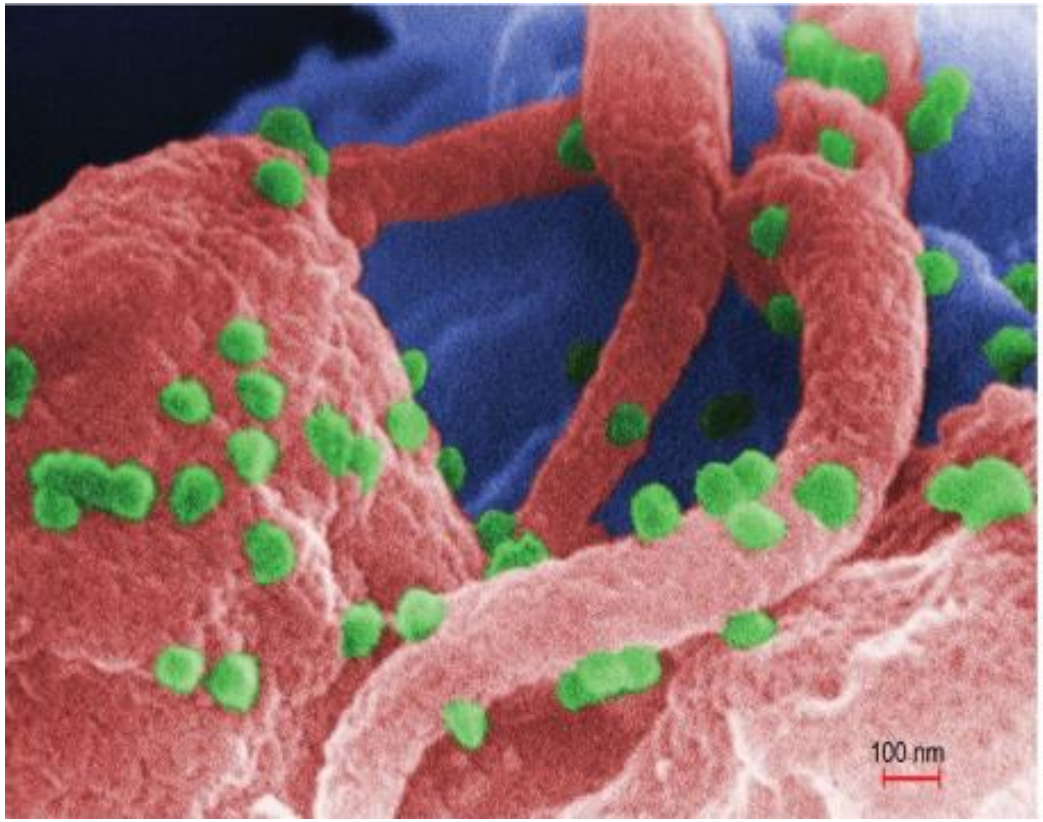
CHAPTER 12

Cellular Organelles and Membrane Trafficking



CDC/ C. Goldsmith, P. Feorino, E. L. Palmer, W. R. McManus / Center for Disease Control and Prevention

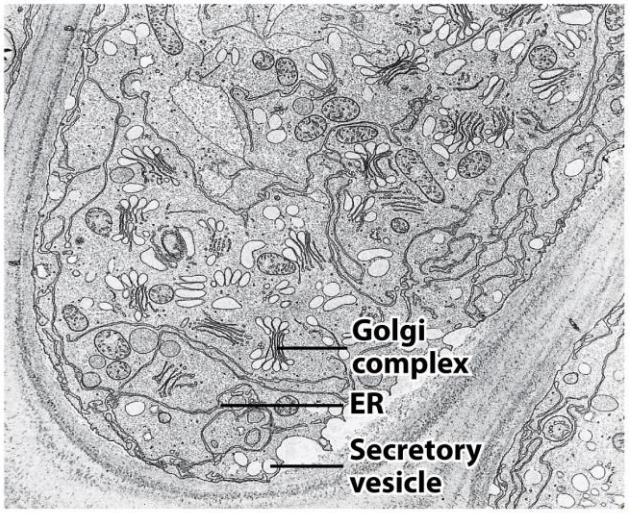
12.1 | An Overview of the Endomembrane System

Eukaryotic cell cytoplasm is subdivided into a variety of distinct compartments bounded by membrane barriers.

Cytoplasmic compartments form different organelles, each containing specialized proteins for particular activities.

Organelles may appear as stable structures, but in fact they are dynamic compartments that are in continual flux.

The ER, Golgi complex, endosomes, lysosomes, and vacuoles form an endomembrane system that act as a coordinated unit.



Courtesy of Hilton H. Mollenhauer

Membrane-bound compartments of the cytoplasm

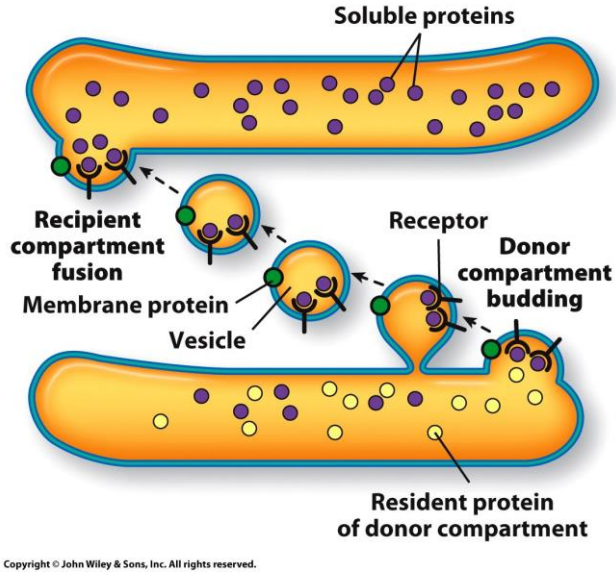
12.1 | An Overview of the Endomembrane System

Organelle boundary membranes probably arise from the ER.

Materials are shuttled between organelles in small, membrane-bounded transport vesicles that bud from a donor membrane compartment.

Transport vesicles move directionally via motor proteins on microtubules and microfilaments of the cytoskeleton.

Vesicles fuse with the membrane of the acceptor compartment, which receives the vesicle's soluble cargo and membranes.



Inside vesicle: orientation remains the same

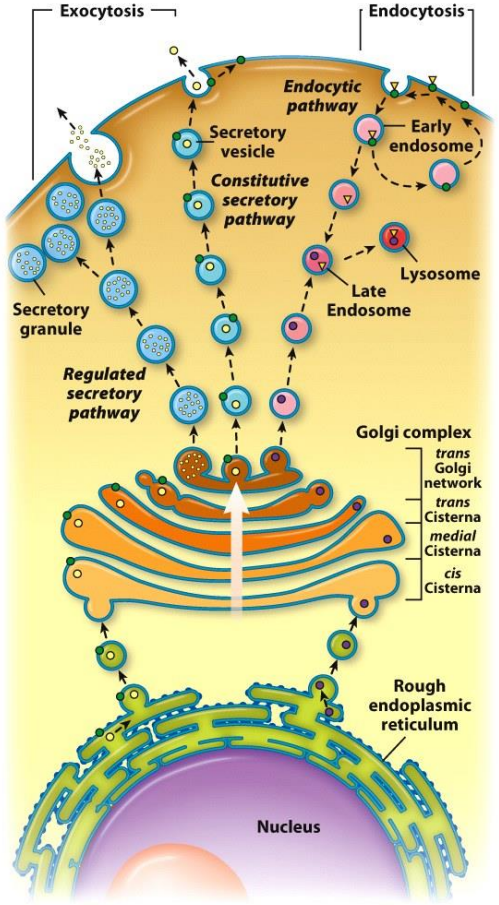
12.1 | An Overview of the Endomembrane System

Biosynthetic pathway: Proteins are synthesized in the ER, modified at the Golgi complex, and transported to various destinations (e.g. plasma membrane, lysosome, plant vacuole).

Secretory pathway: Proteins synthesized in the ER are discharged (secreted or exocytosed) from the cell.

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.



Endocytic pathways that unite endomembranes into a dynamic, interconnected network.

12.1 | An Overview of the Endomembrane System

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.

With the endocytic pathway, materials move from the outer surface of the cell to compartments, such as endosomes and lysosomes, located within the cytoplasm.

12.1 | An Overview of the Endomembrane System

The various types of cargo (e.g. secreted proteins, lysosomal enzymes, and membrane proteins) are routed to their appropriate cellular destinations by sorting signals encoded in the amino acid sequence of the proteins or in the attached oligosaccharides.

The sorting signals are recognized by specific receptors that reside in the membranes or surface coats of budding vesicles, ensuring that the protein is transported to the appropriate destination.

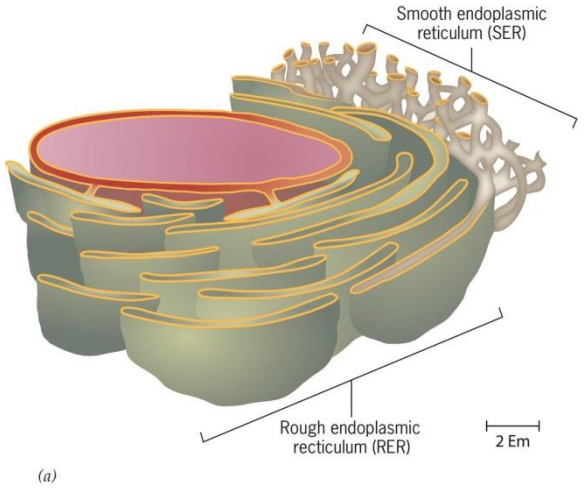
12.3 | The Endoplasmic Reticulum

The ER comprises a network of membranes that penetrates much of the cytoplasm and has a lumen separated from the cytosol by the ER membrane.

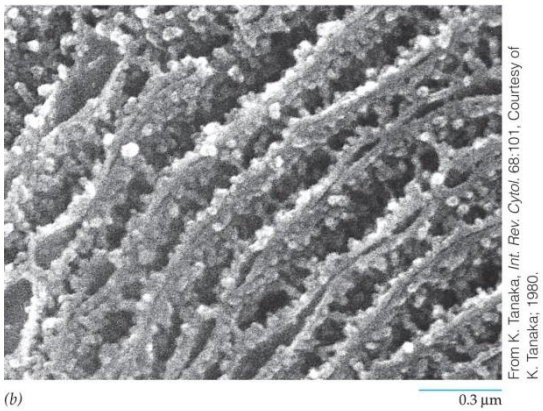
The ER is a highly dynamic structure divided into the rough ER and smooth ER.

The RER has ribosomes bound to its cytosolic surface, whereas the SER lacks associated ribosomes.

The RER typically 1) has flattened sacs (cisternae) connected to neighbors and 2) is continuous with the outer membrane of the nuclear envelope.



Schematic diagram of the rough ER



SEM of the rough ER

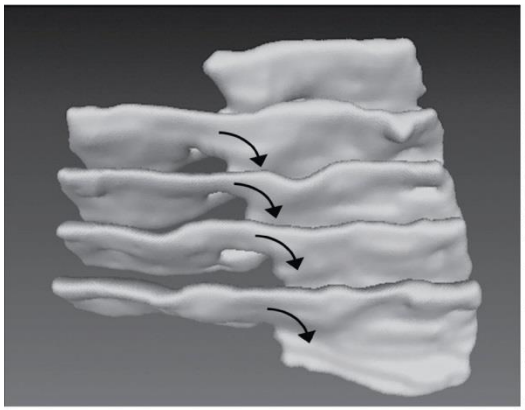
12.3 | The Endoplasmic Reticulum

SER membranes are highly curved and tubular, and continuous with the RER.

