Organelles and Protein Sorting

Protein Targeting

The cell cytoplasm contains many different specialized compartments

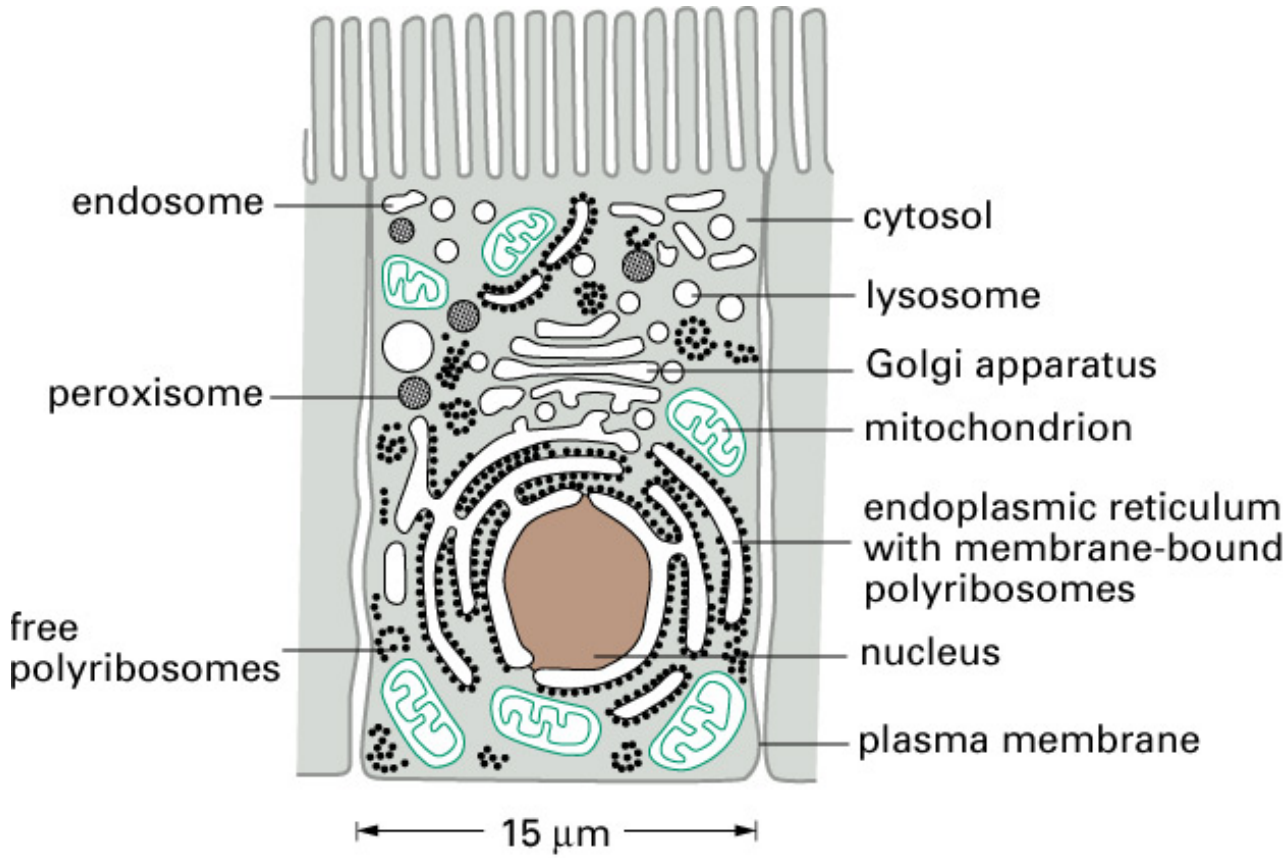


Figure 12–1. Molecular Biology of the Cell, 4th Edition.

The cell cytoplasm contains many different specialized compartments

Electron micrograph of the cytoplasm of a liver cell

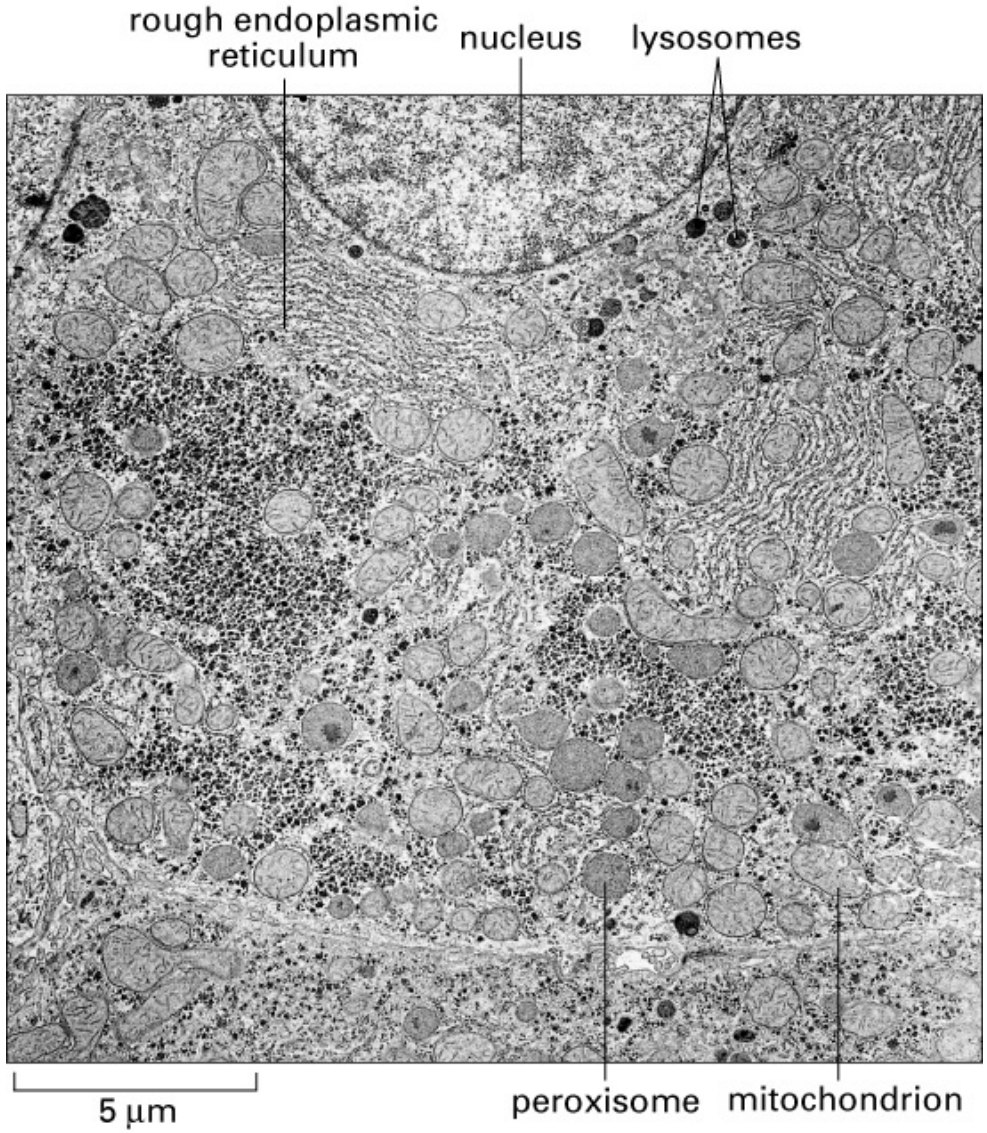


Figure 12–2. Molecular Biology of the Cell, 4th Edition.

Intracellular transport is often critical for the function of the differentiated cell

A Mast cell before (A) and after (B) histamine release

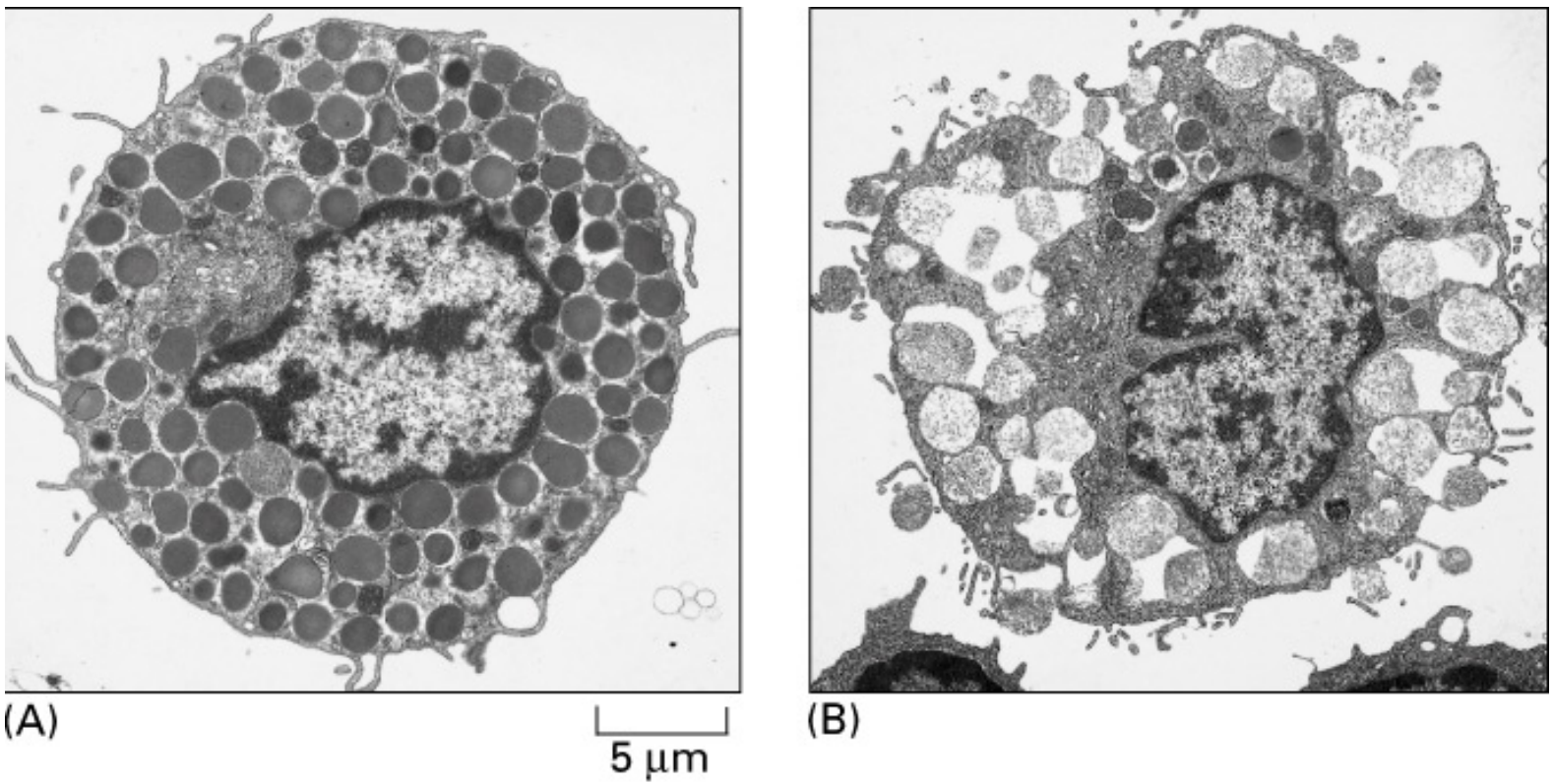
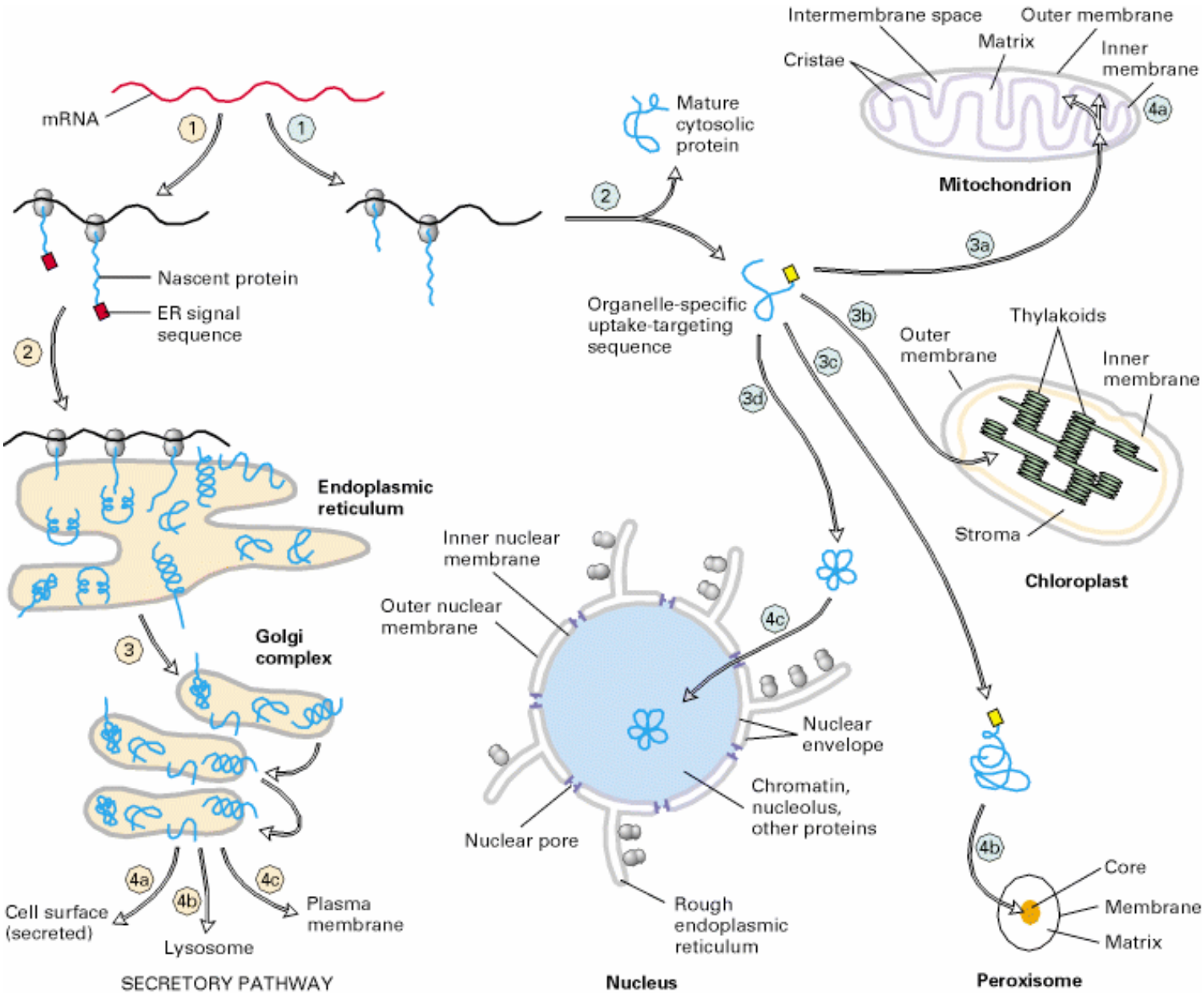


Figure 13–59. Molecular Biology of the Cell, 4th Edition.

