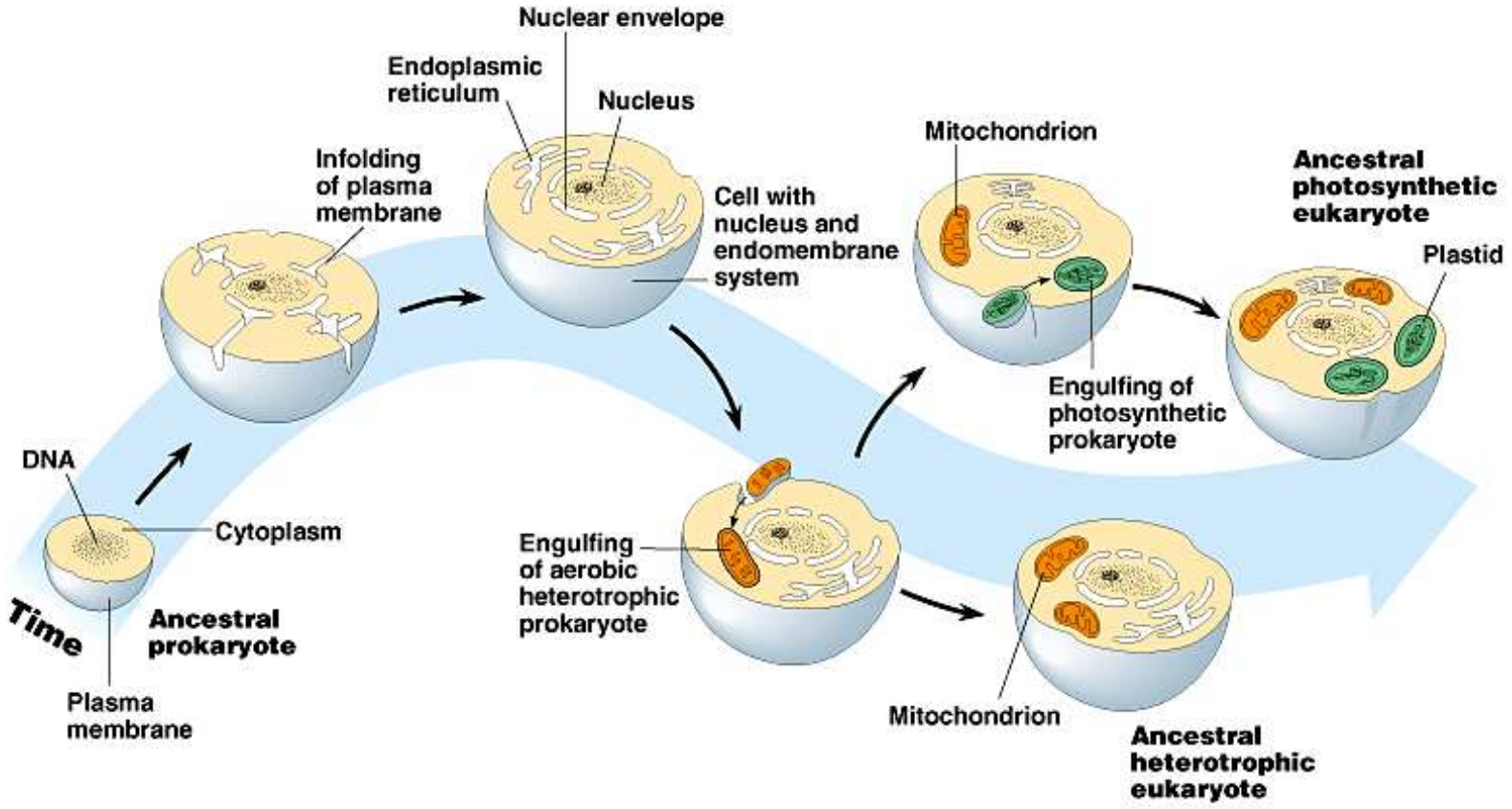
Internal Organization of the Cell

(cellular organelles, protein sorting, vesicular transport, endo- and exocytosis, apoptosis)

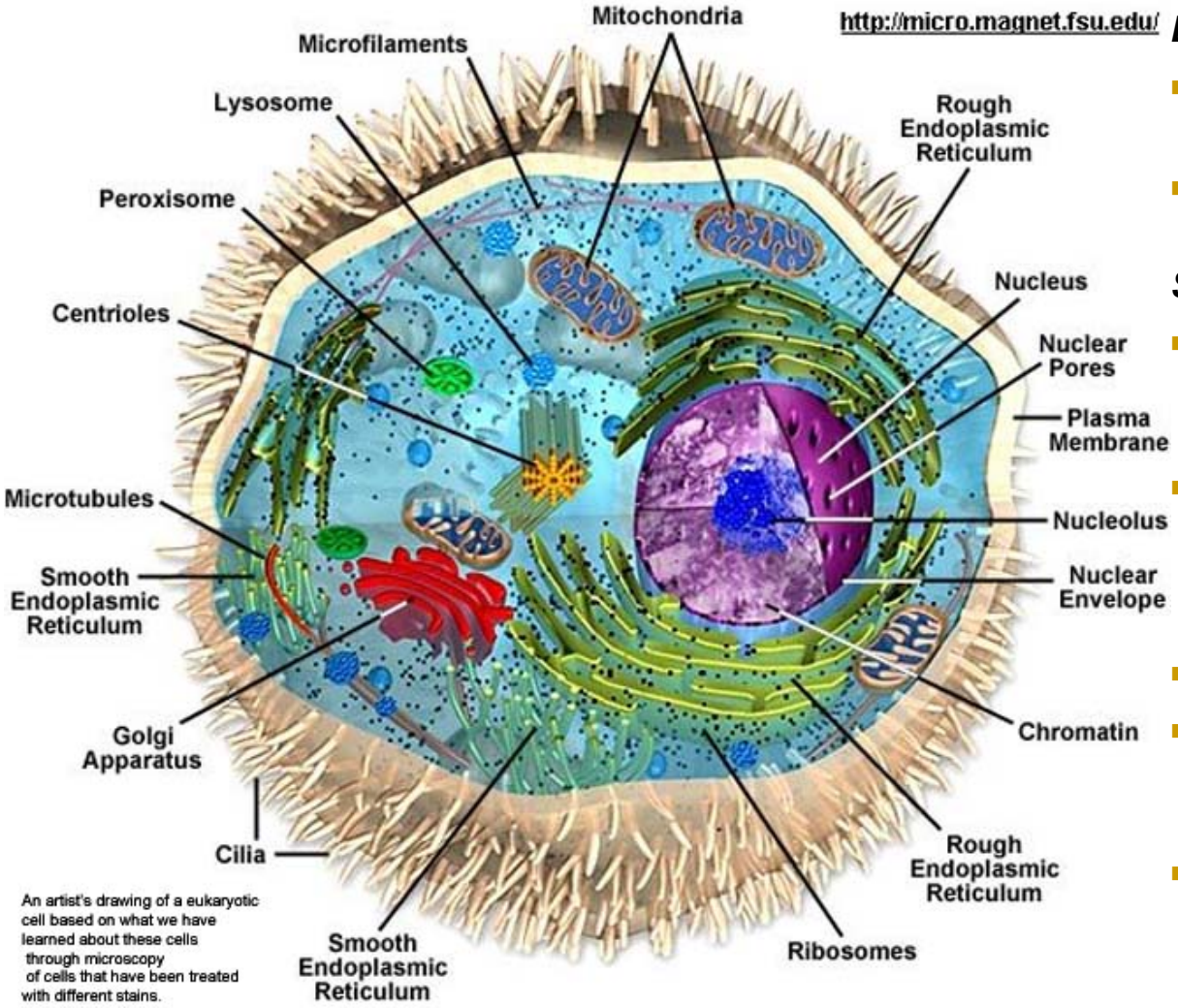
Dushyant Mukkamala

A brief history of membrane-bound organelles



- Invagination of the plasma membrane to form the endomembrane system (nucleus, endoplasmic reticulum, Golgi apparatus)
- Theory of endosymbiosis (mitochondria, chloroplast): small prokaryotes were engulfed by larger predatory cells