SER and RER share many of the same proteins and common activities (e.g. synthesis of certain lipids and cholesterol).

Numerous proteins are ER-specific, like the membrane-bending proteins (reticulons) in the SER; RER-specific proteins help move nascent proteins into the ER lumen.

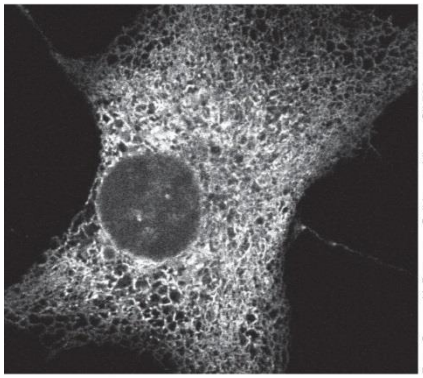
Different types of cells contain markedly different ratios of the two types of ER, depending on the activities of the cell.



From Terasaki et al., *Cell* 154:285, 2013.

(c)

EM reveals ER sheets connected by helicoidal ramps



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(d)

Visualization of ER by GFP fluorescence

12.3 | The Endoplasmic Reticulum

The Smooth Endoplasmic Reticulum

The SER is highly developed in skeletal muscle, kidney tubules, and steroid-producing endocrine glands.

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. Hydrophobic compounds are converted into more hydrophilic ones for excretion.

3) Sequestering Ca^{2+} within the cytoplasm; its regulated release in skeletal and cardiac muscle cells from the *sarcoplasmic reticulum* triggers contraction.

Don Fawcett/Photo Researchers, Inc.

Leydig cell: extensive SER where steroid hormones are synthesized

12.3 | The Endoplasmic Reticulum

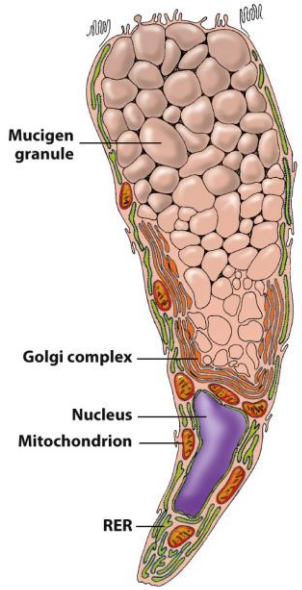
The Rough Endoplasmic Reticulum

Organelles in epithelial secretory cells are positioned with a distinct polarity, which reflects protein movement.

The nucleus and RER are near the basal surface, facing the blood supply; the Golgi complex is centrally located; secretory granules are near the apical surface near ducts.

The RER is the starting point of the biosynthetic pathway: synthesized proteins, carbohydrates, and phospholipids then journey through the membranous cell compartments.

Polarized structure of a secretory cell, a mucus secreting goblet cell.



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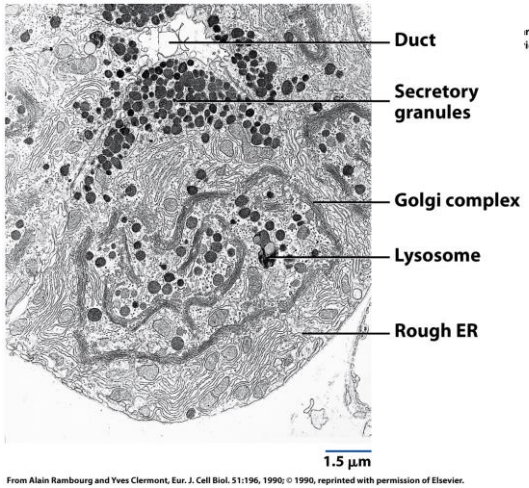

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Mucus-secreting cell from mouse small intestine

12.4 | Functions of The Endoplasmic Reticulum

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.

12.4 | Functions of The 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.

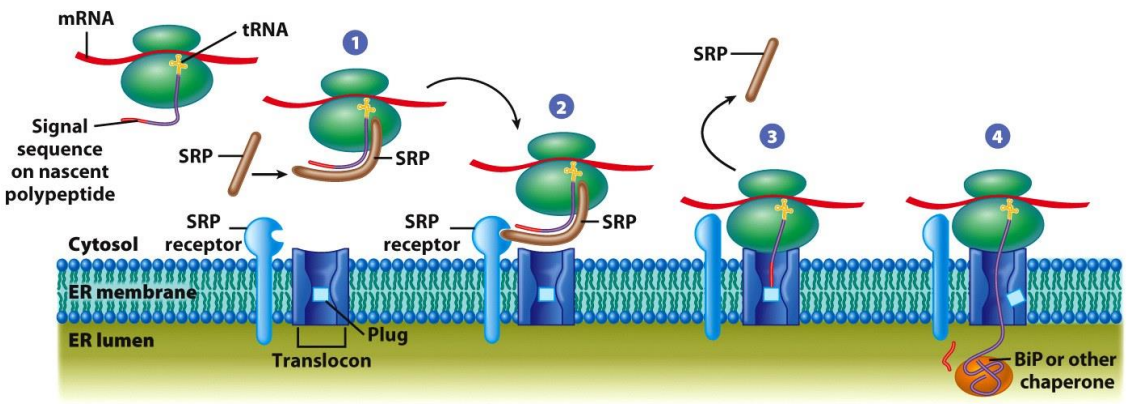
Secretory proteins contain a signal sequence at their N-terminus that directs the emerging polypeptide and ribosome to the ER membrane.

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.

12.4 | Functions of The Endoplasmic Reticulum

Synthesis of Secretory, Lysosomal, or Plant Vacuolar Proteins



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

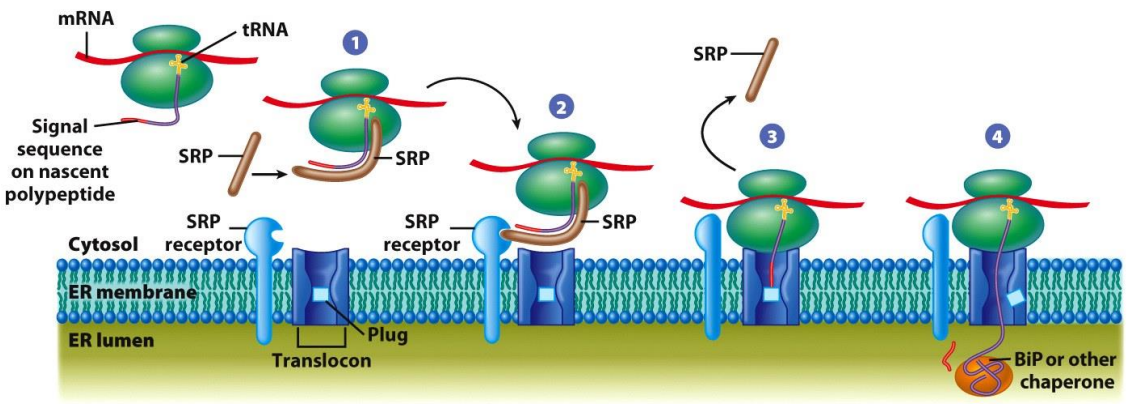
Co-translational translocation deposits protein into the ER lumen by a ribosome that is attached to the ER membrane.

These polypeptides contain a signal sequence, a stretch of 6–15 hydrophobic amino acid residues, that targets the nascent polypeptide to the ER membrane.

The hydrophobic signal sequence is recognized by a signal recognition particle (SRP).

12.4 | Functions of The Endoplasmic Reticulum

Synthesis of Secretory, Lysosomal, or Plant Vacuolar Proteins



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

The SRP binds to both the signal sequence on the nascent polypeptide and the ribosome arresting synthesis.

This complex is recruited to the ER membrane through an interaction between the SRP and the SRP receptor on the ER membrane.

The ribosome is then handed off from the SRP to the translocon, a protein channel embedded in the ER membrane. Upon attachment, the signal sequence is recognized, and the polypeptide is inserted into the channel of the translocon.

12.4 | Functions of The Endoplasmic Reticulum

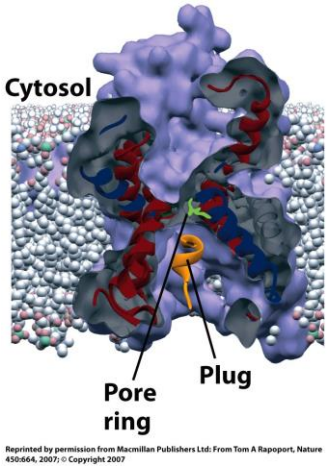
Synthesis of Secretory, Lysosomal, or Plant Vacuolar Proteins

Several the steps involved in the synthesis and trafficking of secretory proteins are regulated by the binding or hydrolysis of GTP.

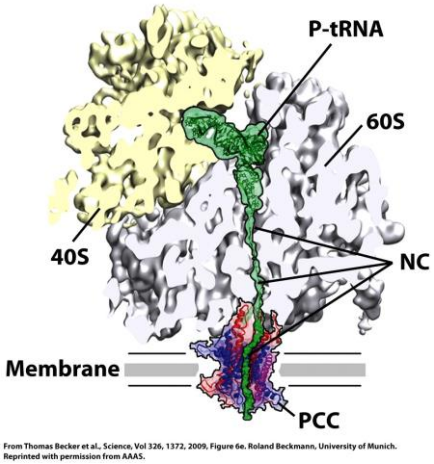
GTP-bound and GDP-bound G protein have different conformations.

The GTP-bound protein typically turns the process on, and hydrolysis of the bound GTP turns it off.

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.



Cross-sectional view of the translocon channel from X-ray crystal structure



Ribosome translocon complex in the act of protein synthesis

12.4 | Functions of The Endoplasmic Reticulum

Processing of Newly Synthesized Proteins in the Endoplasmic Reticulum

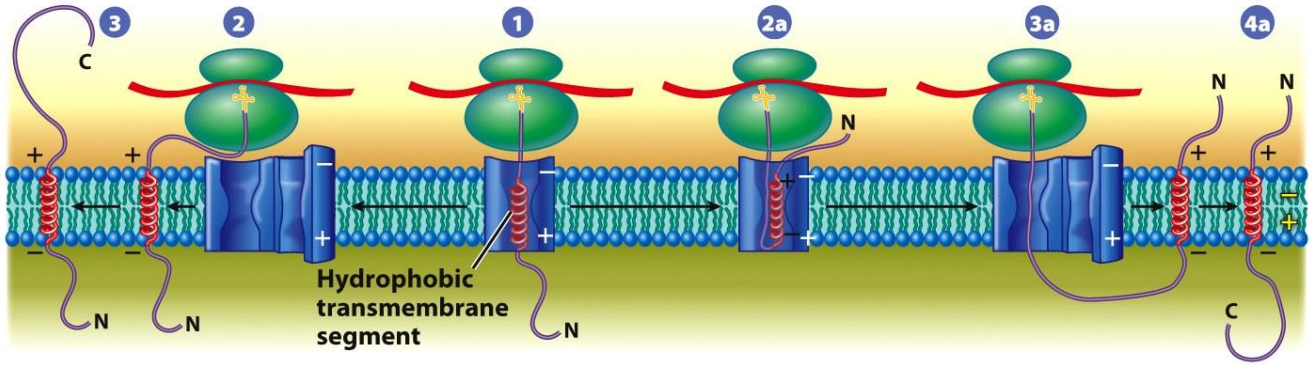
The signal peptide is removed from most nascent polypeptides by *signal peptidase*, while carbohydrates are added by *oligosaccharyltransferase*. Both enzymes are integral membrane proteins associated with the translocon.