Protein sorting in the cytosol establishes organelle identity



A protein’s fate in the cytosol

1. General requirements for protein sorting: *the signal hypothesis*
2. Protein import into the nucleus, mitochondria and peroxisomes
3. Protein import into the ER

What determines the identity of an organelle?

-Maintenance of specialized cellular architecture and function requires that cellular proteins be arranged properly within the cell

-Regulation of where a given protein functions within a cell can be as important as the function of the protein itself.

Transport pathways control the movement of proteins into and out of particular intracellular compartments

What is required for protein sorting?

a signal (address), intrinsic to the protein

a receptor that recognizes the signal and which directs it to the correct membrane

a translocation machinery

energy transfer the protein to its new place

Organelle sorting signals

Nucleus	
Internal	***import**:* One cluster of 5 basic amino acids, or two smaller clusters of basic residues separated by ≈10 amino acids ***export**:* Leucine rich: eg LQLPPLERLTL (rev protein of HIV-1)
Mitochondrion	
N-terminal	3 – 5 nonconsecutive Arg or Lys residues (--> amphiphatic helix) often with Ser and Thr; no Glu or Asp residues
Chloroplast	
N-terminal	No common sequence motifs; generally rich in Ser, and Thr and small hydrophobic amino acids, poor in Glu and Asp residues
Peroxisome	
C-terminal	Usually Ser-Lys-Leu at extreme C-terminus
ER	
N-terminus	hydrophilic domain (often basic) followed by 6 to 12 hydrophobic residues
Internal	16 to 30 hydrophobic residues

Examples of different types of signal sequence

TABLE 12–3 Some Typical Signal Sequences

FUNCTION OF SIGNAL SEQUENCE	EXAMPLE OF SIGNAL SEQUENCE
Import into nucleus	-Pro-Pro-Lys-Lys-Lys-Arg-Lys-Val-
Export from nucleus	-Leu-Ala-Leu-Lys-Leu-Ala-Gly-Leu-Asp-Ile-
Import into mitochondria	$^{+}H_3N$-Met-Leu-Ser-Leu-Arg-Gln-Ser-Ile-Arg-Phe-Phe-Lys-Pro-Ala-Thr-Arg-Thr-Leu-Cys-Ser-Ser-Arg-Tyr-Leu-Leu-
Import into plastid	$^{+}H_3N$-Met-Val-Ala-Met-Ala-Met-Ala-Ser-Leu-Gln-Ser-Ser-Met-Ser-Ser-Leu-Ser-Leu-Ser-Ser-Asn-Ser-Phe-Leu-Gly-Gln-Pro-Leu-Ser-Pro-Ile-Thr-Leu-Ser-Pro-Phe-Leu-Gln-Gly-
Import into peroxisomes	-Ser-Lys-Leu-COO^{-}
Import into ER	$^{+}H_3N$-Met-Met-Ser-Phe-Val-Ser-Leu-Leu-Leu-Val-Gly-Ile-Leu-Phe-Trp-Ala-Thr-Glu-Ala-Glu-Gln-Leu-Thr-Lys-Cys-Glu-Val-Phe-Gln-
Return to ER	-Lys-Asp-Glu-Leu-COO^{-}

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

Common Features of Transport Mechanisms

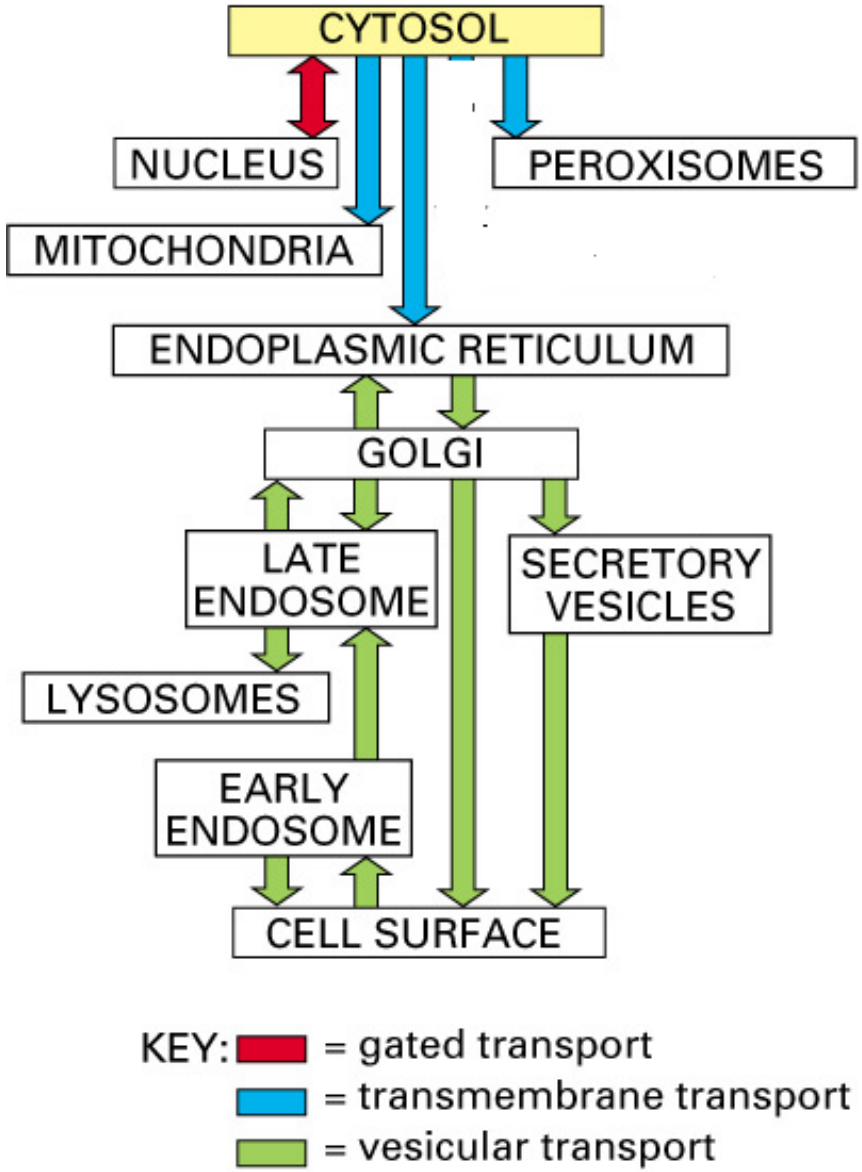
Signal sequences --

Short regions of a protein that act as targeting signals to direct the protein to specific subcellular localization

Receptors that recognize particular signal sequences

Require energy (ATP or GTP)

Road Map of Transport Routes in the Cell



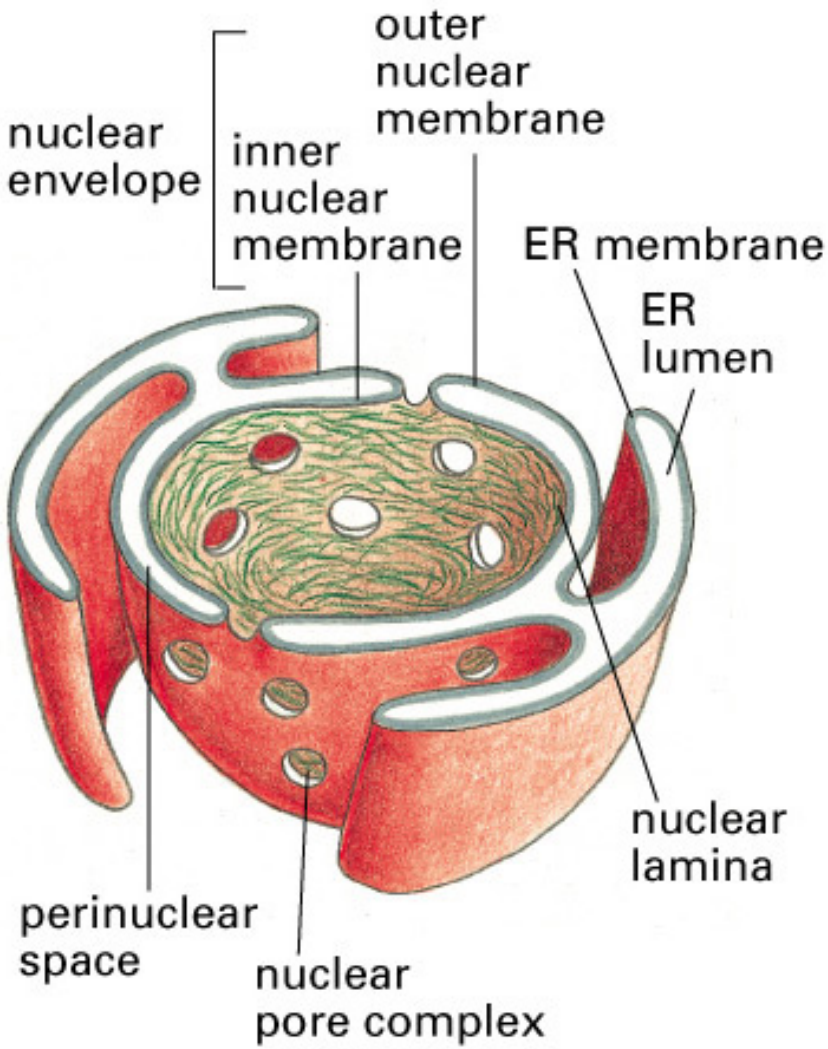
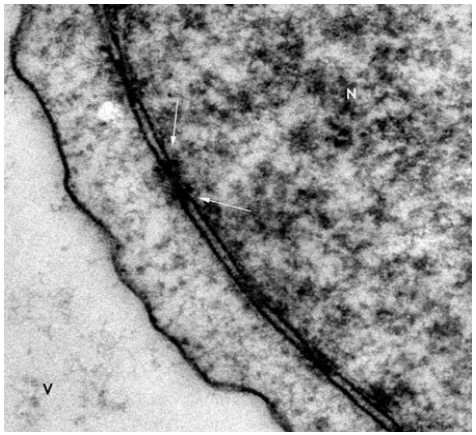
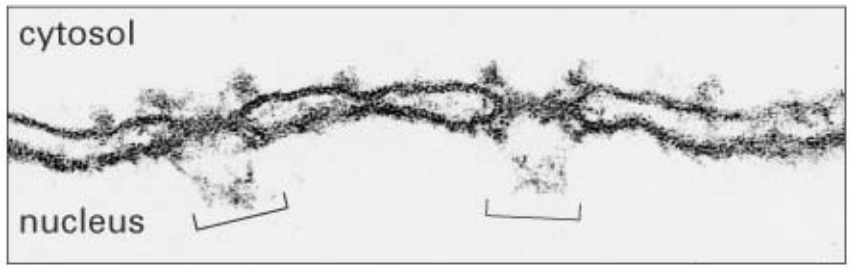


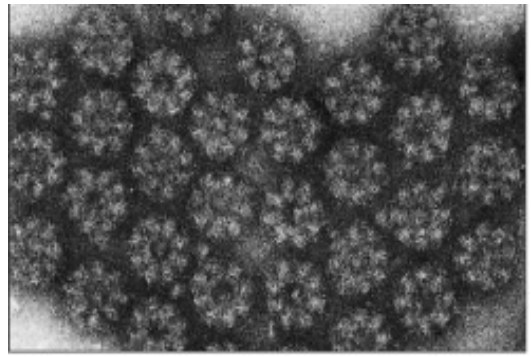
Figure 12–9. Molecular Biology of the Cell, 4th Edition.

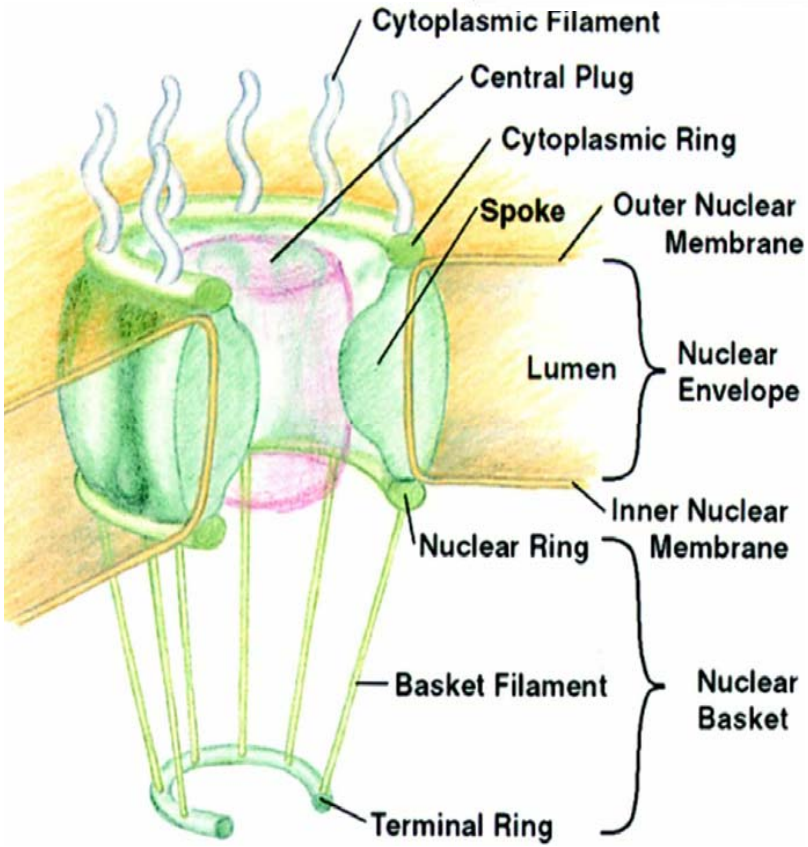
The nucleus is bounded by double membrane, the Nuclear Envelope, that is continuous with the ER.