Membrane-bound organelles



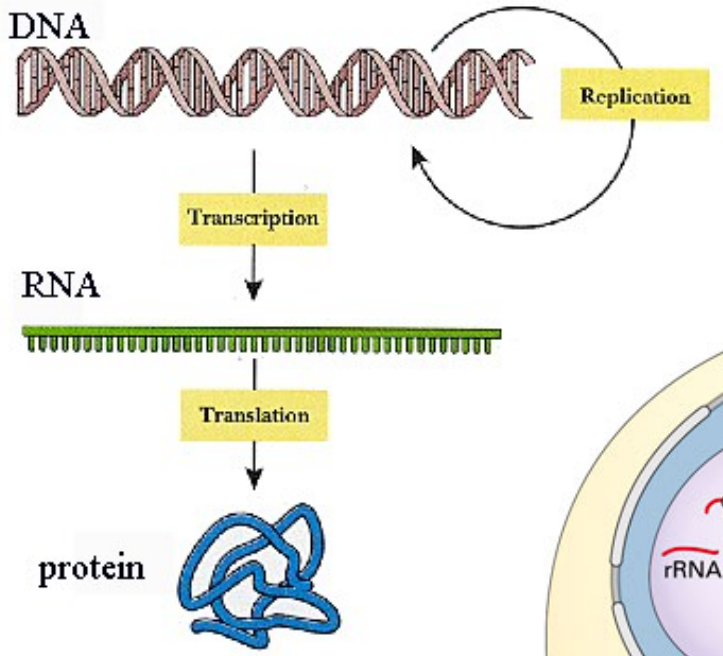
Double membrane bounded

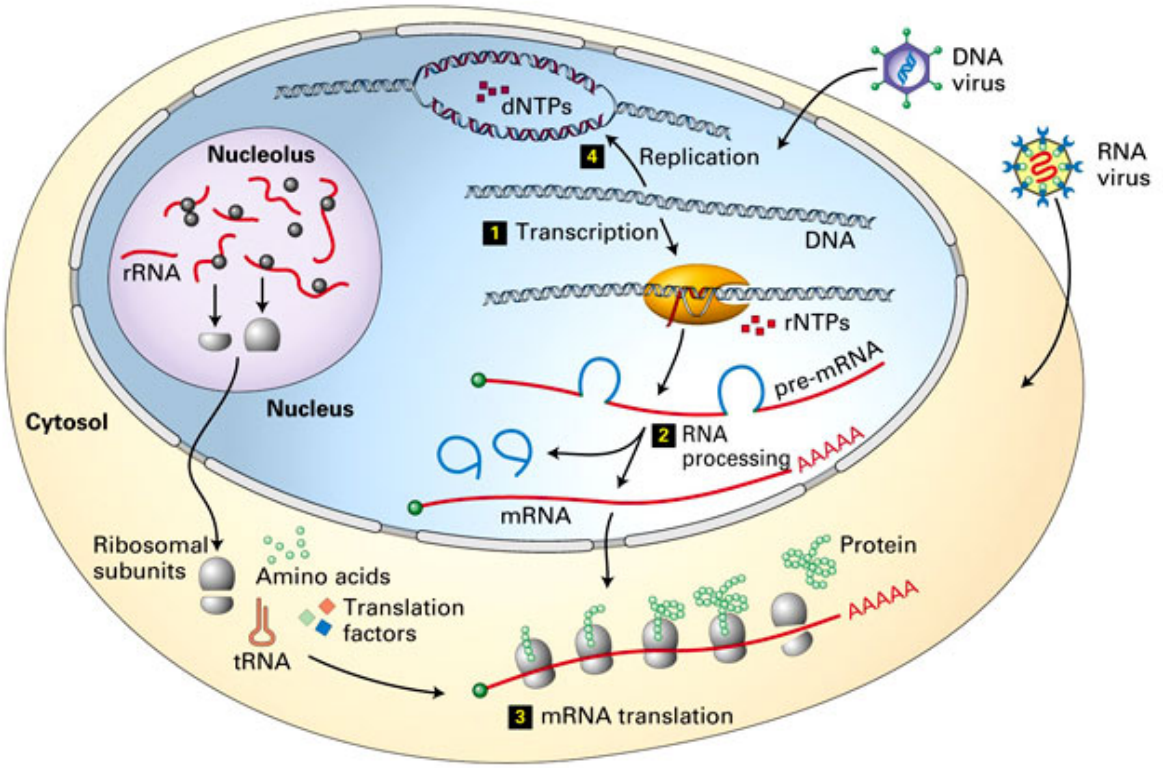
- **Nucleus**: DNA, RNA synthesis and main genome
- **Mitochondria**: main site for oxidative phosphorylation

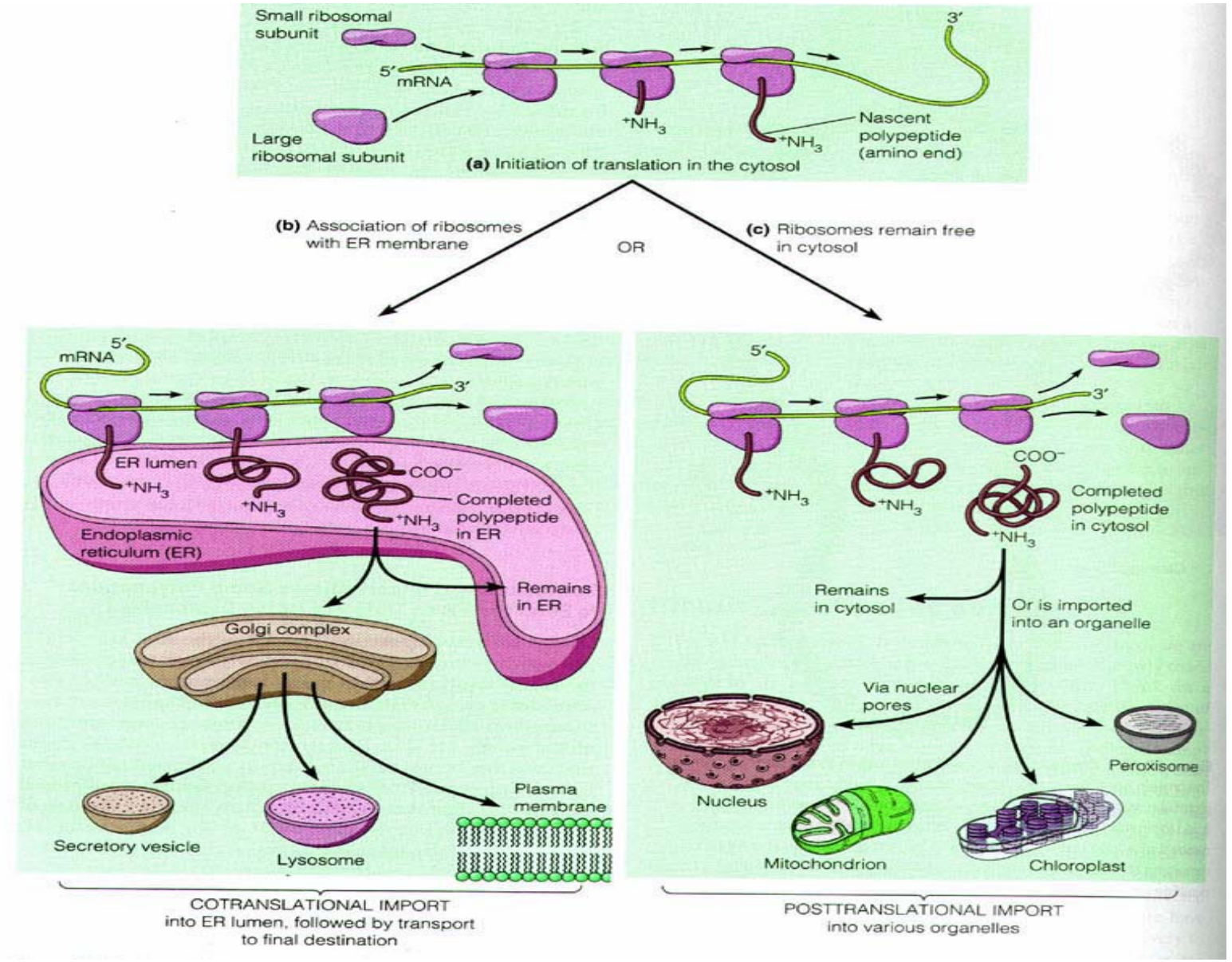
Single membrane bounded

- **Endoplasmic Reticulum** (rough, smooth): lipid and protein synthesis
- **Golgi Apparatus**: modification, sorting and packing of proteins for delivery
- **Lysosomes**: degradation
- **Peroxisomes**: oxidation of molecules which are toxic for the cell
- **Endosomes**: sorting and delivery of proteins and other materials