The RER lumen has molecular chaperones to help unfolded or misfolded proteins attain their correct 3D structure, and *protein disulfide isomerase* stabilizes proteins by forming disulfide bonds.

The ER membrane provides a large surface area for ribosomes to attach, and the lumen gives a specialized local environment that favors protein processing.

12.4 | Functions of The Endoplasmic Reticulum

Synthesis of Integral Membrane Proteins on ER-Bound Ribosomes



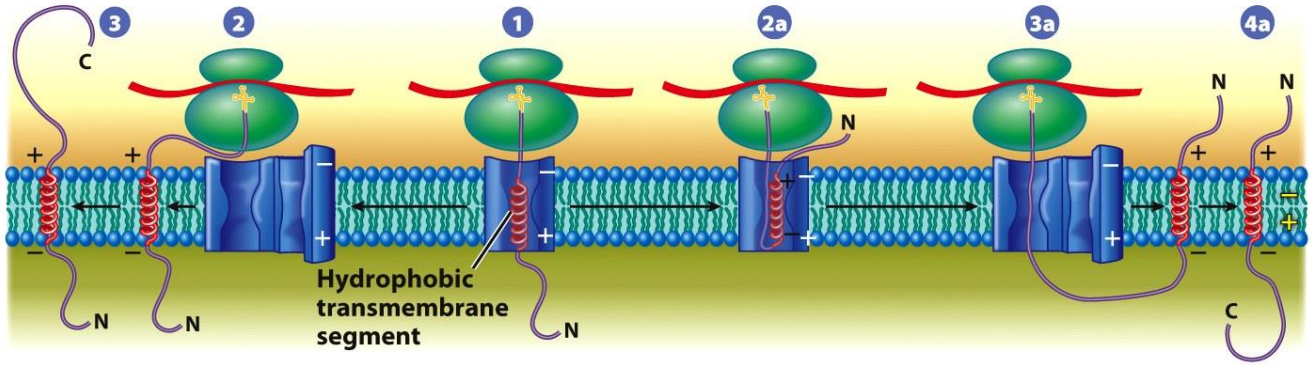
A schematic model for the synthesis of an integral membrane protein

Integral membrane proteins are synthesized co-translationally, and their hydrophobic transmembrane segments are shunted from the translocon into the lipid bilayer.

Single-spanning membrane proteins can have an orientation with their N-terminus facing either the cytosol or the lumen of the ER.

12.4 | Functions of The Endoplasmic Reticulum

Synthesis of Integral Membrane Proteins on ER-Bound Ribosomes



A schematic model for the synthesis of an integral membrane protein

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.

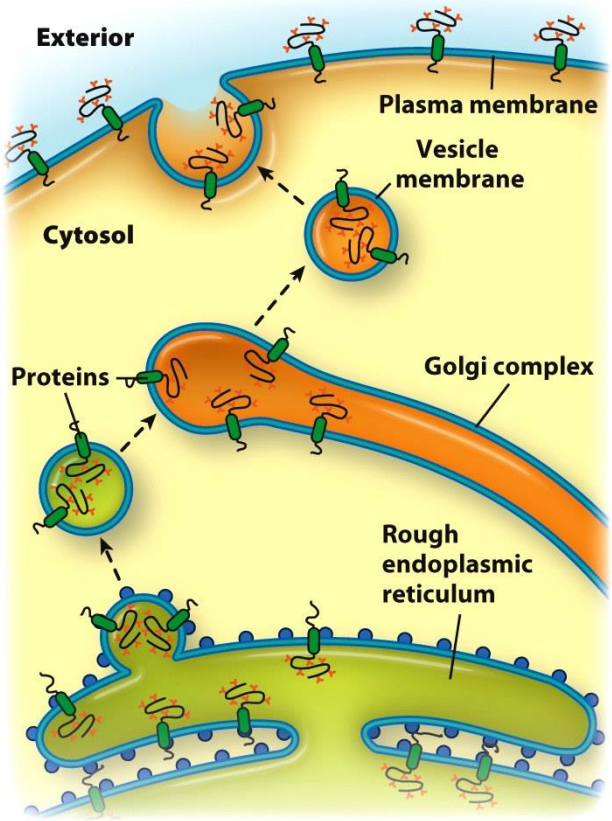
12.4 | Functions of The Endoplasmic Reticulum

Synthesis of Integral Membrane Proteins on ER-Bound Ribosomes

Membranes arise from pre-existing membranes; proteins and lipids are inserted into existing ER membranes and modified by enzymes in various organelles.

Membranes are asymmetric with a cytosolic face and a luminal/extracellular face.

Membrane lipids are solely synthesized in the ER with the exception of sphingomyelin and glycolipids (both completed in the Golgi complex) and unique mitochondrial lipids.



Maintenance of membrane asymmetry

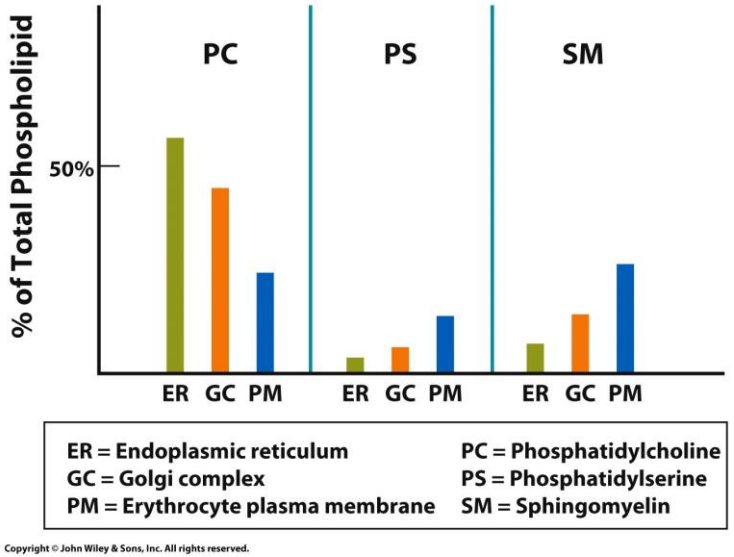
12.4 | Functions of The Endoplasmic Reticulum

Synthesis of Integral Membrane Proteins on ER-Bound Ribosomes

Enzymes that synthesize phospholipids are integral membrane ER proteins with their active sites facing the cytosol.

Newly synthesized phospholipids are inserted into the half of the bilayer facing the cytosol, and some are later flipped into the opposite leaflet by *flippases*.

Membranes of different organelles have markedly different lipid composition, which indicates that changes take place as membrane flows through the cell.



Histogram indicating percentage of each of three phospholipids in three different cellular membranes

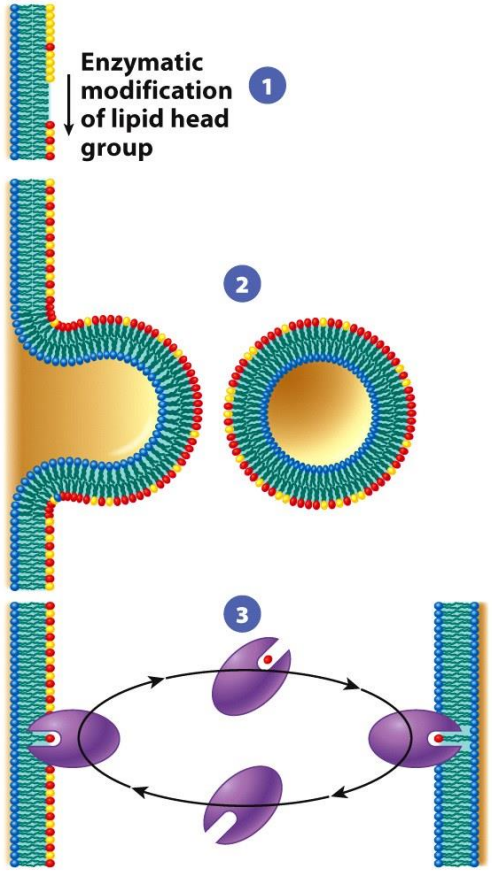
12.5 | Membrane Synthesis in the Endoplasmic Reticulum

Changes in lipid composition could occur by several different mechanisms.

1) Most membranous organelles have lipid-modifying enzymes to convert a phospholipid to another (e.g. phosphatidyl-serine to phosphatidylcholine).

1) When vesicles bud from a compartment, phospholipids may be preferentially included or excluded.

2) Cells contain lipid-transfer proteins that can bind and directly transport lipids through the aqueous cytosol from one membrane compartment to another.



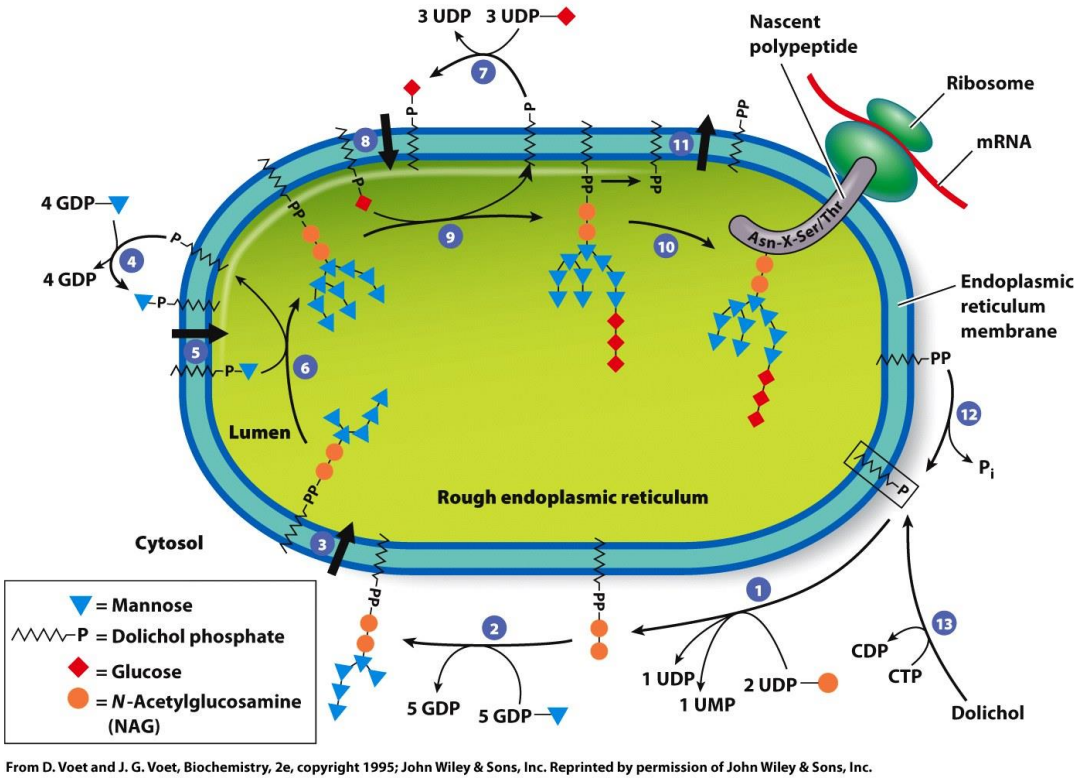
Schematic diagram showing three distinct mechanisms

12.6 | Glycosylation in the Rough Endoplasmic Reticulum

Nearly all proteins produced on membrane-bound ribosomes become glycoproteins.