The nuclear pore



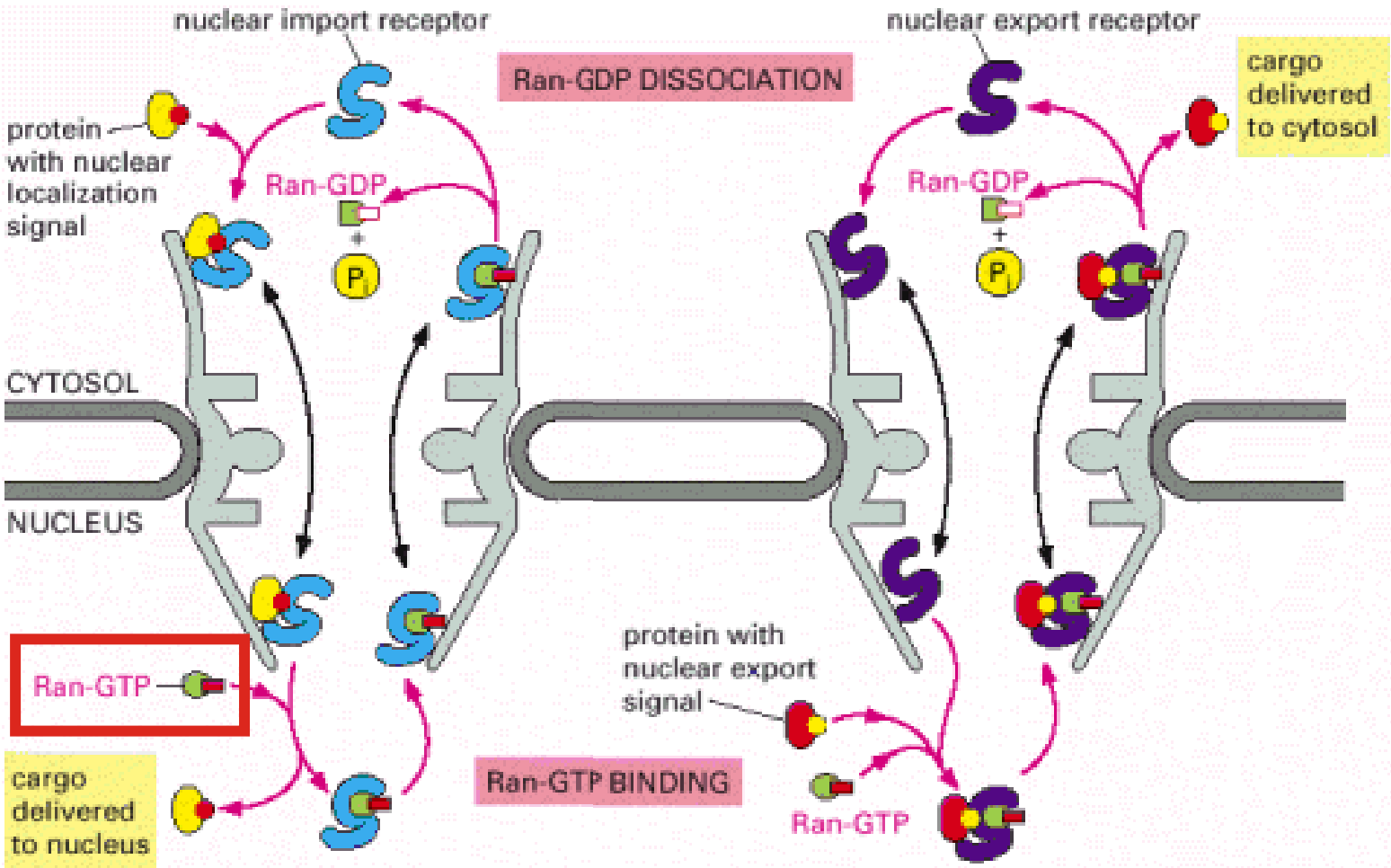






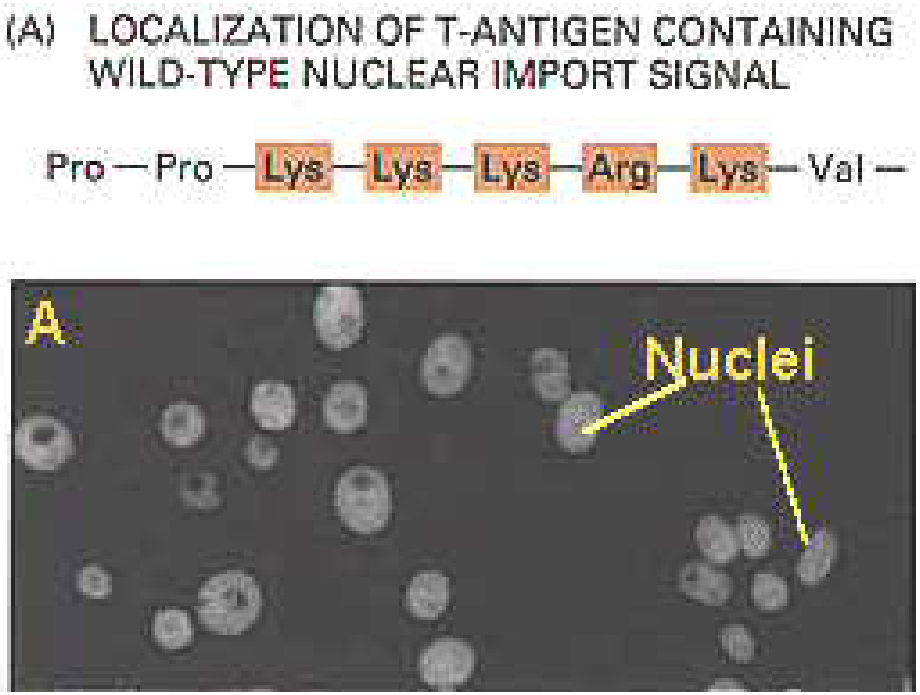
- Bridges the inner and outer membrane
- 8-fold rotational symmetry
- 3 concentric rings
- complex (a least 30 proteins)
- 3000 to 5000 nuclear pores/cell
- site of protein and RNA transport into and out of the nucleus

How is nuclear transport regulated?

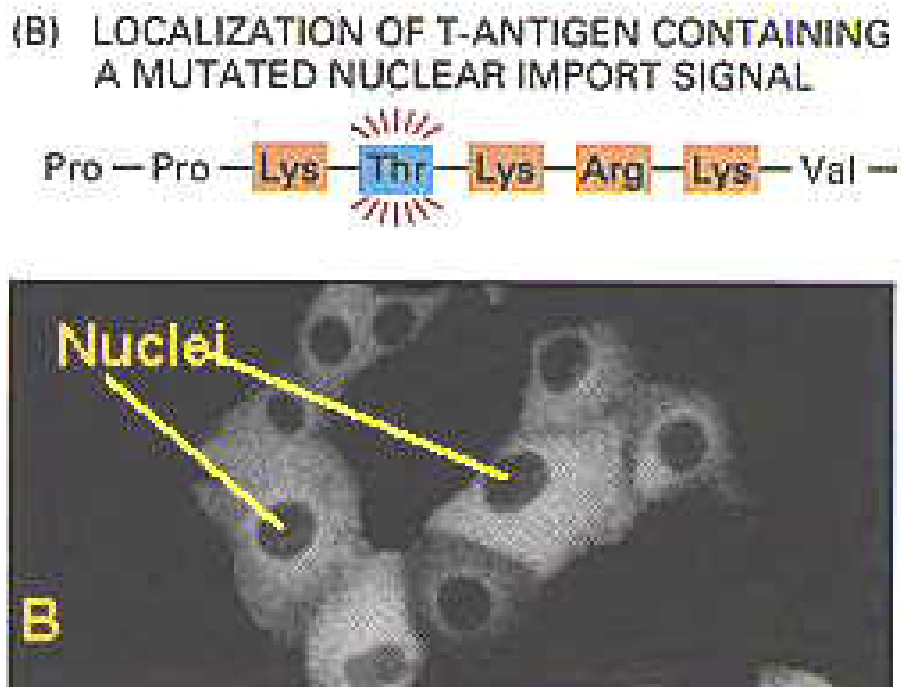


Asymmetric import/export cycles,
Cargo-receptor interaction depend on their environment

What happens if the nuclear localization signal is mutated?

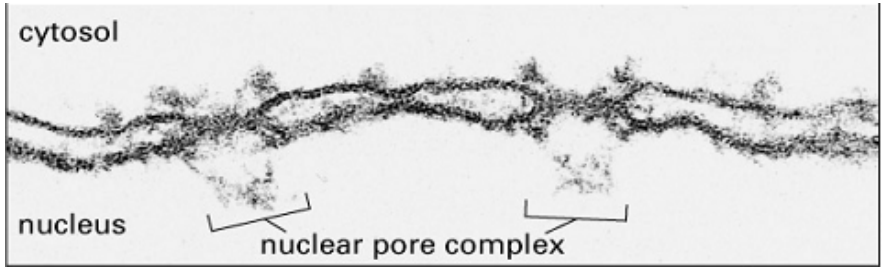


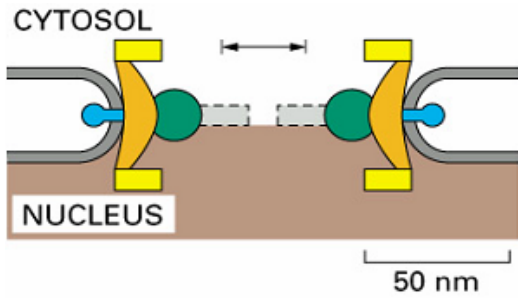
The protein T-antigen with a wild-type NLS localizes to the nucleus

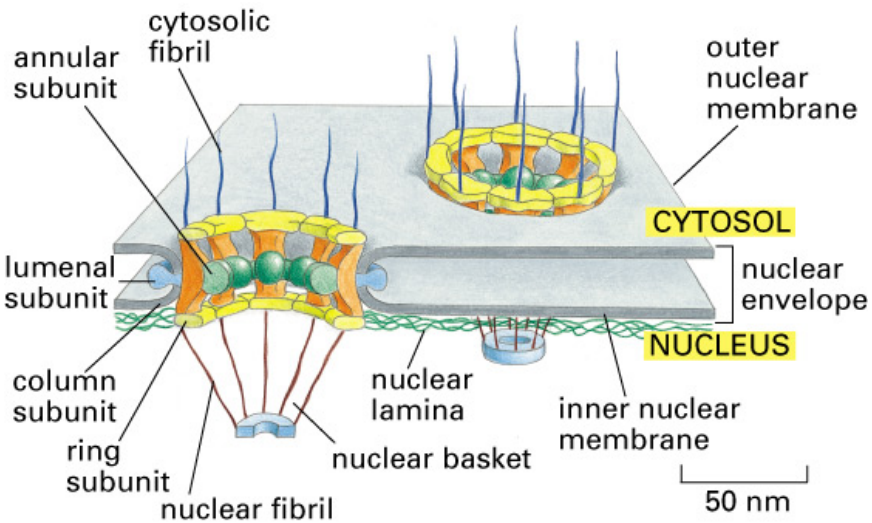


A mutation in the NLS of T-antigen leads to cytosolic localization of the protein (shown as clearly absent from the nucleus)

A mutation in the nuclear localization signal leads to *cytosolic* localization of the protein.







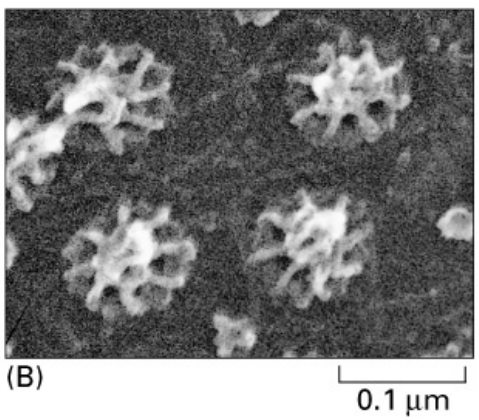
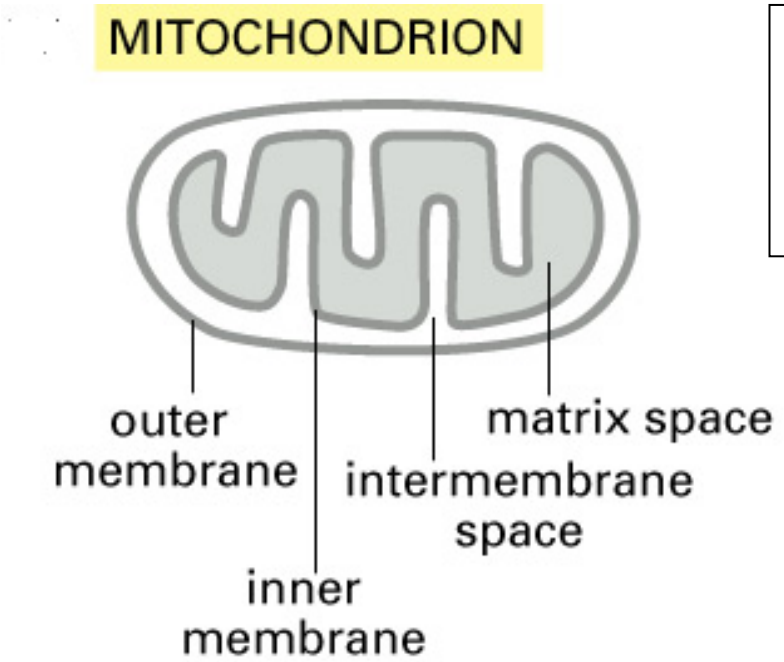
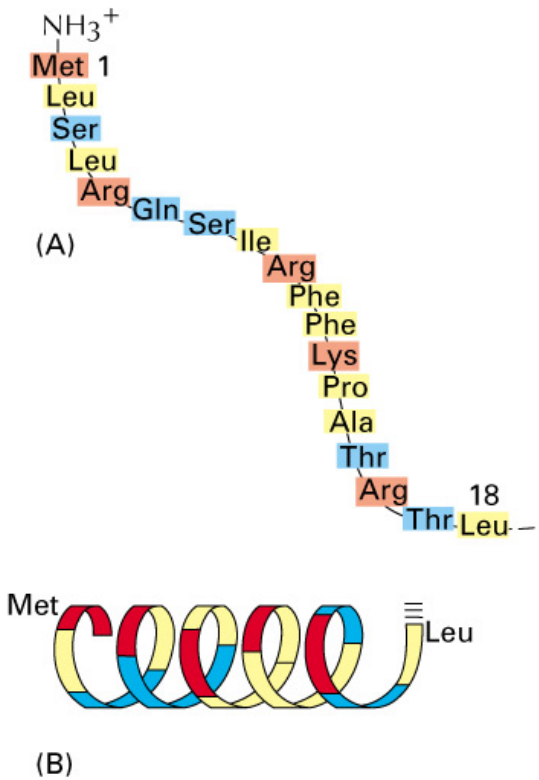


Figure 12–10 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

The openings in the Nuclear Envelope that allow the passage of material in and out of the nucleus are called Nuclear Pores



-Cytoplasmic organelle
-Bounded by a double membrane



An amphipathic alpha helix at the Amino-terminus of a protein can act as a Mitochondrial Targeting Sequence

Figure 12–23. Molecular Biology of the Cell, 4th Edition.

Transport from the Cytoplasm to the Mitochondrial Matrix requires two distinct translocation complexes Tom & Tim.

