Cell and Molecular Biology

Ninth Edition

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Chapter 8

Cytoplasmic Membrane Systems: Structure, Function, and Membrane Trafficking

8.1 | An Overview of the Endomembrane System (1 of 5)

- The ER, Golgi complex, endosomes, lysosomes, and vacuoles form an **endomembrane system** that act as a coordinated unit.
- They are distinct compartments bounded by membrane barriers ad contain specialized proteins for particular activities.

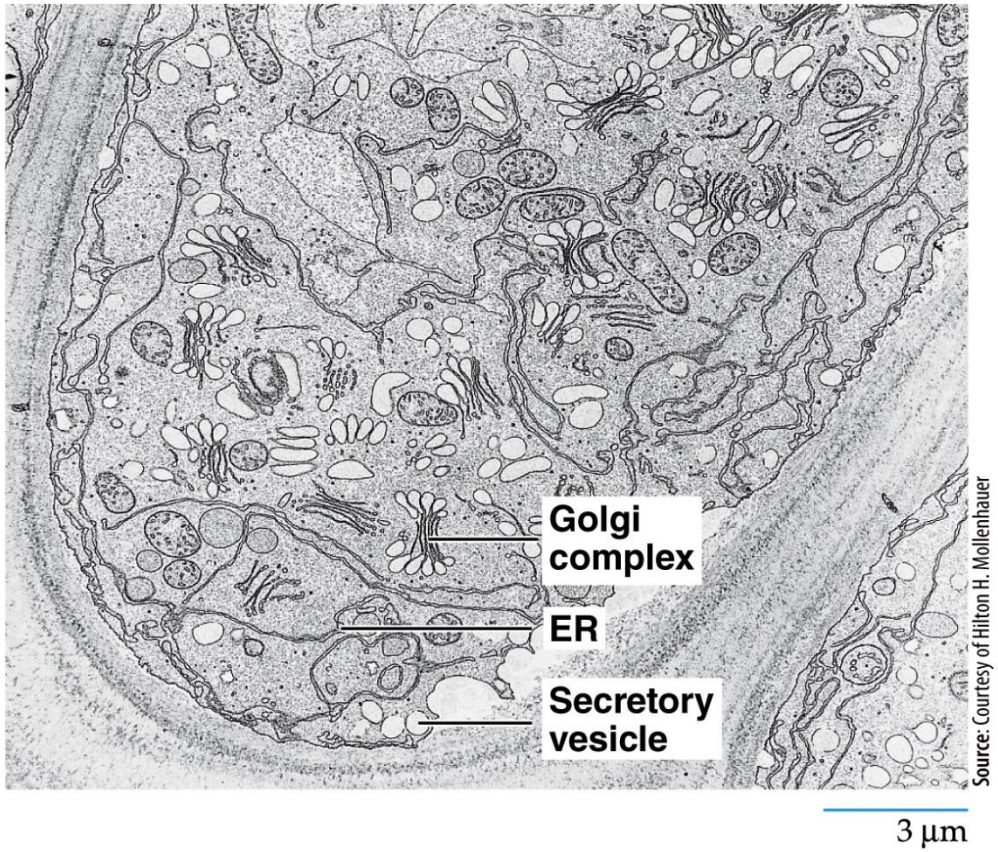


Fig. 8.1 Membrane-bound compartments of the cytoplasm

8.1 | An Overview of the Endomembrane System (2 of 5)

Materials packaged in small, membrane-bounded transport vesicles:

- Bud from a donor membrane compartment.
- Move via motor proteins on microtubules and microfilaments of the cytoskeleton.
- Fuse with the membrane of the acceptor compartment

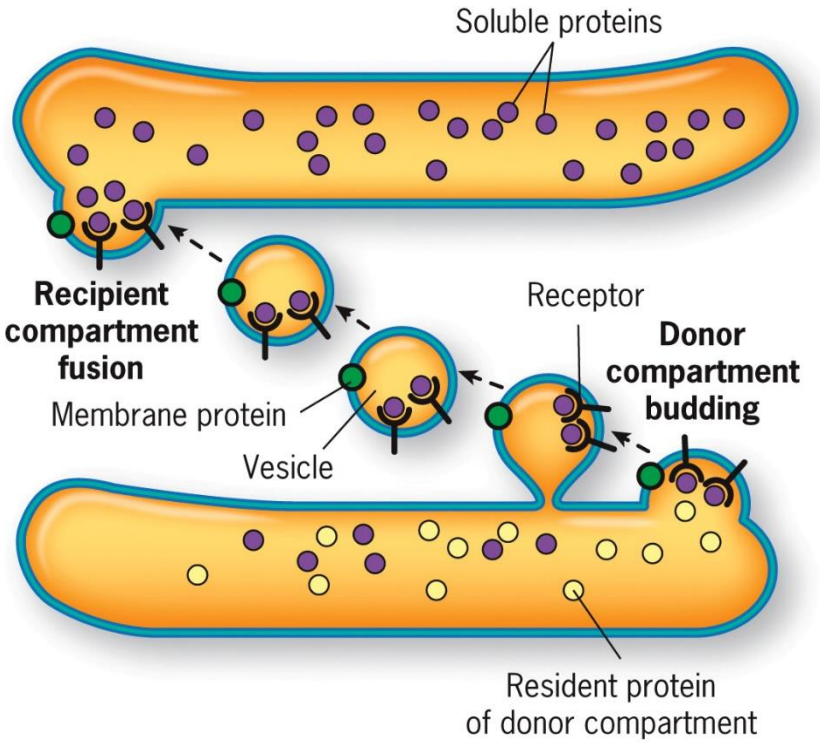


Fig. 8.2a An overview of the biosynthetic/secretory pathways that unite endomembranes into a dynamic interconnected network

8.1 | An Overview of the Endomembrane System (3 of 5)

Pathways:

- **Biosynthetic pathway**: Proteins are synthesized in the ER, modified at the Golgi complex, and transported to various destinations.
- **Secretory pathway**: Proteins synthesized in the ER are discharged from the cell.
- **Endocytic pathway**, materials move from the outer surface of the cell to compartments, such as endosomes and lysosomes

Secretion modes:

- **Constitutive secretion**: Materials are transported in secretory vesicles and discharged in a continual manner.
- **Regulated secretion**: Materials are stored in vesicles and discharged in response to a stimulus.

8.1 | An Overview of the Endomembrane System (4 of 5)

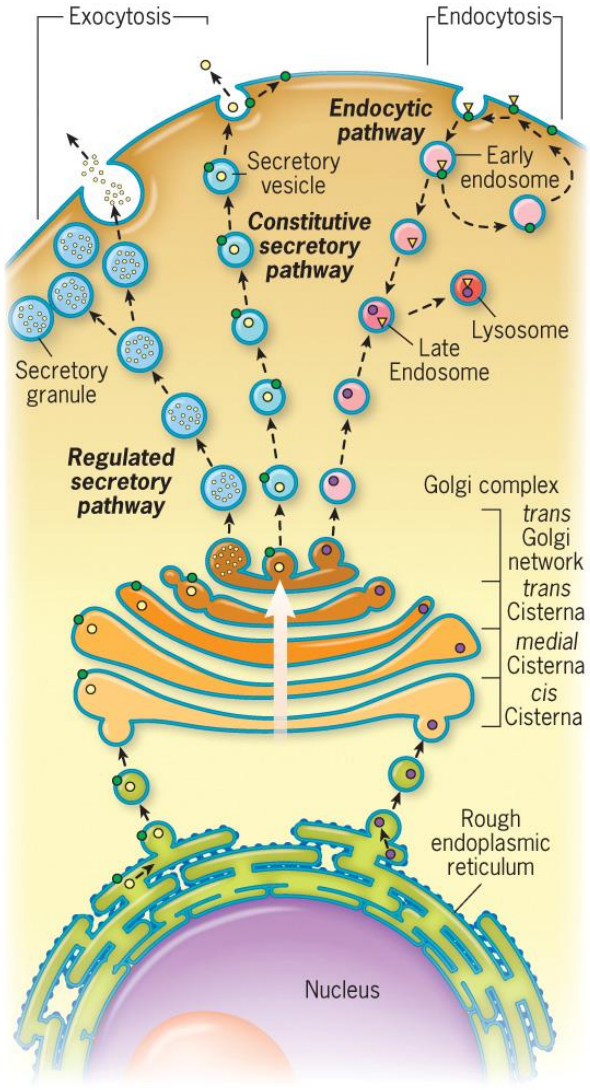


Fig. 8.2b An overview of the biosynthetic/secretory pathways that unite endomembranes into a dynamic interconnected network

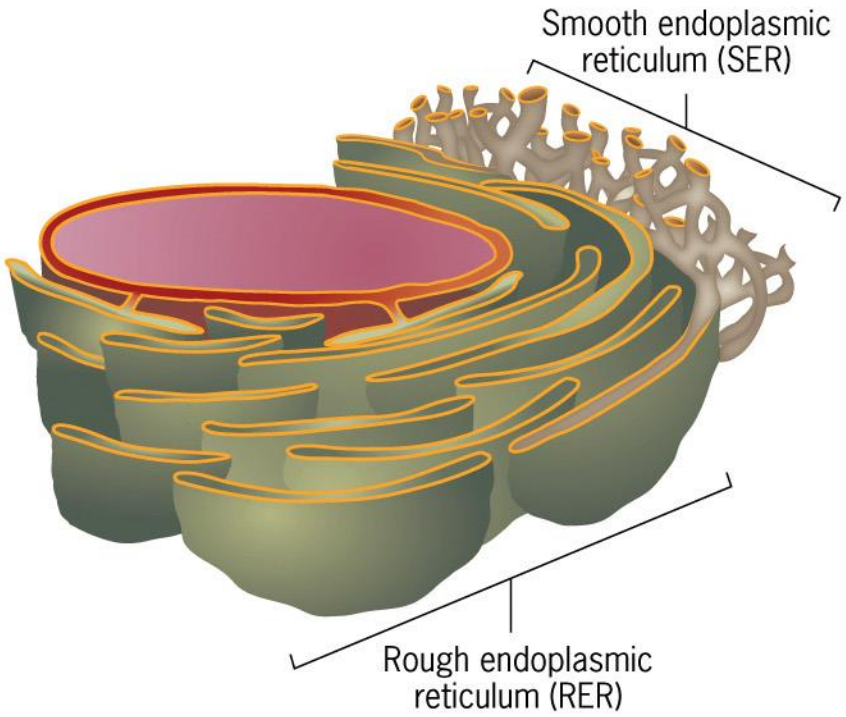
8.1 | An Overview of the Endomembrane System (5 of 5)

- **Regulated secretion** occurs in endocrine cells (hormones), pancreatic acinar cells (digestive enzymes), and nerve cells (neurotransmitters).
- Secreted materials can be stored in large, densely packed, membrane-bound **secretory granules**.
- Proteins, lipids, and complex polysaccharides are transported through the cell along the biosynthetic or secretory pathway.
- The various types of cargo are routed to their appropriate cellular destinations by **sorting signals** encoded in the amino acid sequence of the proteins or in the attached oligosaccharides.

8.3 | The Endoplasmic Reticulum (1 of 21)

- Network of membranes that penetrates much of the cytoplasm and has a lumen separated from the cytosol by the ER membrane.
- Highly dynamic structure, 2 compartments share some proteins and activities
- RER (Rough ER) :
  1. ribosomes bound to its cytosolic surface
  2. flattened sacs (**cisternae**) connected to neighbors by helicoidal membranes
  3. is continuous with the outer membrane of the nuclear envelope.
- SER (Smooth ER:
  1. lacks ribosomes
  2. membranes are highly curved and tubular
  3. is continuous with the RER.

8.3 | The Endoplasmic Reticulum (2 of 21)



Source: From K. Tanaka, *Int. Rev. Cytol.* 68:1010, 1980.
Courtesy K. Tanaka.

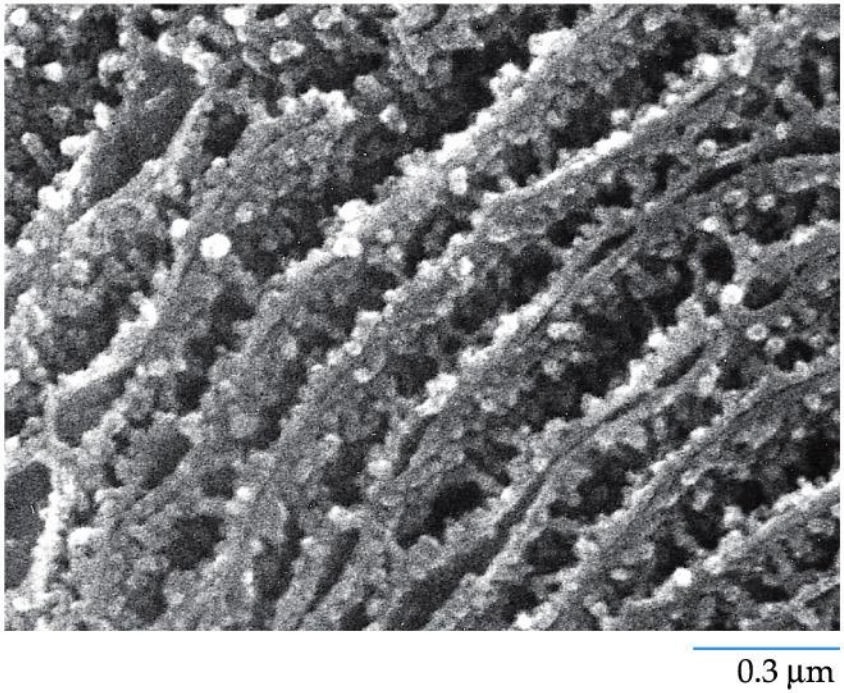


Fig. 8.10a,b The rough endoplasmic reticulum (RER)

8.3 | The Endoplasmic Reticulum (3 of 21)

The Smooth Endoplasmic Reticulum

SER functions include:

1. Steroid hormone synthesis in endocrine cells of the gonad and adrenal cortex.
2. Detoxification of organic compounds in the liver via oxygenases including the **cytochrome P450** family.
3. Calcium ion sequestration and regulated release

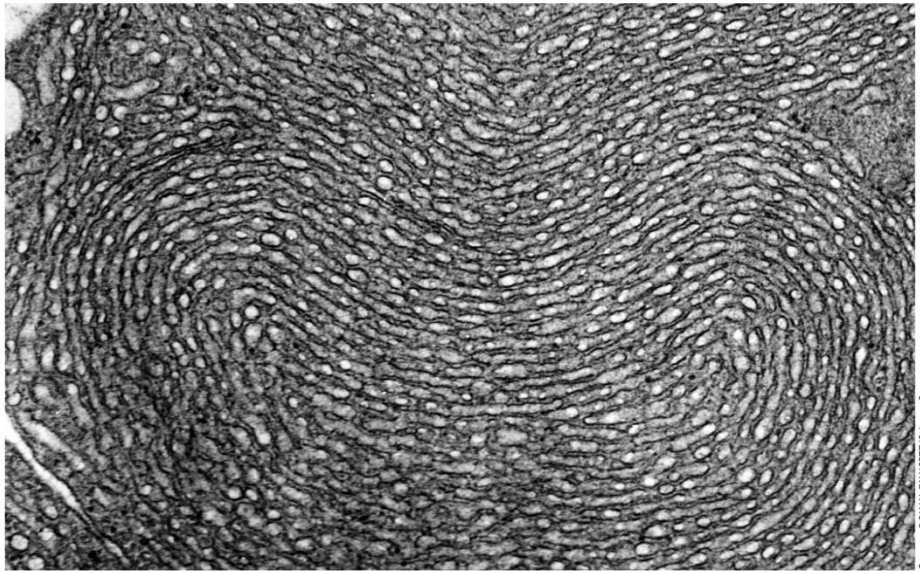


Fig. 8.11 The smooth ER (SER)

8.3 | The Endoplasmic Reticulum (4 of 21)

The Rough Endoplasmic Reticulum

- The nucleus and RER are near the basal surface, facing the blood supply
- The RER is the starting point of the biosynthetic pathway for secretory proteins.
- About one-third of the proteins are synthesized at the RER and released into the ER lumen in a process called co-translational translocation.
- Remaining polypeptides synthesized on “free” ribosomes in the cytosol.