Carbohydrate groups function as macromolecule binding sites and aid in protein folding and stabilization.

Addition of sugars to an oligosaccharide is catalyzed by *glycosyltransferases*, each transfers a specific mono-saccharide to the growing end of the carbohydrate chain.

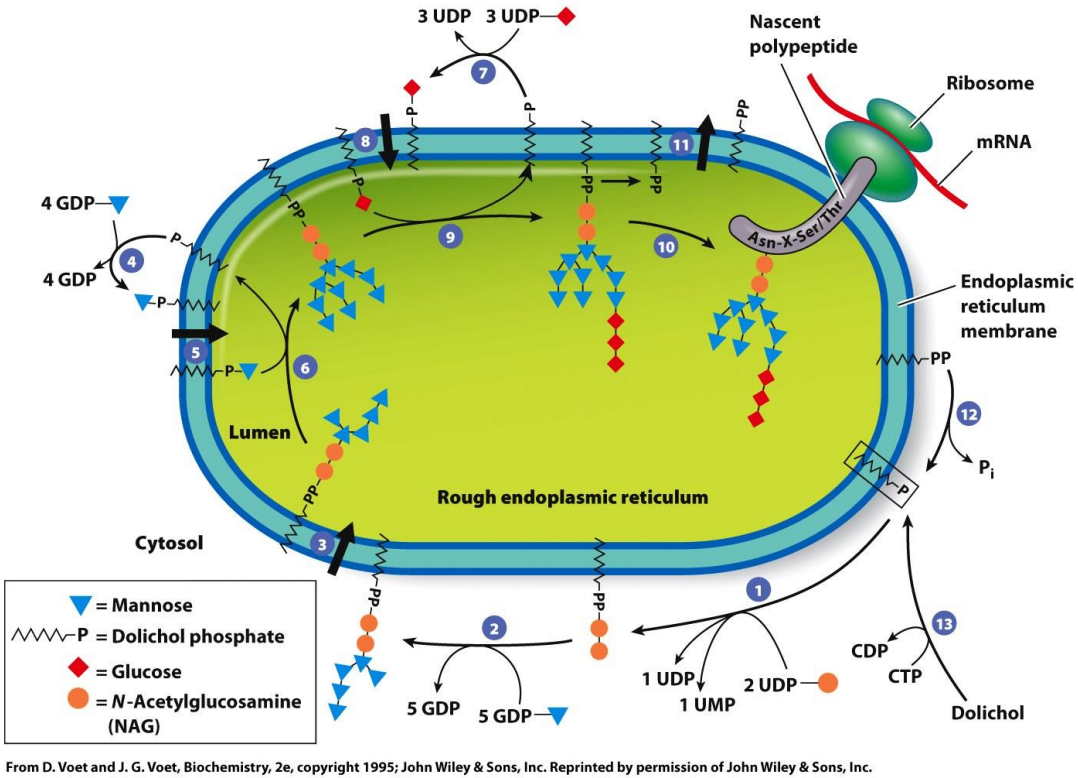


Steps in the synthesis of the core portion of an *N*-linked oligosaccharide in the rough ER

12.6 | Glycosylation in the Rough Endoplasmic Reticulum

The sugar arrangement in the oligosaccharide chains of a glycoprotein depends on the spatial localization of enzymes in the assembly line.

For *N*-linked oligosaccharides, the core 14 sugar chain is assembled on the lipid carrier dolichol phosphate and then transferred to specific asparagine residues by *oligosaccharyltransferase*.



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

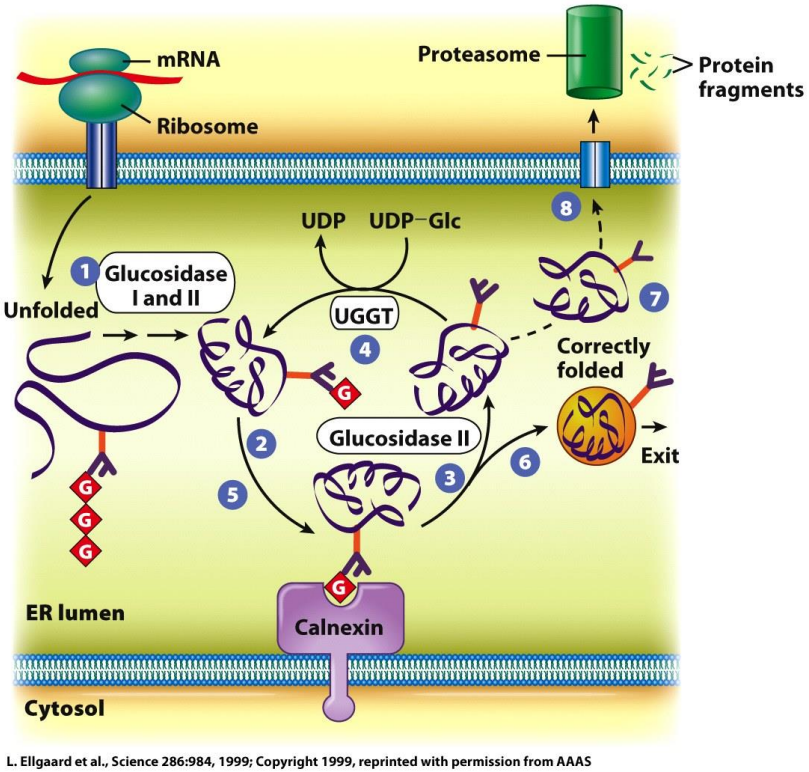
12.6 | Glycosylation in the Rough Endoplasmic Reticulum

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.

Misfolded proteins are tagged by a terminal glucose and recognized by chaperones for refolding.

If the protein does not correctly fold, it is translocated to the cytosol and destroyed.



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

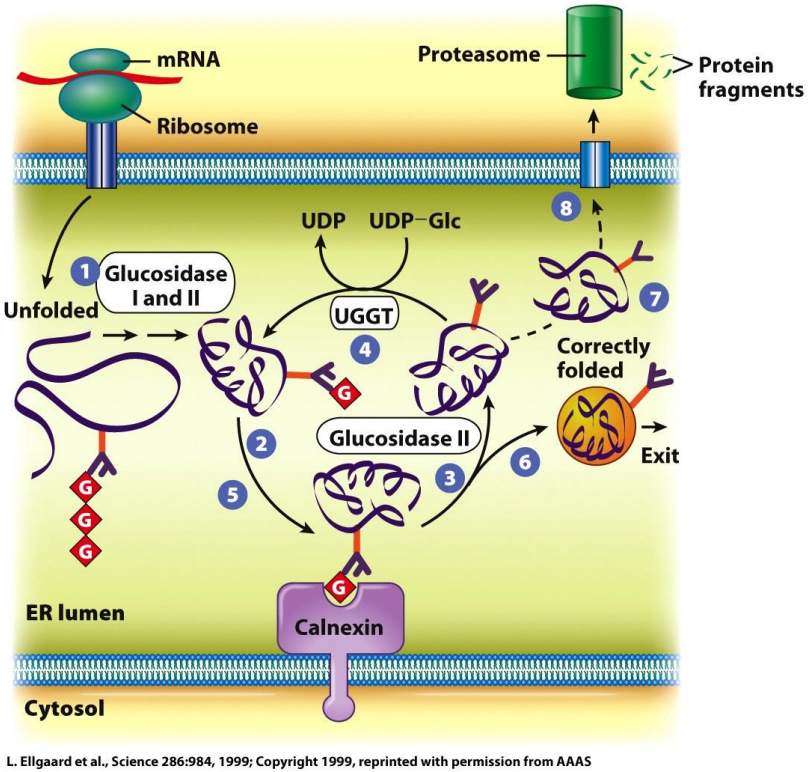
Quality control: ensuring that misfolded proteins do not proceed forward.

12.6 | Glycosylation in the Rough Endoplasmic Reticulum

Mechanisms ensure the destruction of misfolded proteins.

Misfolded proteins are not destroyed in the ER; instead they are transported into the cytosol where they are destroyed in proteasomes.

This process is called ER-associated degradation (ERAD), and ensures the misfolded proteins do not reach the cell surface.



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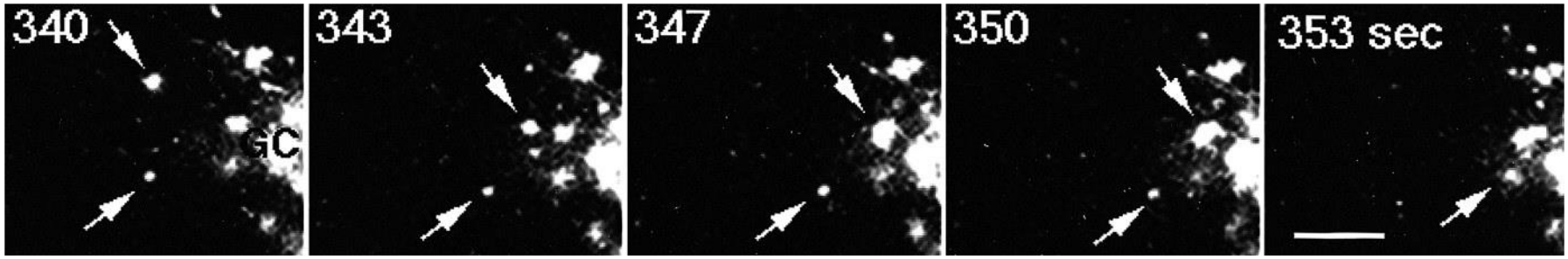
Quality control: ensuring that misfolded proteins do not proceed forward.

12.8 | ER to Golgi Vesicular Transport



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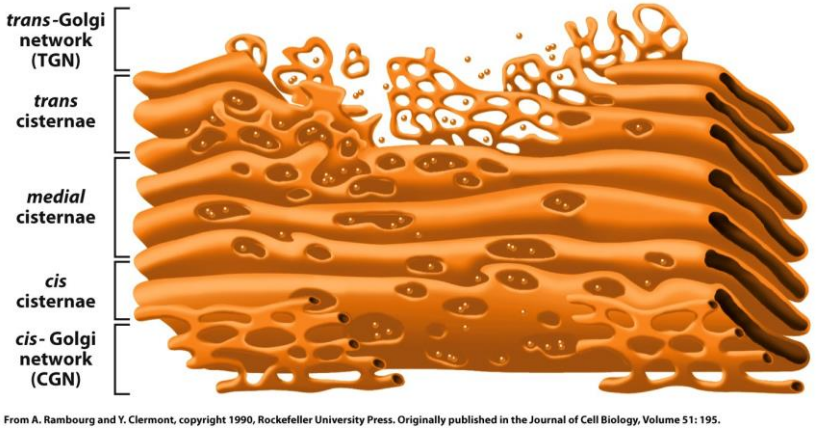
Visualizing membrane traffic with the use of a fluorescent tag:
Movement of vesicular-tubular carriers: ER to Golgi with VSV-G:GFP tag

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.

