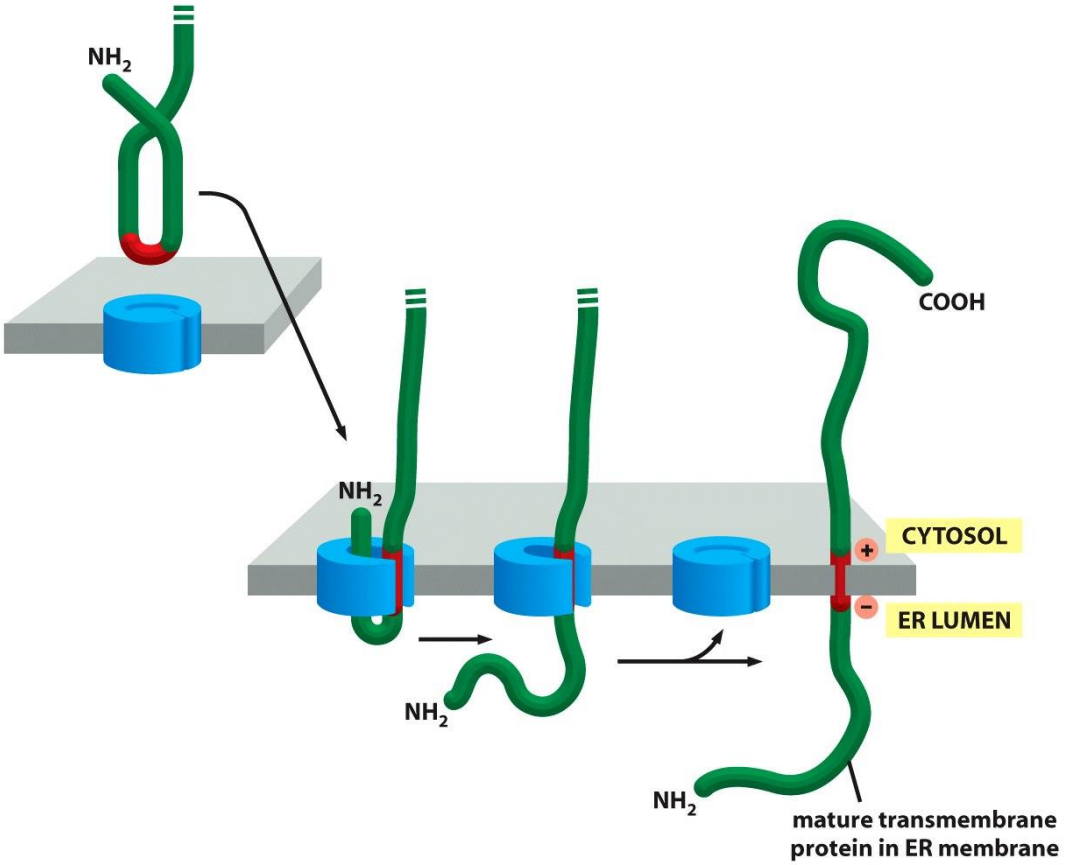
- Proteins are directed into <u>either pathway</u> by features in the polypeptide chain flanking the internal start-transfer sequence:

✓ if there are **more positively charged amino acids immediately preceding** the hydrophobic core of the start-transfer sequence than there are following it, the membrane protein is inserted into the translocator in the orientation shown in pathway A,



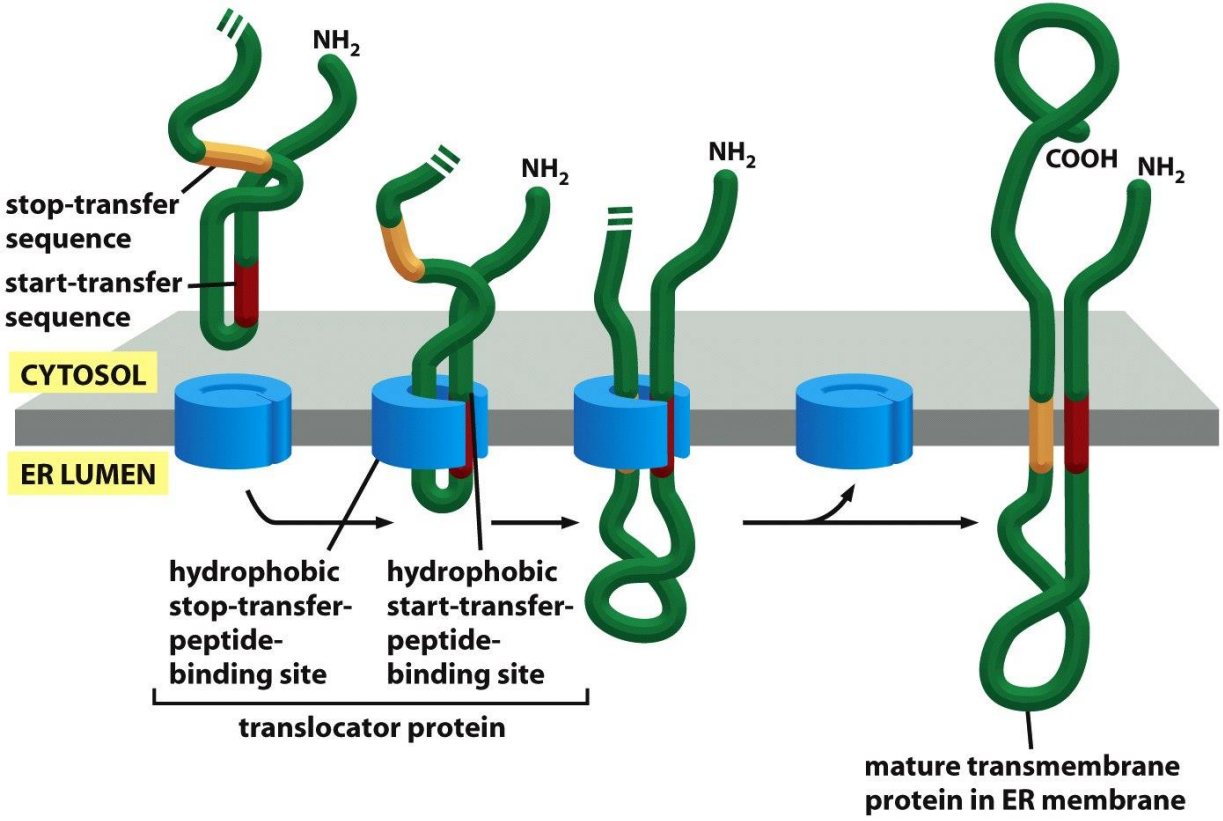
Integration of a singlepass transmembrane protein with an internal signal sequence into the ER membrane

- ✓ whereas if there are **more positively charged amino acids immediately following** the hydrophobic core of the start-transfer sequence than there are preceding it, the membrane protein is inserted into the translocator in the orientation shown in pathway B.