Protein synthesis

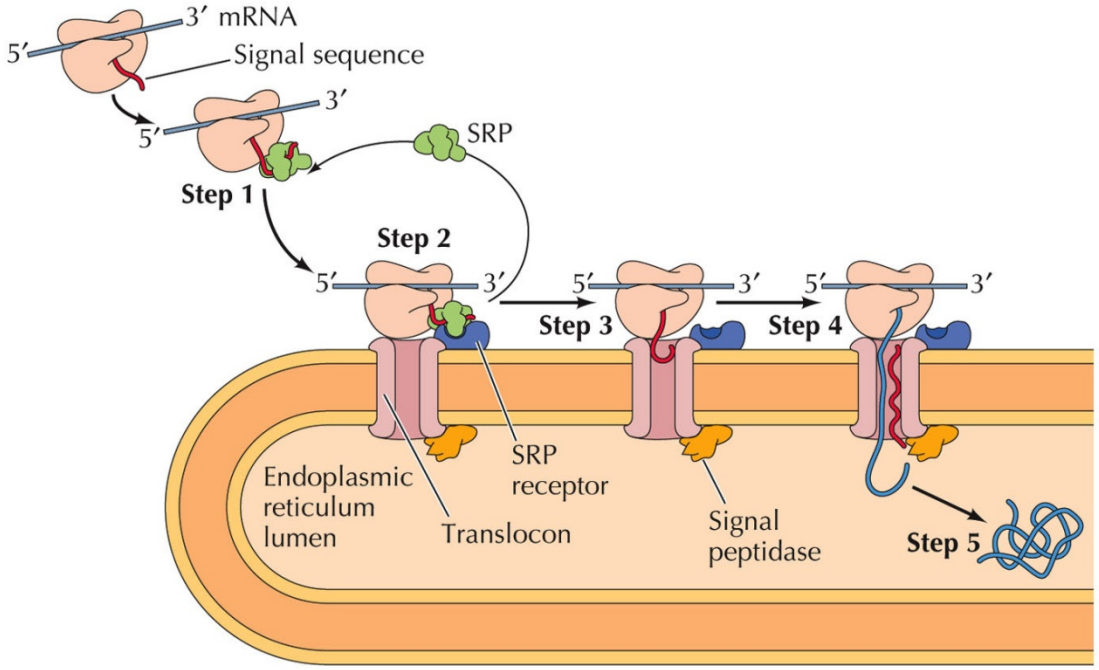





Small ribosomal subunit
5′
mRNA
3′
Large ribosomal subunit
+NH3
+NH3
Nascent polypeptide (amino end)
(a) Initiation of translation in the cytosol
(b) Association of ribosomes with ER membrane
OR
(c) Ribosomes remain free in cytosol
5′
mRNA
3′
ER lumen
+NH3
+NH3
COO−
+NH3
Completed polypeptide in ER
Endoplasmic reticulum (ER)
Remains in ER
Golgi complex
Secretory vesicle
Lysosome
Plasma membrane
COTRANSLATIONAL IMPORT
into ER lumen, followed by transport
to final destination
5′
3′
+NH3
+NH3
COO−
+NH3
Completed polypeptide in cytosol
Remains in cytosol
Or is imported into an organelle
Via nuclear pores
Nucleus
Peroxisome
Mitochondrion
Chloroplast
POSTTRANSLATIONAL IMPORT
into various organelles

Cotranslational and Posttranslational Translocation

Cotranslational translocation



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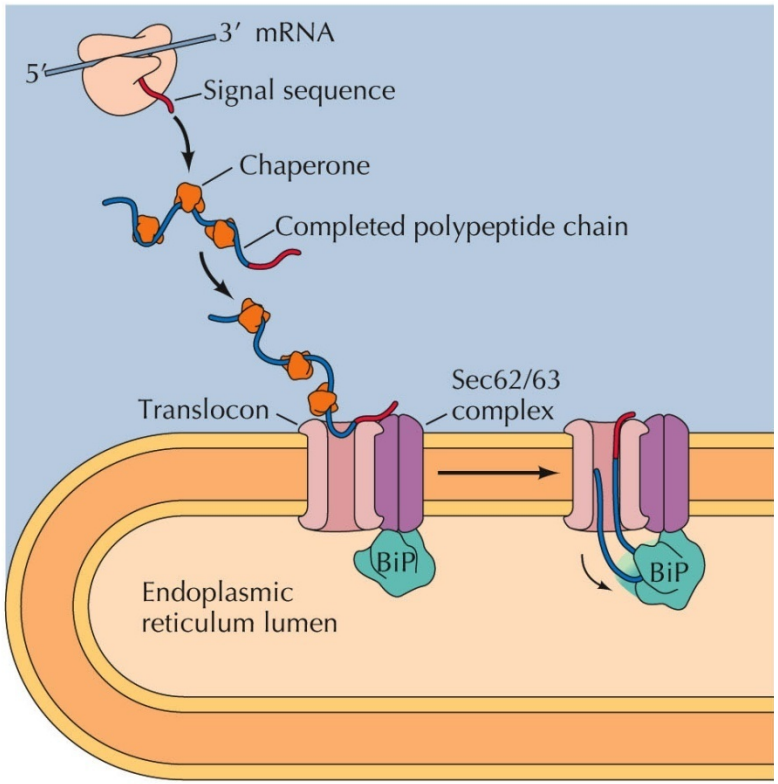
Posttranslational translocation



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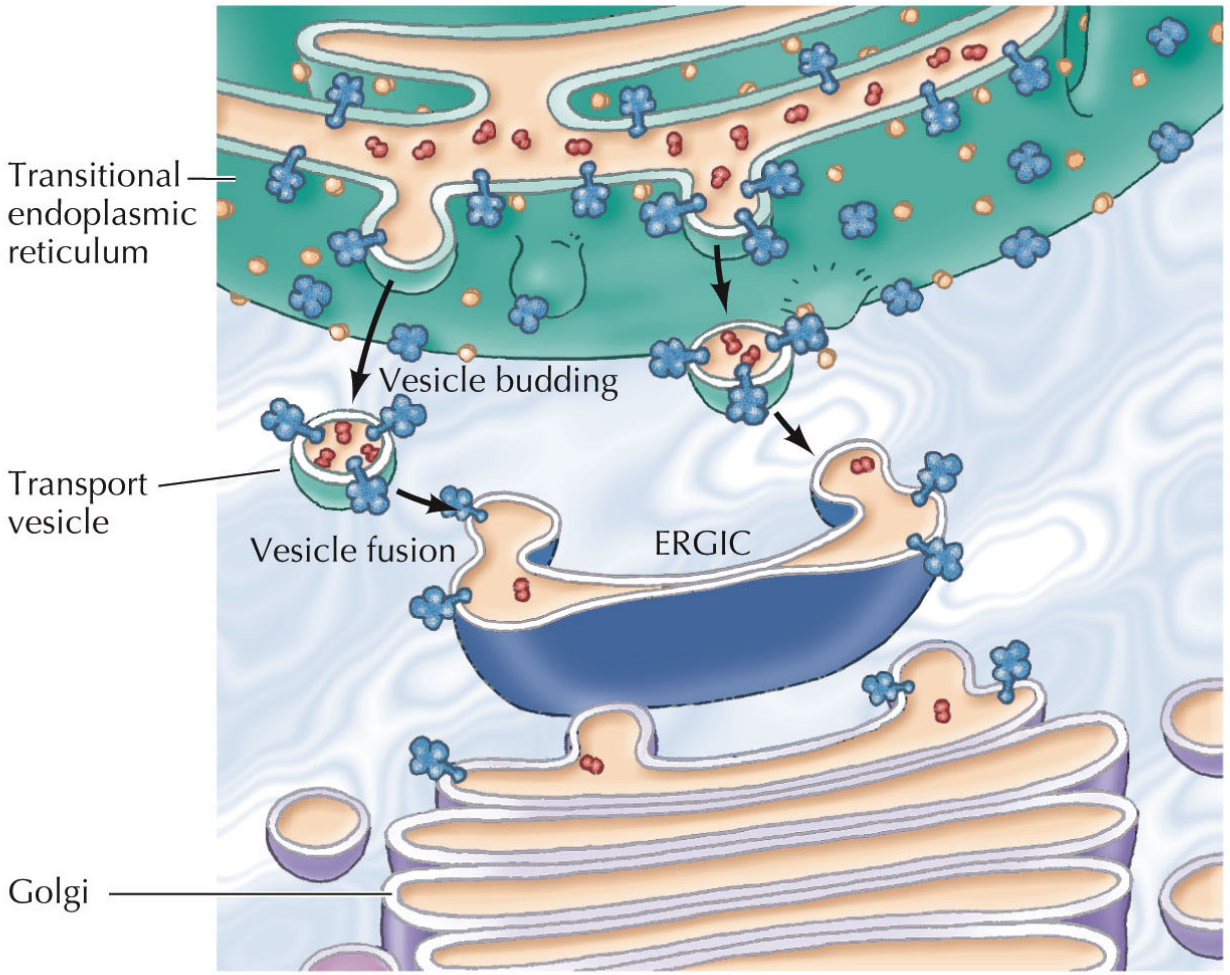
Export from the ER to Golgi apparatus