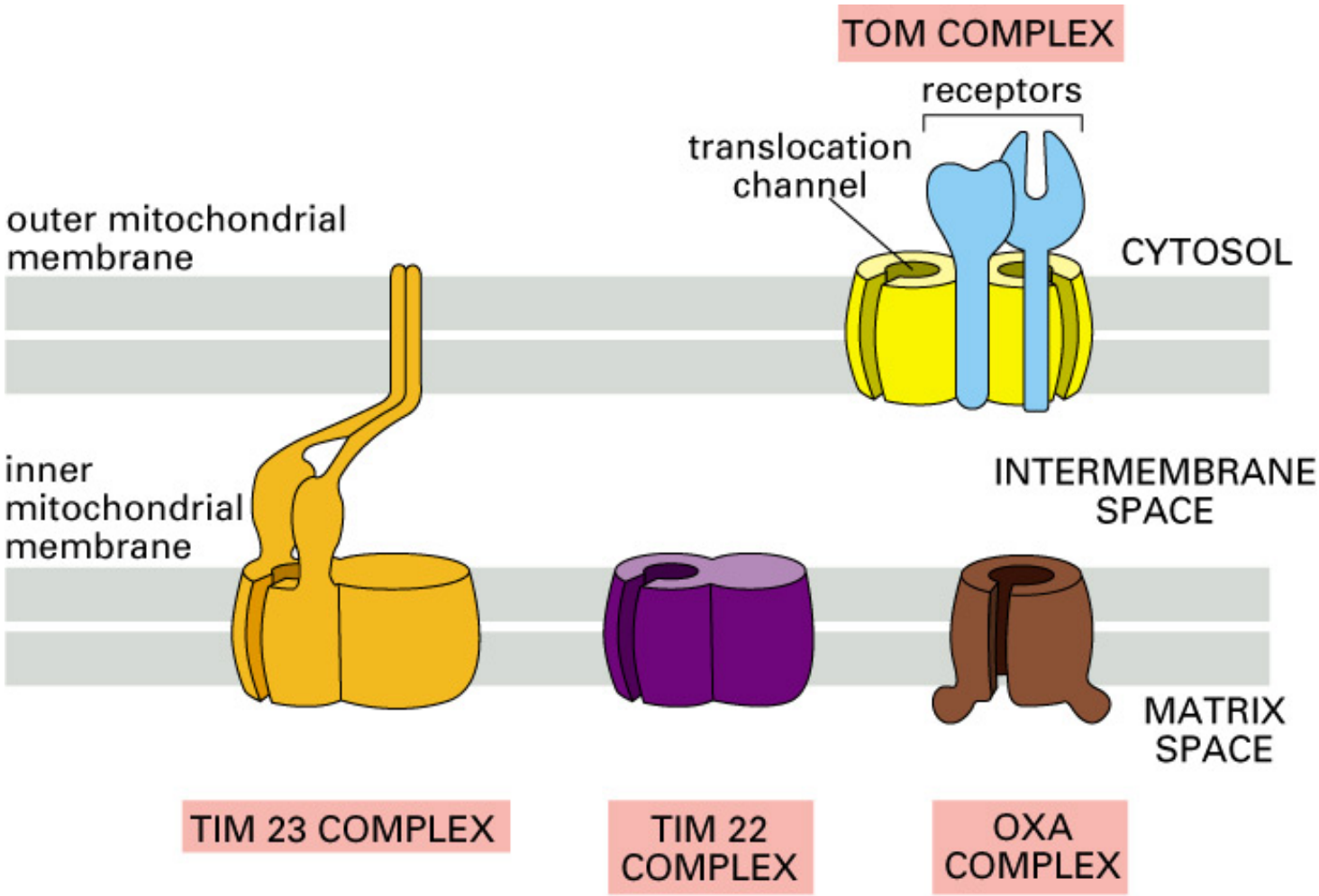
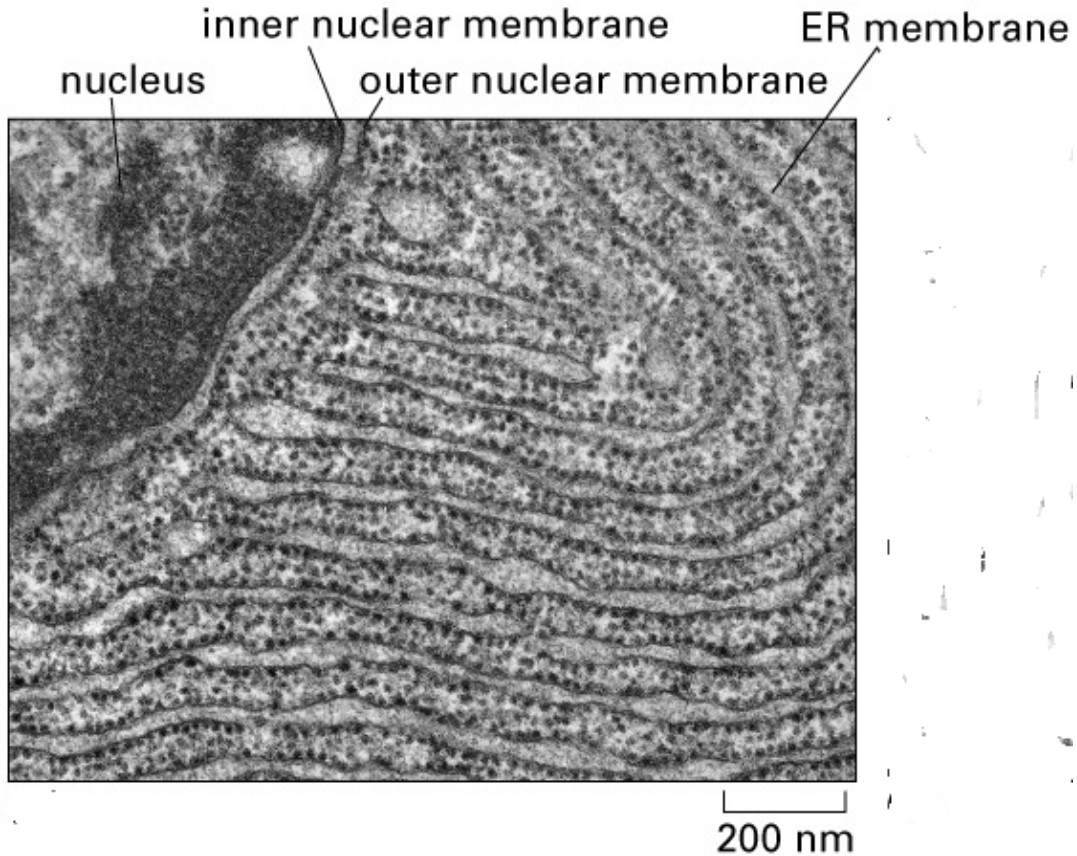


Figure 12–24. Molecular Biology of the Cell, 4th Edition.

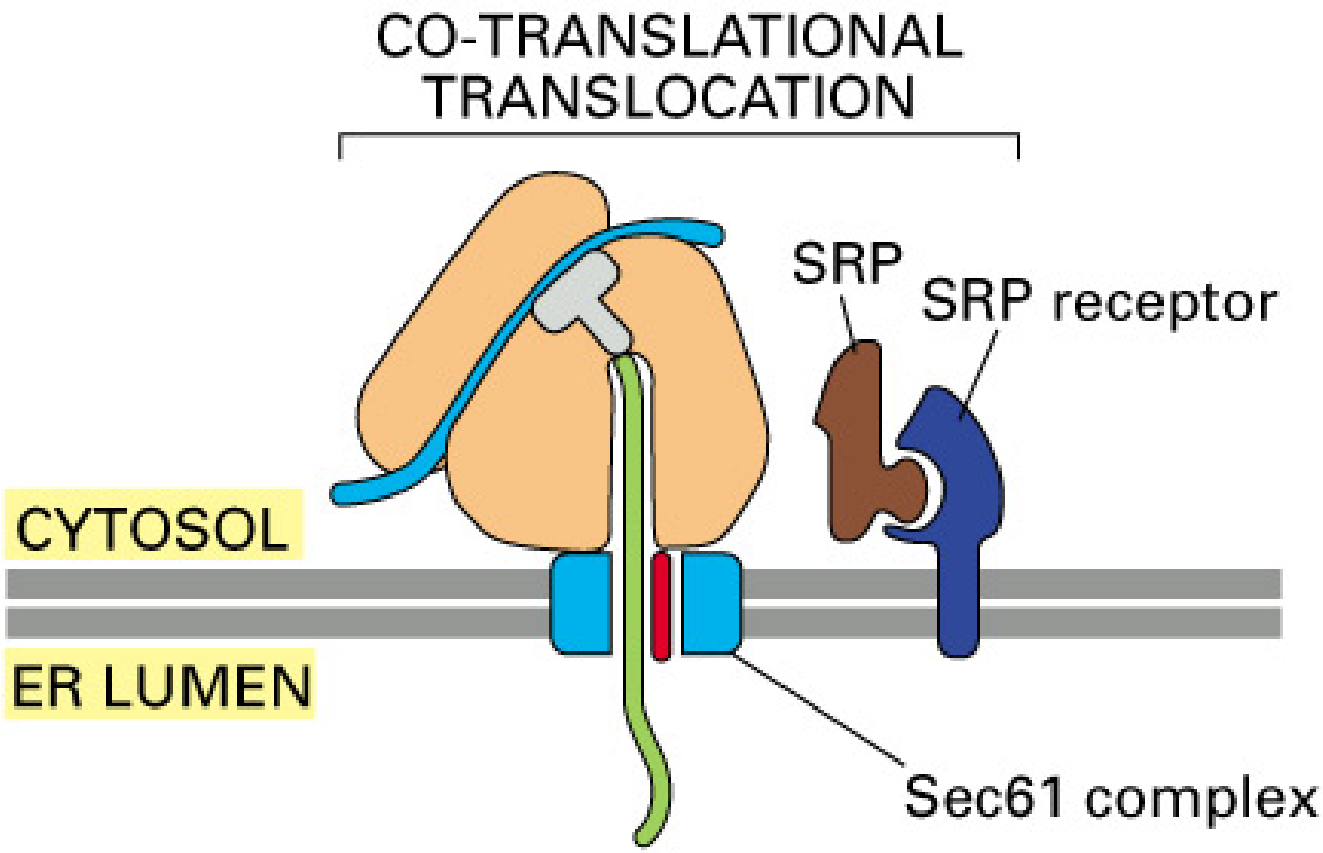
Post-translational translocation

Transmembrane transport into the Endoplasmic Reticulum
(Gateway to the Secretory Pathway)

The “Rough ER” - Endoplasmic Reticulum with ribosomes attached
is the site of co-translational translocation of proteins into the ER



In co-translational translocation, the nascent protein crosses the ER membrane as it leaves the ribosome



This ER quality control is important in numerous genetic diseases

One we've seen before -- Tay-Sachs

the mutations in the hexosaminidase gene that lead to Tay Sachs disease cause changes in the protein that result in its retention and degradation in the ER. Thus, the hexosaminidase protein never reaches the lysosome, leading to the accumulation of gangliosides and disease.

The Endoplasmic Reticulum is the gateway for protein transport into all the other membrane -bound organelles of the secretory pathway.

Proteins and lipids are trafficked through the secretory pathway in small carriers called vesicles.

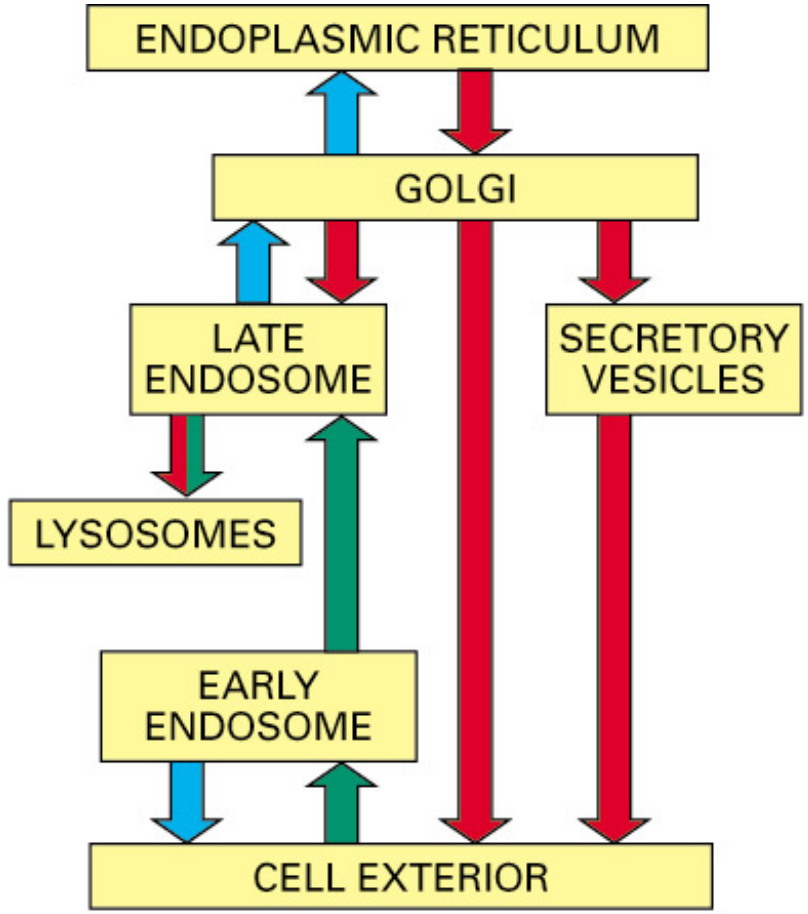


Figure 13–1. Molecular Biology of the Cell, 4th Edition.

Every Vesicle Transport step requires:

1) Coat proteins to generate the vesicle on the 1st compartment
2) Fusion proteins to allow the vesicle to fuse with the acceptor compartment

Formation of a transport vesicle requires coat proteins

Different coat complexes function at different points in the secretory pathway

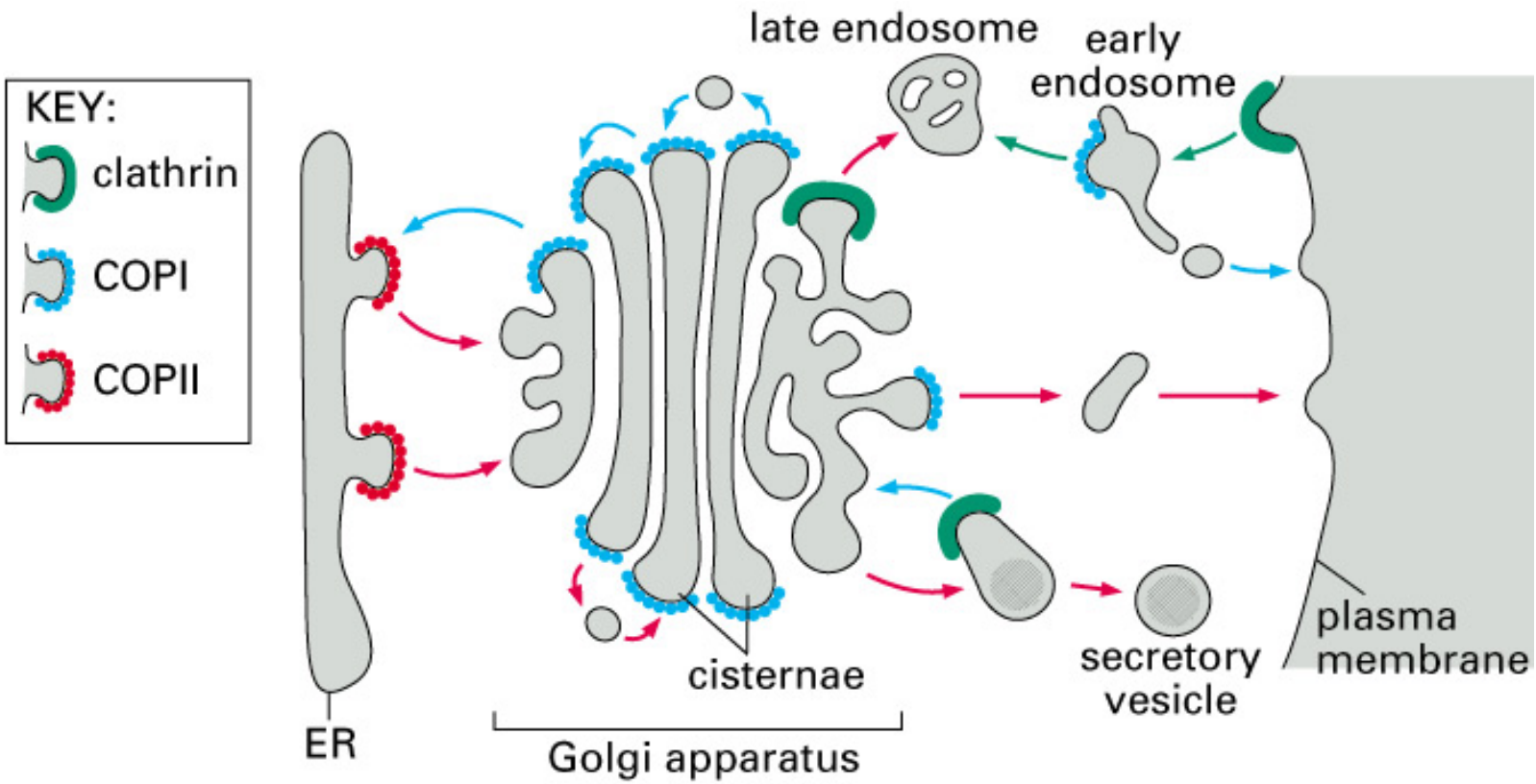


Figure 13–5. Molecular Biology of the Cell, 4th Edition.