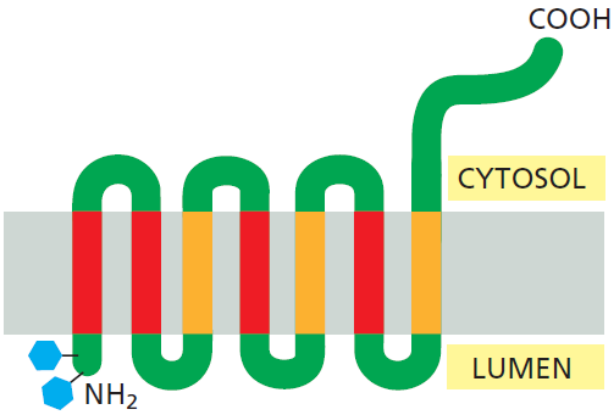
- Because translocation cannot start before a start-transfer sequence appears outside the ribosome, translocation of the N-terminal portion of the protein shown in (B) can occur only after this portion has been fully synthesized.

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



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

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



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

- Hydrophobic start-transfer and stop-transfer signal sequences both act to **fix the topology of the protein in the membrane** by locking themselves into the membrane as membrane-spanning α helices; and they can do this in either orientation.

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

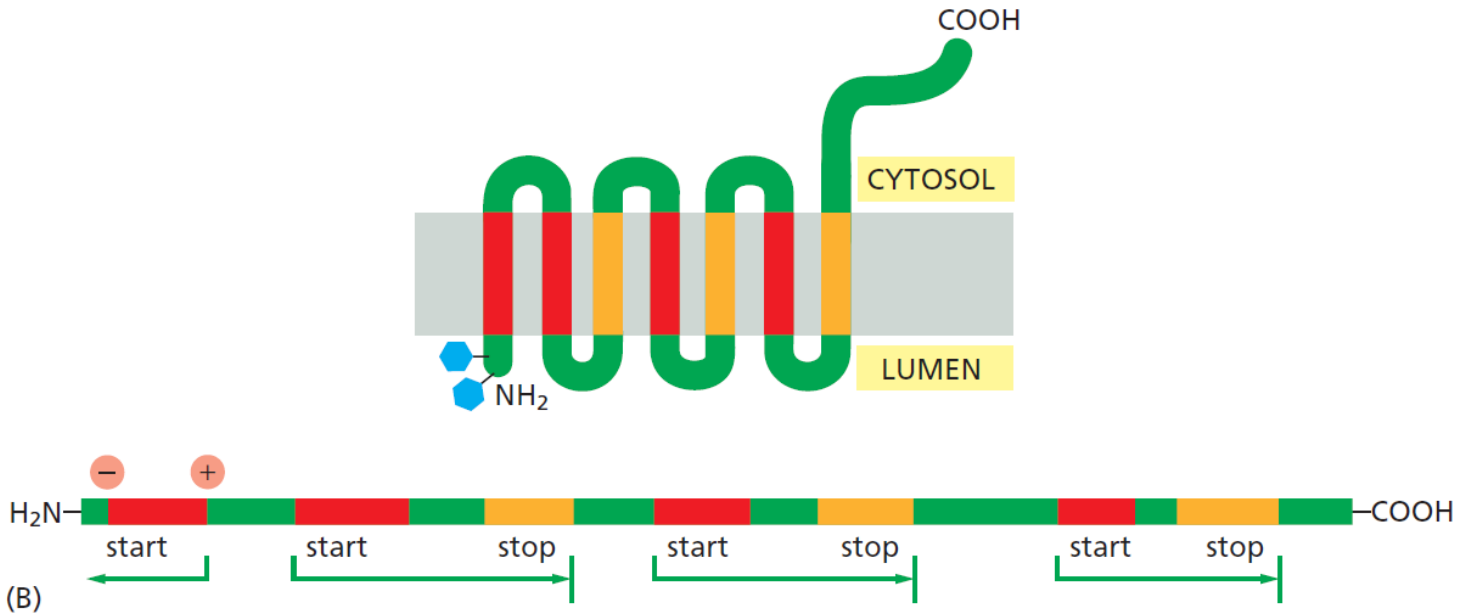
- Whether a given hydrophobic signal sequence functions as a start-transfer or stop-transfer sequence must **depend on its location** in a polypeptide chain.

- Thus, the **distinction** between start-transfer and stop-transfer sequences results mostly from **their relative order** in the growing polypeptide chain.

- It seems that the SRP begins scanning an unfolded polypeptide chain for hydrophobic segments at its N-terminus and proceeds toward the C-terminus, in the direction that the protein is synthesized.
- By recognizing the **first appropriate hydrophobic segment to emerg**e from the ribosome, the SRP sets the "**reading frame**" for membrane integration:
- after the SRP initiates translocation, the translocator recognizes the next appropriate hydrophobic segment in the direction of transfer as a **stop-transfer sequence**, causing the region of the polypeptide chain in between to be threaded across the membrane.

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

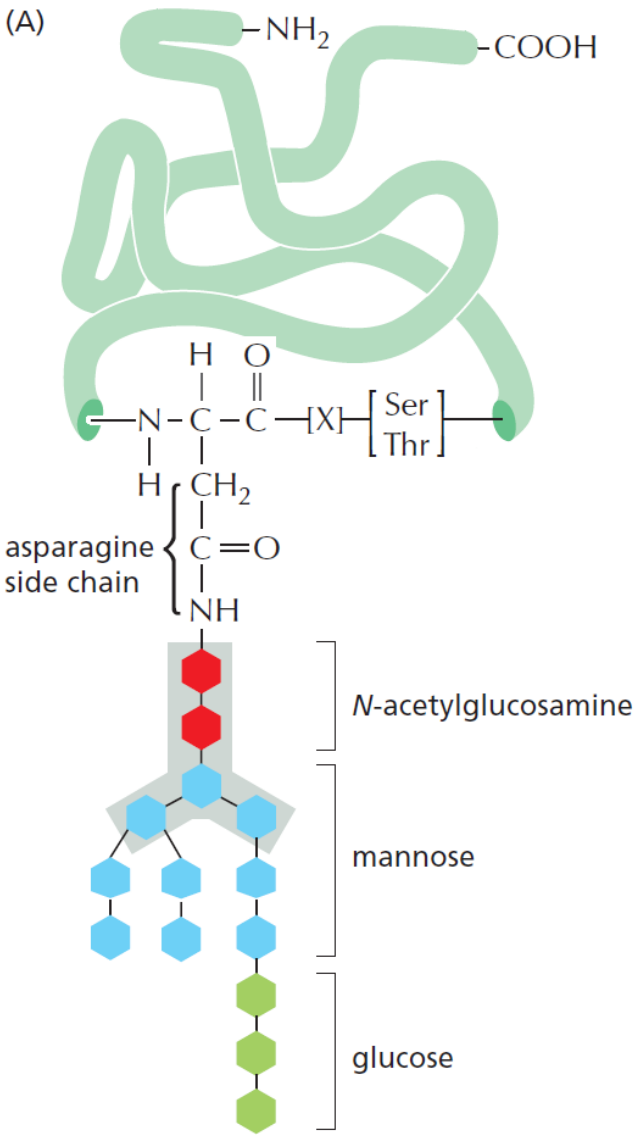
Combinations of Start-Transfer and Stop-Transfer Signals Determine the Topology of Multipass Transmembrane Proteins



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.