8.3 | The Endoplasmic Reticulum (5 of 21)

The Rough Endoplasmic Reticulum

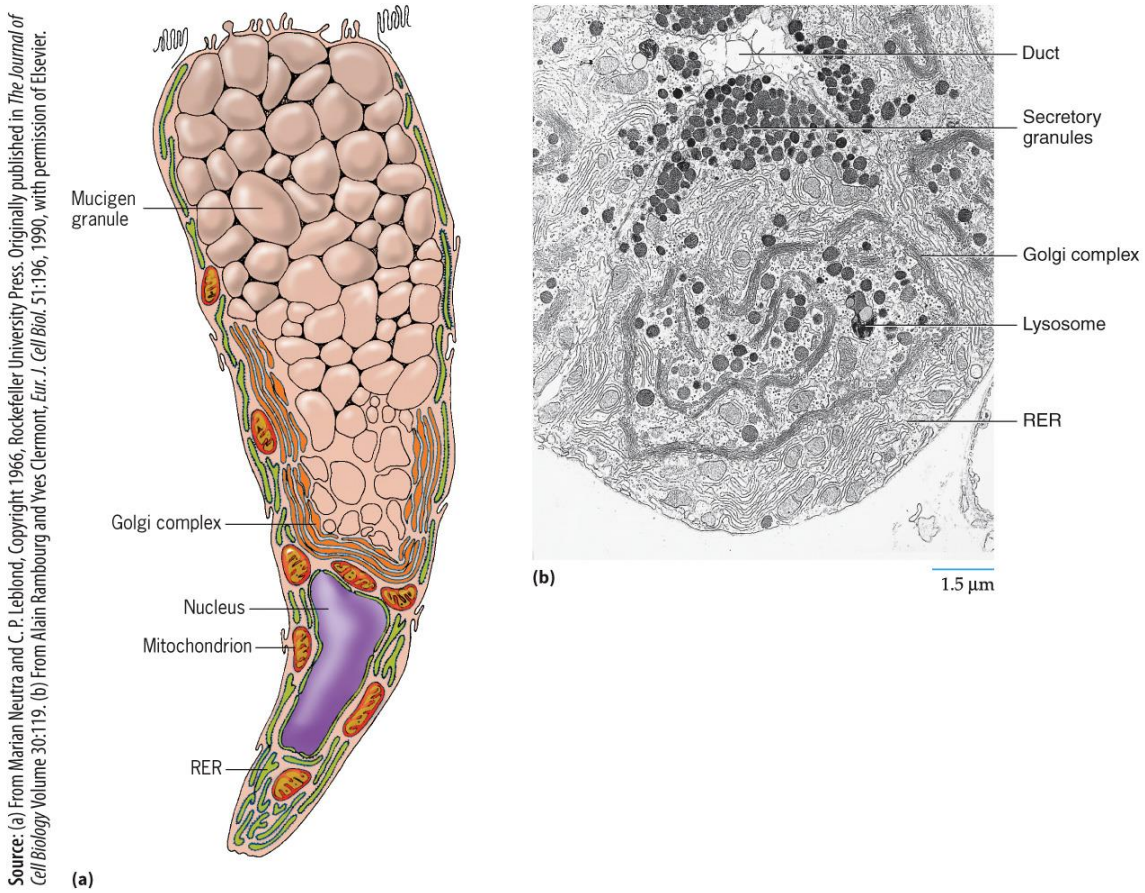


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Fig. 8.12 Polarized structure of a secretory cell

Synthesis of Proteins on Membrane-Bound versus Free Ribosomes

Polypeptides are synthesized at two distinct location within the cell.

About one-third of the proteins are synthesized at the RER and released into the ER lumen in a process called co-translational translocation:

(a) secreted proteins
(b) integral membrane proteins
(c) soluble proteins that reside in the ER, Golgi complex, lysosomes, endosomes, vesicles, and plant vacuoles.

Polypeptides synthesized on “free” ribosomes in the cytosol include:

(a) proteins destined to remain in the cytosol
(b) peripheral proteins of the cytosolic surface of membranes
(c) proteins that are transported to the nucleus and those incorporated into peroxisomes, chloroplasts, and mitochondria. These are synthesized *posttranslationally* into the appropriate organelle.

8.3 | The Endoplasmic Reticulum (6 of 21)

The Rough Endoplasmic Reticulum: Synthesis of Proteins on Membrane-Bound versus Free Ribosomes

- The site of protein synthesis is determined by the sequence of amino acids in the N-terminal portion of the polypeptide.
- Secretory proteins contain a **signal sequence** at their N-terminus that directs the emerging polypeptide and ribosome to the ER membrane.
- The polypeptide moves into the cisternal space of the ER through a protein-lined, aqueous channel in the ER membrane, as it is being synthesized (*co-translationally*).
- Proteins contain built-in “address codes” for protein trafficking pathways throughout the cell. (Signal hypothesis)

8.3 | The Endoplasmic Reticulum (7 of 21)

Synthesis of Secretory, Lysosomal, or Plant Vacuolar Proteins

- Co-translational translocation deposits protein into the ER lumen.
- Polypeptide signal sequence 6–15 hydrophobic amino acid residues, targeting polypeptide to ER membrane.
- Signal sequence recognized by **signal recognition particle (SRP)**.
- SRP binds polypeptide and the ribosome, arresting synthesis.
- Complex is recruited to ER membrane through interactions between the SRP and the SRP receptor on the ER membrane.
- The ribosome is handed off to the **translocon**, a protein channel embedded in the ER membrane. Upon attachment, the signal sequence is recognized, the polypeptide is inserted into the translocon channel.

8.3 | The Endoplasmic Reticulum (8 of 21)

Synthesis of Secretory, Lysosomal, or Plant Vacuolar Proteins

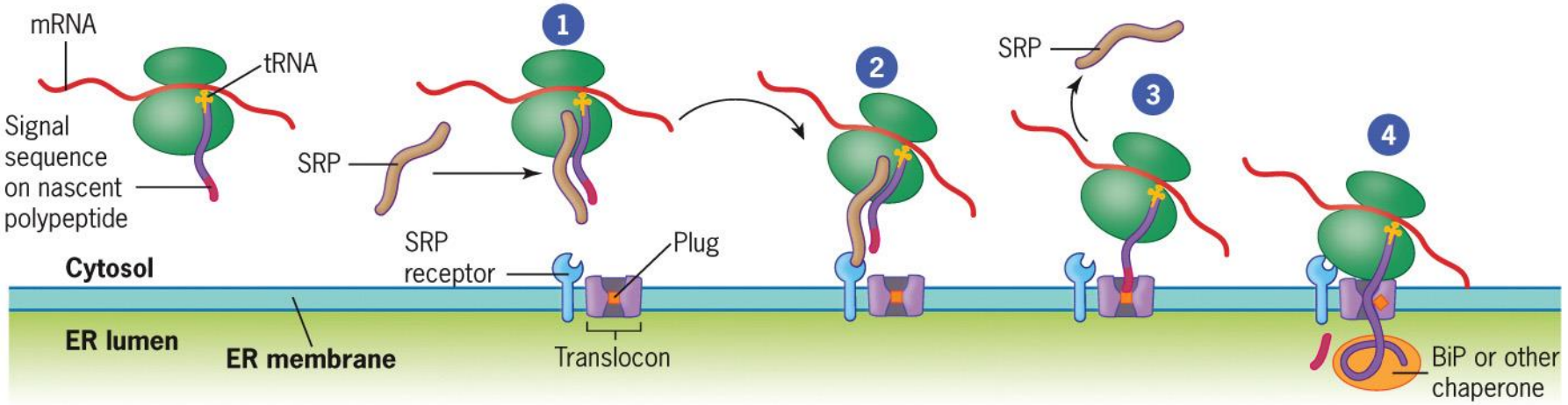

Fig. 8.13a A schematic model of the synthesis of a secretory protein (or a lysosomal enzyme) on a membrane-bound ribosome of the RER

8.3 | The Endoplasmic Reticulum (9 of 21)

Synthesis of Secretory, Lysosomal, or Plant Vacuolar Proteins

- Several of the steps involved in the synthesis and trafficking of secretory proteins are regulated by the binding or hydrolysis of GTP.
- SRP and the SRP receptor are G proteins that interact with one another in their GTP-bound states; GTP hydolysis triggers the release of the signal sequence by the SRP.

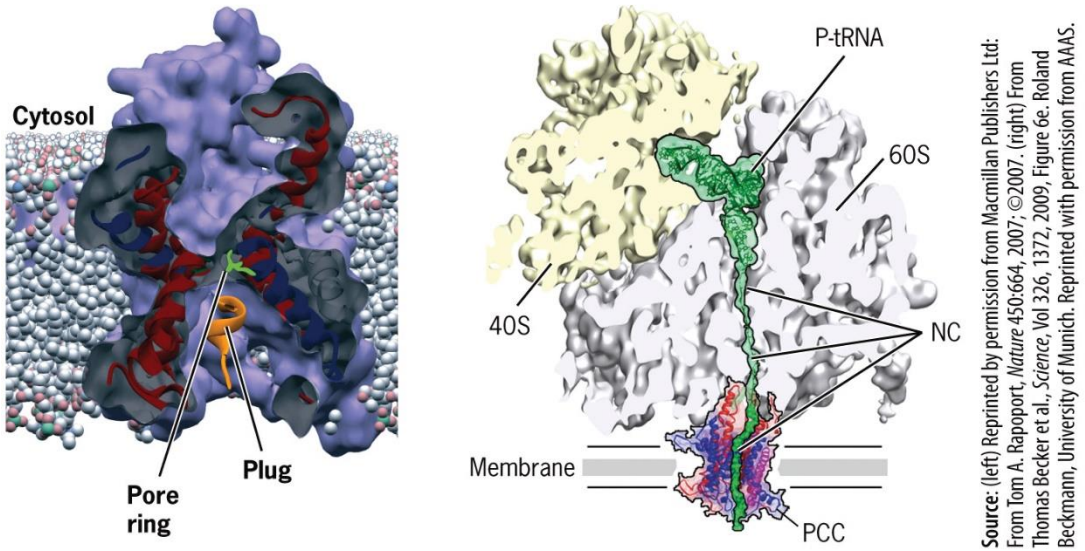

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Fig. 8.13b,c A schematic model of the synthesis of a secretory protein (or a lysosomal enzyme) on a membrane-bound ribosome of the RER

8.3 | The Endoplasmic Reticulum (10 of 21)

The Rough Endoplasmic Reticulum: Processing of Newly Synthesized Proteins in the Endoplasmic Reticulum

- The signal peptide is removed from most nascent polypeptides by **signal peptidase**, while carbohydrates are added by **oligosaccharyltransferase**. Both enzymes are integral membrane proteins associated with the translocon.
- The ER membrane provides a large surface area for ribosomes to attach, and the lumen gives a specialized local environment that favors protein processing.

8.3 | The Endoplasmic Reticulum (11 of 21)

The Rough Endoplasmic Reticulum: Synthesis of Integral Membrane Proteins on ER-Bound Ribosomes

- Integral membrane proteins are synthesized co-translationally, and their hydrophobic transmembrane segments are shunted from the **translocon** into the lipid bilayer.
- During membrane protein synthesis, the inner lining of the translocon orients the nascent polypeptide so that the more positive end faces the cytosol.

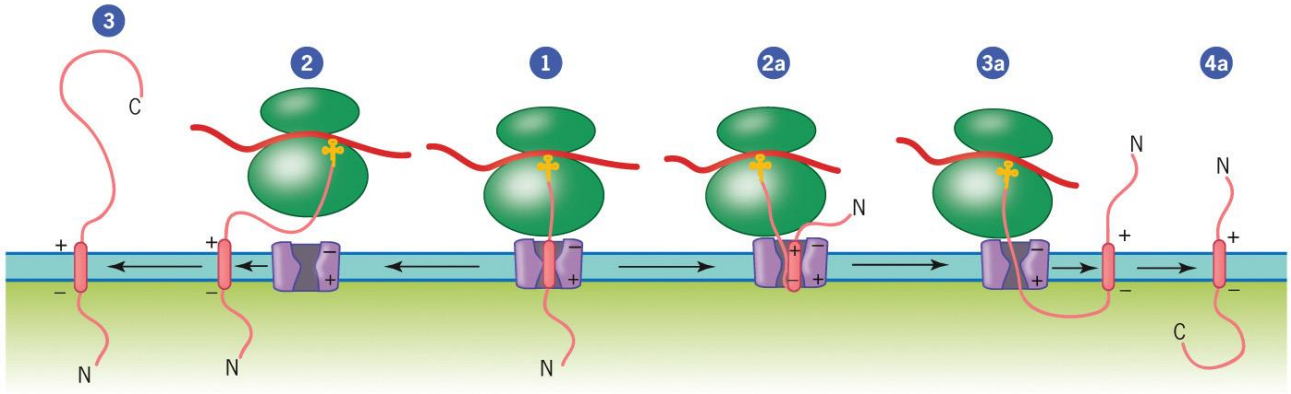

Fig. 8.14 A schematic model for the synthesis of an integral membrane protein

8.3 | The Endoplasmic Reticulum (12 of 21)

The Rough Endoplasmic Reticulum: Synthesis of Integral Membrane Proteins on ER-Bound Ribosomes

- In multispanning proteins, sequential transmembrane segments typically have opposite orientations, so their arrangement in the membrane is determined by the direction in which the first segment is inserted.
- **Tail-anchored proteins** lack a signal sequence, but are synthesized in the cytoplasm, and targeted to the ER through interactions with proteins in the Get (Guided Entry of Tail-Anchored proteins) pathway.