12.9 | The Golgi Complex



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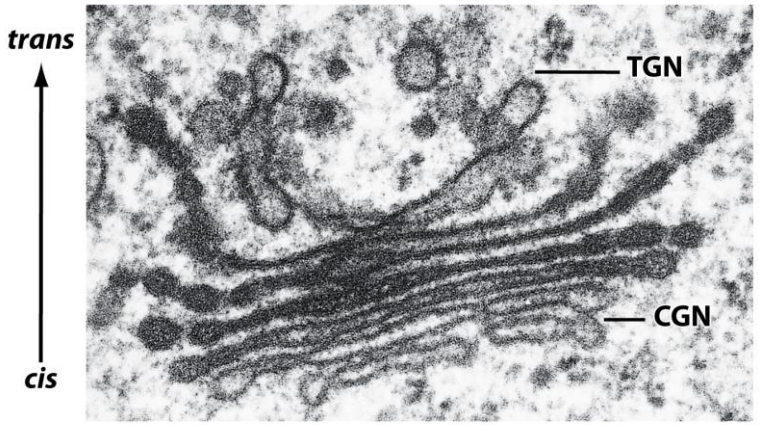
Schematic model of a Golgi complex



Courtesy of Thomas H. Giddings and Andrew Staehelin

EM: Golgi cisterna showing a concave central domain and an irregular peripheral domain

The **Golgi complex** is a stack of flattened cisternae.

It is divided into several functionally distinct compartments; the *cis* face of the Golgi faces the ER; the *trans* face is on the opposite side of the stack.

12.9 | The Golgi Complex

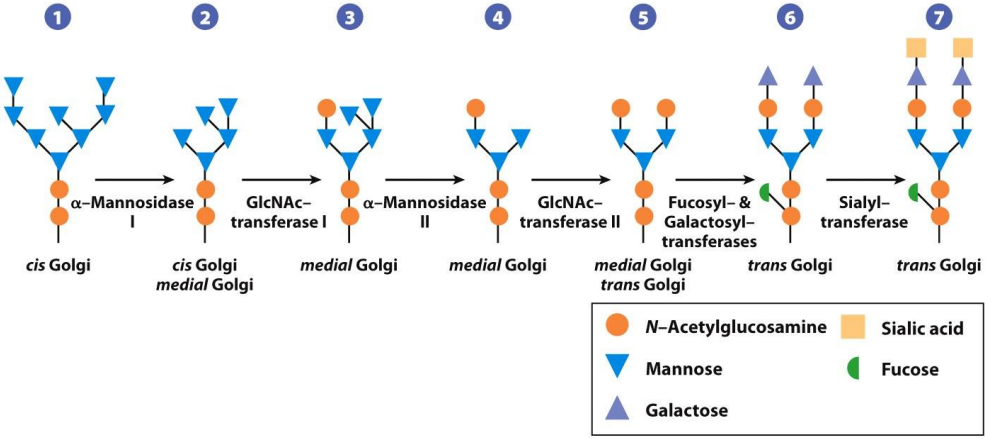
The ***cis* Golgi network (CGN)** functions to sort proteins for the ER or the next Golgi station.

The ***trans* Golgi network** functions in sorting proteins either to the 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.

12.9 | The Golgi Complex

Glycosylation in the Golgi Complex



Glycosylation steps of a typical mammalian *N*-linked oligosaccharide in the Golgi

Assembly of carbohydrates found in glycolipids and glycoproteins takes place in the Golgi.

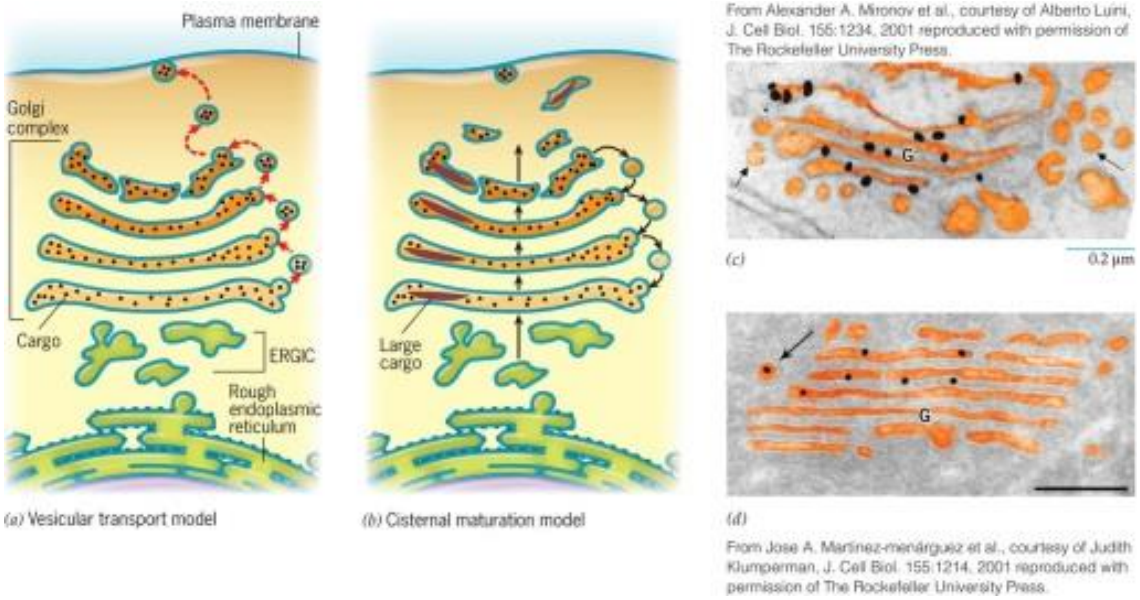
Sequence of incorporation of sugars into oligosaccharides is determined by glycosyltransferases.

Glycosylation steps can be diverse and complex.

12.9 | The Golgi Complex

Movement of Materials Through the Golgi Complex

The dynamics of transport through the Golgi complex



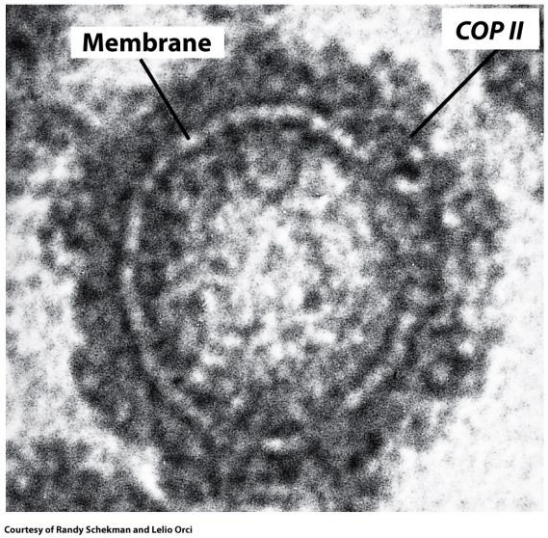
In the *vesicular transport model*, cargo is shuttled from the CGN to the TGN in vesicles.

In the *cisternal maturation model*, each cistern "matures" as it moves from the *cis* face to the *trans* face.

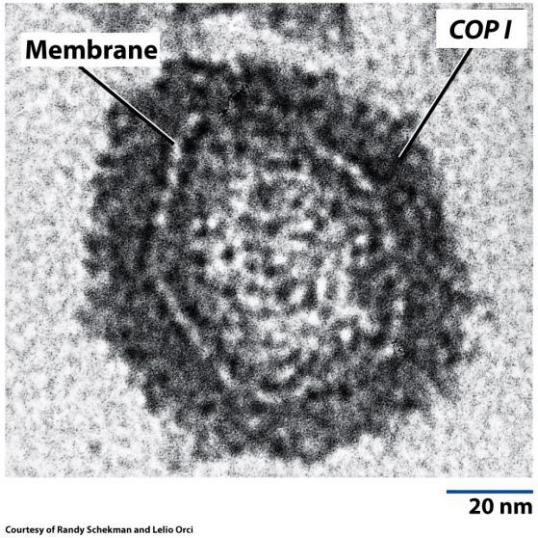
Current model: similar to cisternal maturation model but with vesicle retrograde transport. Golgi cisternae serve an primary anterograde carriers.

12.10 | Types of Vesicle Transport

Electron micrograph: COPII-coated vesicle



Courtesy of Randy Schekman and Lelio Orci



Courtesy of Randy Schekman and Lelio Orci

Electron micrograph: COPI-coated vesicle

Materials are carried between compartment using **coated vesicles**.

Protein coats have two distinct functions:

1) Cause the membrane to curve and form a vesicle.

2) Select the components to be carried by vesicle.

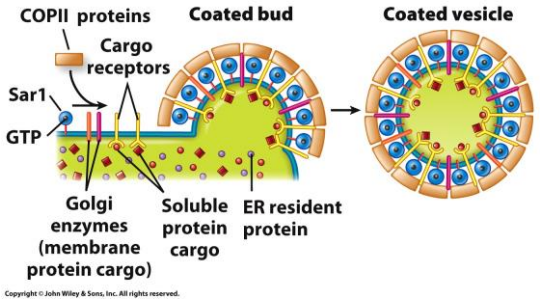
12.10 | Types of Vesicle Transport

Movement of Materials Through the Golgi Complex

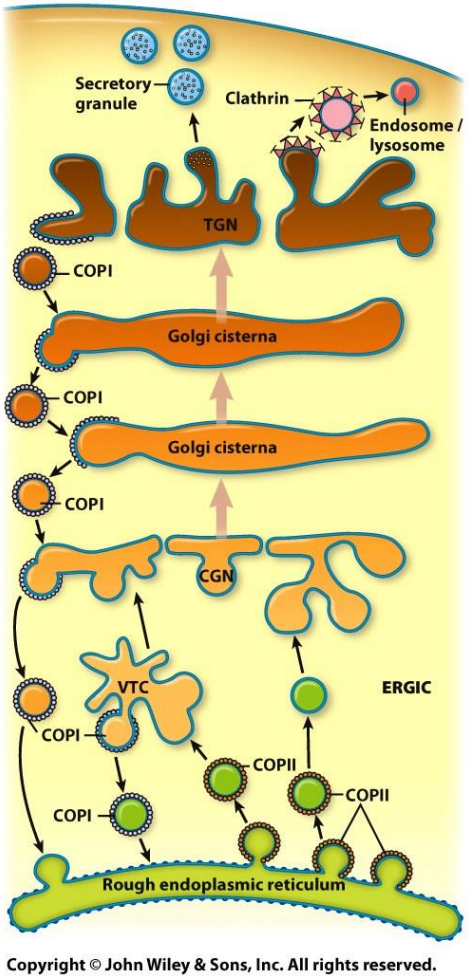
COPII-coated vesicles – move materials from the ER "forward" to the ERGIC and Golgi complex.

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.