(B) The hydrophobic region nearest the N-terminus serves as a start-transfer sequence that causes the preceding N-terminal portion of the protein to pass across the ER membrane. Subsequent hydrophobic sequences function in alternation as start-transfer and stop-transfer sequences. The green arrows indicate the paired start and stop signals inserted into the translocator.
(C) The final integrated rhodopsin has its N-terminus located in the ER lumen and its C-terminus located in the cytosol.

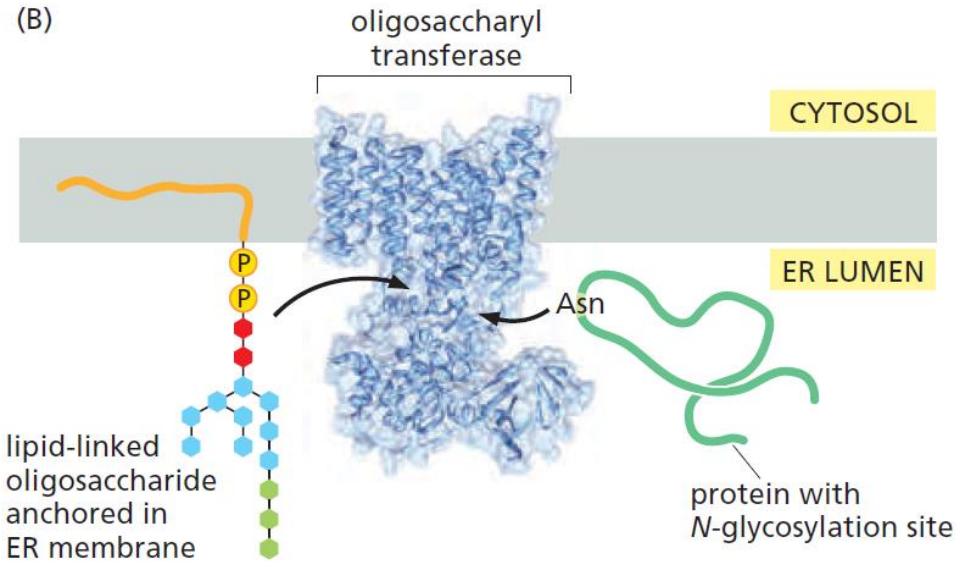
Most Proteins Synthesized in the Rough ER Are Glycosylated by the Addition of a Common N-Linked Oligosaccharide



- The **covalent addition of oligosaccharides to proteins** is one of the major biosynthetic functions of the ER.
- About **half** of the soluble and membrane-bound proteins that are processed in the ER—including those destined for transport to the Golgi apparatus, lysosomes, plasma membrane, or extracellular space—are glycoproteins that are modified in this way.

- Many proteins in the cytosol and nucleus are also glycosylated, but not with oligosaccharides: they carry a much simpler sugar modification, in which a single N-acetylglucosamine group is added to a **serine or threonine of the protein**.
- During the most common form of protein glycosylation in the ER, a preformed precursor oligosaccharide (composed of **N-acetylglucosamine**, **mannose**, and **glucose**, and containing a total of 14 sugars) is transferred en bloc to proteins.
- Because this oligosaccharide is transferred to the side-chain NH2 group of an asparagine in the protein, it is said to be **N-linked or asparagine-linked**.

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



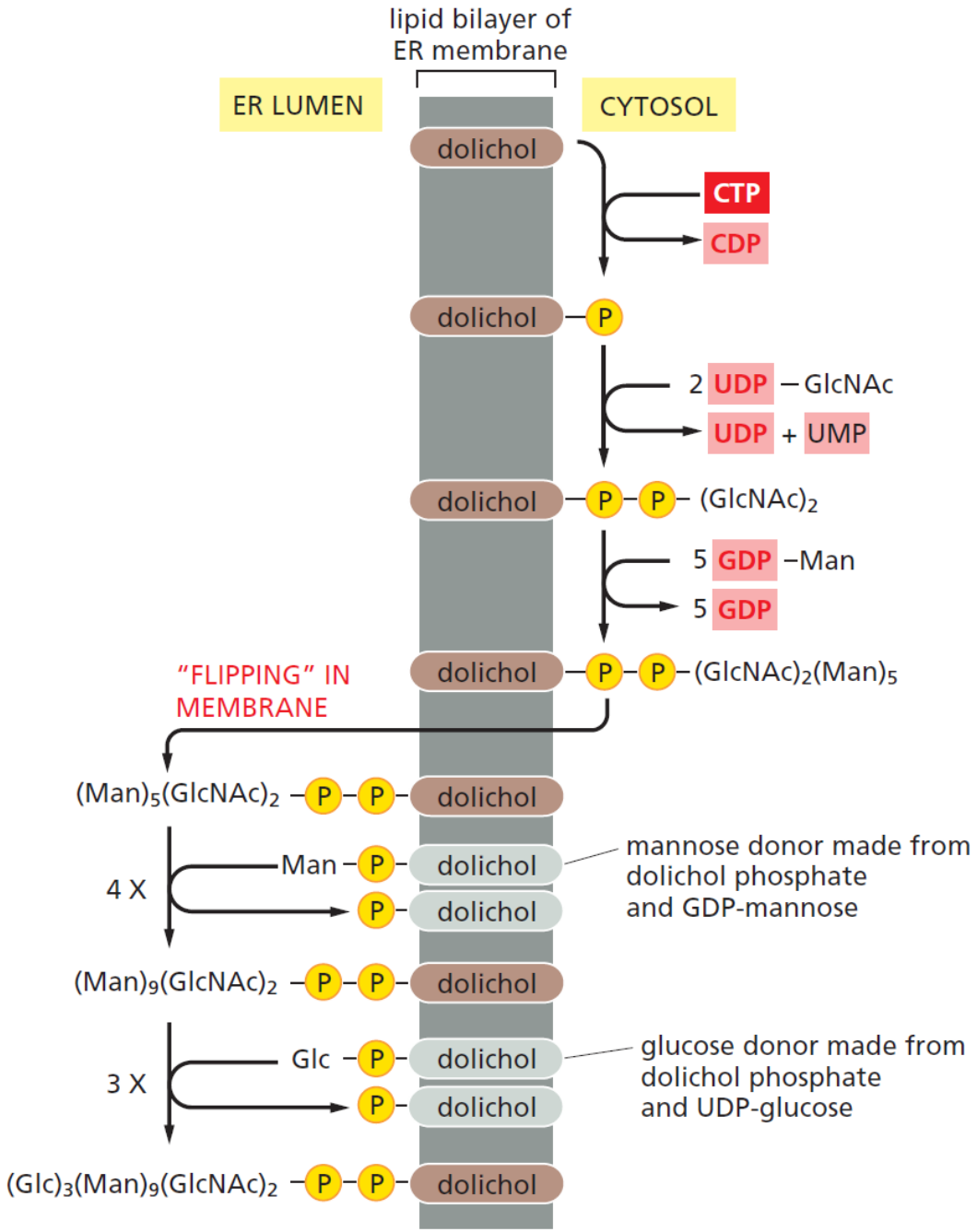
- The transfer is catalyzed by a **membrane-bound enzyme complex**, an **oligosaccharyl transferase**, which has its active site exposed on the lumenal side of the ER membrane; this explains why cytosolic proteins are not glycosylated in this way.
- A special lipid molecule called **dolichol** anchors the precursor oligosaccharide in the ER membrane.
- The precursor oligosaccharide is transferred to the target asparagine in a single enzymatic step immediately after that amino acid has reached the ER lumen during protein translocation.
- The precursor oligosaccharide is linked to the dolichol lipid by a **high-energy pyrophosphate bond**, which provides the activation energy that drives the glycosylation reaction.