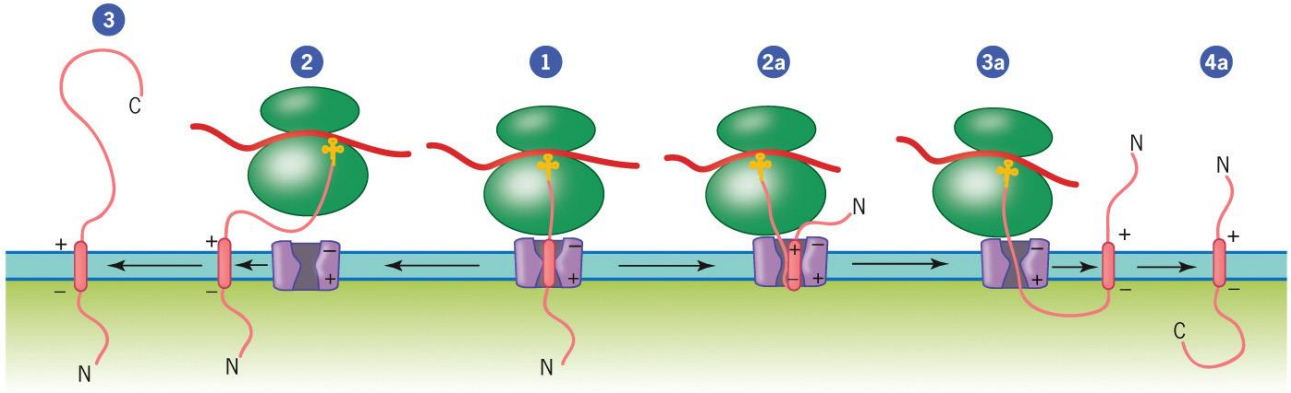

Fig. 8.14 A schematic model for the synthesis of an integral membrane protein

8.3 | The Endoplasmic Reticulum (13 of 21)

The Rough Endoplasmic Reticulum: Membrane Biosynthesis in the Endoplasmic Reticulum

- Membranes arise from pre-existing membranes
- Membranes are enzymatically modified as they move from ER into other cellular compartments
- Membranes are asymmetric with a cytosolic face and a luminal/extracellular face established in the ER

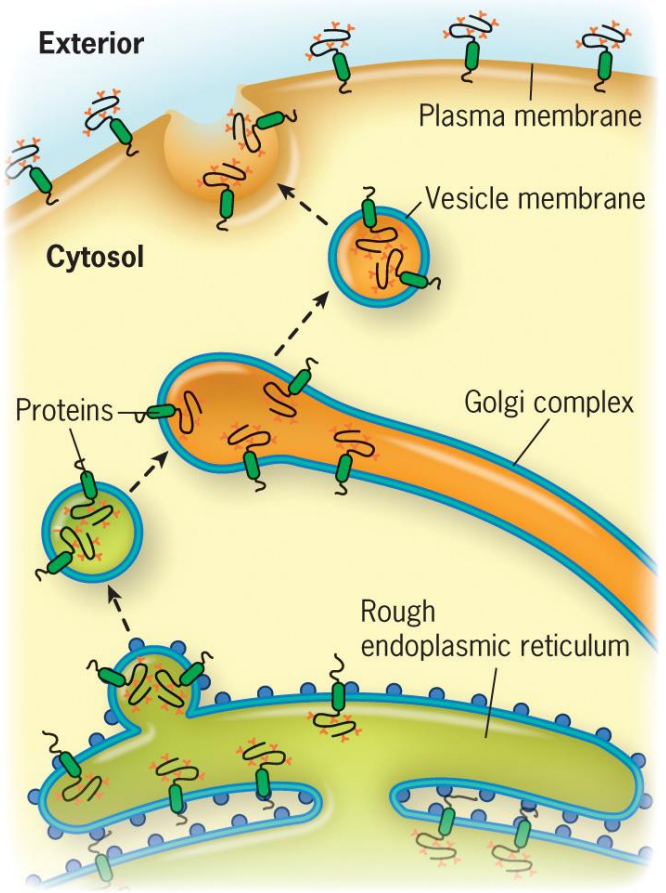

Fig. 8.15 Maintenance of membrane asymmetry

8.3 | The Endoplasmic Reticulum (14 of 21)

The Rough Endoplasmic Reticulum: Membrane Biosynthesis in the Endoplasmic Reticulum

- Membranes of different organelles have different lipid compositions. They can change their lipid composition by:
- Using lipid-modifying enzymes to convert a phospholipid to another
- Preferentially including or excluding phospholipid vesicles
- Exchanging lipids between organellar compartments using lipid transfer proteins

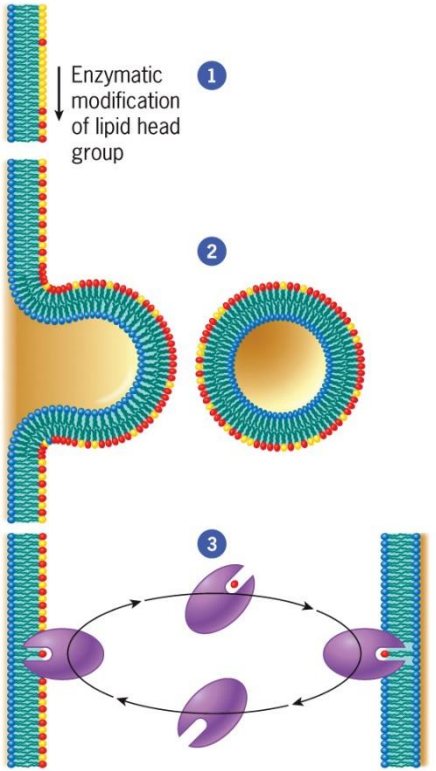

Fig. 8.16 Modifying the lipid composition of membranes

8.3 | The Endoplasmic Reticulum (15 of 21)

The Rough Endoplasmic Reticulum: Glycosylation in the Rough Endoplasmic Reticulum

- Nearly all proteins produced on RER become glycoproteins.
- Addition of sugars to an oligosaccharide is catalyzed by **glycosyltransferases**, each transfers a specific monosaccharide to the growing end of the carbohydrate chain.
- The sugar arrangement in the oligosaccharide chains of a glycoprotein depends on the spatial localization of enzymes in the assembly line.

8.3 | The Endoplasmic Reticulum (16 of 21)

The Rough Endoplasmic Reticulum: Glycosylation in the Rough Endoplasmic Reticulum

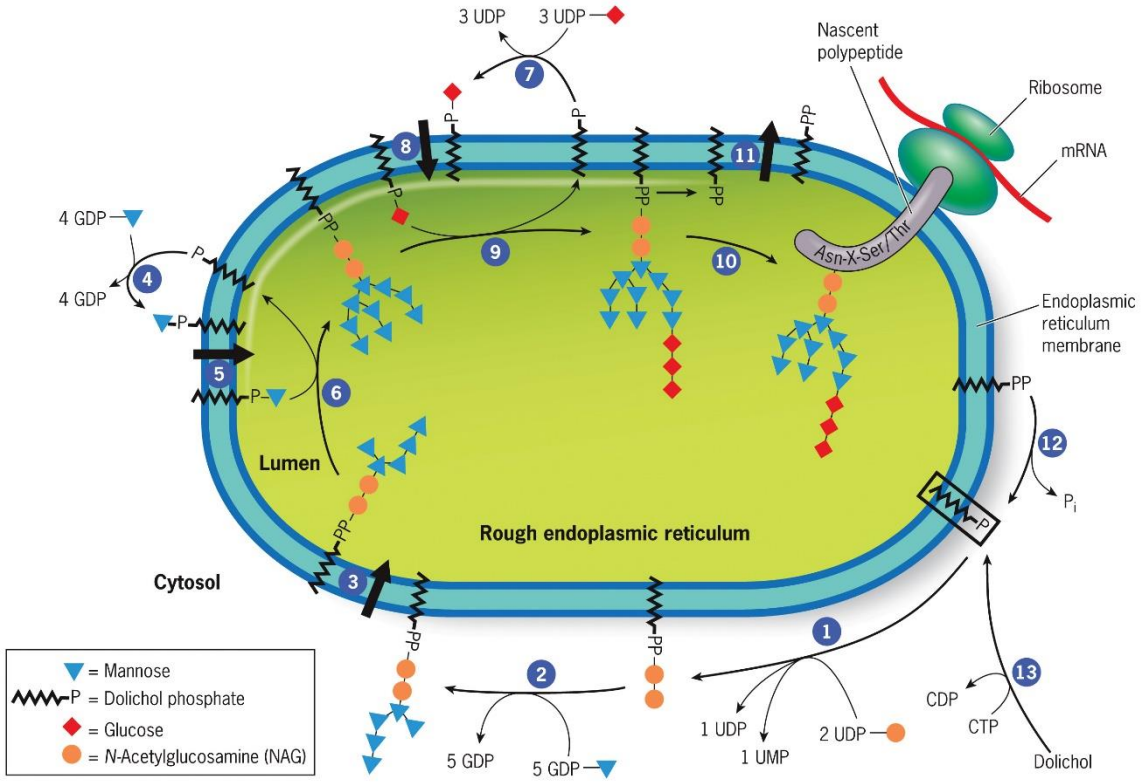

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Fig. 8.17 Steps in the synthesis of the core portion of an *N*-linked oligosaccharide in the rough ER

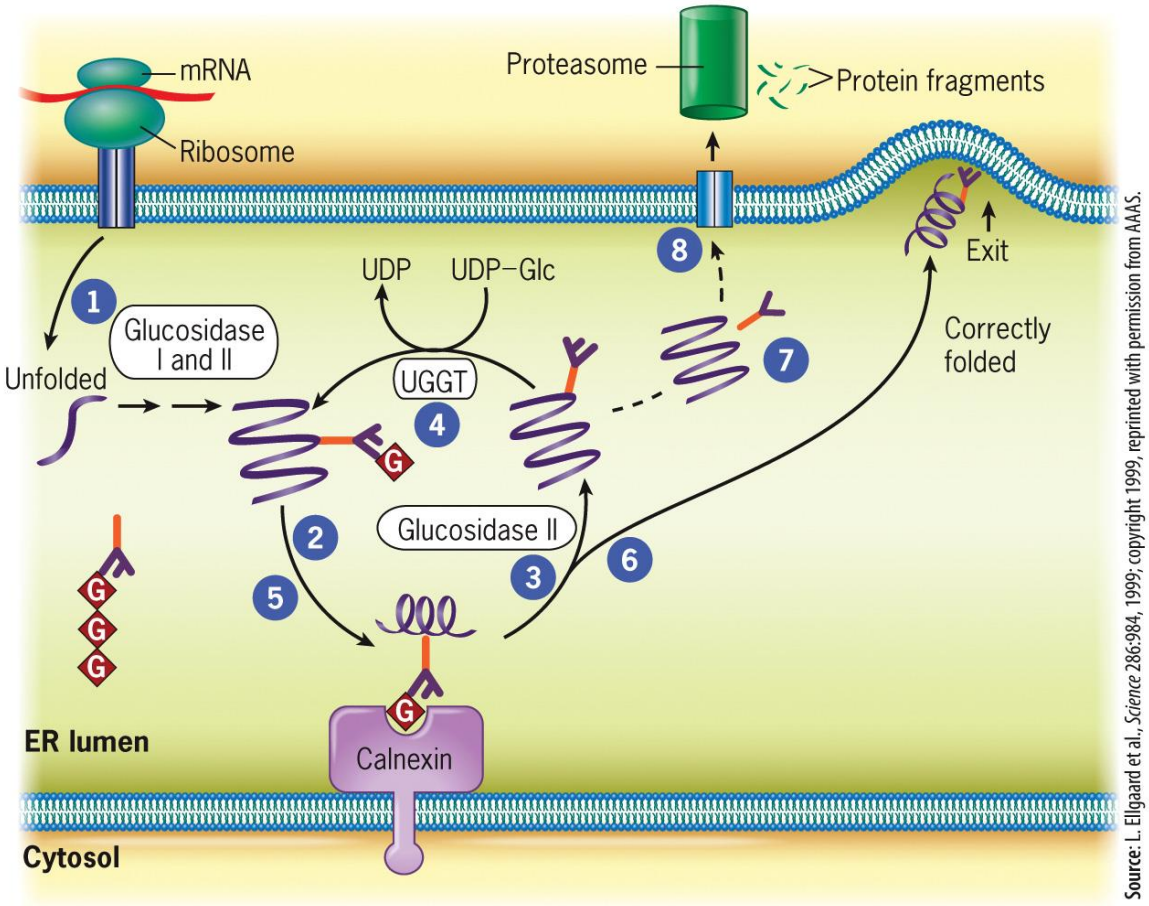
8.3 | The Endoplasmic Reticulum (17 of 21)

The Rough Endoplasmic Reticulum: Glycosylation in the Rough Endoplasmic Reticulum

- Soon after transfer to the polypeptide, the oligosaccharide is gradually modified.
- A glycoprotein goes through a system of **quality control** to determine its fitness for a specific compartment. (UGGT)
- Misfolded proteins will be glucose tagged, mannose deficient and ultimately degraded by proteasomes (ER-associated degradation

8.3 | The Endoplasmic Reticulum (18 of 21)

The Rough Endoplasmic Reticulum: Glycosylation in the Rough Endoplasmic Reticulum



Source: L. Ellgaard et al., *Science* 286:984, 1999; copyright 1999, reprinted with permission from AAAS.

Fig. 8.18 Quality control: ensuring that misfolded proteins do not proceed forward

8.3 | The Endoplasmic Reticulum (19 of 21)

The Rough Endoplasmic Reticulum: Mechanisms That Ensure the Destruction of Misfolded Proteins

- Accumulation of misfolded proteins triggers the **unfolded protein response (UPR)**.
- Sensors in the ER are kept inactive by the chaperone BiP.
- When misfolded proteins accumulate, BiP is incapable of inhibiting the sensors.
- Activated sensors send signals to trigger proteins involved in destruction of misfolded proteins.

8.3 | The Endoplasmic Reticulum (20 of 21)

The Rough Endoplasmic Reticulum: Glycosylation in the Rough Endoplasmic Reticulum

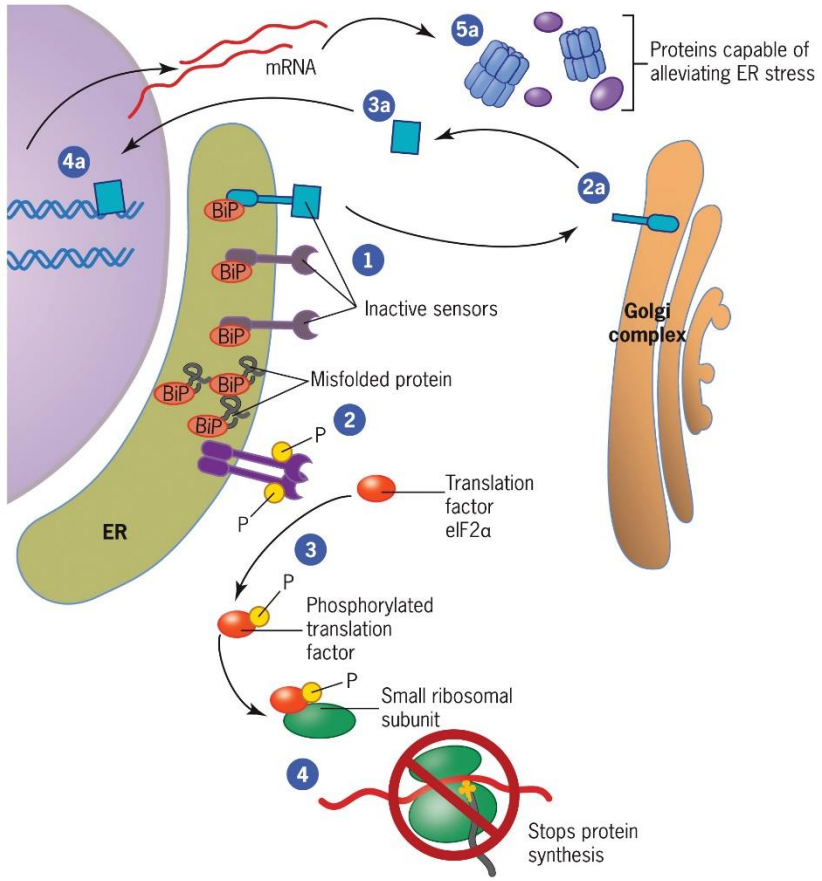


Fig. 8.19 A model of the mammalian unfolded protein response (UPR)

8.3 | The Endoplasmic Reticulum (21 of 21)

The ER to Golgi Vesicular Transport

- The ER to the Golgi Complex is the first step in vesicular transport.
- RER have specialized exit sites where transport vesicles are formed (no ribosomes).
- Transport vesicles fuse with one another and form the *ERGIC* (endoplasmic reticulum Golgi intermediate compartment) toward the Golgi complex.

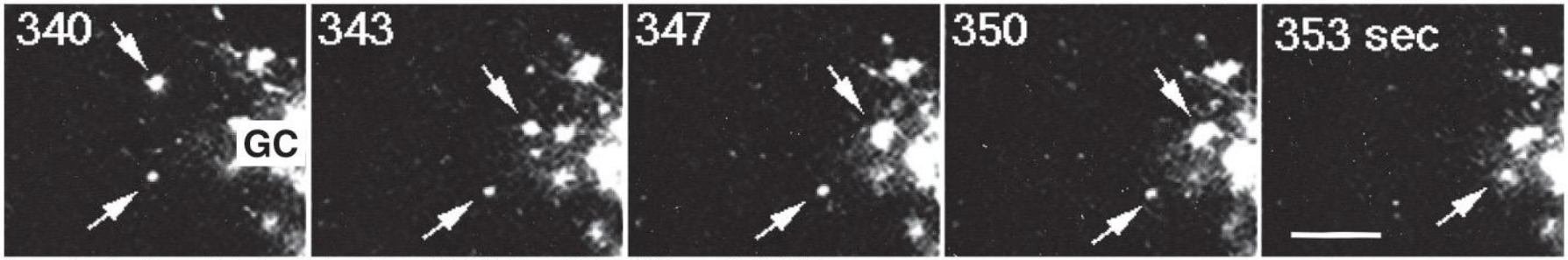


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Fig. 8.20 Visualizing membrane traffic with the use of a fluorescent tag

8.4 | The Golgi Complex (1 of 4)

- The **Golgi complex** is a stack of flattened cisternae.
- Several functionally distinct compartments:
  - *cis* face of the Golgi faces the ER
  - *trans* face is on the opposite side of the stack.