Arrows: red = anterograde transport steps
blue = retrograde transport steps
green = endocytic transport steps

Vesicle formation and budding is driven by coat formation

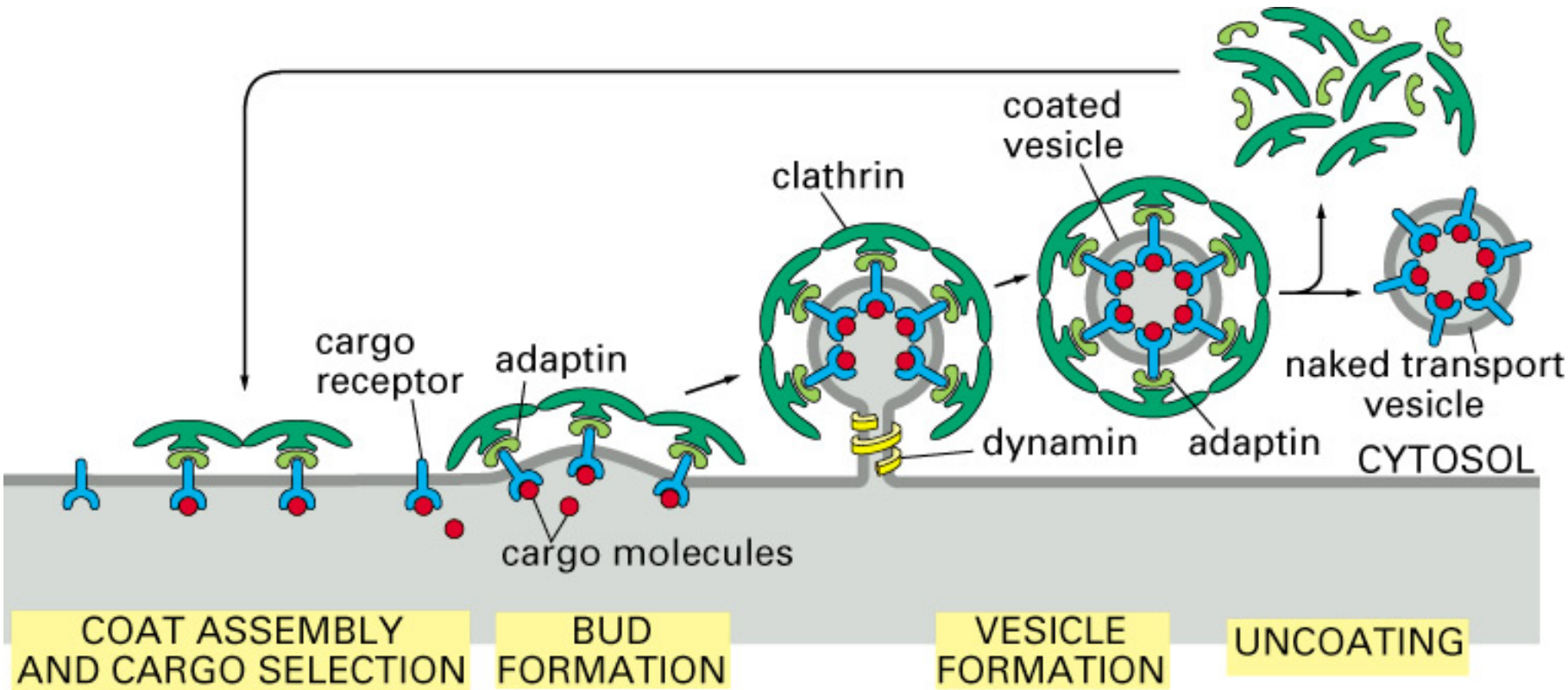


Figure 13–8. Molecular Biology of the Cell, 4th Edition.

Lysosomes are
a specialized
compartment of
the secretory pathway
that acts as a
degradative
organelle

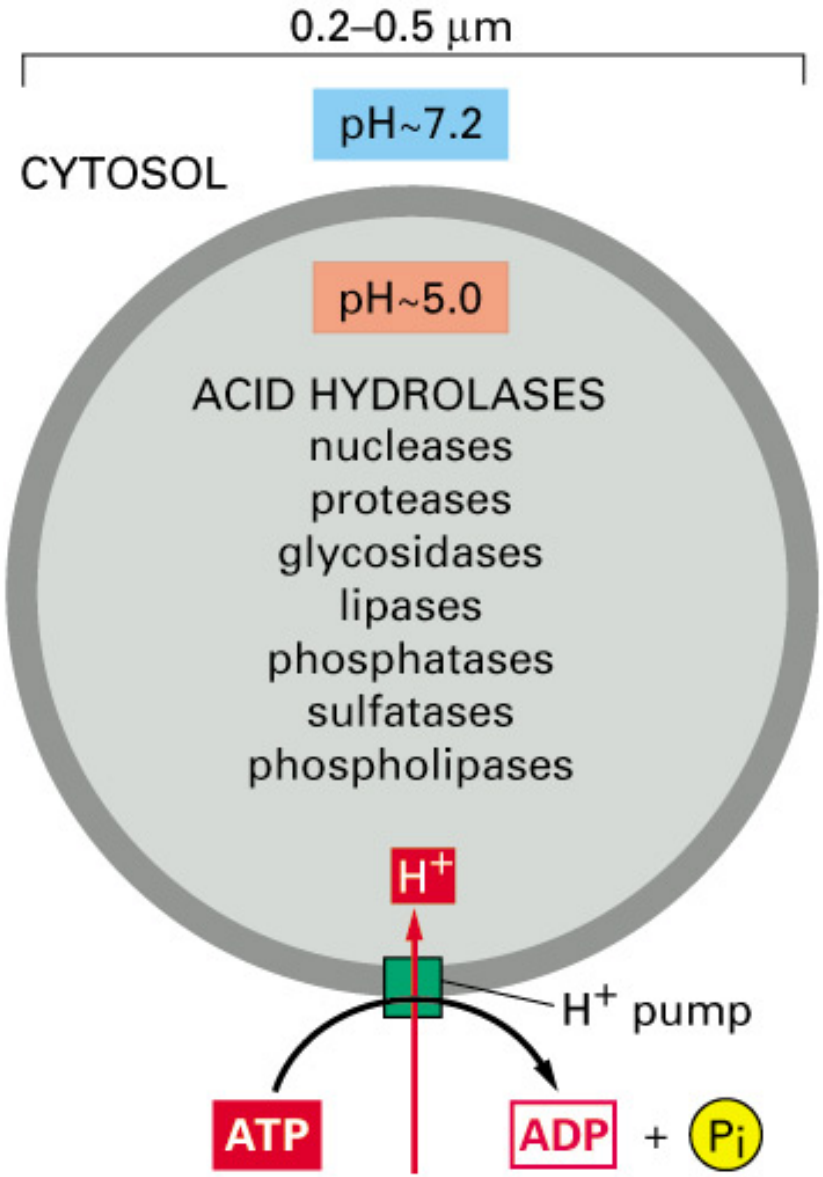


Figure 13–31. Molecular Biology of the Cell, 4th Edition.

A specific sugar acts as a sorting signal to target proteins to the lysosome

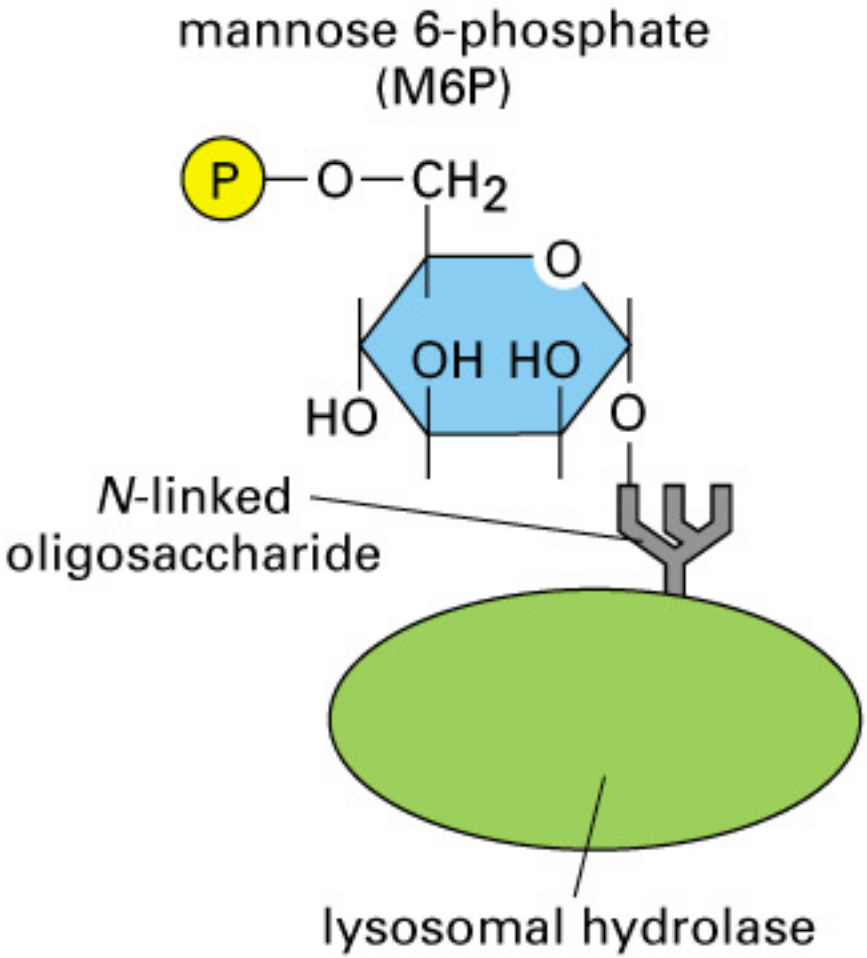


Figure 13–36. Molecular Biology of the Cell, 4th Edition.

A M6P receptor in the trans-Golgi sorts lysosomal hydrolyases into the proper vesicles for delivery to the lysosome

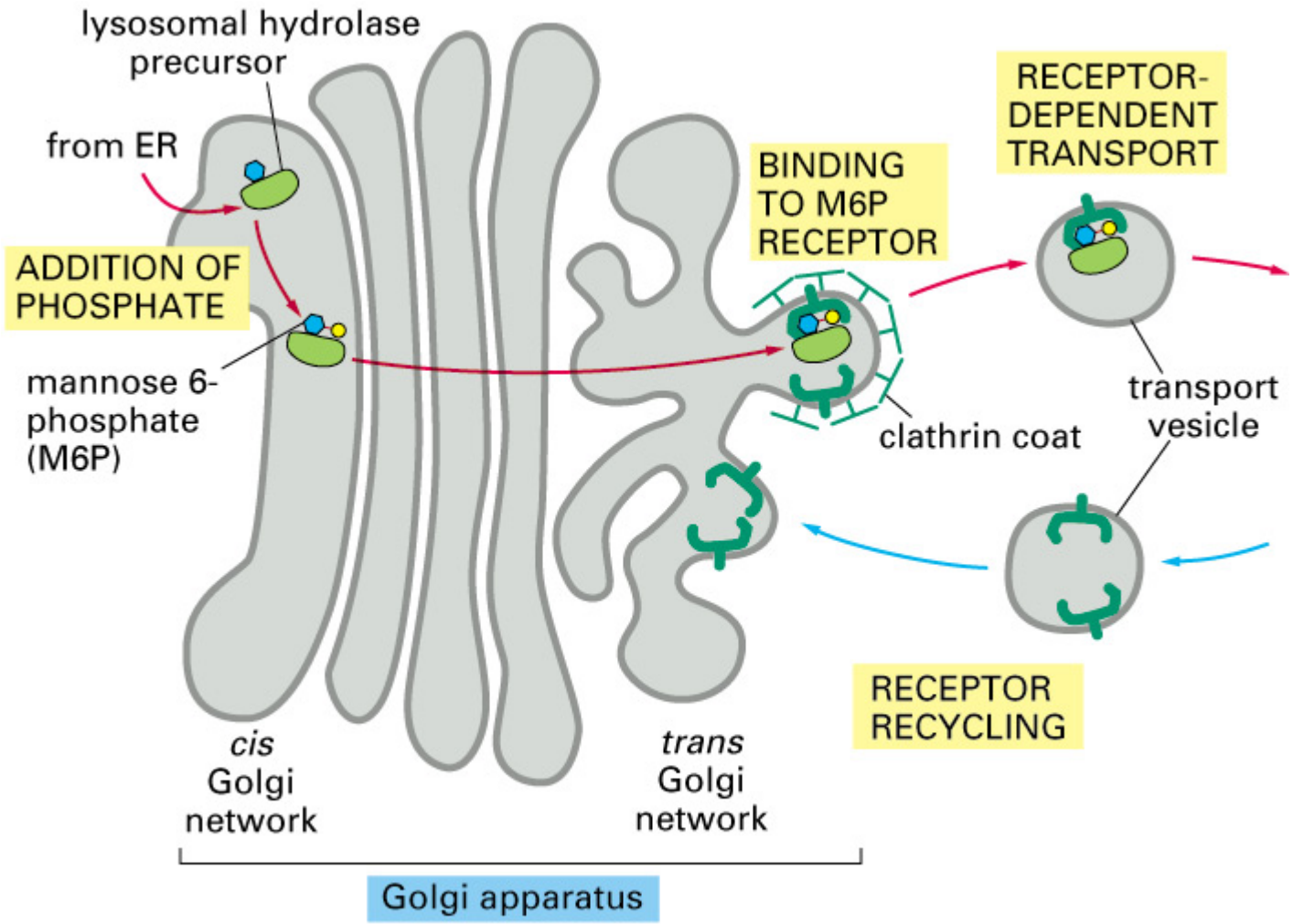


Figure 13–37 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

A M6P receptor in the trans-Golgi sorts lysosomal hydrolyases into the proper vesicles for delivery to the lysosome