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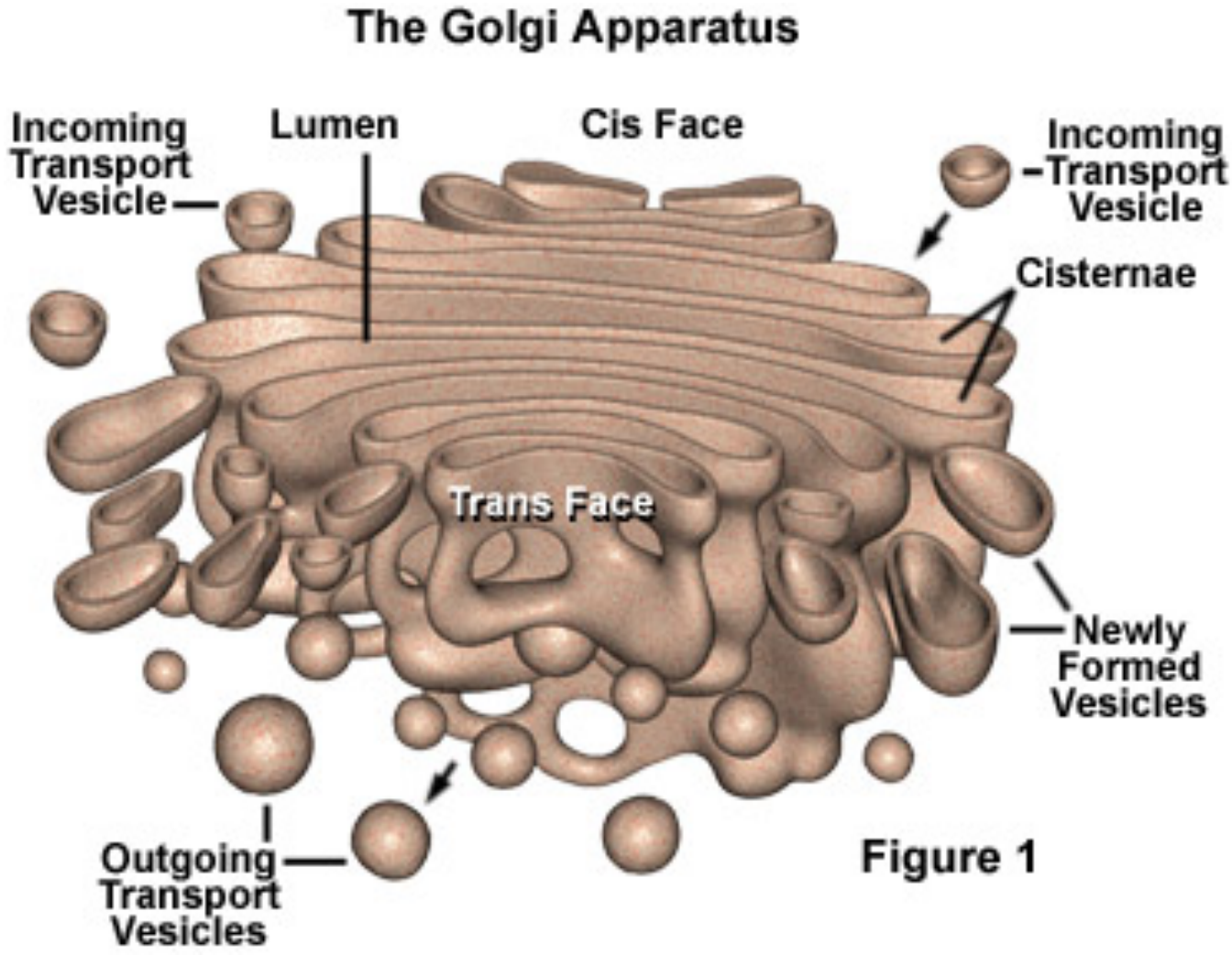
ERGIC: ER-Golgi Intermediate Compartment

Golgi apparatus - discovered by Camillo Golgi (1889)

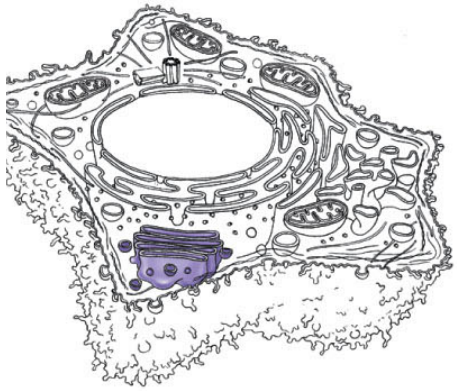
„*Cis*" – Facing to the Endoplasmic Reticulum

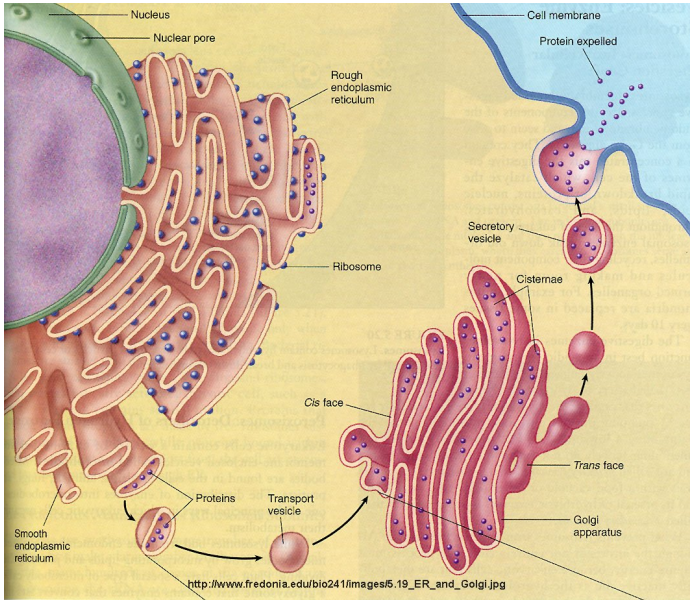


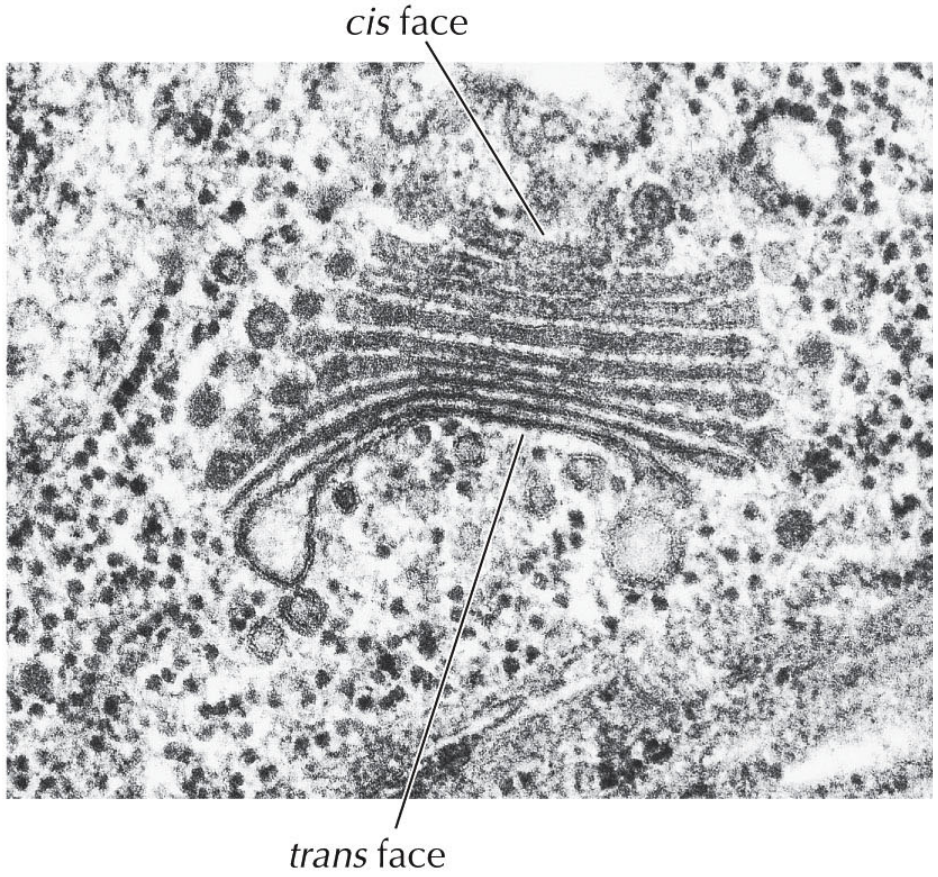
Polarization in the subcompartments of the Golgi apparatus:

1. *cis* Golgi
   - network (fused incoming vesicles)
   - cisternae
2. Medial Golgi
   - cisternae
3. *trans* Golgi
   - cisternae
   - network (tubular elements with outgoing vesicles

Golgi apparatus under TEM







***THE CELL, Fourth Edition,* Figure 10.26**

Functions of the Golgi body

„Post office function" –enzymes in different cis-trans levels: **Completing posttranslational modifications** of proteins synthesized in the rER → **packaging** → **addressing**

1. Processes such as **phosphorylation** and **glycosylation** are used to modify the various cargo proteins received from the endoplasmic reticulum.