Proposed transport between membrane compartments of the biosynthetic-secretory pathway



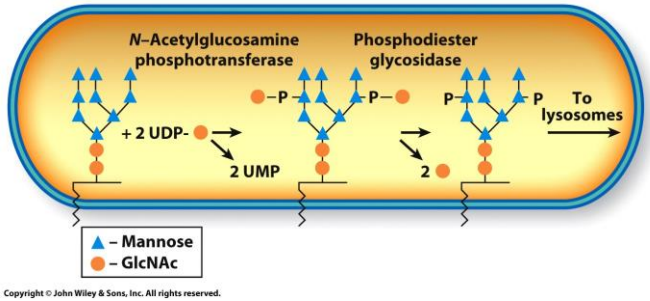
12.11 | Beyond the Golgi Complex: Sorting Proteins at the TGN

Sorting and Transport of Lysosomal Enzymes

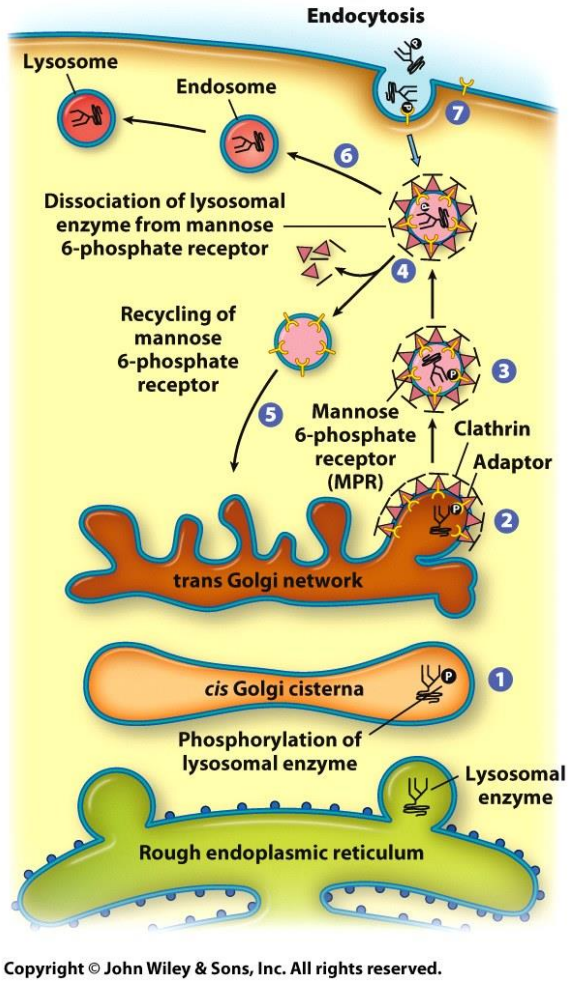
Beyond the Golgi Complex: Sorting and transport of lysosomal enzymes utilizes clathrin-coated vesicles.

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.



Targeting lysosomal enzymes to lysosomes: tagging with mannose 6-phosphate



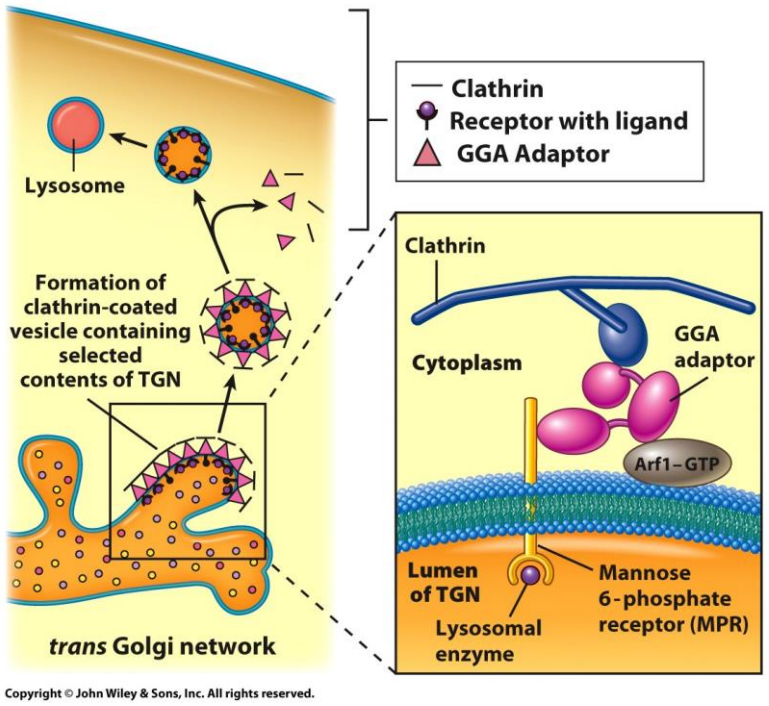
12.11 | Beyond the Golgi Complex: Sorting Proteins at the TGN

Sorting and Transport of Lysosomal Enzymes

Lysosomal enzymes are transported from the TGN in clathrin-coated vesicles.

The coats of these vesicles contain:

1) An outer lattice composed of clathrin.
2) An inner shell composed of **GGA** adaptor proteins, which interacts with clathrin, MPR, and G-protein.
3) The G-protein Arf1-GTP, used to initiate membrane curvature and recruit adaptors.



Formation of clathrin-coated vesicles at the TGN

12.11 | Beyond the Golgi Complex: Sorting Proteins at the TGN

Sorting and Transport of Nonlysosomal Proteins

Plasma membrane proteins are also transported from the TGN.

Proteins 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.

Sorting signals are found in the cytoplasmic domains of the membrane proteins. In polarized cells, membrane proteins destined to reside in the apical plasma membrane contain different sorting signals from those destined for the lateral or basal portion.

12.12 | Targeting Vesicles to a Particular Compartment

Vesicle fusion requires specific interactions between different membranes, and selective fusion is one of the factors that ensures a directed flow through the membranous compartments of the cell.

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.***

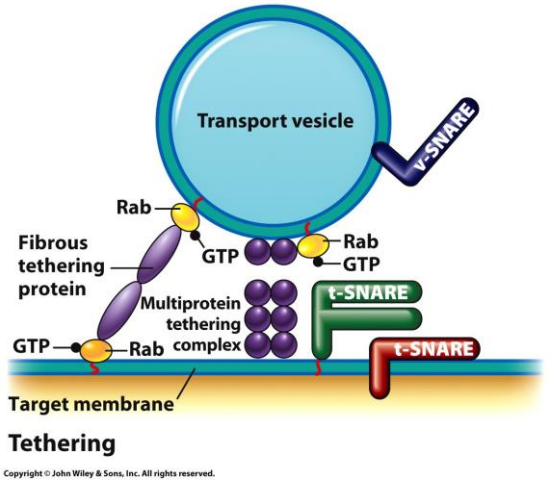
12.12 | Targeting Vesicles to a Particular Compartment

Two groups of tethering proteins: rod-shaped, fibrous proteins and large multiprotein complexes.

Specificity between vesicle and target may be conferred G proteins called Rabs; GTP-bound Rabs associate with membranes by a lipid anchor.

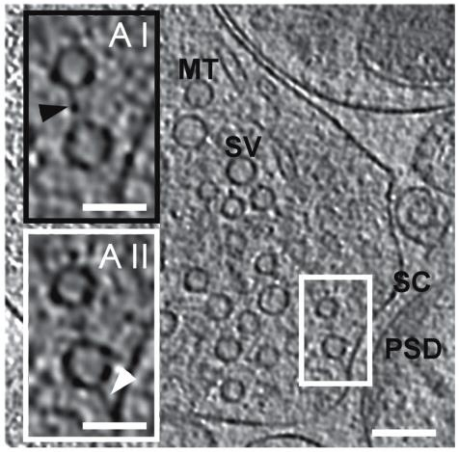
Over 60 different Rab genes identified in humans; different Rabs become associated with different membrane compartments.

Rabs also help recruit motor proteins that move membranous vesicles through the cytoplasm.



Tethering

Rab proteins are involved in recruiting tethering proteins



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Synaptic vesicles associated with plasma membrane

12.13 | Exocytosis

Exocytosis – discharge of a secretory vesicle or granule after fusion with plasma membrane.

Process is triggered by an increase in [Ca^{2+}].

Contacts between vesicle and plasma membrane lead to formation "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.

12.14 | Lysosomes

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 where the pH=4.6, maintained by a proton pump (a V-type H+-ATPase).

Lysosomal membranes have highly glycosylated integral proteins that form a barrier against the enclosed enzymes.

TABLE 8.1 A Sampling of Lysosomal Enzymes

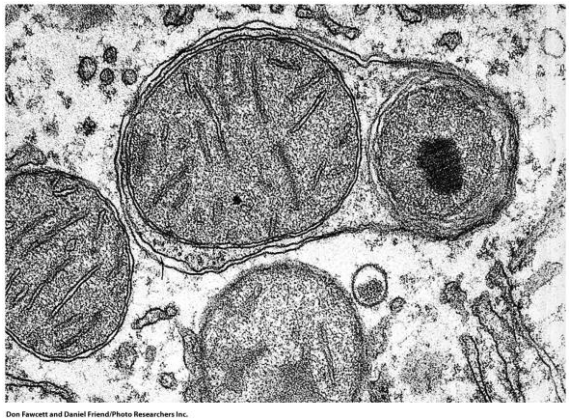
Enzyme	Substrate
Phosphatases	
Acid phosphatase	Phosphomonoesters
Acid phosphodiesterase	Phosphodiesters
Nucleases	
Acid ribonuclease	RNA
Acid deoxyribonuclease	DNA
Proteases	
Cathepsin	Proteins
Collagenase	Collagen
GAG-hydrolyzing enzymes	
Iduronate sulfatase	Dermatan sulfate
β-Galactosidase	Keratan sulfate
Heparan *N*-sulfatase	Heparan sulfate
α-*N*-Acetylglucosaminidase	Heparan sulfate
Polysaccharidases and oligosaccharidases	
α-Glucosidase	Glycogen
Fucosidase	Fucosyloligosaccharides
α-Mannosidase	Mannosyloligosaccharides
Sialidase	Sialyloligosaccharides
Sphingolipid hydrolyzing enzymes	
Ceramidase	Ceramide
Glucocerebrosidase	Glucosylceramide
β-Hexosaminidase	G_{M_2} ganglioside
Arylsulfatase A	Galactosylsulfatide
Lipid hydrolyzing enzymes	
Acid lipase	Triacylglycerols
Phospholipase	Phospholipids

12.14 | Lysosomes

Lysosomes play a role in the regulated process of organelle turnover, known as *autophagy*.

A double membrane structure (or *phagophore*) envelops an organelle to produce a double-membrane sequestering vesicle called an *autophagosome*.

The autophagosome fuses with a lysosome, generating an *autolysosome*, in which both the inner membrane of the autophagosome and the enclosed contents are degraded.



EM: a mitochondrion and peroxisome enclosed in a double membrane wrapper.

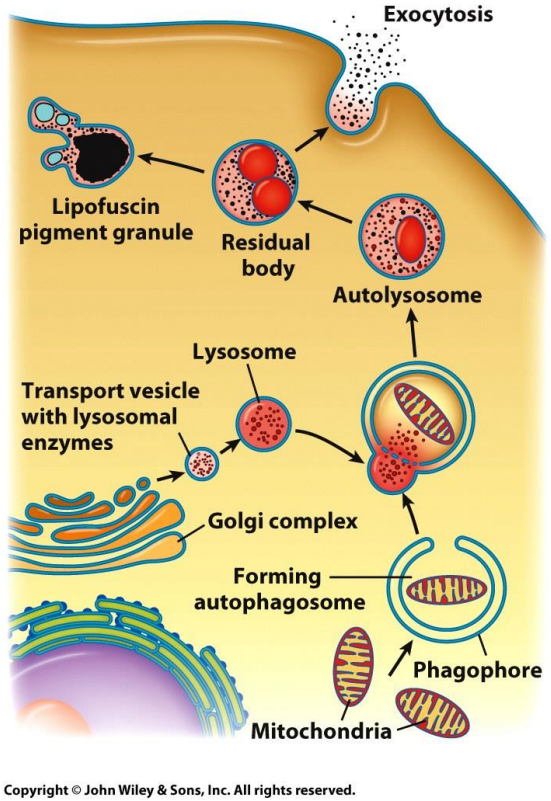
12.14 | Lysosomes

Autophagy probably evolved in early eukaryotic organisms as a response to nutrient deprivation.