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

- One copy of oligosaccharyl transferase is associated with each protein translocator, allowing it to scan and glycosylate the incoming polypeptide chains efficiently.
- The precursor oligosaccharide is built up sugar by sugar on the membrane-bound dolichol lipid and is then transferred to a protein.

- The sugars are first activated in the cytosol by the formation of nucleotide (UDP or GDP)-sugar intermediates, which then donate their sugar (directly or indirectly) to the lipid in an orderly sequence. Part way through this process, the lipid-linked oligosaccharide is flipped, with the help of a transporter, from the cytosolic to the lumenal side of the ER membrane.
- All of the diversity of the N-linked oligosaccharide structures on mature glycoproteins results from the later modification of the original precursor oligosaccharide.
- While still in the ER, three glucoses and one mannose are quickly removed from the oligosaccharides of most glycoproteins.
- We shall return to the importance of glucose trimming shortly. This oligosaccharide "trimming," or "processing," continues in the Golgi apparatus.
- The N-linked oligosaccharides are by far the most common oligosaccharides, being found on 90% of all glycoproteins.
- Less frequently, oligosaccharides are linked to the hydroxyl group on the side chain of a serine, threonine, or hydroxylysine amino acid. A first sugar of these O-linked oligosaccharides is added in the ER and the oligosaccharide is then further extended in the Golgi apparatus.

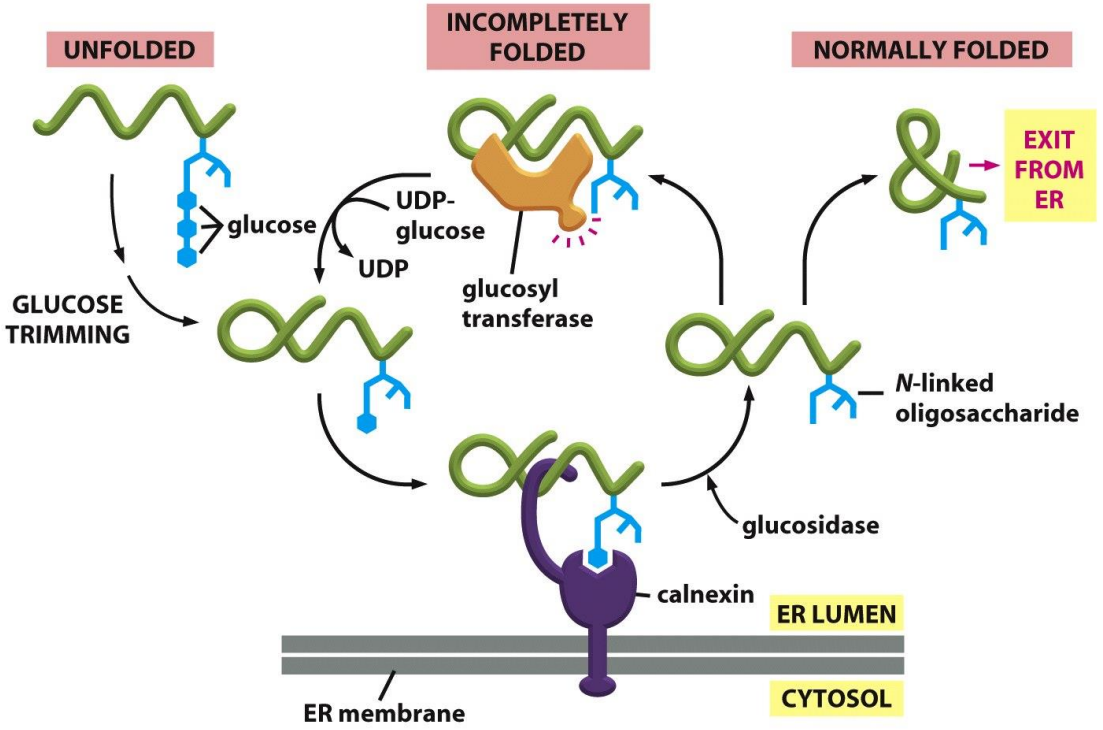
Synthesis of the lipidlinked precursor oligosaccharide in the rough ER membrane



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

- It has long been debated why glycosylation is such a **common modification** of proteins that enter the ER??
- One particularly puzzling observation has been that some proteins require N-linked glycosylation for **proper folding in the ER**, yet the precise location of the oligosaccharides attached to the protein's surface does not seem to matter.
- A clue to the role of glycosylation in protein folding came from studies of two ER chaperone proteins, which are called **calnexin** and **calreticulin** because they require **Ca2+** for their activities.
- These chaperones are carbohydrate-binding proteins, or **lectins**, which bind to oligosaccharides on **incompletely folded proteins** and retain them in the ER.
- They prevent incompletely folded proteins from irreversibly aggregating.
- Both calnexin and calreticulin also promote the association of incompletely folded proteins with another ER chaperone, which binds to cysteines that have not yet formed disulfide bonds.