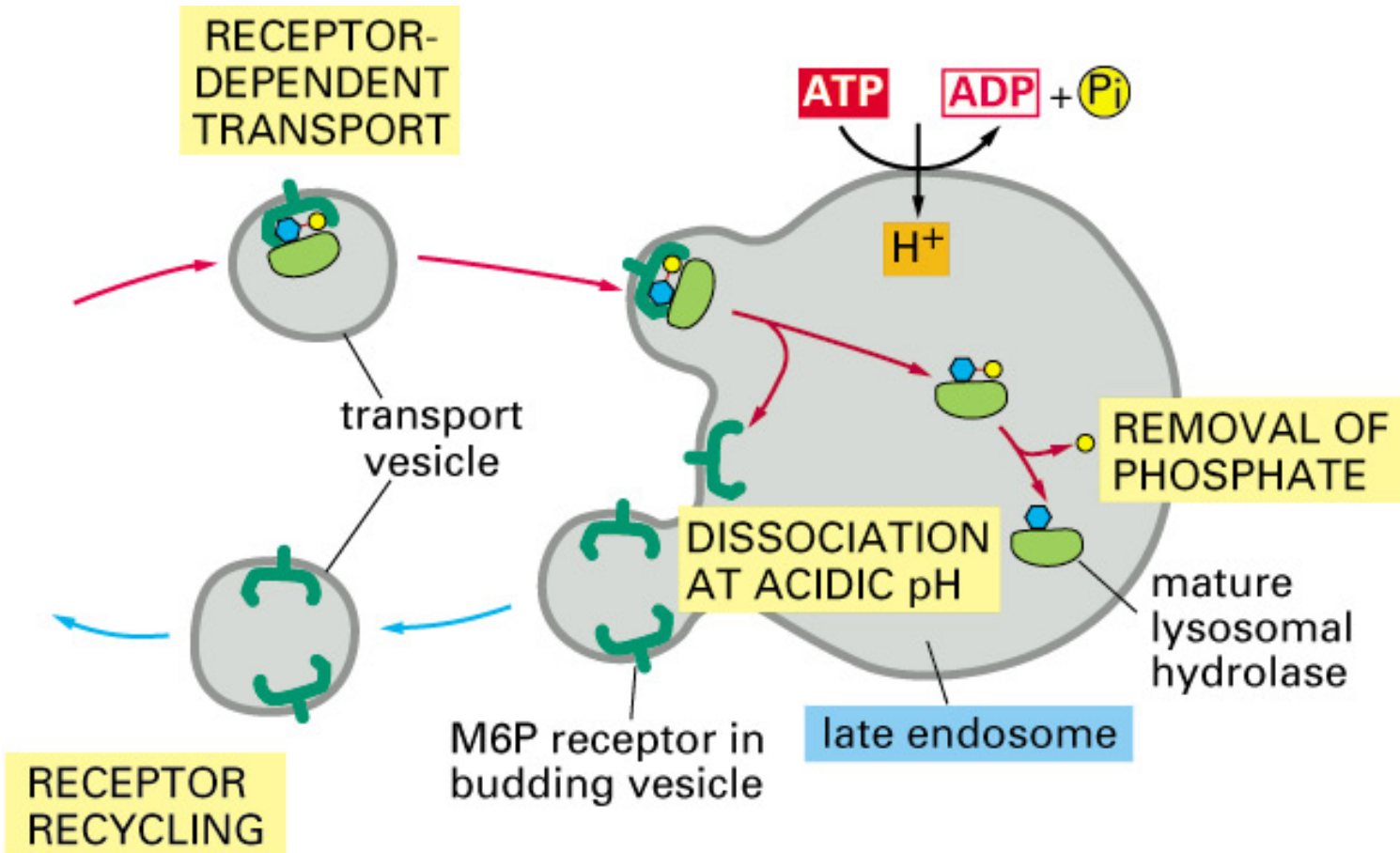
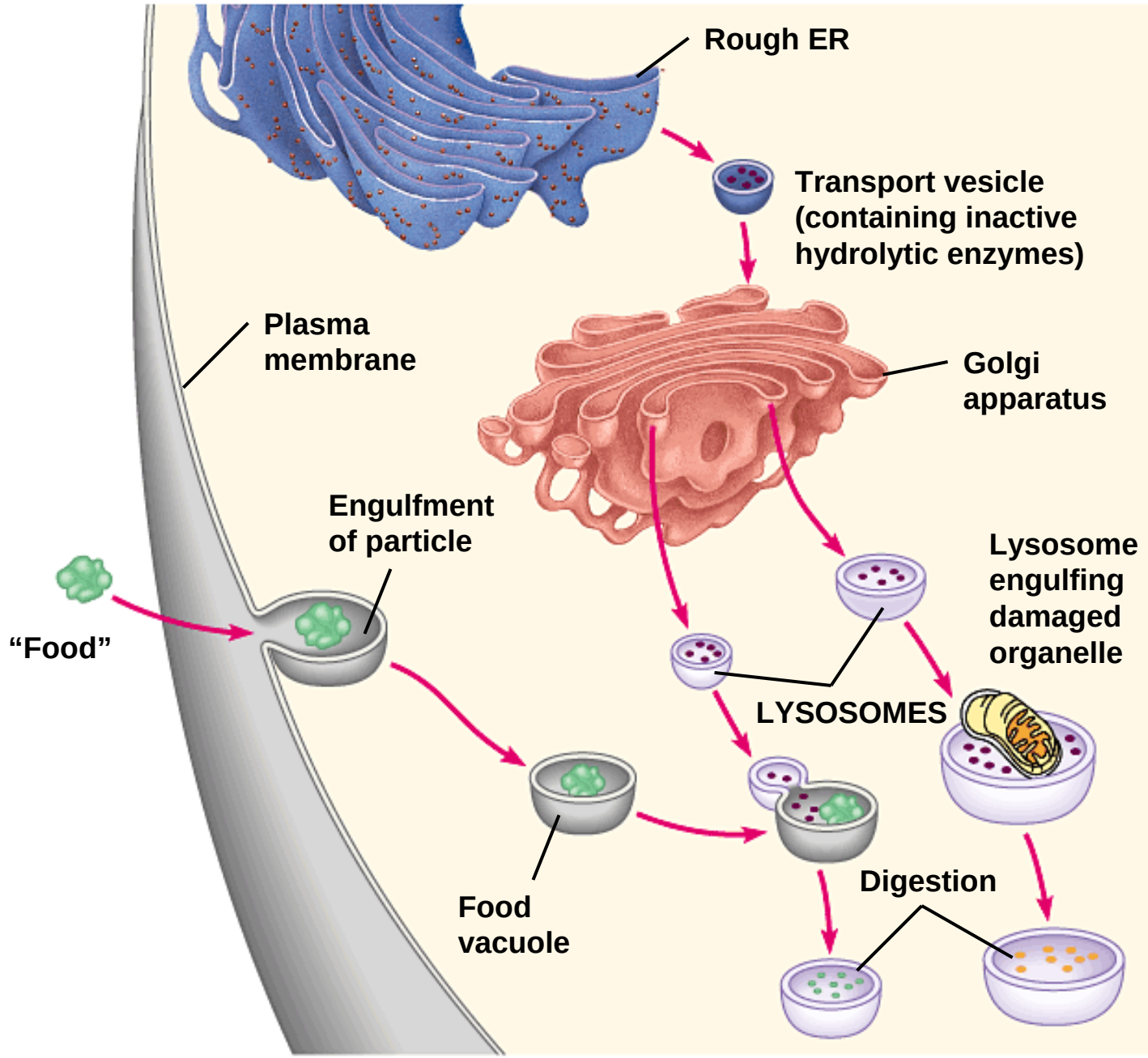
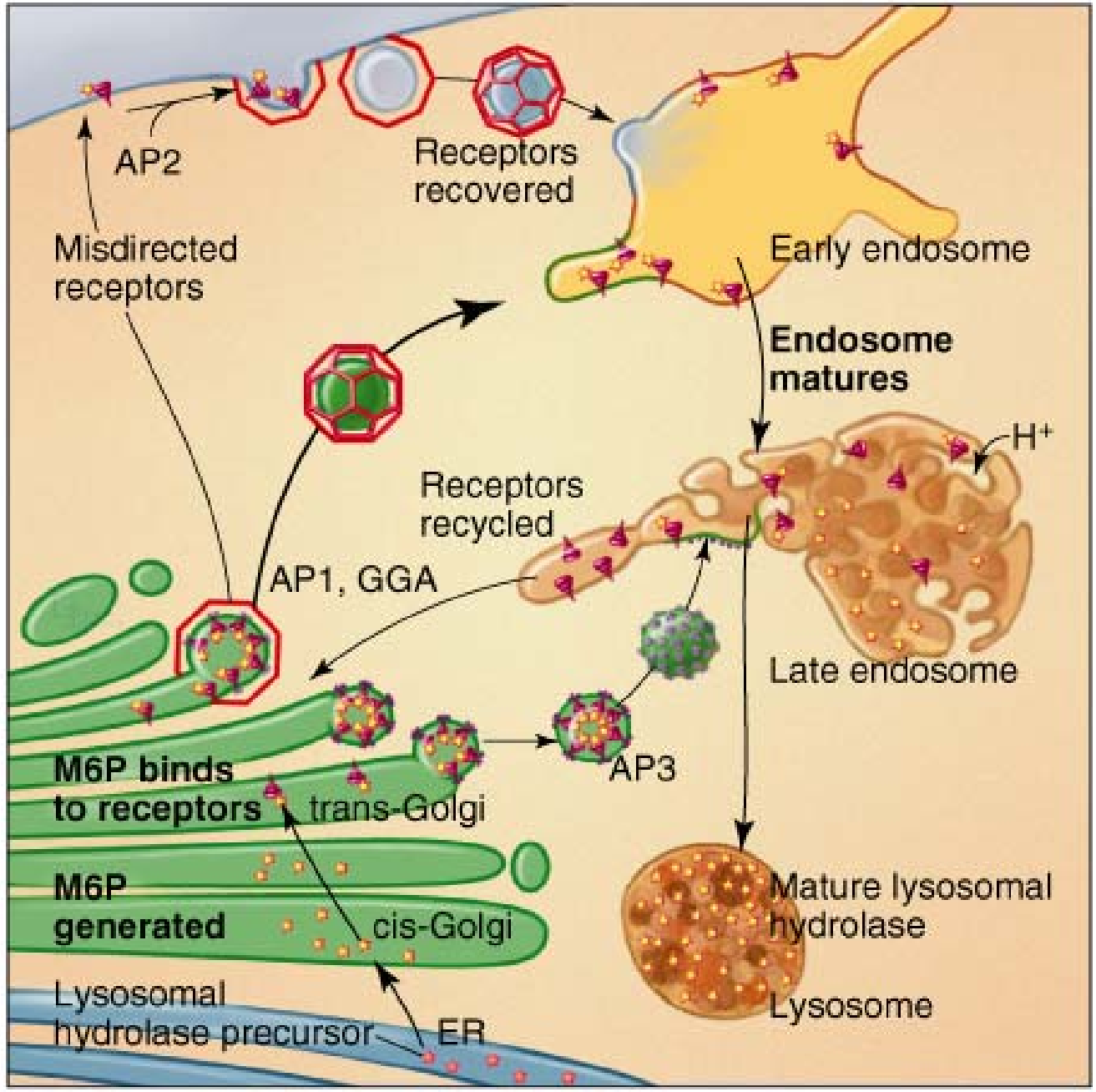
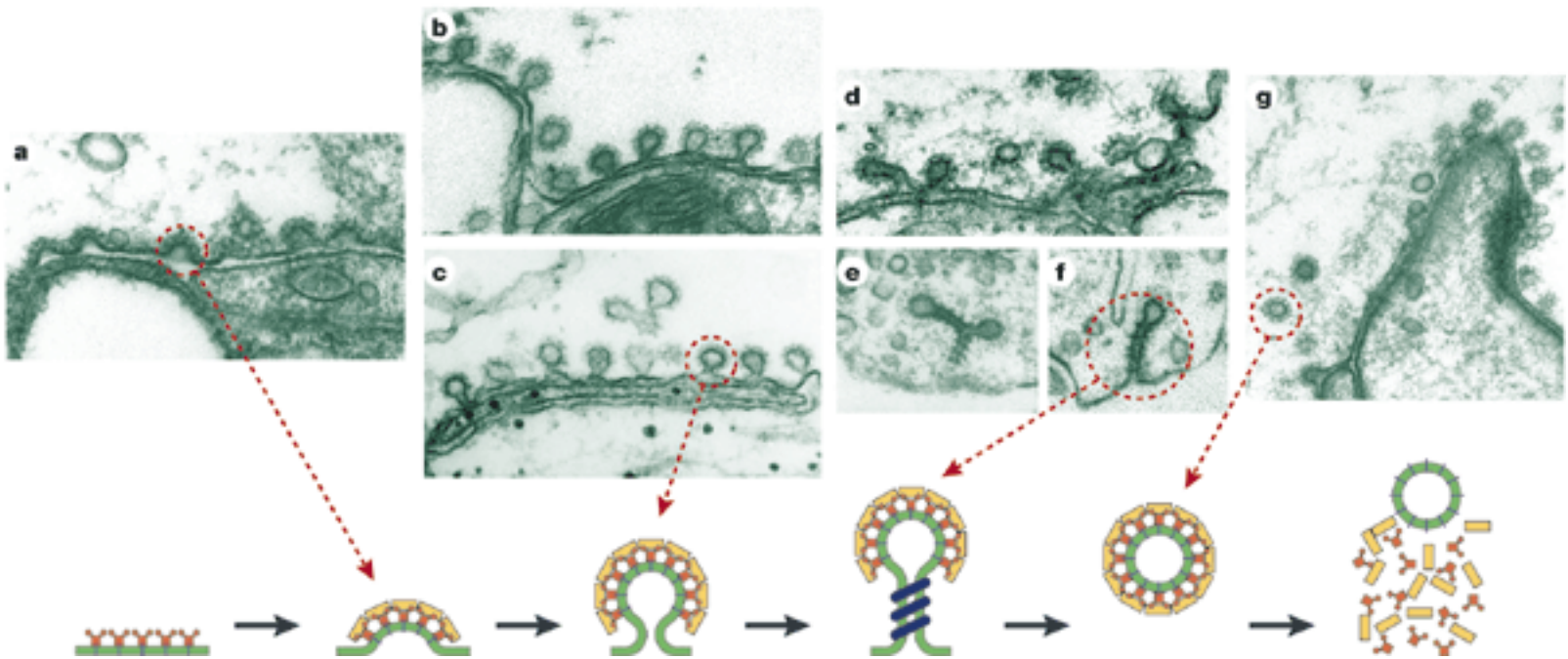