Autophagy helps protect an organism against intracellular threats ranging from abnormal protein aggregates to invading bacteria and parasites.

Autophagy may even play a role in the prevention of certain types of cancers and slowing the body's aging process.

A *residual body* results from the degradation process, which may be exocytosed or retained as a *lipofuscin granule*.



A summary of the autophagic pathway

12.16 | Endocytosis

Endocytosis is primarily a process by which the cell internalizes cell-surface receptors and bound extracellular ligands.

Endocytosis can be divided broadly into two categories: bulk-phase endocytosis and receptor-mediated endocytosis.

Bulk-phase endocytosis (*pinocytosis*) is the nonspecific uptake of extracellular fluids and may function primarily in the recycling of membrane between the cell-surface and interior compartments.

In contrast, receptor-mediated endocytosis (RME) or clathrin-mediated endocytosis, brings about the uptake of specific ligands following their binding to receptors on the external surface of the plasma membrane.

12.16 | Endocytosis

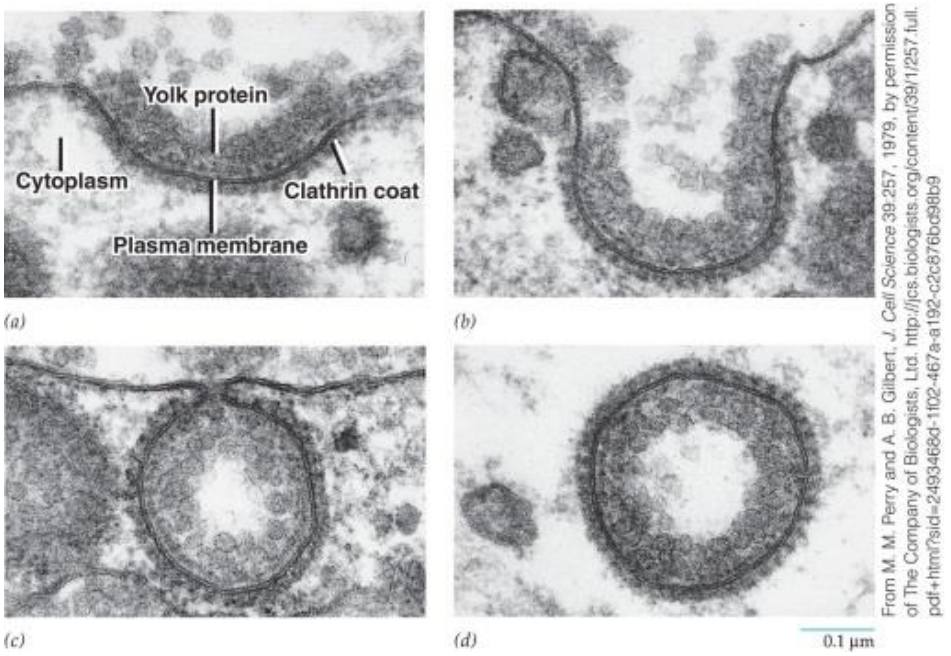
Receptor-Mediated Endocytosis and the Role of Coated Pits

RME provides a selective and efficient uptake of macromolecules (e.g. hormones, growth factors, enzymes) mediated by receptors.

Receptors are concentrated in coated pits at 10–20 times their level in the remainder of the plasma membrane.

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.

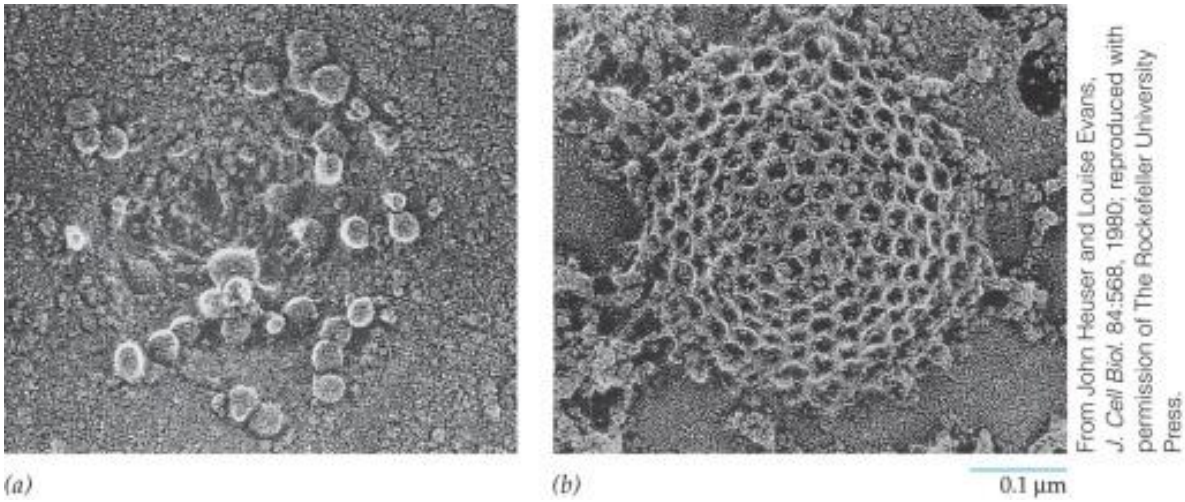


From M. M. Perry and A. B. Gilbert, *J. Cell Science* 39:257, 1979, by permission of The Company of Biologists, Ltd. http://jcs.biologists.org/content/39/1/257.full.pdf+html?sid=2493468d-1f02-467a-a192-c2c876bd98b9

Receptor-mediated endocytosis: Uptake of yolk lipoproteins by hen oocyte

12.16 | Endocytosis

Receptor-Mediated Endocytosis and the Role of Coated Pits



LDL-cholesterol droplets on extracellular surface

Cytosolic surface showing invagination of clathrin coated pit.

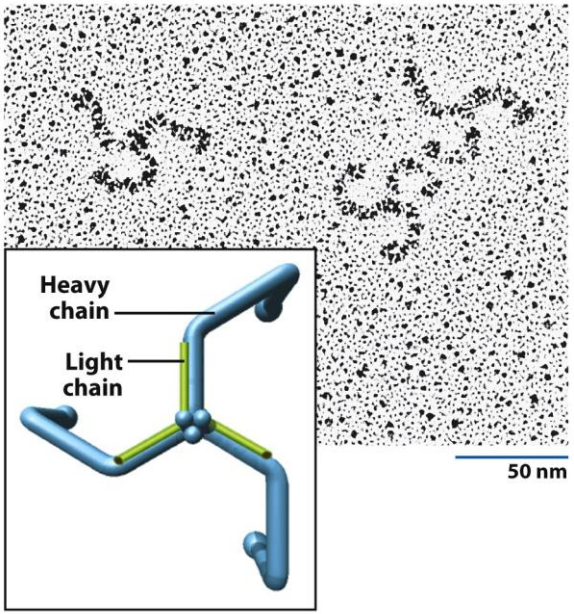
When coated pits are viewed from its cytoplasmic surface, the bristly coat appears as a network of polygons.

The geometric construction of the coat is derived from the structure of its clathrin building blocks.

12.16 | 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.



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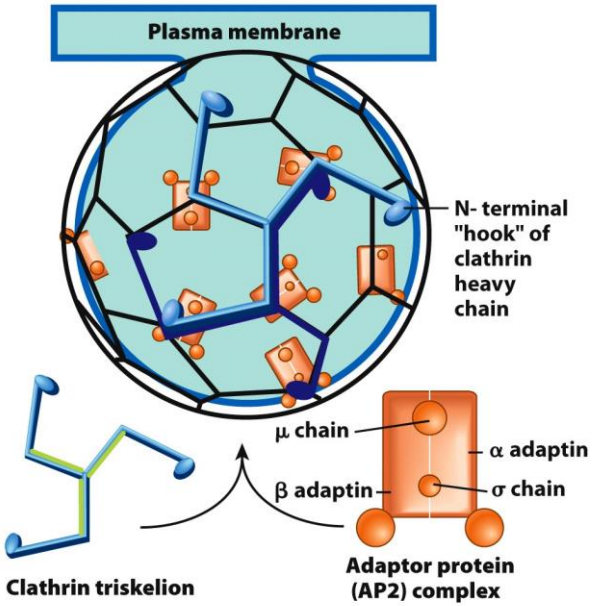
EM of a metal-shadowed preparation of clathrin triskelions. **Inset**: triskelion composed of three heavy chains with inner portion linked to a smaller light chain.

12.16 | 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 engage cytoplasmic tails of specific receptors to select bound cargo molecules, and bind and recruit the clathrin molecules of the overlying lattice.



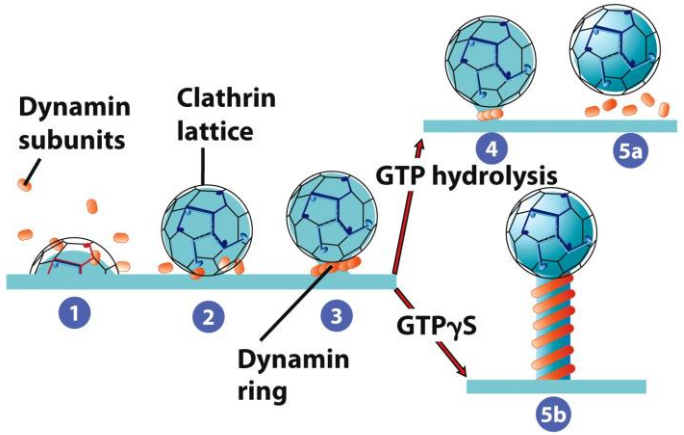
Schematic of a coated vesicle surface showing arrangement of triskelions and adaptors in the outer clathrin coat.

12.16 | Endocytosis

Receptor-Mediated Endocytosis and the Role of Coated Pits

Dynamin is a G protein required for the release of a clathrin-coated vesicle from the membrane where it forms.

It self-assembles into a helical collar around the neck of an invaginated coated pit.



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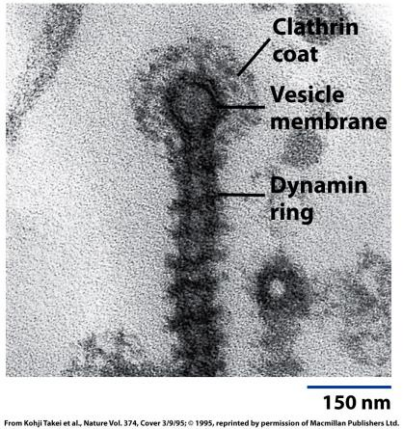
Dynamin promotes a GTP-mediated fission of the coated pit from the plasma membrane followed by disassembly of the dynamin ring

EM: coated vesicle forming in presence of non-hydrolyzable GTP(S)



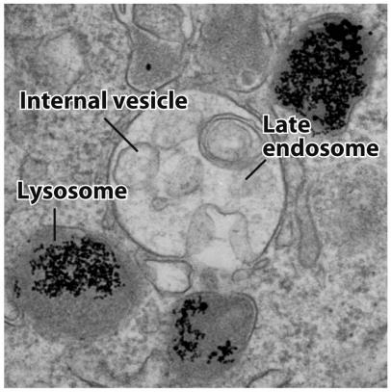
From Kohji Takei et al., Nature Vol. 374, Cover 3/9/95; © 1995, reprinted by permission of Macmillan Publishers Ltd.