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

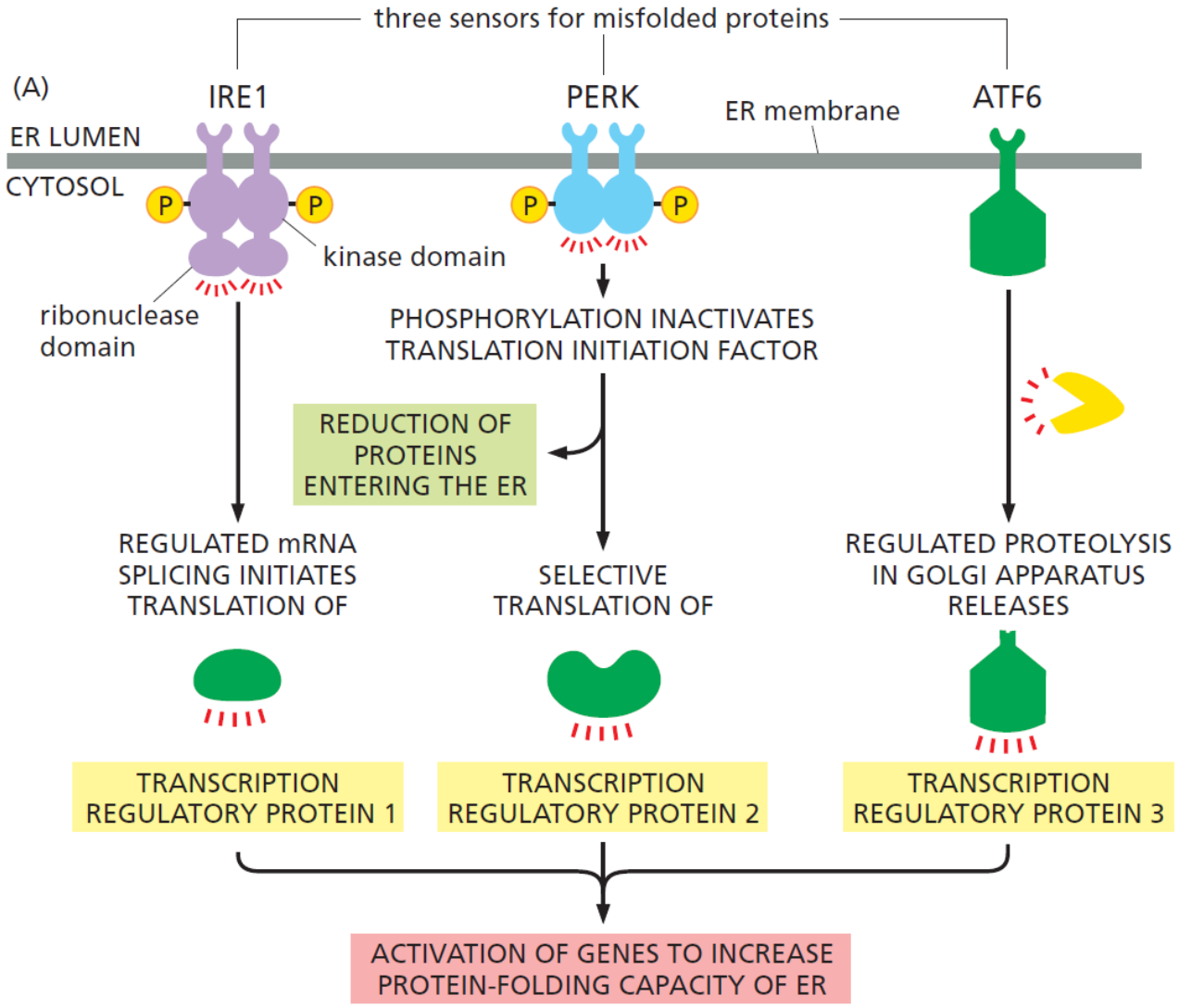


- The ER-membrane-bound chaperone protein **calnexin** binds to incompletely folded proteins containing **one terminal glucose** on N-linked oligosaccharides, trapping the protein in the ER.
- Removal of the terminal glucose by a **glucosidase** releases the protein from calnexin.
- A **glucosyl transferase**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

Misfolded Proteins in the ER Activate an Unfolded Protein Response

- Cells carefully monitor the amount of misfolded protein in various compartments.
- An accumulation of misfolded proteins in the cytosol, for example, triggers a heat-shock response, which stimulates the transcription of genes encoding cytosolic chaperones that help to refold the proteins.
- Similarly, an accumulation of misfolded proteins in the ER triggers an **unfolded protein response (UPR)**, which includes an increased transcription of genes encoding proteins involved in **retrotranslocation** and **protein degradation in the cytosol**, **ER chaperones**, and many other proteins that help to increase the **protein-folding capacity** of the ER.
- **How do misfolded proteins in the ER signal to the nucleus?**
- There are three parallel pathways that execute the unfolded protein response.