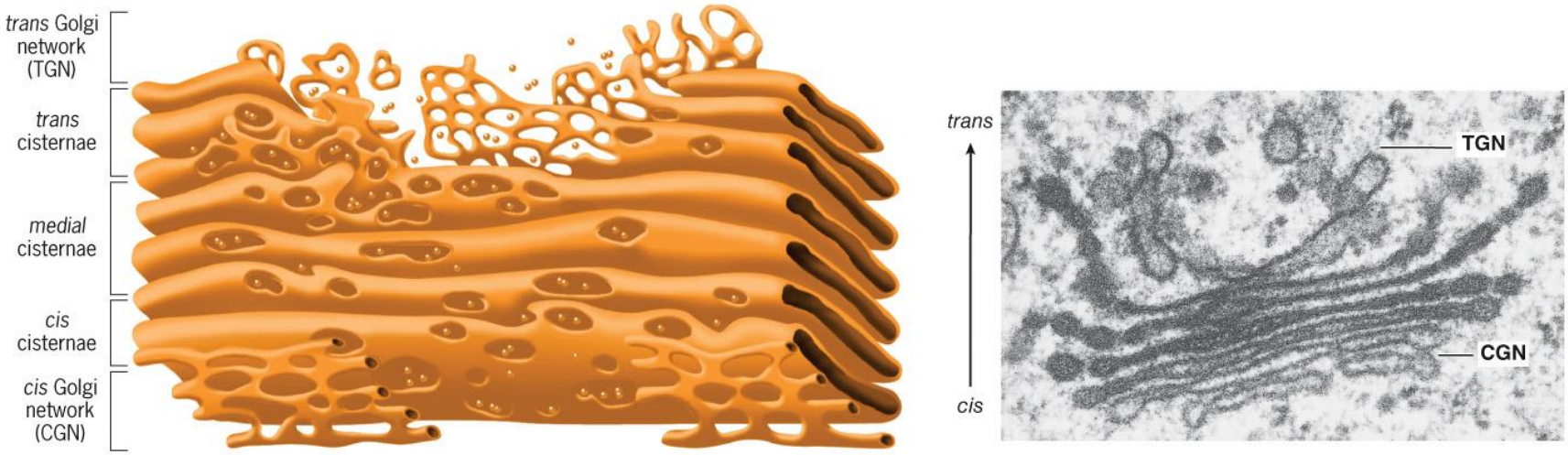

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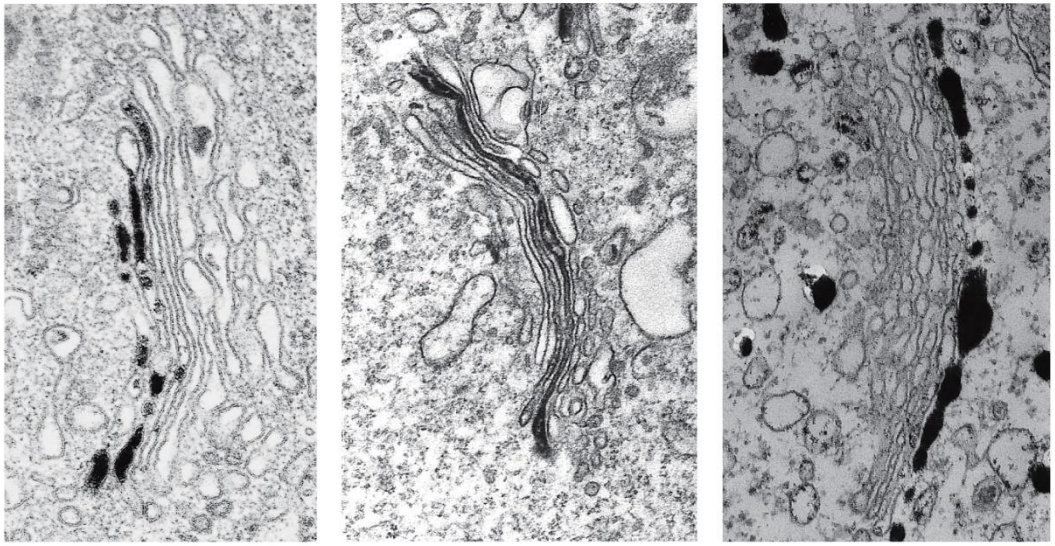
Fig. 8.21a,b The Golgi complex

8.4 | The Golgi Complex (2 of 4)

- The ***cis* Golgi network (CGN)** functions to sort proteins for the ER or the next Golgi station.
- The ***trans* Golgi network (TGN)** functions in sorting proteins to the plasma membrane or various intracellular destinations.
- The Golgi complex is not uniform in composition; there are differences in composition from the *cis* to the *trans* face.



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8.22 Regional differences in membrane composition across the Golgi stack

8.4 | The Golgi Complex (3 of 4)

- Assembly of carbohydrates found in glycolipids and glycoproteins takes place in the Golgi.
- Sequence of incorporation of sugars into oligosaccharides is determined by glycosyltransferases.

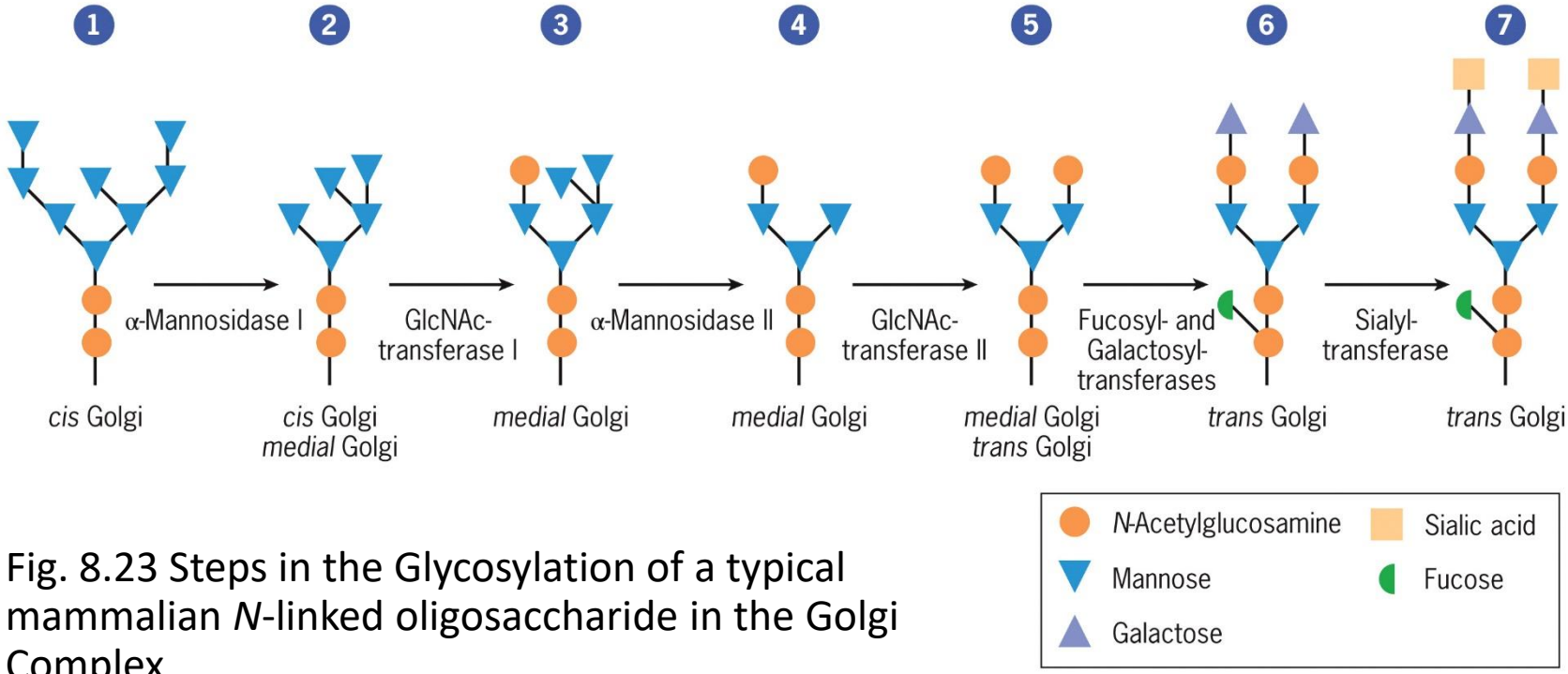
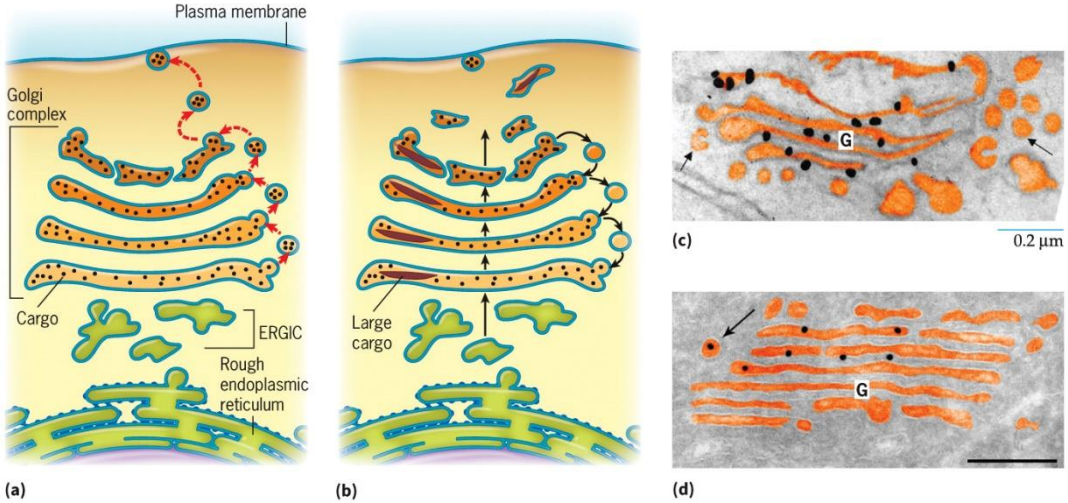


Fig. 8.23 Steps in the Glycosylation of a typical mammalian *N*-linked oligosaccharide in the Golgi Complex

8.4 | The Golgi Complex (4 of 4)

- In the **vesicular transport model**, cargo is shuttled from the CGN to the TGN in vesicles.
- In the **cisternal maturation model**, each cistern "matures" as it moves from the *cis* face to the *trans* face.
- **Current model**: similar to cisternal maturation model but with vesicle retrograde transport. Golgi cisternae serve an primary anterograde carriers.



Source: (a) Vesicular transport model. From Alexander A. Mironov et al., courtesy of Alberto Luini, *J. Cell Biol.* 155:1234, 2001 reproduced with permission of The Rockefeller University Press; (b) Cisternal maturation model. From Jose A. Martinez-menárguez et al., courtesy of Judith Klumperman, *J. Cell Biol.* 155:1214, 2001 reproduced with permission of The Rockefeller University Press; (c) ©2001 Alexander A. Mironov.et.al. Originally published in *The Journal of Cell Biology*. https://doi.org/10.1083/jcb.200108073; (d) ©2001 Judith Klumperman et al. Originally published in *The Journal of Cell Biology*. https://doi.org/10.1083/jcb.200108029.

Fig. 8.24 The dynamics of transport through the Golgi complex

8.5 | Types of Vesicle Transport (1 of 16)

- Materials are carried between compartment using **coated vesicles**.
- Protein coats have two functions:
  1. Cause the membrane to curve and form a vesicle.
  2. Select the components to be carried by vesicle.

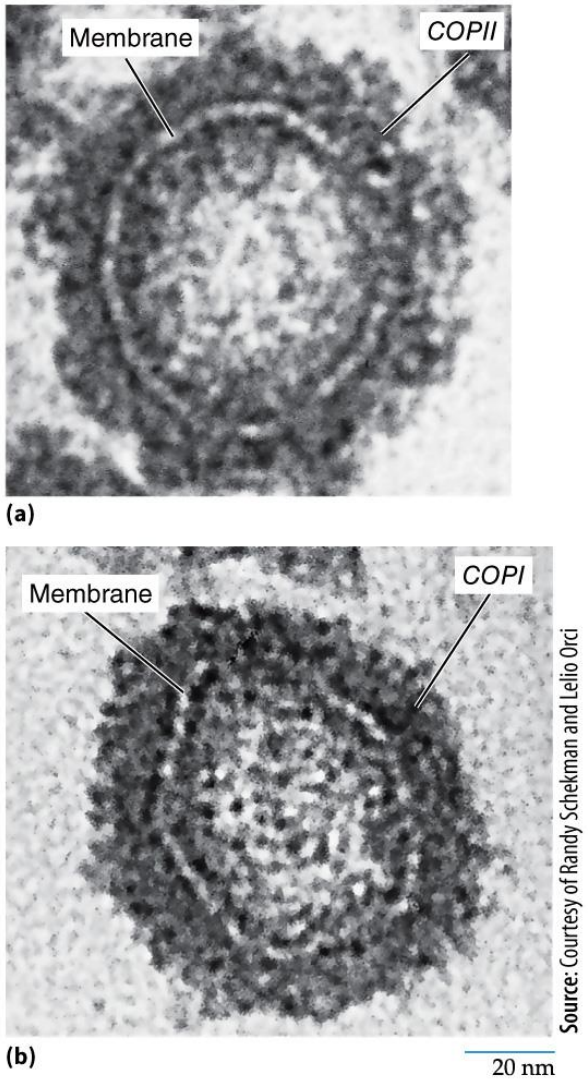
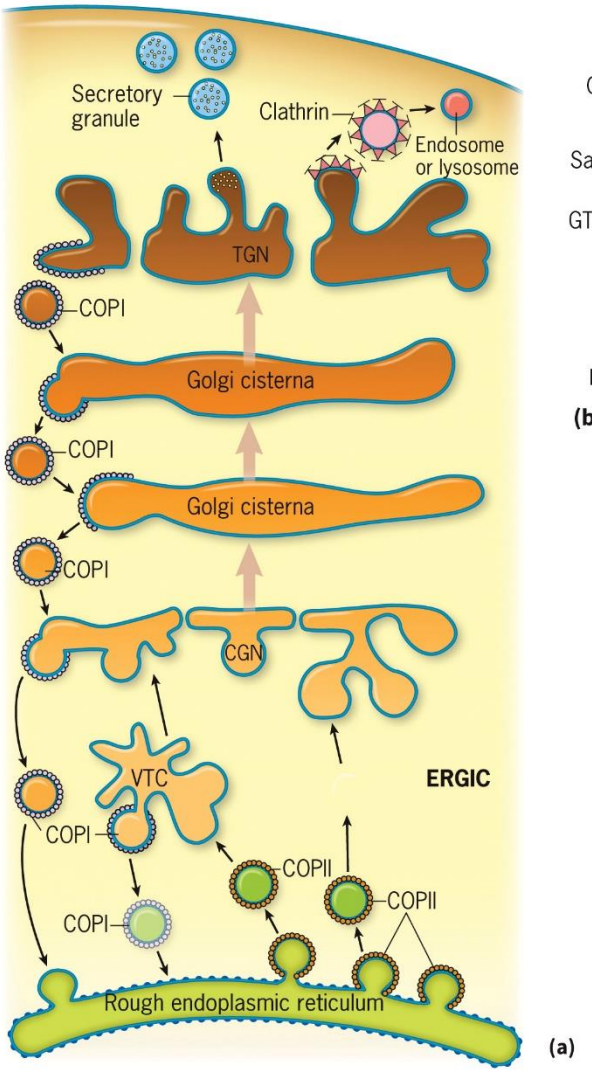


Fig. 8.25 Coated Vesicles

8.5 | Types of Vesicle Transport (2 of 16)

- **COPII-coated vesicles** – move materials from the ER "forward" to the ERGIC intermediate compartment and Golgi complex.
- **COPI-coated vesicles** – move materials from ERGIC and Golgi "backward" to ER, or from the trans Golgi to the cis Golgi cisternae.
- **Clathrin-coated vesicles** – move materials from the TGN to endosomes, lysosomes, and plant vacuoles.