12.17 | The Endocytic Pathway

After internalization, vesicle-bound materials are transported in vesicles and tubules known as **endosomes**.

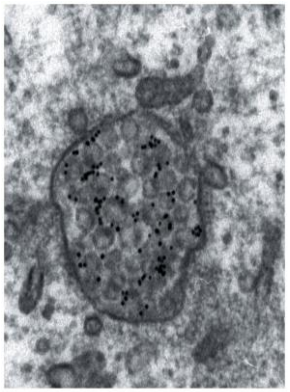
Early endosomes are located near the periphery of the cell. It sorts materials and sends bound ligands to the **late endosomes**.

Late endosomes are near the nucleus, also known as *multivesicular bodies (MVBs).*



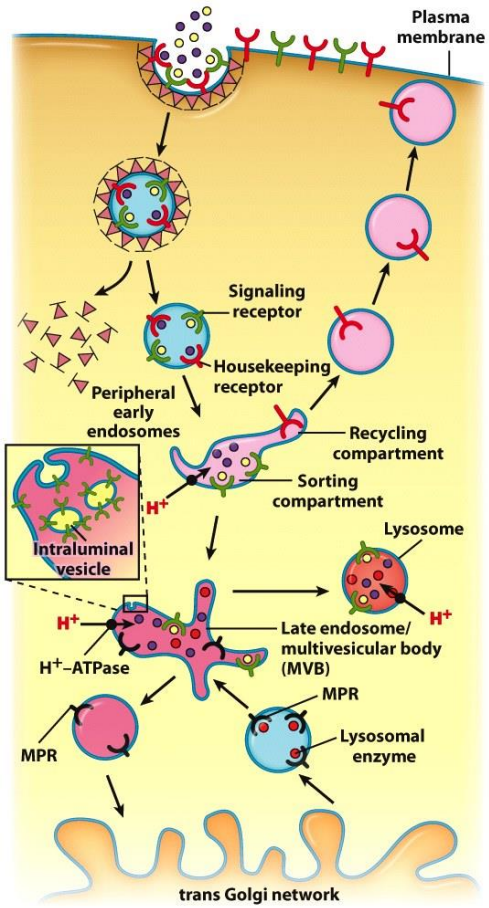
From J. Paul Luzio, Paul R. Pryor, and Nicholas A. Bright, Nature Revs. Mol. Cell Biol. 8: 625, 2007; © 2007, Reprinted by permission of Macmillan Publishers Ltd.

EM: internal vesicles in the lumen of a late endosome



Courtesy of Clare Futter

EM: Gold particles bound to internalized EGF receptors



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Endocytosis of receptor–ligand complexes

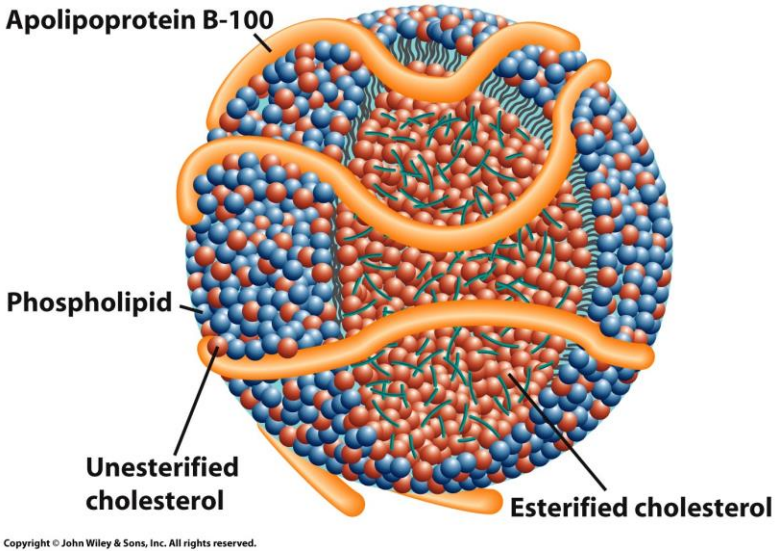
12.17 | The Endocytic Pathway

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.

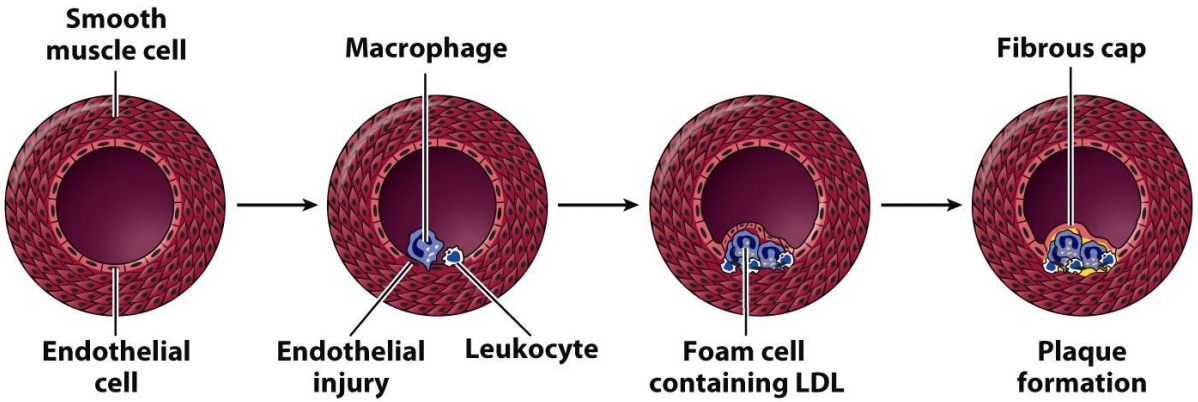
LDLs are taken up by RME and taken to the lysosomes, releasing the cholesterol for use by the cells.

Lowering blood LDL levels is accomplished by administration of drugs called *statins* to block HMG CoA reductase, a key enzyme in the synthesis of cholesterol.



Each particle consists of approximately 1500 cholesterol ester molecules surrounded by a monolayer of P-lipids and cholesterol and a single molecule of the protein apolipoprotein B which interacts specifically with LDL receptor

12.17 | The Endocytic Pathway



A model for atherosclerotic plaque formation

High-density lipoproteins (HDLs) transport cholesterol from tissues to the liver for excretion.

HDLs associated with lowering cholesterol levels; LDLs associated with high blood cholesterol.

LDL deposition leads to plaques on inner lining of blood vessels.

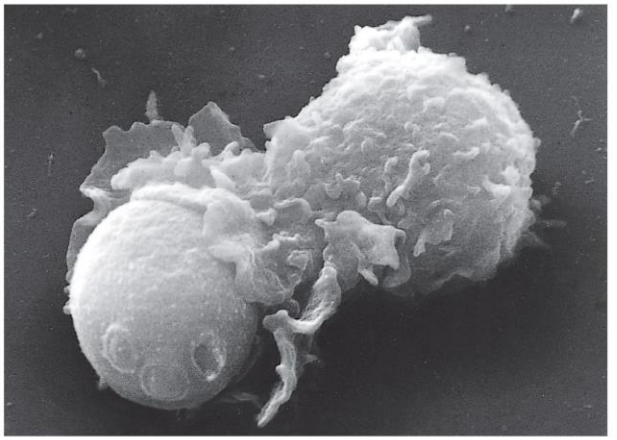
12.18 | Phagocytosis

Phagocytosis is carried out by cells specialized for the uptake of relatively large particles.

Amoebas and ciliates trap food particles and smaller organisms and enclosing them within folds of the plasma membrane.

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.



From Janet Boyles and Dorothy F. Bainton, Cell 24:906, 1981, reprinted by permission of Elsevier

Phagocytosis: The process of engulfment as illustrated by a polymorphonuclear leukocyte ingesting a yeast cell (lower left).

12.18 | Phagocytosis

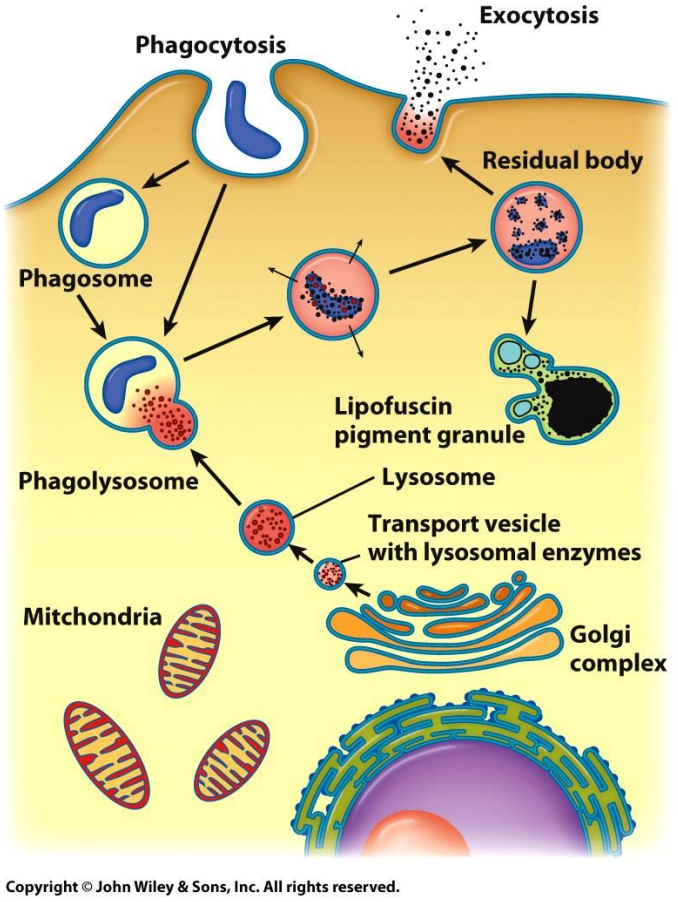
The engulfment of particles by phagocytosis is driven by actin-containing microfilaments.

Not all bacteria engulfed by phagocytic cells are destroyed; some species hijack the phagocytic machinery for their own survival.

Mycobacterium tuberculosis (affects fusion with lysosome)

Coxiella burnetti (pH tolerant so can survive in the lysosome)

Listeria monocytogenes (can degrade the lysosome)



Summary of the phagocytic pathway

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.

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.

The Human Perspective

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.

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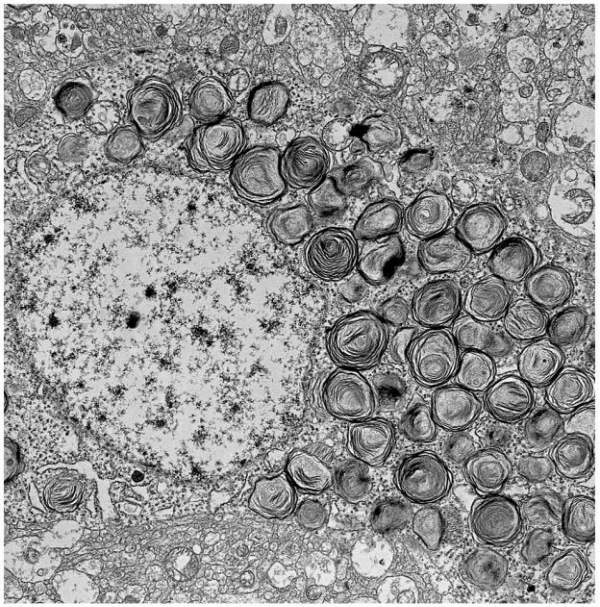

Courtesy of Kinuko Suzuki

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