Misfolded Proteins in the ER Activate an Unfolded Protein Response

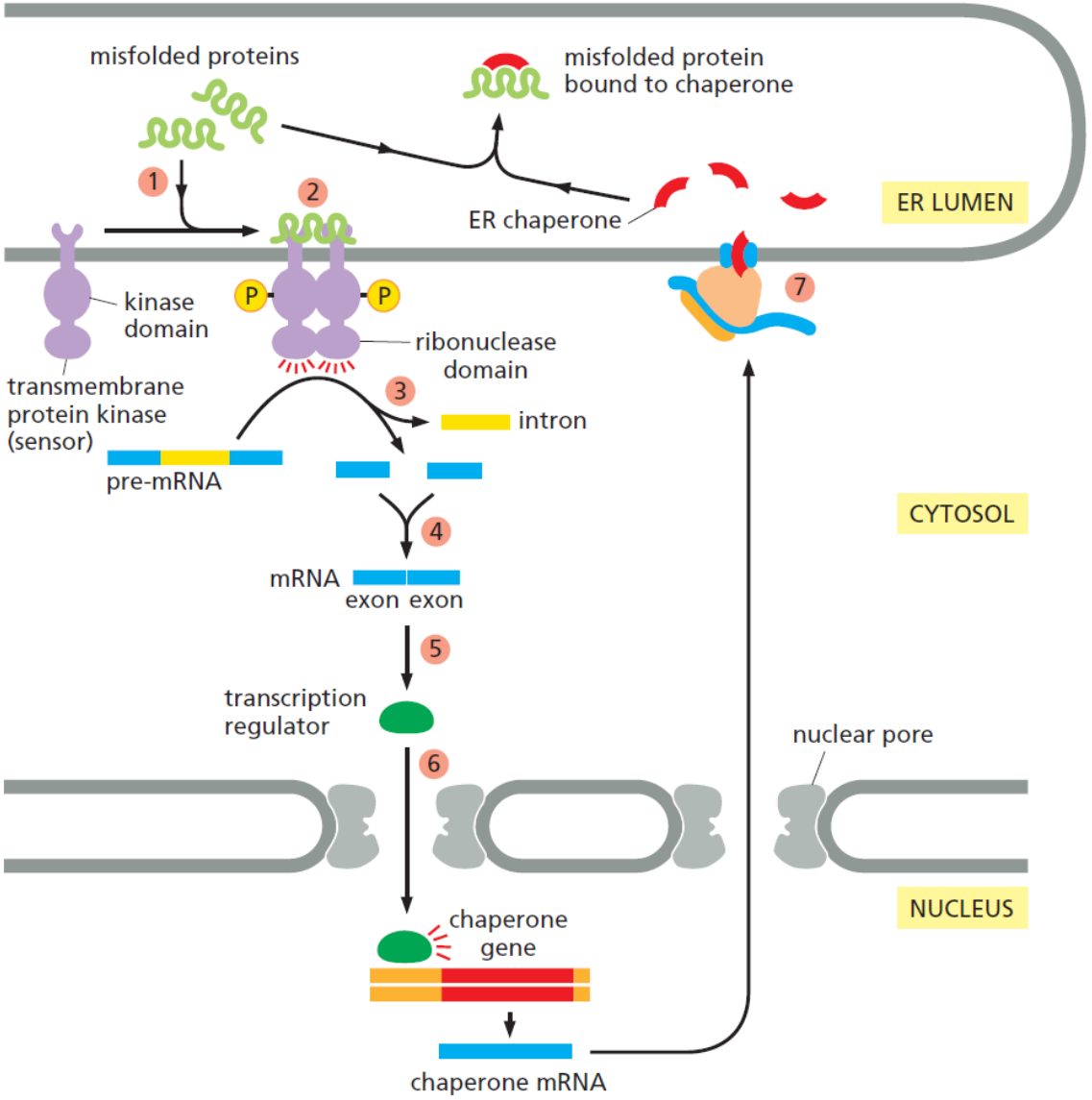


Misfolded Proteins in the ER Activate an Unfolded Protein Response

The first pathway, which was initially discovered in **yeast** cells, is particularly remarkable.

(B)

1. MISFOLDED PROTEINS IN ER SIGNAL THE NEED FOR MORE ER CHAPERONES. THEY BIND TO AND ACTIVATE A TRANSMEMBRANE KINASE
2. ACTIVATED KINASE UNMASKS AN ENDORIBONUCLEASE ACTIVITY
3. ENDORIBONUCLEASE CUTS SPECIFIC RNA MOLECULES AT TWO POSITIONS, REMOVING AN INTRON
4. TWO EXONS ARE LIGATED TO FORM AN ACTIVE mRNA
5. mRNA IS TRANSLATED TO MAKE A TRANSCRIPTION REGULATOR
6. TRANSCRIPTION REGULATOR ENTERS NUCLEUS AND ACTIVATES GENES ENCODING ER CHAPERONES
7. CHAPERONES ARE MADE IN ER, WHERE THEY HELP FOLD PROTEINS



Misfolded Proteins in the ER Activate an Unfolded Protein Response

- Misfolded proteins also activate a second transmembrane kinase in the ER, **PERK**, which inhibits a translation initiation factor by **phosphorylating** it, thereby reducing the production of new proteins throughout the cell.

- One consequence of the reduction in protein synthesis is **to reduce the flux of proteins into the ER**, thereby reducing the **load of proteins** that need to be folded there.

- Some proteins, however, are preferentially translated when translation initiation factors are scarce, and one of these is **a transcription regulator** that helps activate the transcription of the genes encoding proteins active in the unfolded protein response.

Misfolded Proteins in the ER Activate an Unfolded Protein Response

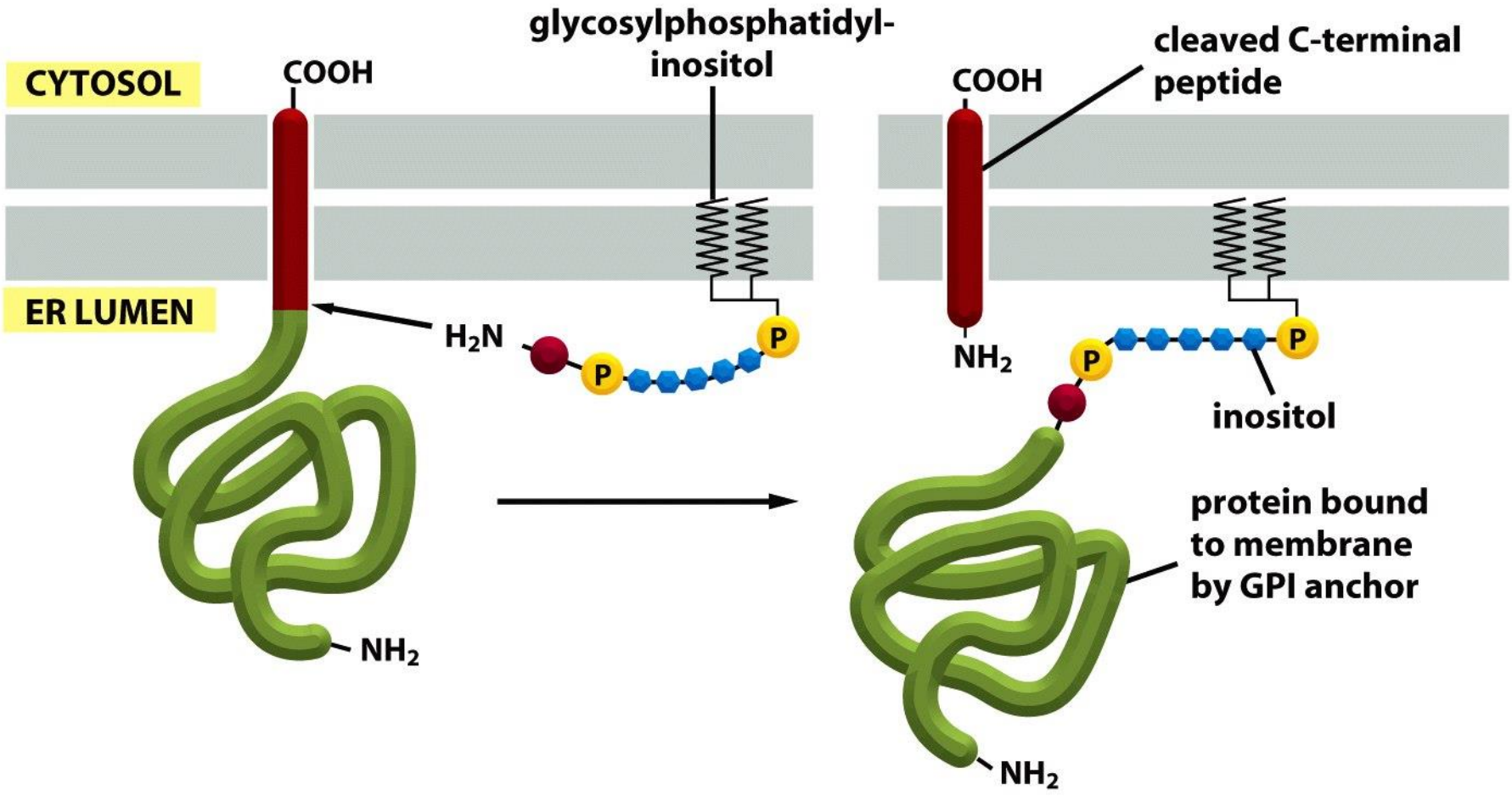
- A third transcription regulator, **ATF6**, is initially synthesized as a transmembrane ER protein.