8.5 | Types of Vesicle Transport (3 of 16)



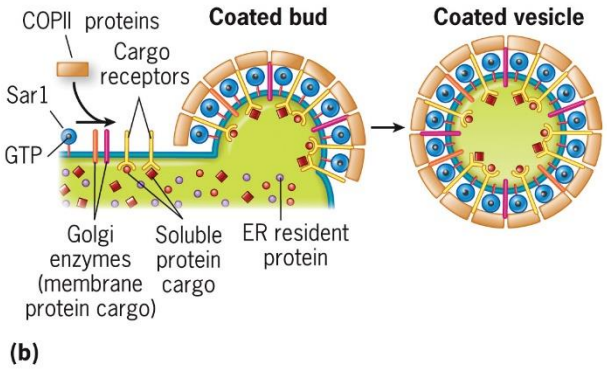
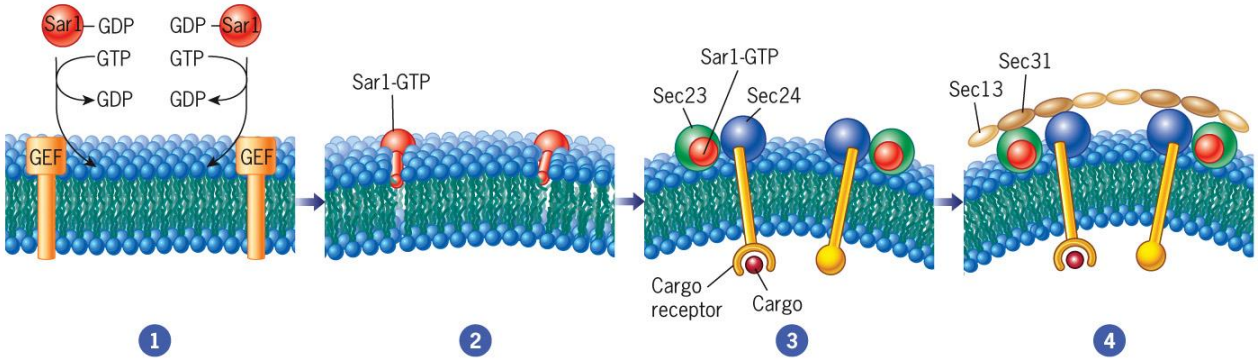


Fig. 8.26 Proposed transport between membrane compartments of the biosynthetic-secretory pathway

8.5 | Types of Vesicle Transport (4 of 16)

COPII-Coated Vesicles: Transporting Cargo from the ER to the Golgi Complex

- COPII coated vesicles bud off specialized domains of the ER called **ER exit sites (ERESs)**. This begins the biosynthetic pathway.
- **ER export signals** found in cytosolic tails of transported proteins
- COPII coats select and concentrate enzymes such as glycosyl-transferases, vesicle docking proteins and cargo selecting proteins



Source: Adapted from Stephen Stephan Fath et al., by Jonathan Goldberg, *Cell* 129 : 1333, 2007.

Fig. 8.27 Proposed roles of the COPII coat proteins in generating membrane curvature, assembling the protein coat, and capturing cargo

8.5 | Types of Vesicle Transport (5 of 16)

COPII-Coated Vesicles: Transporting Cargo from the ER to the Golgi Complex

- All vesicles have two distinct layers: an outer scaffold and an inner layer of adaptor (or adaptor-like) proteins.
- The structure of the outer scaffolds are very different;
    1. the subunits of the clathrin lattice (three-legged clathrin complexes) overlap extensively
    2. the COPII lattice does not overlap. Each vertex of a COPII coat is formed by four edges rather than three (clathrin and perhaps the COPI coat).

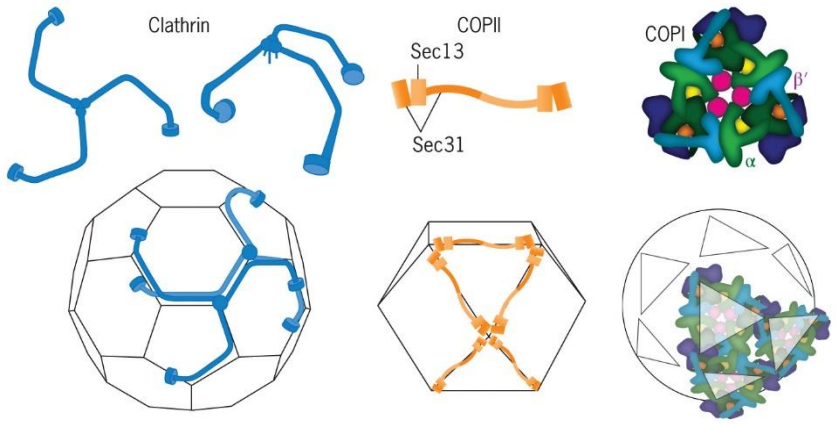


Fig. 8.28c Structure of vesicle coats

8.5 | Types of Vesicle Transport (6 of 16)

COPI-Coated Vesicles: Transporting Escaped Proteins Back to the ER

- COPI coat is made up of a complex called a **coatamer** that is made up of seven proteins.
- COPI-coated vesicles have been most clearly implicated in the retrograde transport of proteins, including the movement of:
  1. Golgi resident enzymes in a trans-to-cis direction
  2. ER resident enzymes from the ERGIC and the Golgi complex back to the ER.

8.5 | Types of Vesicle Transport (7 of 16)

COPI-Coated Vesicles: Transporting Escaped Proteins Back to the ER

- Organelle proteins are maintained by:
  1. **Retention** of resident molecules excluded from transport vesicles.
  2. **Retrieval** of "escaped" molecules back to their normal compartment.
- Resident proteins of the ER contain an amino acid sequence at the C-terminus serving as a **retrieval signal**.
- Specific receptors capture the molecules and bring them to the ER in COPI-coated vesicles. Each membrane compartment may have its own retrieval signals.

8.5 | Types of Vesicle Transport (8 of 16)

COPI-Coated Vesicles: Transporting Escaped Proteins Back to the ER

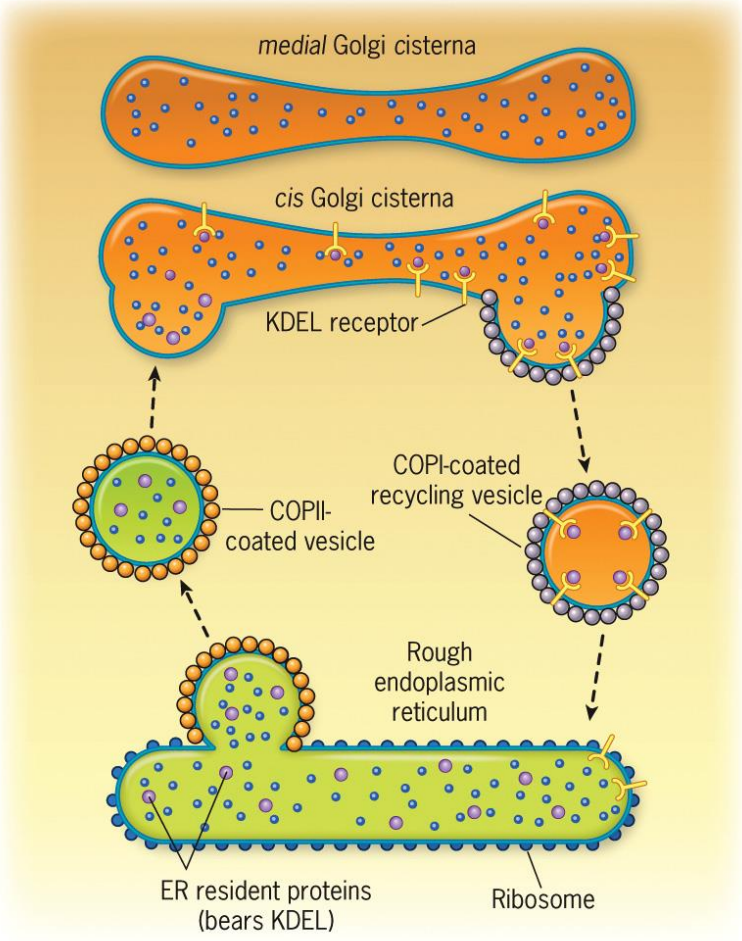
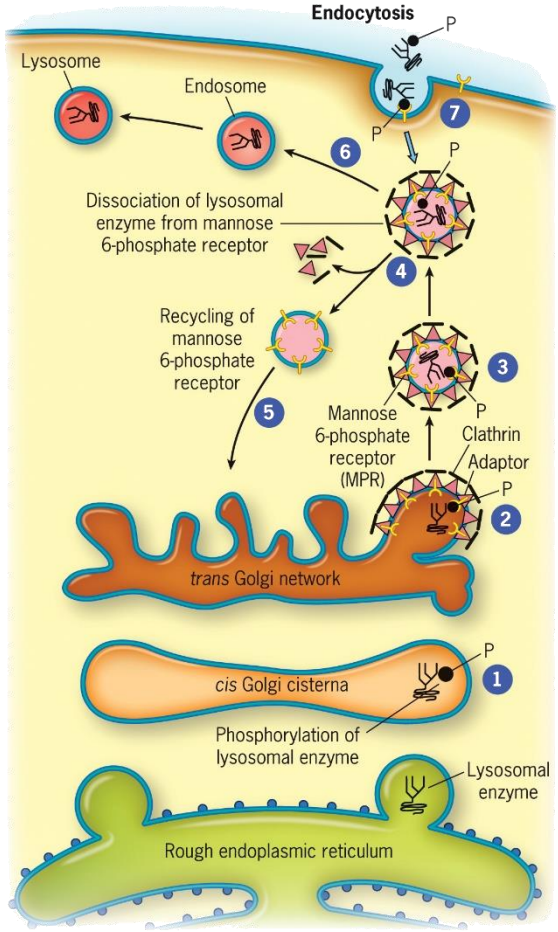


Fig. 8.29 Retrieving ER proteins

8.5 | Types of Vesicle Transport (9 of 16)

Beyond the Golgi Complex: Sorting Proteins at the TGN

- Sorting and transport of lysosomal enzymes utilizes clathrin-coated vesicles.
- Lysosomal proteins are tagged in the *cis*-Golgi with phosphorylated mannose residues.
- Tagged lysosomal enzymes are recognized and captured by **mannose 6-phosphate receptors (MPRs)**, which are bound by coat proteins.



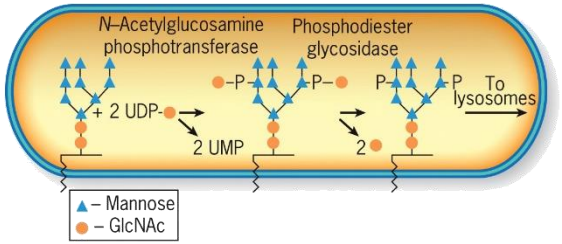


Fig. 8.30 Targeting lysosomal enzymes to lysosomes

8.5 | Types of Vesicle Transport (10 of 16)

Beyond the Golgi Complex: Sorting Proteins at the TGN—Sorting and Transport of Lysosomal Enzymes

- Clathrin-coated vesicles contain:
  - An outer lattice composed of clathrin.
  - An inner shell composed of **GGA** adaptor proteins, which interacts with clathrin, MPR, and G-protein.
  - The G-protein Arf1-GTP, used to initiate membrane curvature and recruit adaptors

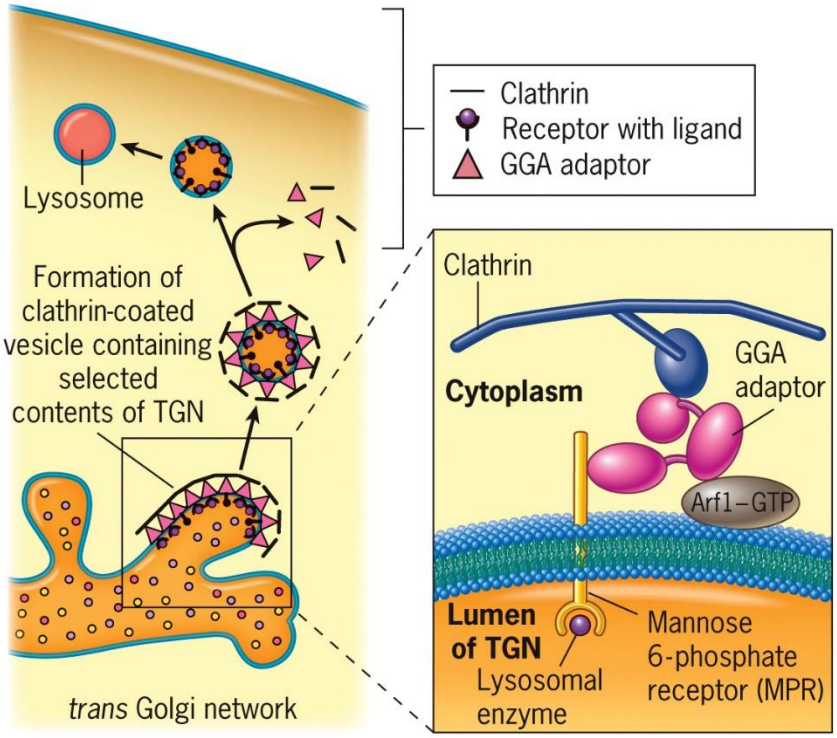


Fig. 8.31 The Formation of clathrin-coated vesicles at the TGN

8.5 | Types of Vesicle Transport (11 of 16)

Beyond the Golgi Complex: Sorting Proteins at the TGN—Sorting and Transport of Nonlysosomal Proteins

- Secreted proteins which are released by regulated secretion may form selective aggregates that eventually become contained in large, densely packed secretory granules.
- Mature granules are stored in the cytoplasm until their contents are released following stimulation of the cell by a hormone or nerve impulse.
- Plasma membrane proteins may use constitutive secretion or one of two TGN membrane carrier systems

8.5 | Types of Vesicle Transport (12 of 16)

Beyond the Golgi Complex: Sorting Proteins at the TGN—Targeting Vesicles to a Particular Compartment

- The steps that occur between vesicle budding and vesicle fusion include:

1. **Movement of the vesicle toward the specific target compartment**, mediated largely by microtubules and their associated motor proteins.
2. **Tethering vesicles to the target compartment**, mediated by a diverse collection of "tethering" proteins.
3. **Docking vesicles to the target compartment**, vesicle and target compartment membranes come into close contact via interaction between integral proteins of the two membranes.
4. **Fusion between vesicle and target membranes**.

8.5 | Types of Vesicle Transport (13 of 16)

Beyond the Golgi Complex: Sorting Proteins at the TGN—Targeting Vesicles to a Particular Compartment

- **Rabs** are a family of small G proteins which cycle between an active GTP bound state and an inactive GDP bounds state.
- GTP-bound Rabs associate with membranes by a lipid anchor.
- Over 60 different Rab genes identified in humans
- Different Rabs become associated with different membrane compartments.