2. Nucleotide sugars are imported from the cytosol and used in the phosphorylation and glycosylation processes.

3. Some cargo proteins are broken down into smaller **actively functioning fragments**.

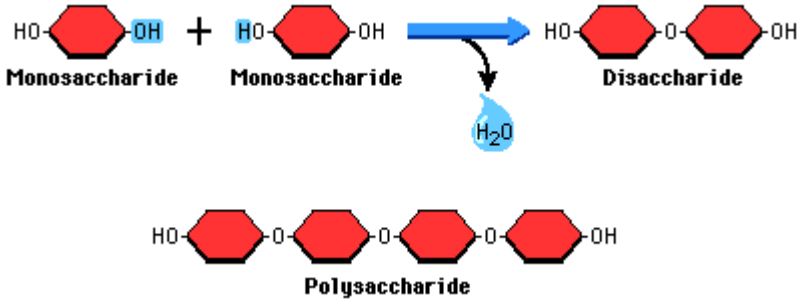
4. The **addition of phosphate groups** to some proteins The addition of sulfate groups to some proteins via the help of *sulfotransferases.* This gives the molecule a negative charge which is an important part of the signalling properties of the molecule.

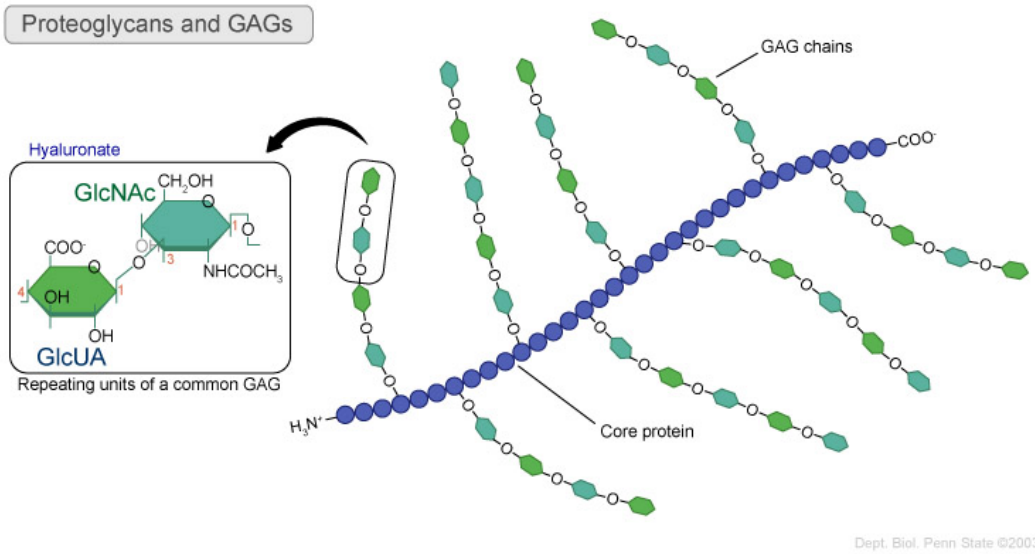
5. Production of **polysaccharides** and **glycosaminoglycans** *(long chain polysaccharides composed of a disaccharide repeat)* to be used in the manufacturing of carbohydrates. Some of the glycosaminoglycans are used elsewhere to form parts of connective tissues.

Functions of the Golgi body

6. Synthesis of **proteoglycans** by attaching the long non-branched polysaccharides and glycosaminoglycans to some proteins.

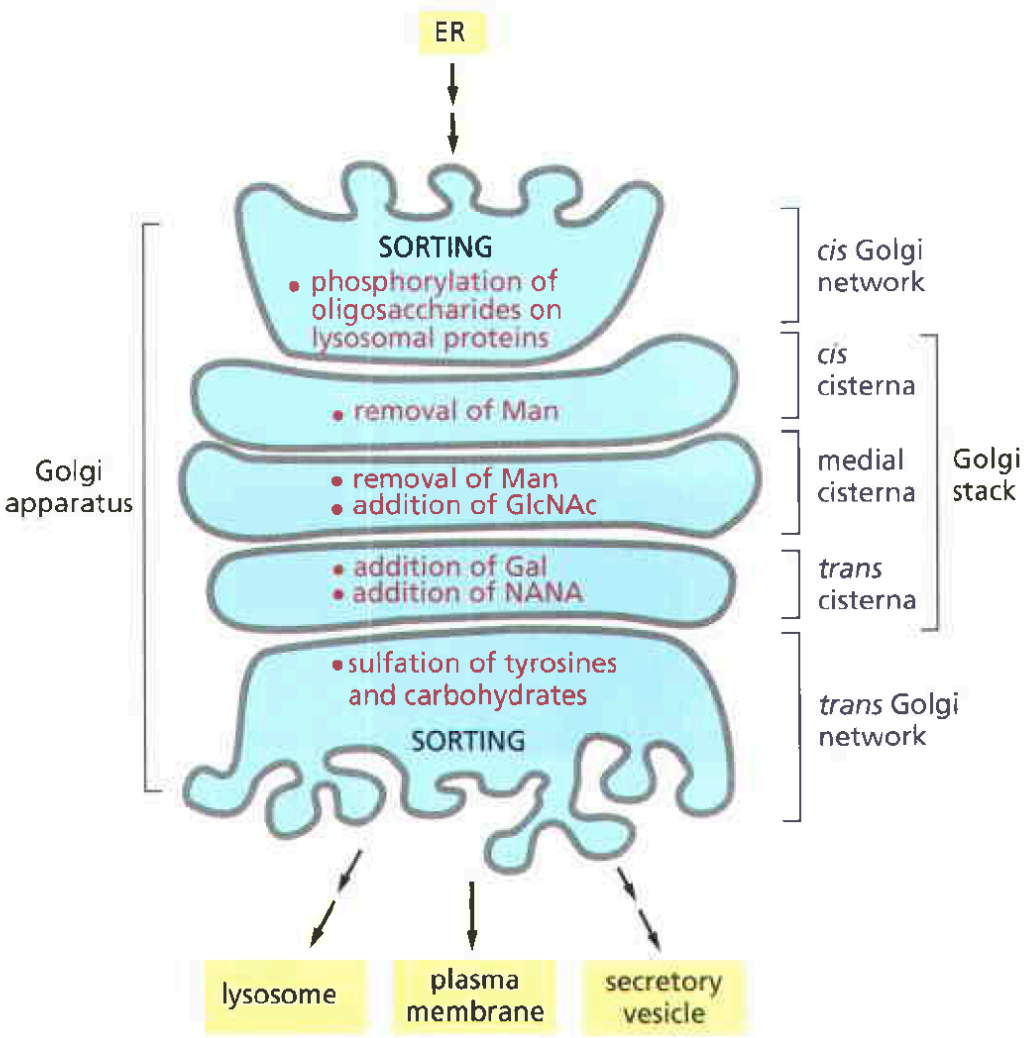
7. Formation of core proteins by the polymerization of the **glycosaminoglycans**