- Because it is embedded in the ER membrane, it cannot activate the transcription of genes in the nucleus.

- When misfolded proteins accumulate in the ER, however, the ATF6 protein is transported to the Golgi apparatus, where it encounters proteases that cleave off its cytosolic domain, which can now migrate to the nucleus and help activate the transcription of genes encoding proteins involved in the unfolded protein response.

The relative importance of each of these three pathways in the unfolded protein response differs in different cell types, enabling each cell type to tailor the unfolded protein response to its particular needs.

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



The ER Assembles Most Lipid Bilayers

- The ER membrane is the site of synthesis of nearly all of the cell's major classes of lipids, including both **phosphoipids** and **cholesterol**, required for the production of new cell membranes.

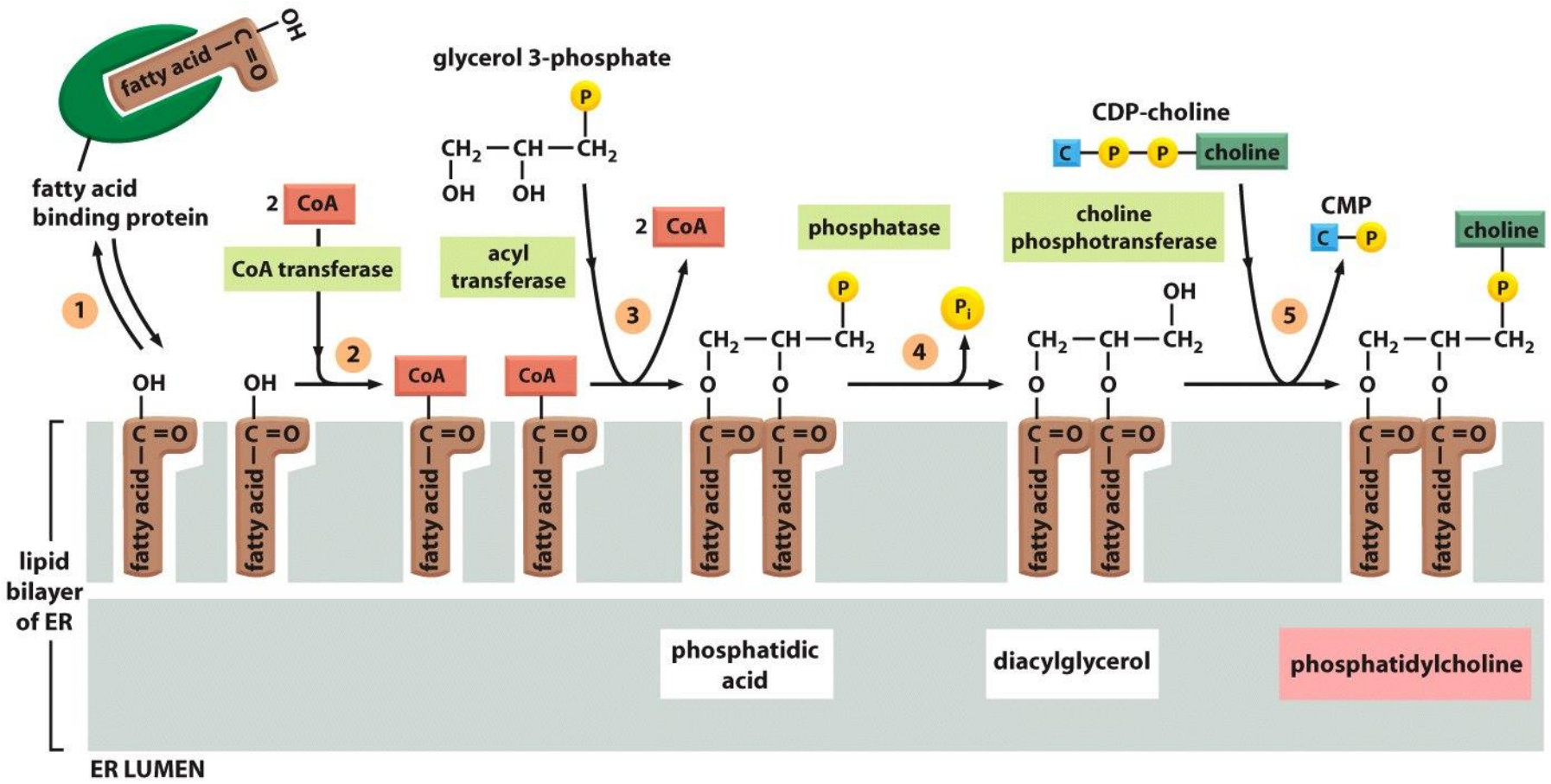
- The major phospholipid made is **phosphatidylcholine**, which can be formed in three steps from choline, two fatty acids, and glycerol phosphate.

- Each step is catalyzed by enzymes in the ER membrane, which have their active sites facing the cytosol, where all of the required metabolites are found.
- Thus, phospholipid synthesis occurs exclusively in the cytosolic leaflet of the ER membrane.
- Because fatty acids are not soluble in water, they are shepherded from their sites of synthesis to the ER by a fatty acid binding protein in the cytosol.
- After arrival in the ER membrane and activation with **CoA**, **acyl transferases** successively add two fatty acids to glycerol phosphate to produce phosphatidic acid.

- It is therefore this first step that enlarges the ER lipid bilayer.

- The later steps determine the head group of a newly formed lipid molecule and therefore the **chemical nature of the bilayer**, but they do not result in net membrane growth.
- The two other major membrane phospholipids—**phosphatidylethanolamine** and **phosphatidylserine**—as well as the minor phospholipid **phosphatidylinositol (PI)**, are all synthesized in this way

The ER Assembles Most Lipid Bilayers



The ER Assembles Most Lipid Bilayers

- Because phospholipid synthesis takes place in the **cytosolic leaflet** of the ER lipid bilayer, there needs to be a mechanism that transfers some of the newly formed phospholipid molecules to the **lumenal leaflet** of the bilayer.

- In synthetic lipid bilayers, lipids do not "**flip-flop**" in this way.

- 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 "flipflop."

- This rapid **trans-bilayer movement** is mediated by a poorly characterized phospholipid translocator called a **scramblase**, which nonselectively equilibrates phospholipids between the two leaflets of the lipid bilayer.