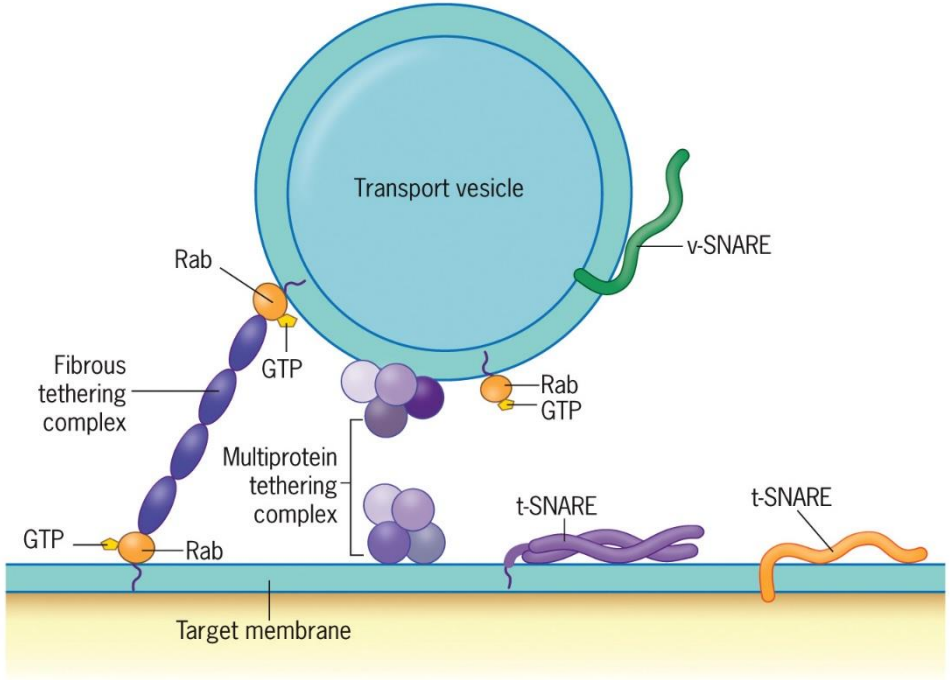


Fig. 8.32a Proposed steps in the targeting of transport vesicles to target membranes

8.5 | Types of Vesicle Transport (14 of 16)

Beyond the Golgi Complex: Sorting Proteins at the TGN—Targeting Vesicles to a Particular Compartment

- **SNAREs** constitute a family of proteins localized to specific subcellular compartments.
- SNAREs are integral proteins that bring the vesicle and target compartment in close contact
- **v-SNAREs** are found in transport vesicles and **t-SNAREs** are located in the target compartments.

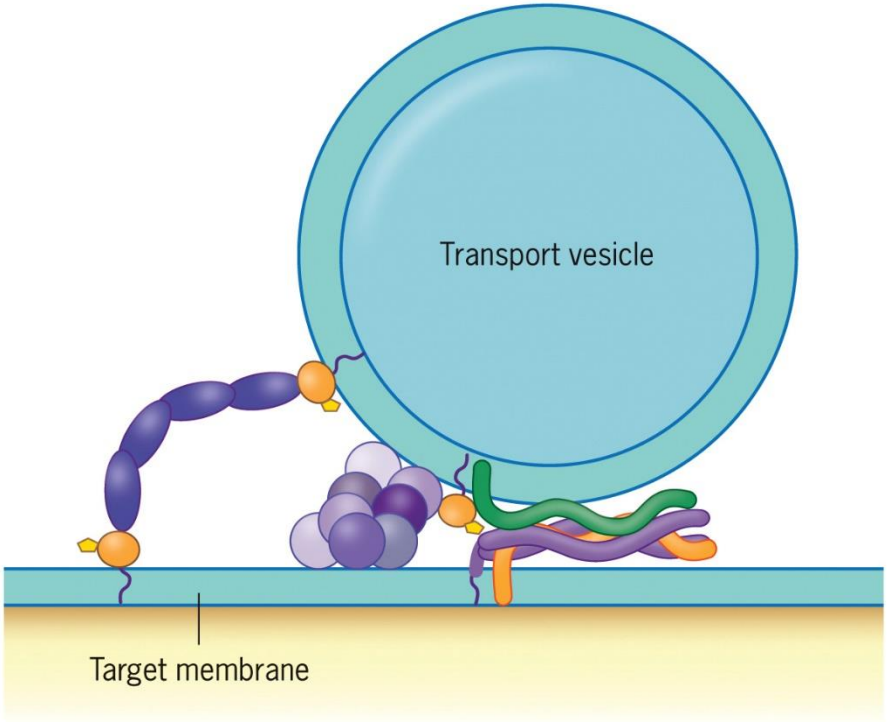


Fig. 8.32c Proposed steps in the targeting of transport vesicles to target membranes

8.5 | Types of Vesicle Transport (15 of 16)

Beyond the Golgi Complex: Sorting Proteins at the TGN—Targeting Vesicles to a Particular Compartment

- In nerve cells:
  - plasma membrane contains two t-SNAREs (syntaxin and SNAP-25)
  - synaptic vesicle membrane contains a single v-SNARE (synaptobrevin)
- T- and v-SNARE molecules interact to form four-stranded bundles, each with four a helices
- These parallel a helices zip together to form a tightly interwoven complex that pulls the two apposing lipid bilayers into very close association.

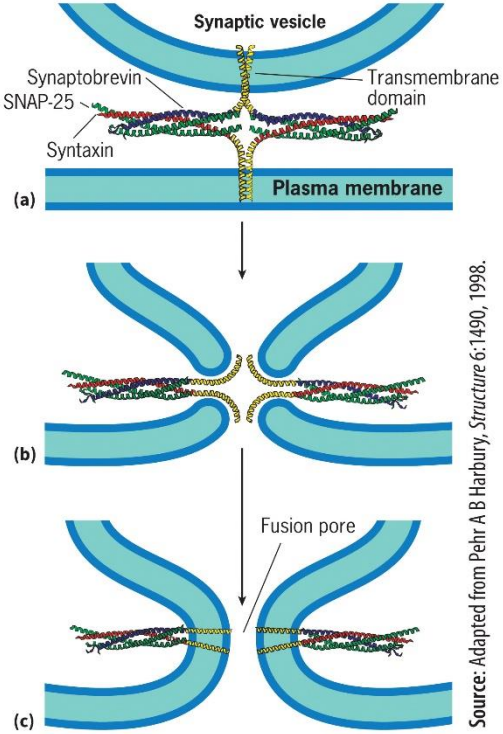


Fig. 8.33 A models of the Interactions between v- and t- SNAREs leading to membrane fusion and exocytosis

8.5 | Types of Vesicle Transport (16 of 16)

Beyond the Golgi Complex: Sorting Proteins at the TGN—Exocytosis

- **Exocytosis** – discharge of a secretory vesicle or granule after fusion with plasma membrane (PM).
- Process is triggered by an increase in [Ca^{2+}].
- Contacts between vesicle and plasma membranes lead to formation of **fusion pore**
- The luminal part of the vesicle membrane becomes the outer surface of the PM, and the cytosolic part becomes part of the inner surface of the PM.

8.6 | Engineering Linkage: Extracellular Vesicles for Drug Delivery

- Extracellular vesicles can move through the body unnoticed by the immune system passing through difficult biological barriers (i.e. blood brain barrier)
- Downside is their short half-life before phagocytosis
- Currently extracellular vesicles can be isolated from cell culture using ultracentrifugation or affinity purification and loaded with therapeutic RNAs or other small molecules
- The challenge now is finding ways to target specific cells in the body.

8.7 | Lysosomes (1 of 5)

- Lysosomes contain at least 50 different hydrolytic enzymes produced in the RER, and can hydrolyze virtually every type of biological macromolecule.
- Lysosomal enzymes (**acid hydrolases**) have optimal activity in the acidic lumen

8.7 | Lysosomes (2 of 5)

Enzyme	Substrate
Phosphatases	
Acid phosphatase	Phosphomonoesters
Acid phosphodiesterase	Phosphodiesters
Nucleases	
Acid ribonuclease	RNA
Acid deoxyribonuclease	DNA
Proteases	
Cathepsin	Proteins
Collagenase	Collagen
GAG-hydrolyzing enzymes	
Iduronate sulfatase	Dermatan sulfate
β-Galactosidase	Keratan sulfate
Heparan *N*-sulfatase	Heparan sulfate
α-*N*-Acetylglucosaminidase	Heparan sulfate

A Sampling of Lysosomal Enzymes (Part 1)

8.7 | Lysosomes (3 of 5)

Enzyme	Substrate
Polysaccharidases and oligosaccharidases	
α-Glucosidase	Glycogen
Fucosidase	Fucosyloligosaccharides
α-Mannosidase	Mannosyloligosaccharides
Sialidase	Sialyloligosaccharides
Sphingolipid-hydrolyzing enzymes	
Ceramidase	Ceramide
Glucocerebrosidase	Glucosylceramide
β-Hexosaminidase	G_{M2} ganglioside
Arylsulfatase A	Galactosylsulfatide
Lipid-hydrolyzing-enzymes	
Acid lipase	Triacylglycerols
Phospholipase	Phospholipids

A Sampling of Lysosomal Enzymes (Part 2)

8.7 | Lysosomes (4 of 5)

- Single-celled organisms and phagocytic white blood cells digest ingested materials by fusing phagosomes and lysosomes
- Lysosomes play a role in the regulated process of organelle turnover, known as **autophagy**.
- A **phagophore** envelops an organelle to produce a double-membrane sequestering vesicle called an **autophagosome**.
- This fuses with a lysosome, generating an **autolysosome**, both the inner membrane of the autophagosome and the enclosed contents are degraded.

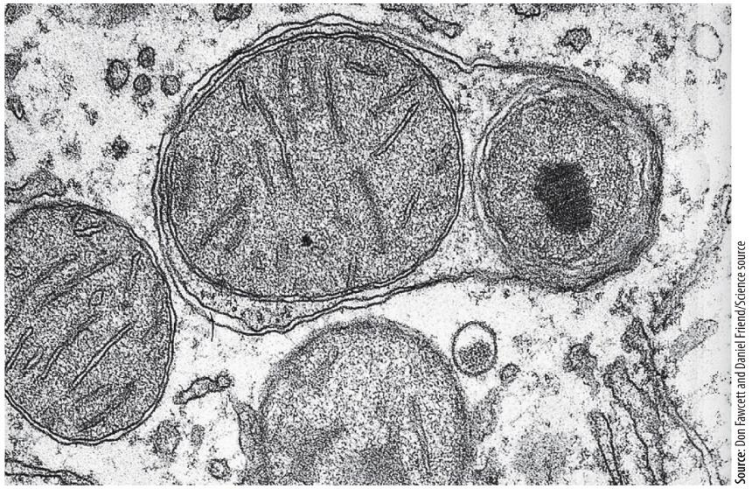


Fig. 8.36 Autophagy

8.7 | Lysosomes (5 of 5)

- Autophagy benefits:
  - Helps protect an organism against intracellular threats (*i.e.*, abnormal protein aggregates or invading bacteria)
  - May play a role in the prevention of certain types of cancers and slowing the body's aging process.

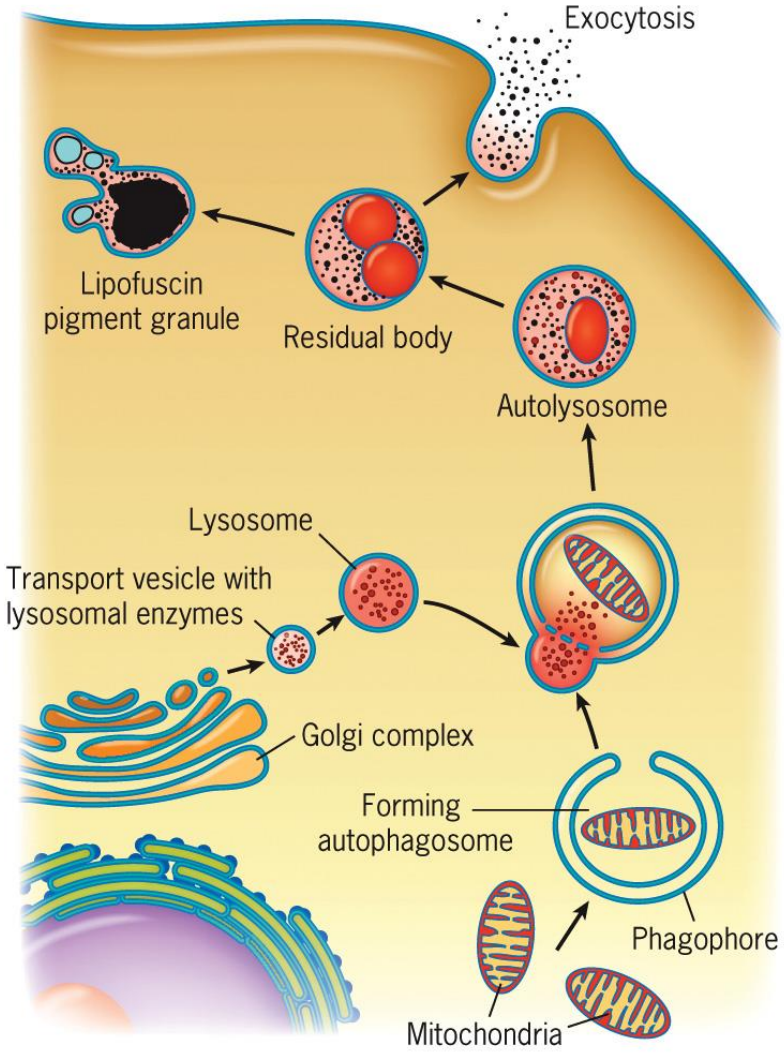


Fig. 8.37 A summary of the autophagic pathway

8.8 | Green Cells: Plant Cell Vacuoles

- A **vacuole** is a membrane-bound, fluid-filled compartment.
- Plant vacuoles have several storage functions:
  - Storage of solutes and macromolecules
  - Storage of toxins
  - Ionic concentration capability
- The vacuole membrane (**tonoplast**) contains an active transport system to keep a high concentration of ions so that water enters by osmosis.
- Plant vacuoles contain acid hydrolases for degradation of biomolecules.

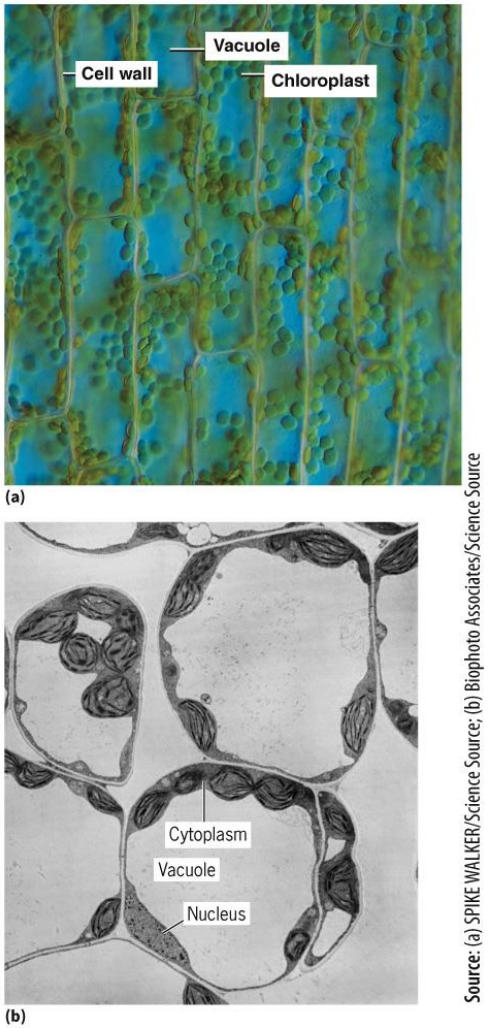


Fig. 8.38 Plant cell vacuoles

8.9 | The Endocytic Pathway: Moving Membrane and Materials into the Cells Interior (1 of 13)

Two basic processes, different mechanisms:

1. **Endocytosis** is primarily a process by which the cell internalizes cell-surface receptors and bound extracellular ligands.
2. **Phagocytosis** is the uptake of particulate matter

8.9 | The Endocytic Pathway: Moving Membrane and Materials into the Cells Interior (2 of 13)

Endocytosis

Endocytosis can be divided broadly into two categories:

1. **Bulk phase endocytosis** (pinocytosis) – non specific uptake of extracellular fluid
2. **Receptor mediated endocytosis** (clathrin mediated endocytosis) – brings about the uptake of specific extracellular macromolecules following binding to receptors on the surface of the plasma membrane.