Figure 13–37 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

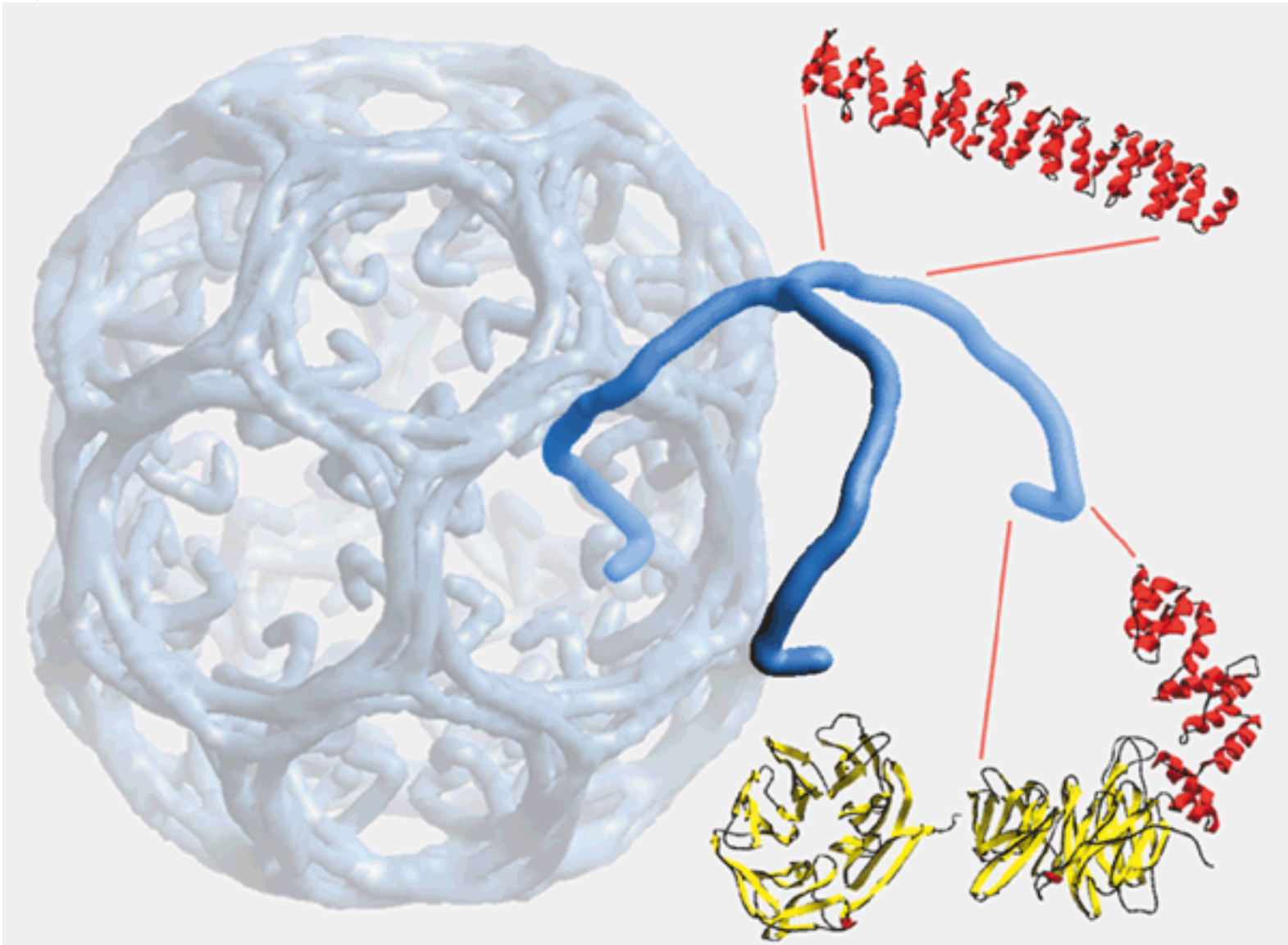

Rough ER
Transport vesicle (containing inactive hydrolytic enzymes)
Plasma membrane
Golgi apparatus
Engulfment of particle
"Food"
Lysosome engulfing damaged organelle
LYSOSOMES
Food vacuole
Digestion



Morphology of steps formation of a clathrin coated vesicle, in this case clathrin-mediated endocytosis.



Structure of clathrin-coated vesicle. Structure of the clathrin trisekelion (heavy chain trimer).



Nat Rev MCB 1:187

A number of disease states are associated with lysosomal defects

Lysosomal Storage Diseases (described earlier by Dr. Schechter)

Big group (~50) of rare diseases often caused by the lack of a particular lysosomal enzyme

eg. Hexosaminidase in Tay-Sachs

but can also be caused by sorting defects

eg. I-cell disease (defect in the mannose-6-phosphate system)

Several of the diseases with more general effects on lysosome biogenesis

eg. Hermansky-Pudlak syndrome

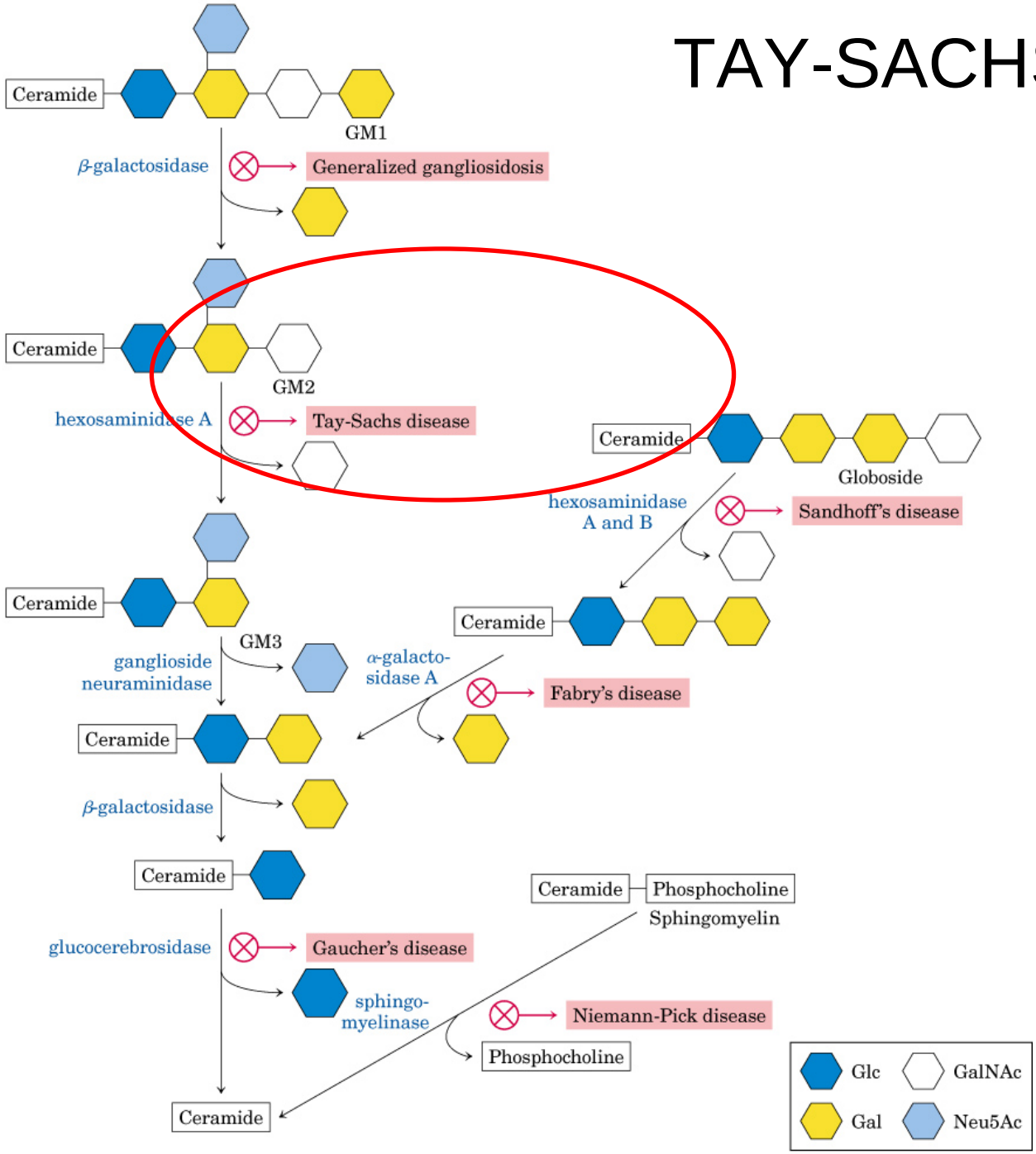
Chediak-Higashi syndrome

share a common manifestation -- albinism

Lysosomal Storage Diseases

Disease Name	Enzyme Defect	Substance Stored
	A. Glycogenosis Disorders	
Pompe Disease	Acid-α1, 4-Glucosidase	Glycogen α1-4 linked Oligosaccharides
	B. Glycolipidosis Disorders	
GM1 Gangliodsidosis	β-Galactosidase	GM1 Ganliosides
Tay-Sachs Disease	β-Hexosaminidase A	GM2 Ganglioside
GM2 Gangliosidosis:	GM2 Activator Protein	GM2 Ganglioside
Sandhoff Disease	β-Hexosamindase A&B	GM2 Ganglioside
Fabry Disease	α-Galactosidase A	Globosides
Gaucher Disease	Glucocerebrosidase	Glucosylceramide
Metachromatic Leukodystrophy	Arylsulfatase A	Sulphatides
Krabbe Disease	Galactosylceramidase	Galactocerebroside
Niemann-Pick	Acid Sphingomyelinase	Sphingomyelin
Niemann-Pick, Type C	Cholesterol Esterification Defect	Sphingomyelin
Nieman-Pick, Type D	Unknown	Sphingomyelin
Farber Disease	Acid Ceramidase	Ceramide
Wolman Disease	Acid Lipase	Cholesteryl Esters
	C. Mucopolysaccharide Disorders	
Hurler Syndrome (MPS IH)	α-L-Iduronidase	Heparan & Dermatan Sulfate
Scheie Syndrome (MPS IS)	α-L-Iduronidase	Heparan & Dermatan Sulfate
Hurler-Scheie (MPS IH/S)	α-L-Iduronidase	Heparan & Dermatan Sulfate
Hunter Syndrome (MPS II)	Iduronate Sulfatase	Heparan & Dermatan Sulfate
Sanfilippo A (MPS IIIA)	Heparan N-Sulfatase	Heparan Sulfate
Sanfilippo B (MPS IIIB)	α-N-Acetylglucosaminidase	Heparan Sulfate
Sanfilippo C (MPS IIIC)	Acetyl-CoA-Glucosaminide Acetyltransferase	Heparan Sulfate
Sanfilippo D (MPS IIID)	N-Acetylglucosamine -6-Sulfatase	Heparan Sulfate
Morquio A (MPS IVA)	Galactosamine-6-Sulfatase	Keratan Sulfate
Morquio B (MPS IVB)	β-Galactosidase	Keratan Sulfate
Maroteaux-Lamy (MPS VI)	Arylsulfatase B	Dermatan Sulfate
Sly Syndrome (MPS VII)	β-Glucuronidase	?
	D. Oligosaccharide/Glycoprorders	
α-Mannosidosis	α-Mannosidase	Mannose/Oligosaccharides
β-Mannosidosis	β-Mannosidase	Mannose/Oligosaccharides
Fucosidosis	α-L-Fucosidase	Fucosyl Oligosaccharides
Asparylglucosaminuria	N-Aspartyl-Asparylglucosaminidase	Asparagine β-Glucosamine
Sialidosis (Mucolipidosis I)	α-Neuraminidase	Sialyloligosaccharides
Galactosialidosis (Goldberg Syndrome)	Lysosomal Protective Protein Deficiency	Sialyloligosaccharides
Schindler Disease	α-N-Acetyl-Galactosaminidase	?
	E. Lysosomal Enzyme Transorders	
Mucolipidosis II (I-Cell Disease)	N-Acetylglucosamine-1-Phosphotransferase	multiple glycan structures
	F. Lysosomal Membrane Disorders	
Cystinosis	Cystine Transport Protein	Free Cystine
Salla Disease	Sialic Acid Transport Protein	Free Sialic Acid and Glucuronic Acid
Infantile Sialic Acid Storage Disease	Sialic Acid Transport Protein	Free Sialic Acid and Glucuronic Acid
	G. Other	
Batten Disease (Juvenile Neuronal Ceroid Lipofuscinosis)	Unknown	Lipofuscins
Infantile Neuronal Ceroid Lipofuscinosis	Palmitoyl-Protein Thioesterase	Lipofuscins
Mucolipidosis IV	Unknown	Gangliosides & Hyaluronic Acid
Prosaposin		Saposins A, B, C or D

TAY-SACHS DISEASE



Glycogen storage diseases

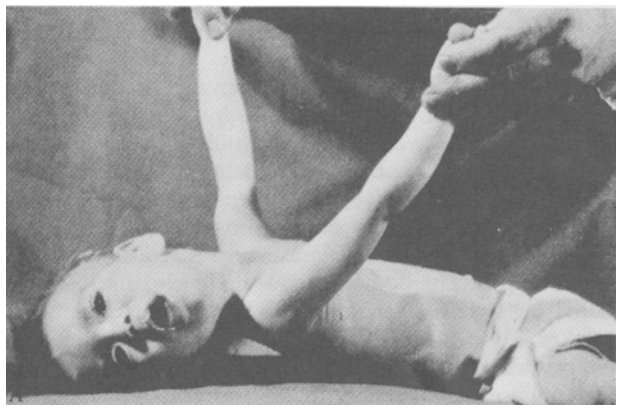
For reference only

Type	Defective enzyme	Organ affected	Glycogen in the affected organ	Clinical features
I Von Gierke disease	Glucose 6-phosphatase or transport system	Liver and kidney	Increased amount; normal structure.	Massive enlargement of the liver. Failure to thrive. Severe hypoglycemia, ketosis, hyperuricemia, hyperlipemia.
II Pompe disease	α-1,4-Glucosidase (lysosomal)	All organs	Massive increase in amount; normal structure.	Cardiorespiratory failure causes death, usually before age 2.
III Cori disease	Amylo-1,6-glucosidase (debranching enzyme)	Muscle and liver	Increased amount; short outer branches.	Like type I, but milder course.
IV Andersen disease	Branching enzyme (α-1,4 $\longrightarrow$ α-1,6)	Liver and spleen	Normal amount; very long outer branches.	Progressive cirrhosis of the liver. Liver failure causes death, usually before age 2.
V McArdle disease	Phosphorylase	Muscle	Moderately increased amount; normal structure.	Limited ability to perform strenuous exercise because of painful muscle cramps. Otherwise patient is normal and well developed.
VI Hers disease	Phosphorylase	Liver	Increased amount.	Like type I, but milder course.
VII	Phosphofructokinase	Muscle	Increased amount; normal structure.	Like type V.
VIII	Phosphorylase kinase	Liver	Increased amount; normal structure.	Mild liver enlargement. Mild hypoglycemia.