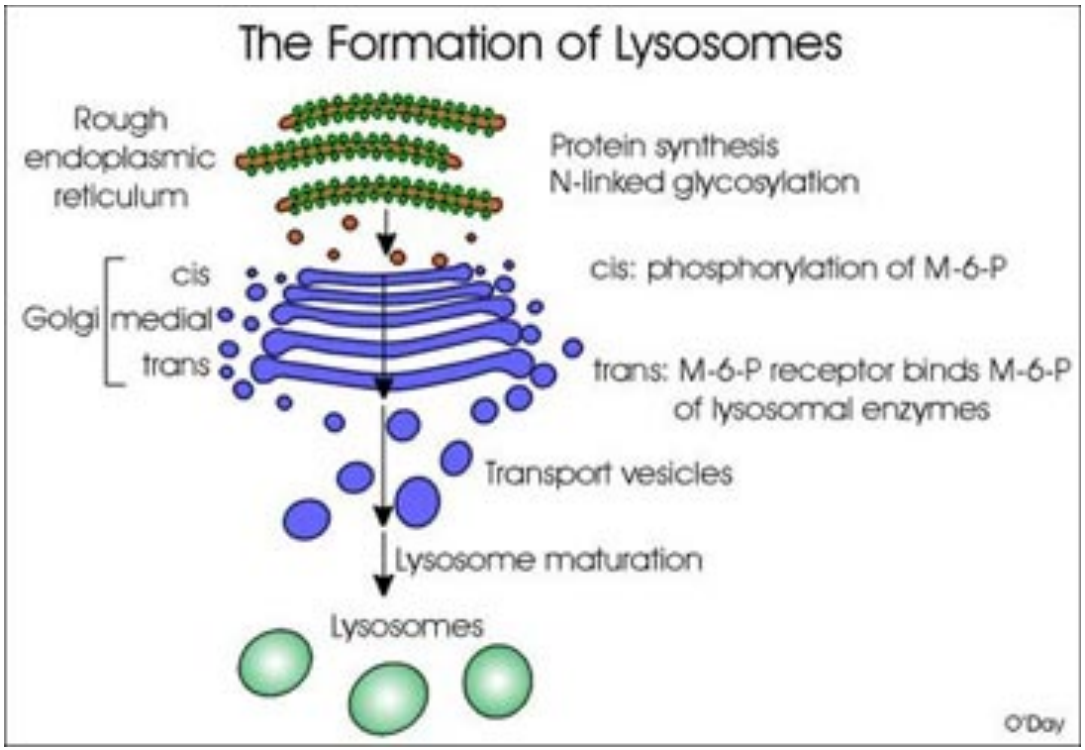


8. The Bcl-2 gene present in the Golgi apparatus plays an important roll in the prevention of apoptosis.

Oligosaccharide processing in Golgi apparatus



Functions of the Golgi body: formation of lysosomes



The lysosome membrane has three special properties:

1. an ATP driven proton [H^+] pump to maintain a low pH (4.5-5.5) in the lysosomal compartment, which is required for the activity of the hydrolytic enzymes

2. a glycoprotein coat, rich in carbohydrates, on its inner surface to protect it against hydrolysis by its own enzymes

3. transporter channels that transport break-down products such as amino acids, glucose, nucleotides and other small molecules out of the lysosome.

Lysosomes: Principal sites of intracellular digestion

- are membrane bound organelles
- digest ingested material and aged or damaged organelles (break down cellular waste products, fats, carbohydrates, proteins, and other macromolecules into simple compounds
- processes: Phagocytosis, Autophagocytosis, formation of endosomes, receptor-mediated endocytosis
- transport lipids to other parts of the cell
- contain (60 different) hydrolytic enzymes (**hydrolases** eg.RNAase, peptidases, sulphatases,)
- have acidic contents (pH 4.5-5.5)
- have electron-dense heterogeneous contents
- Melanosomes are pigment-containing lysosomes in melanocytes that release melanin by exocytosis

Electron microscopy of the lysosome

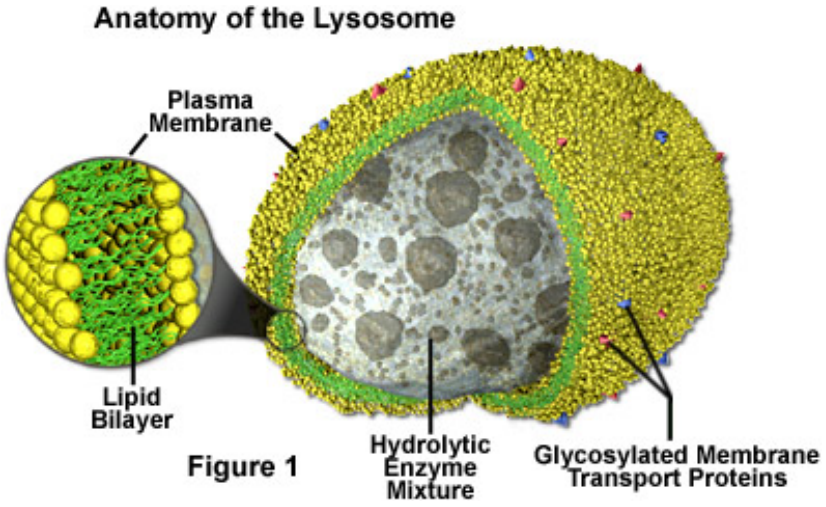
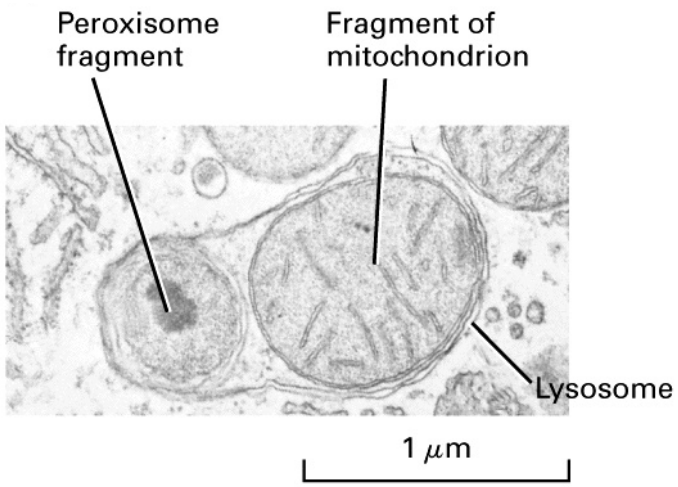
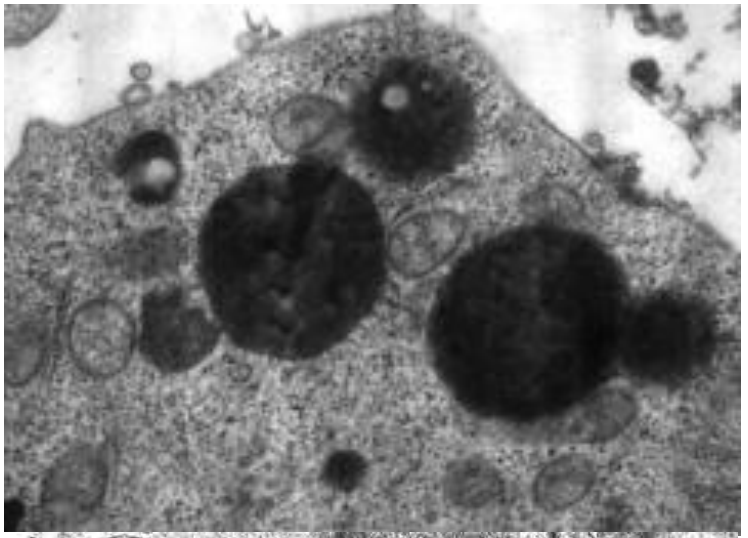
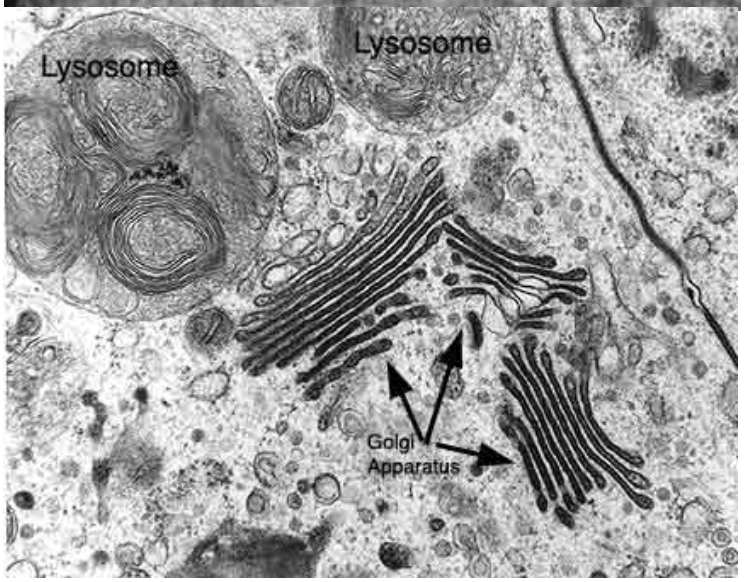