8.9 | The Endocytic Pathway: Moving Membrane and Materials into the Cells Interior (3 of 13)

Endocytosis: Receptor-Mediated Endocytosis (RME) and the Role of Coated Pits

- Substances that enter the cell through clathrin-mediated RME become bound to **coated pits** on the plasma membrane.
- Clathrin-coated regions invaginate into the cytoplasm and then pinch free of the cytoplasm.

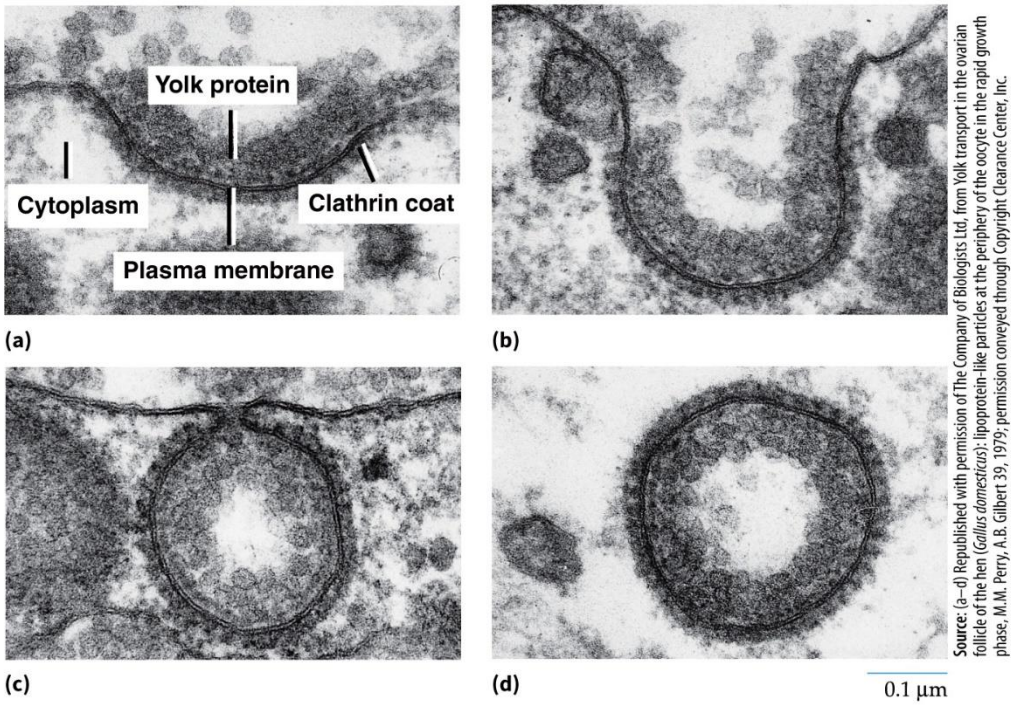


Fig. 8.39 Receptor-mediated endocytosis

8.9 | The Endocytic Pathway: Moving Membrane and Materials into the Cells Interior (4 of 13)

Endocytosis: Receptor-Mediated Endocytosis and the Role of Coated Pits

- Each clathrin molecule has 3 heavy chains and 3 light chains, joined together at the center to form a three-legged assembly called a **triskelion**.
- Triskelions overlap and each leg of extends outward along two edges of a polygon.
- Each vertex of a polygon contains a center of one of the component triskelions

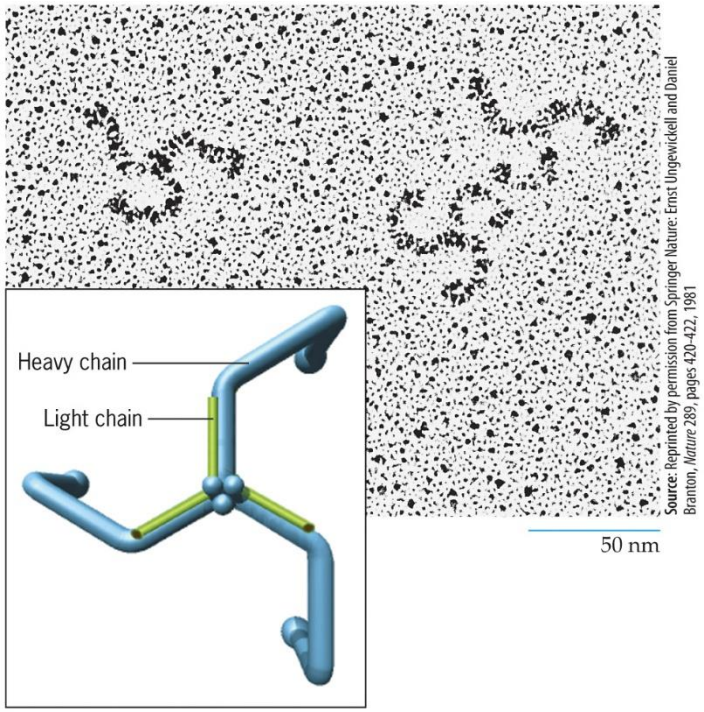


Fig. 8.41 Clathrin triskelions

8.9 | The Endocytic Pathway: Moving Membrane and Materials into the Cells Interior (5 of 13)

Endocytosis: Receptor-Mediated Endocytosis and the Role of Coated Pits

- Coated vesicles contain adaptors between the clathrin lattice and the surface of the vesicle facing the cytosol such as AP2.
- AP2 adaptors contain multiple subunits having different functions.
- AP2 adaptors engage cytoplasmic tails of specific receptors to select bound cargo molecules, and bind and recruit the clathrin molecules of the overlying lattice.

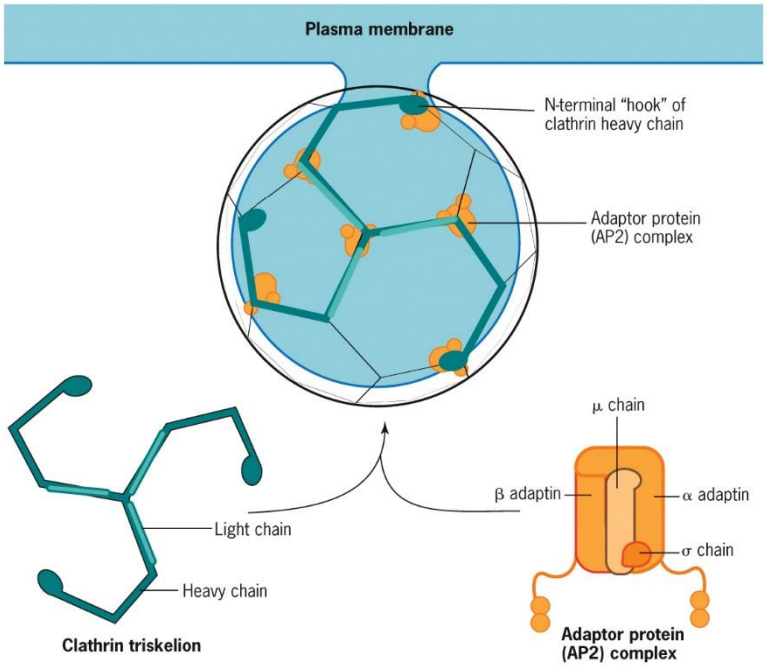
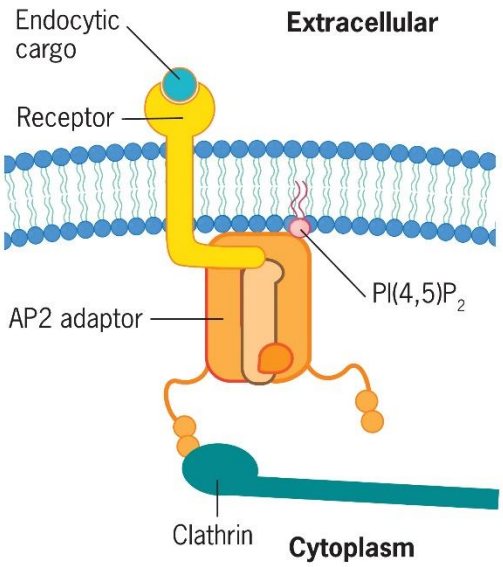


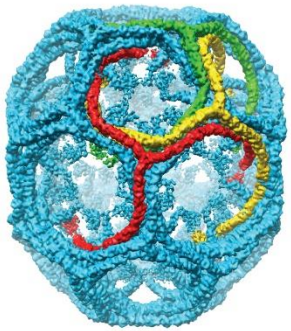
Fig. 8.42a Molecular organization of a coated vesicle

8.9 | The Endocytic Pathway: Moving Membrane and Materials into the Cells Interior (6 of 13)

Endocytosis: Receptor-Mediated Endocytosis and the Role of Coated Pits

- Coated vesicles may contain two dozen different accessory proteins that form a dynamic network of interacting molecules.
- These proteins have roles in cargo recruitment, coat assembly, membrane bending and invagination, interaction with cytoskeletal components, vesicle release, and membrane uncoating.





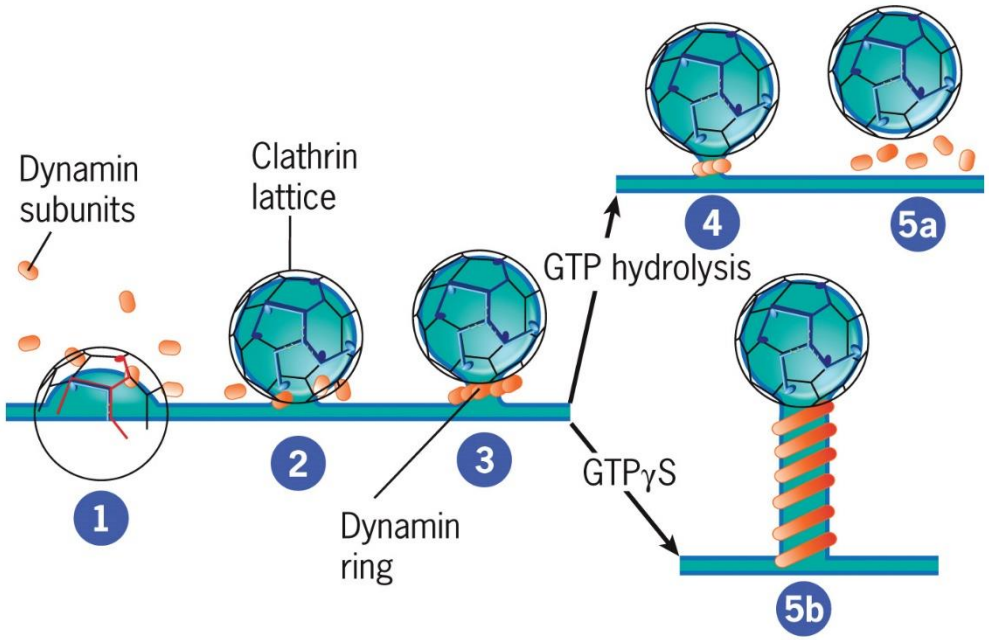
Source: (c) From Alexander Fotin et al., *Nature* 432:574, 2004, courtesy of Stephen C. Harrison; ©2004. Reprinted with permission from Macmillan Publishers Ltd.

Fig. 8.42b,c Molecular organization of a coated vesicle

8.9 | The Endocytic Pathway: Moving Membrane and Materials into the Cells Interior (7 of 13)

Endocytosis: Receptor-Mediated Endocytosis and the Role of Coated Pits

- **Dynamin** is a G protein required for the fission of the vesicle from the membrane on which it forms
- It self-assembles into a helical collar around the neck of an invaginated coated pit.



Source: Adapted From P. De Camilli et al. *Current Opin Neuro-Biol.* Vol 5, p. 562, 1995.

Fig. 8.43a The role of dynamin in the formation of clathrin-coated vesicles

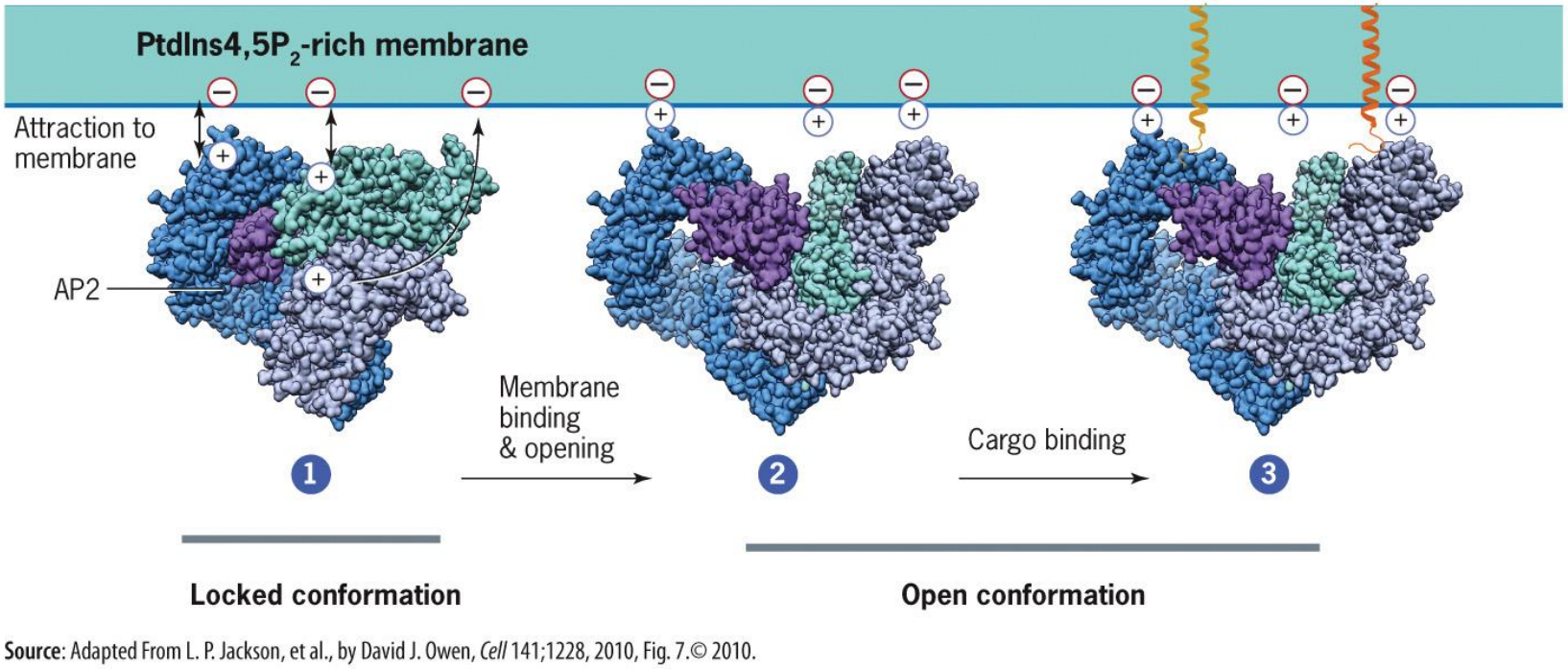
8.9 | The Endocytic Pathway: Moving Membrane and Materials into the Cells Interior (8 of 13)

Endocytosis: The Role of Phosphoinositides in the Regulation of Coated Vesicles

- AP2 adaptors normally exist in the cytosol in a locked conformation.
- Binding of AP2 complex to $PI(4,5)P_2$ causes a conformational change in AP2.
- The AP2 cargo binding site becomes exposed, allowing it to interact with specific membrane receptors.

8.9 | The Endocytic Pathway: Moving Membrane and Materials into the Cells Interior (9 of 13)

Endocytosis: The Role of Phosphoinositides in the Regulation of Coated Vesicles



Source: Adapted From L. P. Jackson, et al., by David J. Owen, *Cell* 141;1228, 2010, Fig. 7.© 2010.