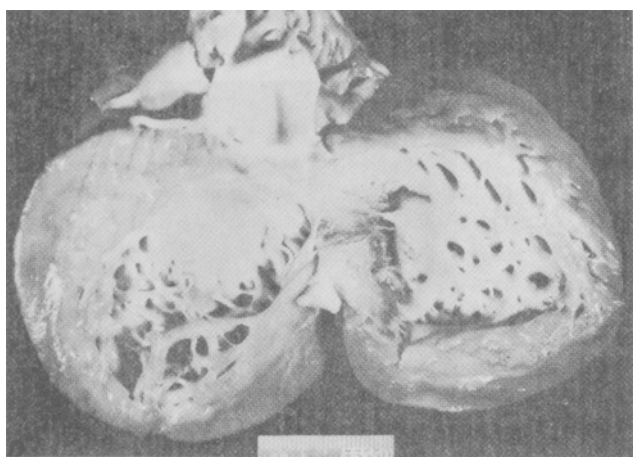
Note: Types I through VII are inherited as autosomal recessives. Type VIII is sex linked.

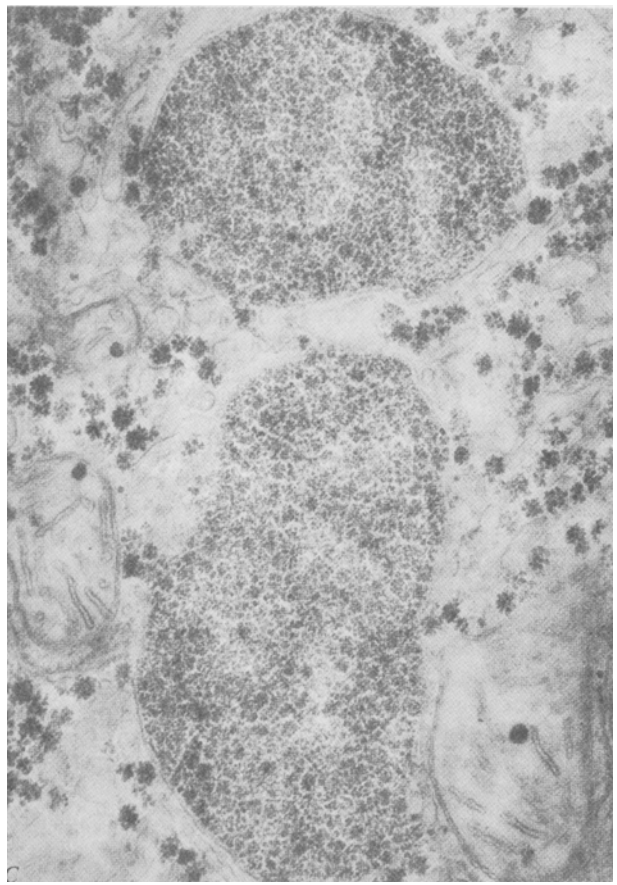
Pompe disease (glycogenosis type II) is a lysosomal storage disease

Lysosomal accumulation of glycogen due to deficiency of lysosomal alpha-glucosidase, which normally degrades glycogen in lysosomes

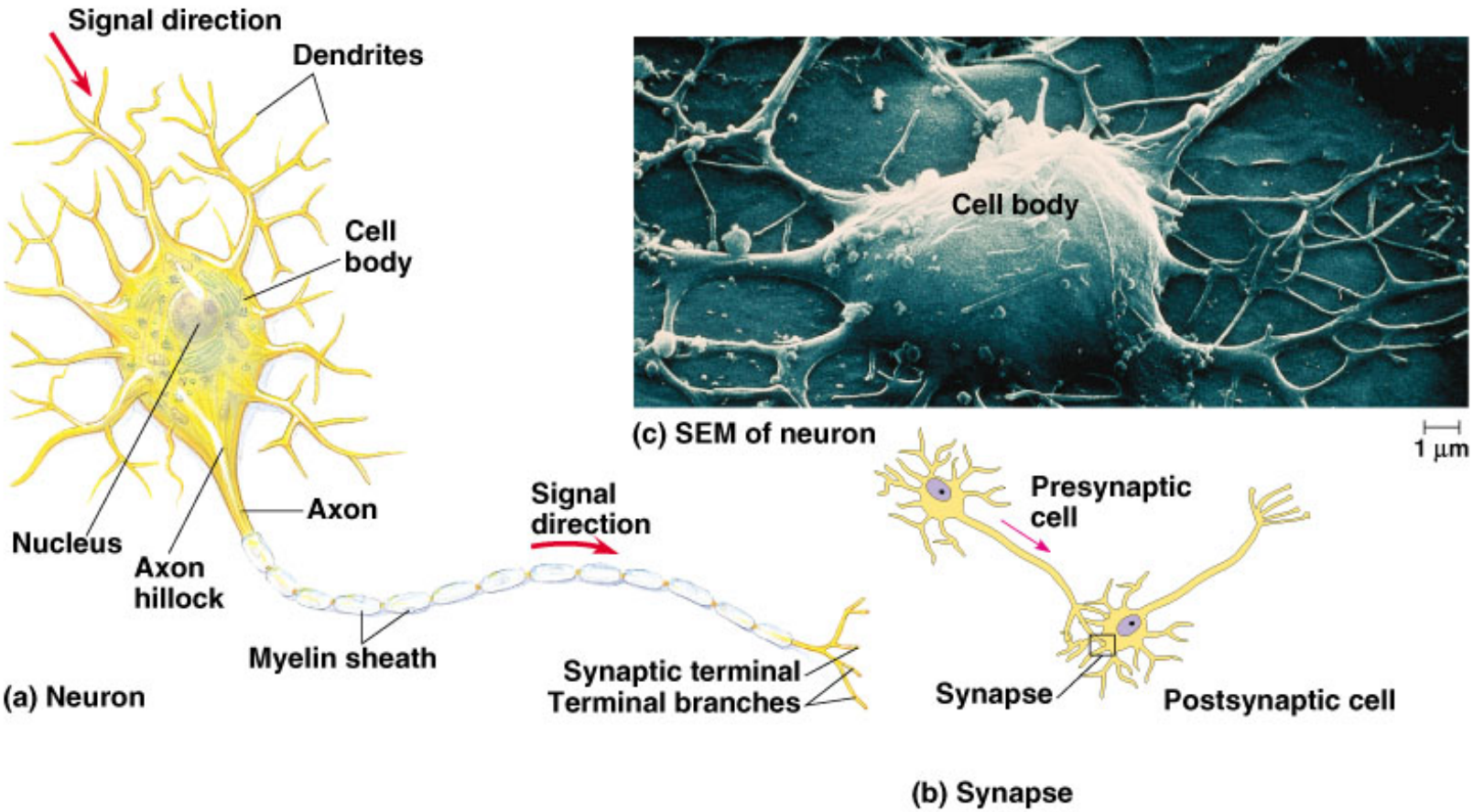


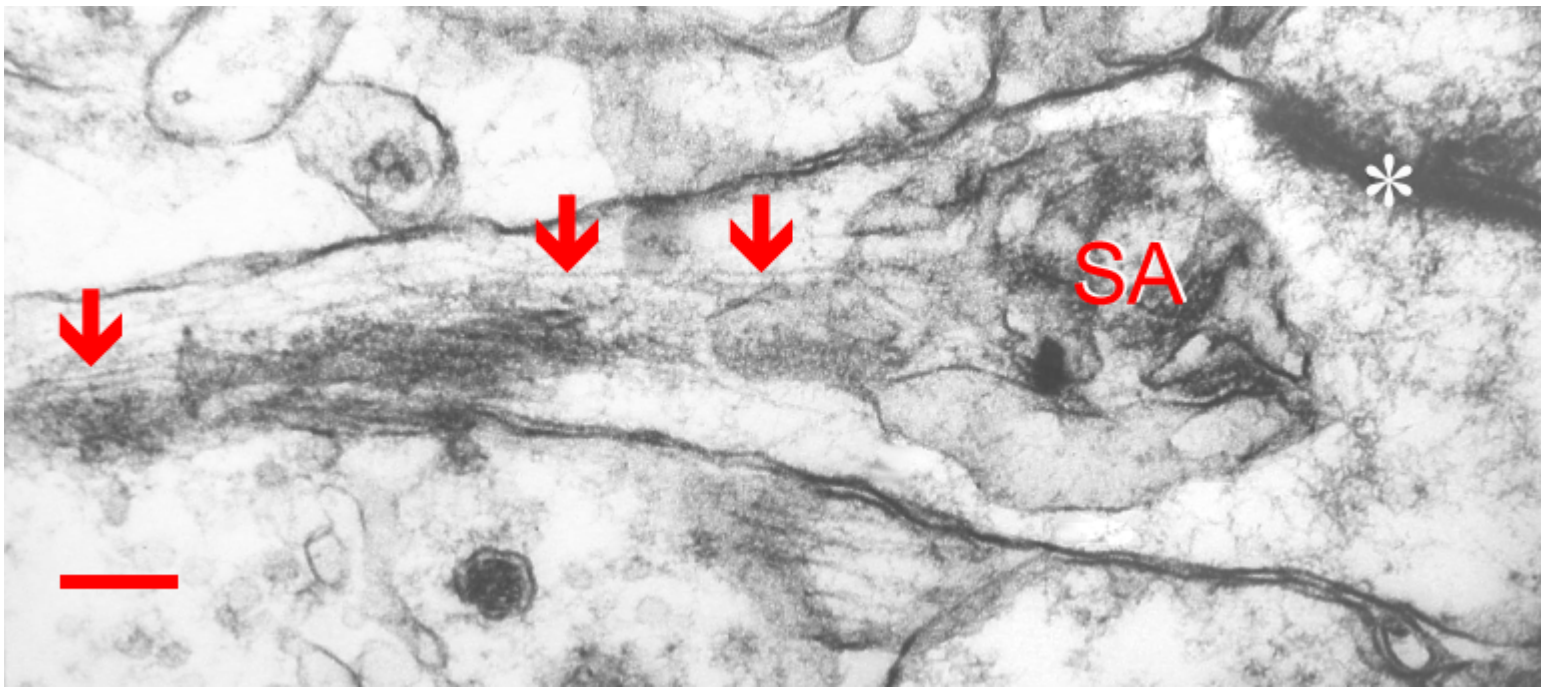
muscle weakness; enlarged heart





(not epinephrine responsive because sequestered by lysosomal membrane)





Microfilaments of actin running in parallel in the dendritic spine neck (arrows). Synapse - asterisk, spine apparatus - SA. Scale = 200 nm. (Human, neocortex.) http://synapse-web.org/index.asp

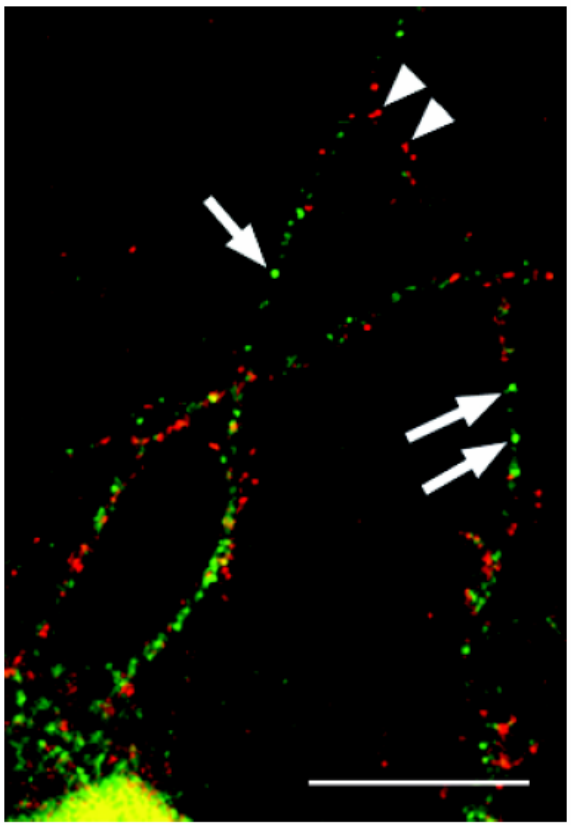


Fig. 3. Confocal laser scanning microscopy image of the neurites of a cultured hippocampal neuron showing granules containing heterogeneous nuclear ribonucleoprotein (hnRNP) A2 (green, arrows) and hnRNP A3 (red, arrowheads). Statistical analysis of the fluorescence of individual granules showed that the majority of granules in the neurites contained either hnRNP A2 or hnRNP A3. Only a small number of granules were yellow, indicating the presence of both proteins. Scale bar = 5 μm.

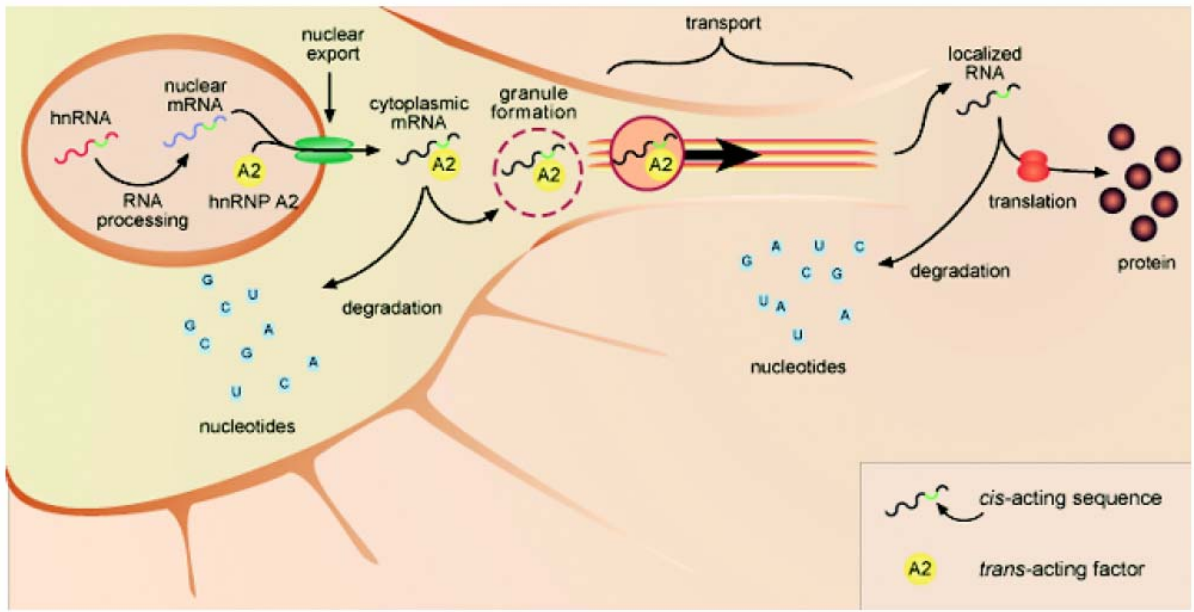


Fig. 2. Model for trafficking of A2 response element (A2RE)-containing RNAs. The A2RE, which is represented in a different color from the remainder of the mRNA, binds heterogeneous nuclear ribonucleoprotein (hnRNP) A2 or hnRNP A3 (yellow circle), and the complex is recruited to transport granules that move along the microtubules (orange unlabeled horizontal lines). The granules contain multiple copies of the RNA-protein complex. At its destination, the mRNA is anchored and translated. Each of the steps in this process, including RNA degradation, is a potential posttranscriptional control point for gene expression.