Figure 1

Digested organelles



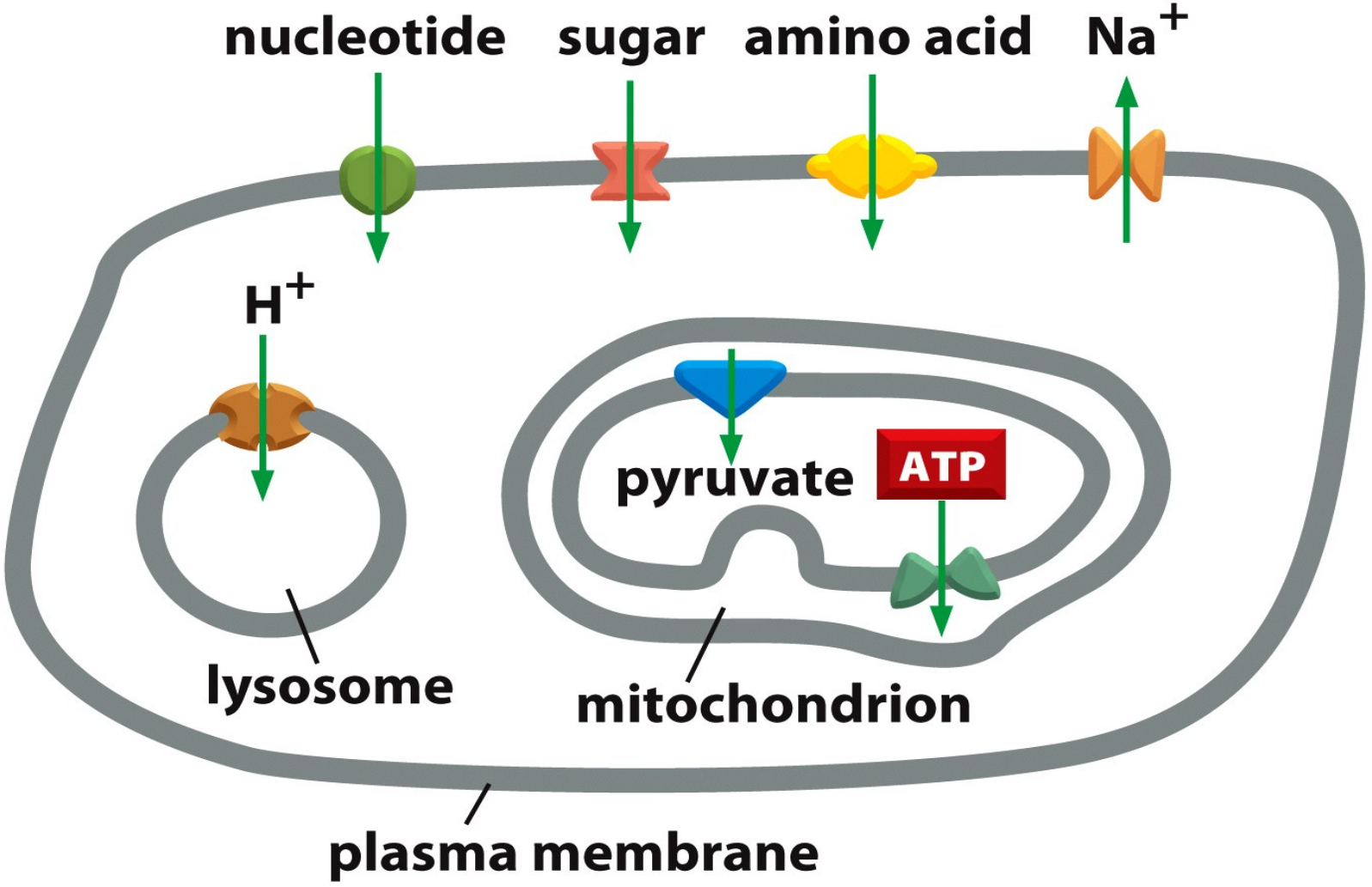




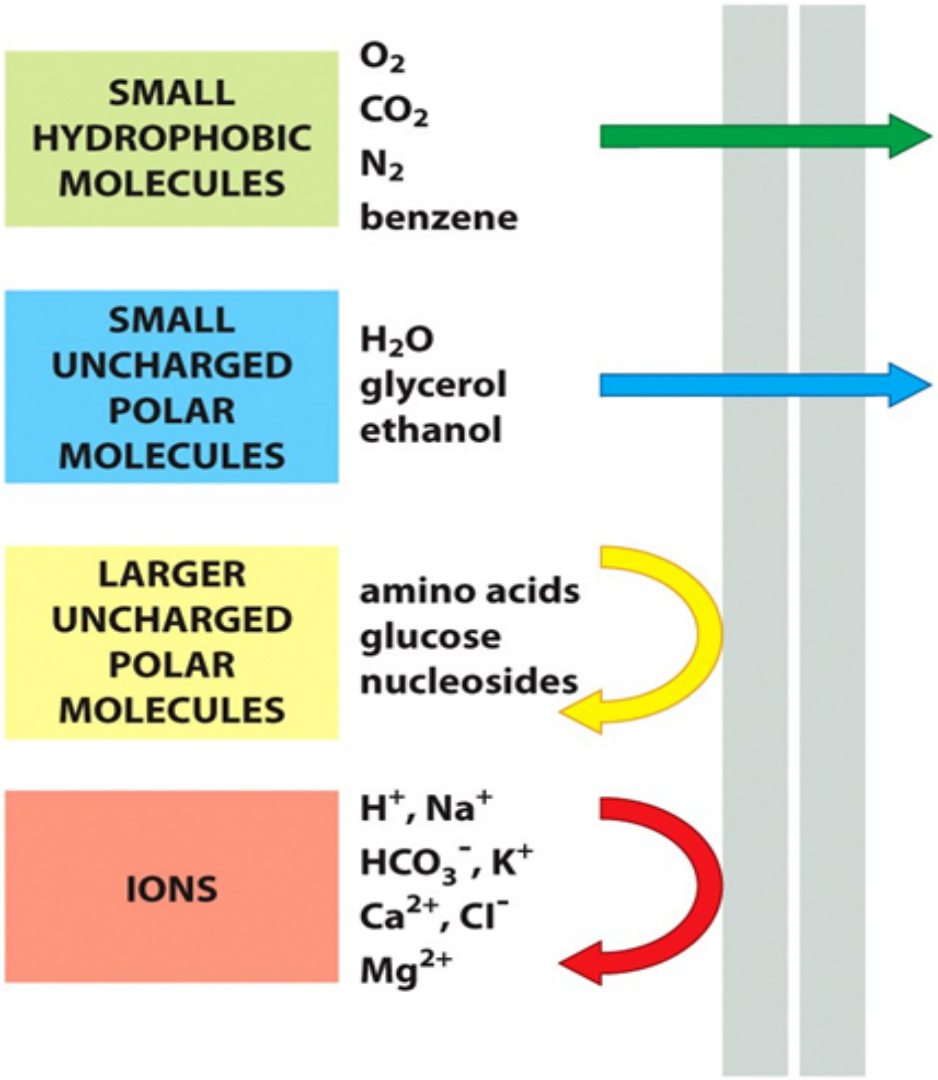
Lysosomal storage diseases

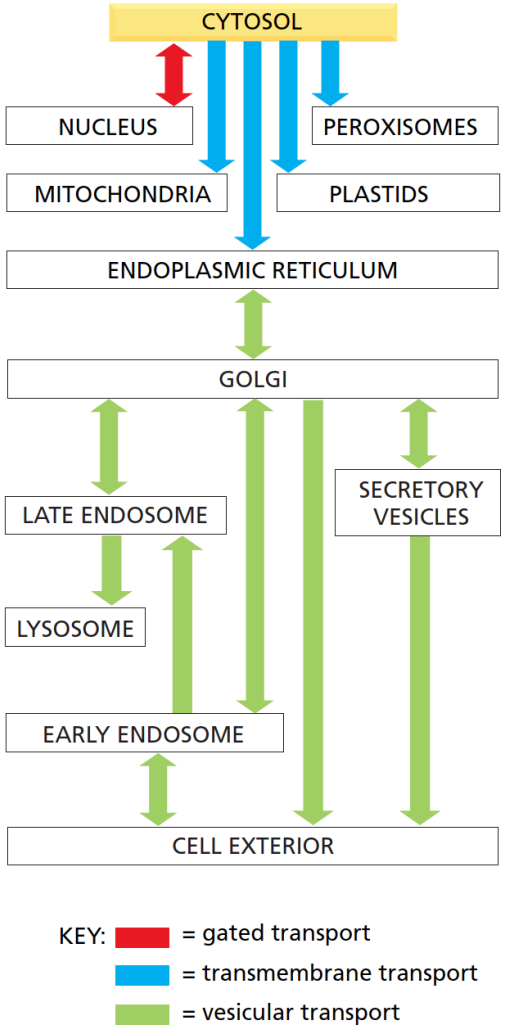
- Fabry disease: α-galactosidase A deficiency
- Gaucher disease: Glucocerebrosidase (β-glucosidase) deficiency
- Niemann-Pick disease: Sphingomyelinase deficiency
- Tay-Sachs disease: Hexosaminidase A deficiency
- Krabbe disease: Galactocerebrosidase deficiency
- Metachromatic leukodystrophy: Arylsulfatase A deficiency
- Hurler syndrome: α-L-iduronidase deficiency
- Hunter syndrome: Iduronate sulfatase deficiency