Fig. 8.44 A structural model depicting the changes in protein conformation that occur upon AP2 binding to the plasma membrane

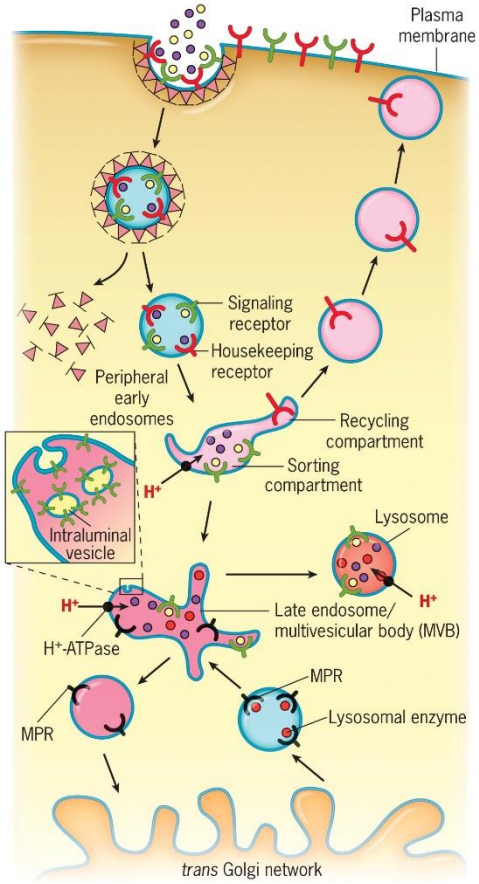
8.9 | The Endocytic Pathway: Moving Membrane and Materials into the Cells Interior (10 of 13)

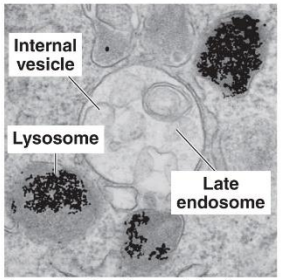
Endocytosis: The Endocytic Pathway

- After internalization, vesicle-bound materials are transported in vesicles and tubules known as **endosomes**.
- **Early endosomes** are located near the periphery of the cell. It sorts materials and sends bound ligands to the **late endosomes**.

8.9 | The Endocytic Pathway: Moving Membrane and Materials into the Cells Interior (11 of 13)

Endocytosis: The Endocytic Pathway





Source: Reprinted by permission from Springer Nature: J. Paul Luzio et al., *Nature Revs. Mol. Cell Biol.* 8, pages 622–632, 2007.

Fig. 8.45 The endocytic pathway

8.9 | The Endocytic Pathway: Moving Membrane and Materials into the Cells Interior (12 of 13)

Endocytosis: The Endocytic Pathway

- **Low-density lipoproteins (LDLs)** are a complex of cholesterol and proteins.
- LDL receptors are transported to the plasma membrane and bound to a coated pit.
- LDLs are taken up by RME and taken to the lysosomes, releasing the cholesterol for use by the cells.

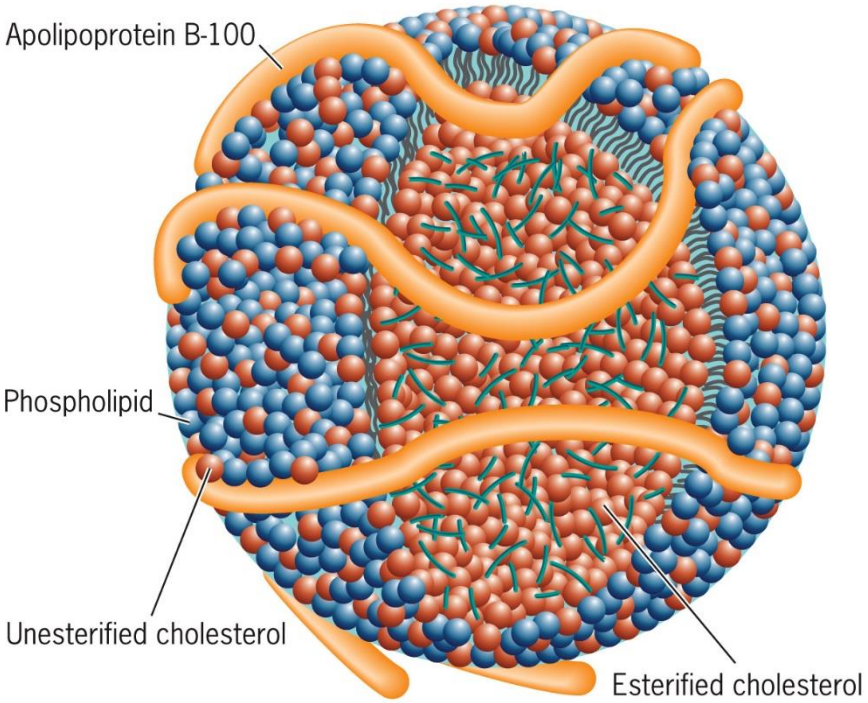


Fig. 8.46 LDL Cholesterol

8.9 | The Endocytic Pathway: Moving Membrane and Materials into the Cells Interior (13 of 13)

Phagocytosis

- Phagocytosis is carried out by cells specialized for the uptake of relatively large particles.
- The folds fuse to produce a vacuole (**phagosome**) that pinches off inwardly from the plasma membrane and fuses with a lysosome (**phagolysosome**).
- Mammals have “professional” phagocytes, *e.g.*, macrophages and neutrophils, that phagocytize invading organisms, damaged and dead cells.

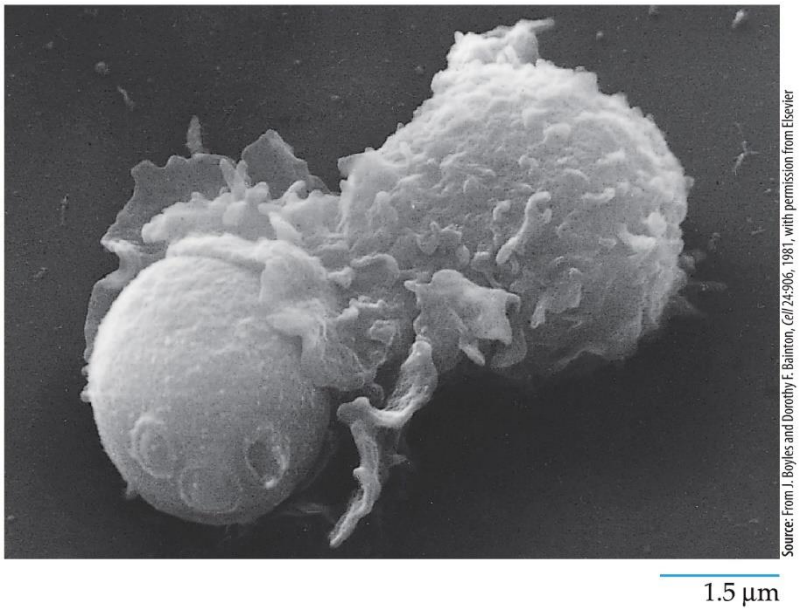


Fig. 8.48a Phagocytosis

8.10 | Posttranslational Uptake of Proteins by Peroxisomes, Mitochondria, and Chloroplasts (1 of 4)

- The nucleus, mitochondria, chloroplasts, and peroxisomes import proteins through one or more outer boundary membranes.
- As in the case of the rough ER, proteins that are imported by these organelles contain amino acid sequences that serve as addresses that are recognized by receptors at the organelle's outer membrane.
- Unlike RER, which generally imports its proteins cotranslationally, the proteins of these other organelles are imported posttranslationally, following their complete synthesis on free ribosomes in the cytosol.

8.10 | Posttranslational Uptake of Proteins by Peroxisomes, Mitochondria, and Chloroplasts (2 of 4)

Uptake of Proteins Into Peroxisomes

- Peroxisomes have two subcompartments in which an imported protein can be placed: the boundary membrane and the internal matrix.
- Peroxisomal proteins possess a peroxisomal targeting signal, either a PTS for a peroxisomal matrix protein or an mPTS for a peroxisomal membrane protein.
- PTS receptors bind to peroxisome-destined proteins in the cytosol and shuttle them to the surface of the peroxisome, where they can enter the organelle.

8.10 | Posttranslational Uptake of Proteins by Peroxisomes, Mitochondria, and Chloroplasts (3 of 4)

Uptake of Proteins Into Mitochondria

- The outer mitochondrial membrane includes a protein-import complex (**TOM complex**) which includes a receptor and channel.
- Proteins destined for the inner mitochondrial membrane engage with another protein-import complex (**TIM complex**).

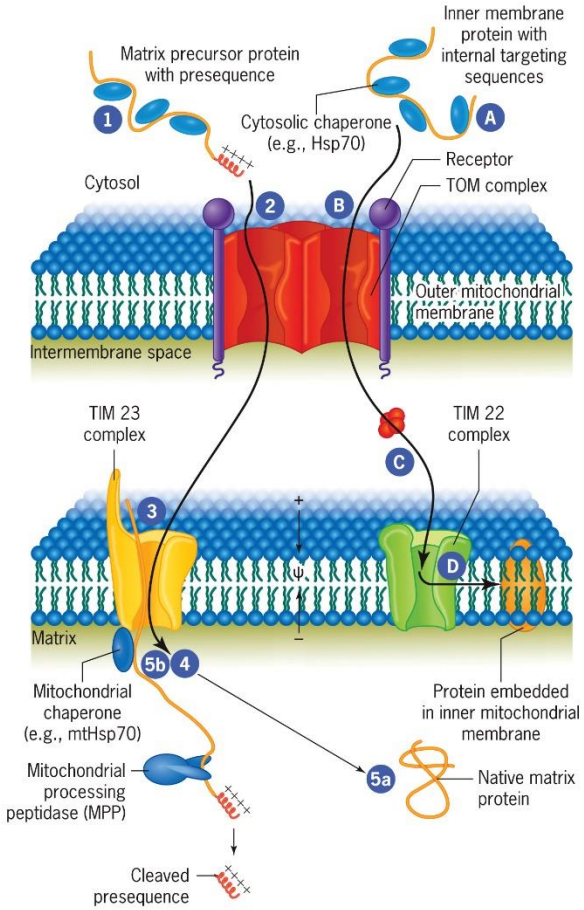


Fig. 8.49 Importing proteins into a mitochondrion

8.10 | Posttranslational Uptake of Proteins by Peroxisomes, Mitochondria, and Chloroplasts (4 of 4)

Uptake of Proteins Into Chloroplasts

- Outer and inner envelope membranes contain translocation complexes (**Toc** and **Tic**) to facilitate import of the proteins.
- Chaperones unfold proteins in cytosol and fold them in the chloroplasts.
- Proteins include a **transit peptide** sequence.

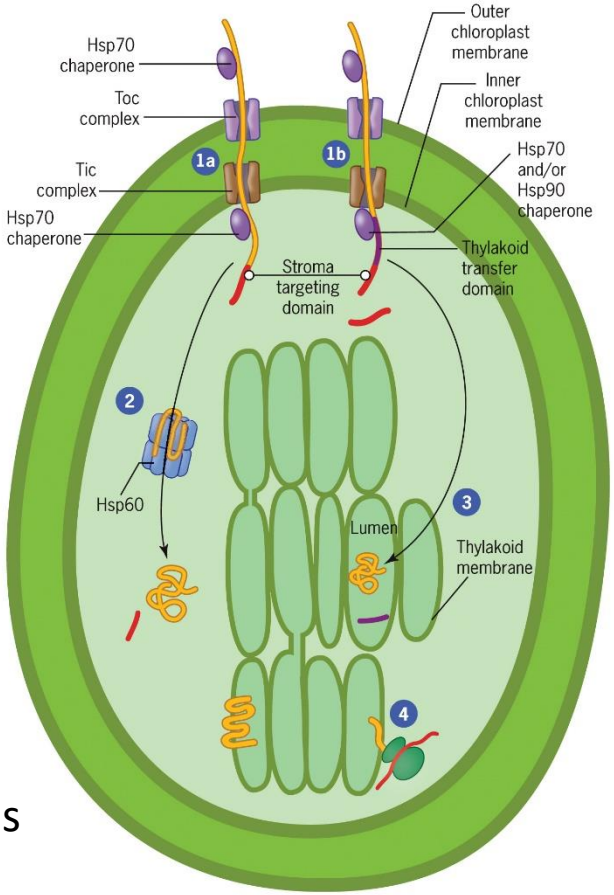


Fig. 8.50 Importing proteins into a chloroplast

The Human Perspective

Disorders Resulting from Defects in Lysosomal Function

Lysosomal malfunctions can have serious effects on human health.

Lysosomal storage disorders result from the absence of specific lysosomal enzymes thus allowing undigested material to accumulate.

Lysosomal storage disorders can have a wide variety of symptoms, and among the best-studied disorders is Tay-Sachs disease.

Tay-Sachs disease results from a deficiency in an enzyme responsible for degrading gangliosides, a major component of cell membranes. It has a high incidence among Jews of eastern European ancestry.

The Human Perspective

Disorders Resulting from Defects in Lysosomal Function

TABLE 1 Sphingolipid Storage Diseases

Disease	Enzyme deficiency	Principal storage substance	Consequences
G_{M1} Gangliosidosis	G_{M1} β-Galactosidase	Ganglioside G_{M1}	Mental retardation, liver enlargement, skeletal involvement, death by age 2
Tay-Sachs disease	Hexosaminidase A	Ganglioside G_{M2}	Mental retardation, blindness, death by age 3
Fabry's disease	α-Galactosidase A	Trihexosylceramide	Skin rash, kidney failure, pain in lower extremities
Sandhoff's disease	Hexosaminidases A and B	Ganglioside G_{M2} and globoside	Similar to Tay-Sachs disease but more rapidly progressing
Gaucher's disease	Glucocerebrosidase	Glucocerebroside	Liver and spleen enlargement, erosion of long bones, mental retardation in infantile form only
Niemann-Pick disease	Sphingomyelinase	Sphingomyelin	Liver and spleen enlargement, mental retardation
Farber's lipogranulomatosis	Ceramidase	Ceramide	Painful and progressively deformed joints, skin nodules, death within a few years
Krabbe's disease	Galactocerebrosidase	Galactocerebroside	Loss of myelin, mental retardation, death by age 2
Sulfatide lipidosis	Arylsulfatase A	Sulfatide	Mental retardation, death in first decade

Over 40 lysosomal storage diseases have been described, affecting about 1 in 5000 infants.

The symptoms of lysosomal storage diseases can range from very severe to barely detectable, depending primarily on the degree of enzyme dysfunction.

Several diseases are linked to mutations in lysosomal membrane proteins that impair transport of substances from the lysosomal lumen to the cytosol.

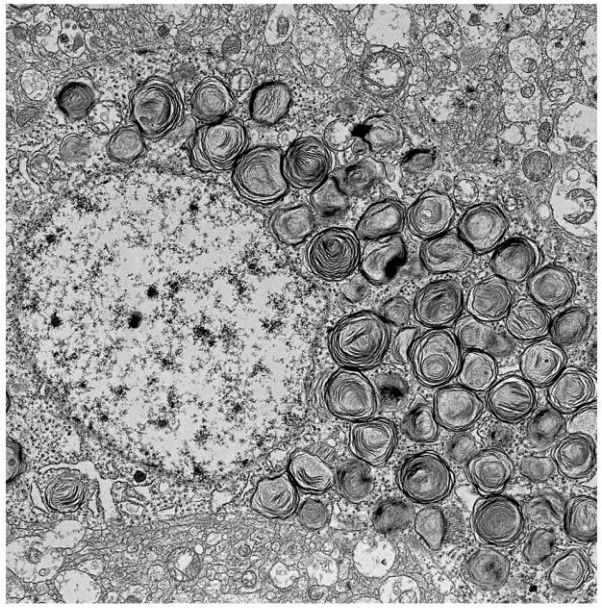
Disorders Resulting from Defects in Lysosomal Function

Enzyme replacement therapy is used to add the corrective enzyme back.

Cerezyme, used to treat Gaucher's disease, is a modified glucocerebrosidase recognized by mannose receptors on the surface of cells for uptake.

Substrate reduction therapy uses drugs to inhibit the synthesis of materials that accumulate.

Miglustat partially inhibits glycosphingolipid biosynthesis, and is used to treat Gaucher and Niemann-Pick type C disease.



Courtesy of Kinuko Suzuki

Electron micrograph of a section through a portion of a neuron of a person with a lysosomal storage