- Thus, the different types of phospholipids are thought to be equally distributed between the two leaflets of the ER membrane.

The ER Assembles Most Lipid Bilayers

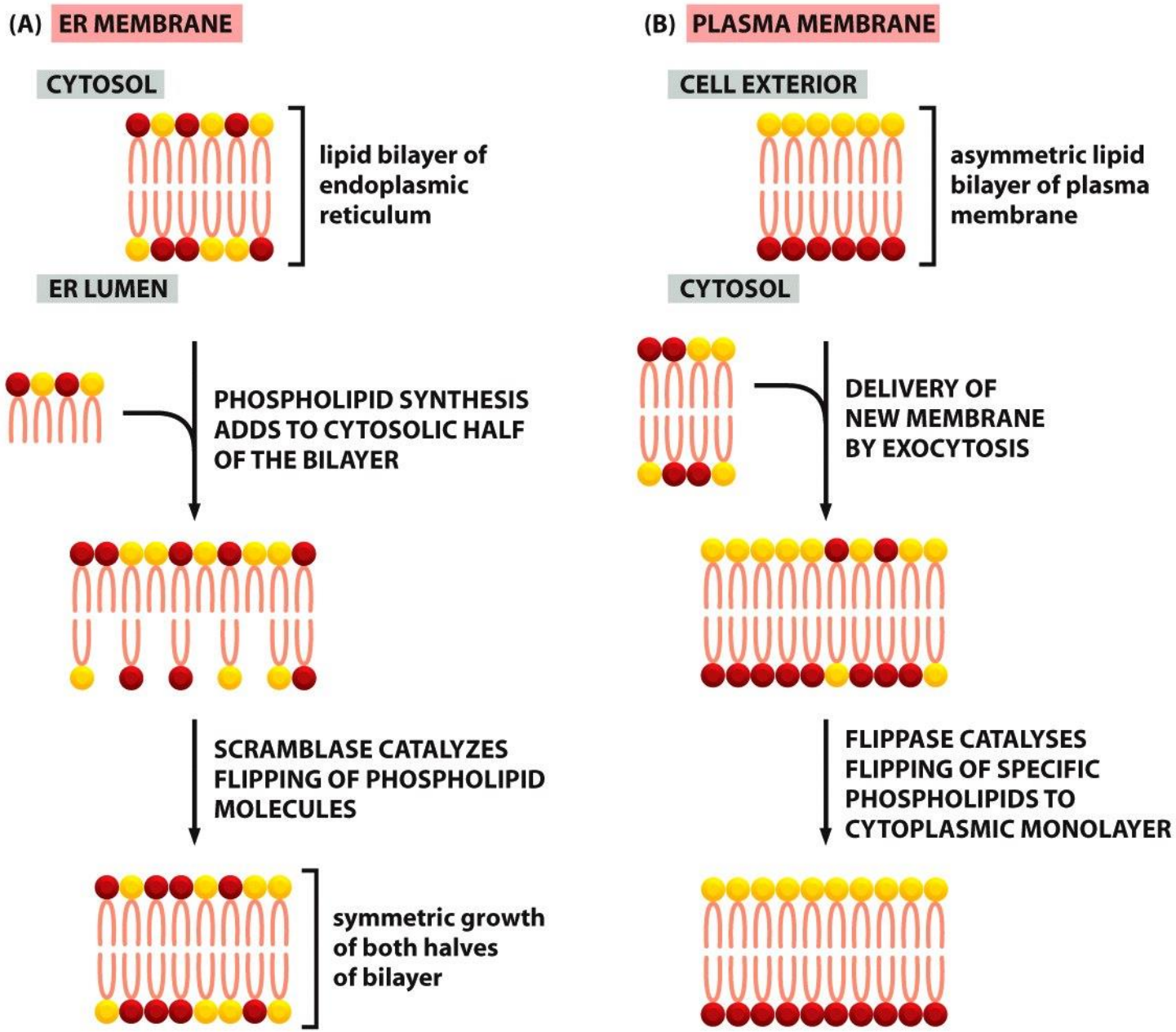
- The **plasma membrane** contains a different type of phospholipid translocator that belongs to the family of **P-type pumps**.

- These flippases (head-group-specific flippase) specifically recognize those phospholipids that contain free amino groups in their head groups (phosphatidylserine and phosphatidylethanolamine) and transfers them from the extracellular to the cytosolic leaflet, using the energy of ATP hydrolysis.

- The plasma membrane therefore has a highly asymmetric phospholipid composition, which is actively maintained by the flippases.

- The plasma membrane also contains a scramblase but, in contrast to the ER scramblase, which is **always active**, the plasma membrane enzyme is regulated and only activated in some situations, such as in **apoptosis** and in **activated platelets**, where it acts to abolish the lipid bilayer asymmetry; the resulting exposure of phosphatidylserine on the surface of apoptotic cells serves as a signal for phagocytic cells to ingest and degrade the dead cell or it serves as a pro-coagulant surface.

The role of phospholipid translocators in lipid bilayer synthesis



The ER Assembles Most Lipid Bilayers

- 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 covalently added to form ceramide.

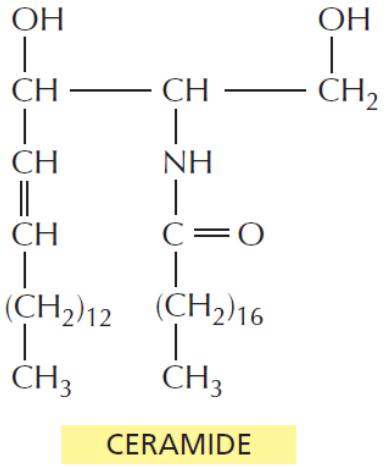


Figure 12–55 The structure of ceramide.

- The ceramide is exported to the Golgi apparatus, where it serves as a precursor for the synthesis of two types of lipids:
  - ✓ oligosaccharide chains are added to form **glycosphingolipids (glycolipids),**
  - ✓ 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 that have their active sites exposed to the Golgi lumen, they are found exclusively in the **noncytosolic leaflet** of the lipid bilayers that contain them.

The ER Assembles Most Lipid Bilayers

- The plasma membrane and the membranes of the Golgi apparatus, lysosomes, and endosomes all form part of a membrane system that communicates with the ER by means of **transport vesicles**, which transfer both proteins and lipids.

- **Mitochondria** and **plastids**, however, do not belong to this system, and they therefore require different mechanisms to import proteins and lipids for growth.
- They import most of their proteins from the cytosol.

- Although mitochondria modify some of the lipids they import, they do not synthesize lipids de novo; instead, **their lipids have to be imported from the ER**, either directly or indirectly by way of other cell membranes.
- In either case, special mechanisms are required for the transfer.

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