Transport mechanisms in Cells

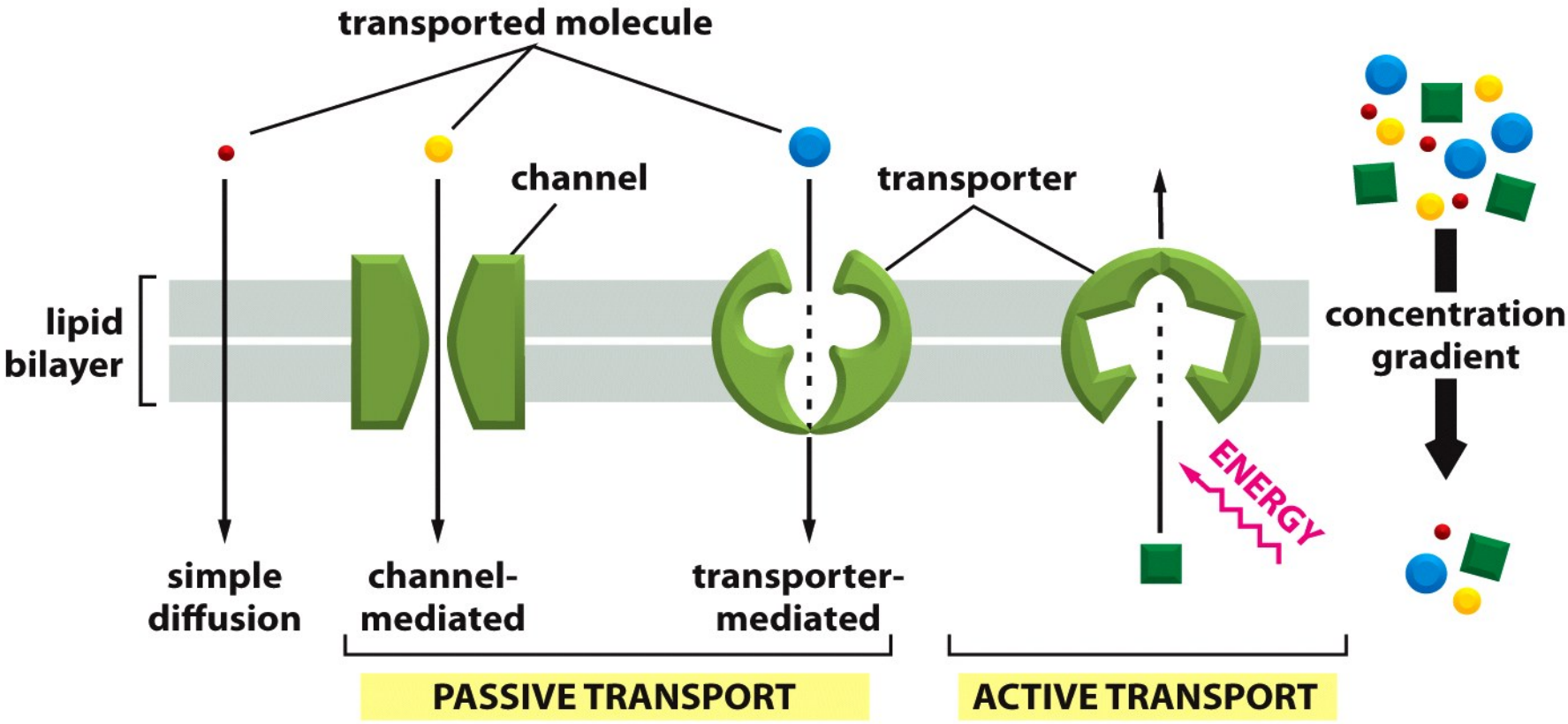


Transport across lipid bilayer





Types of transport across cell membrane



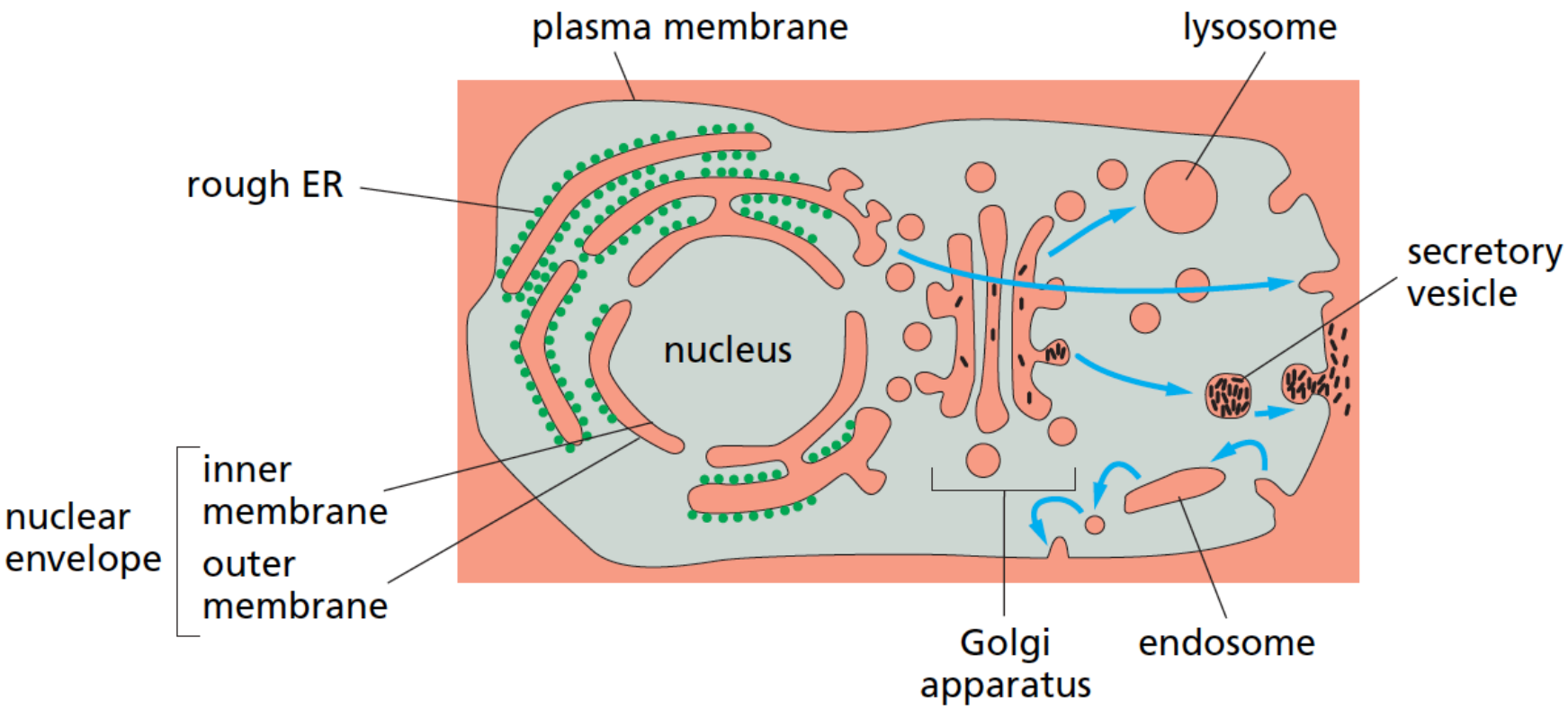
A channel is a hole formed by transmembrane proteins, along which molecules pass the membrane.

A transporter (carrier) catches the molecule-to-be-transported, transports it to the other side of the membrane, and releases it.

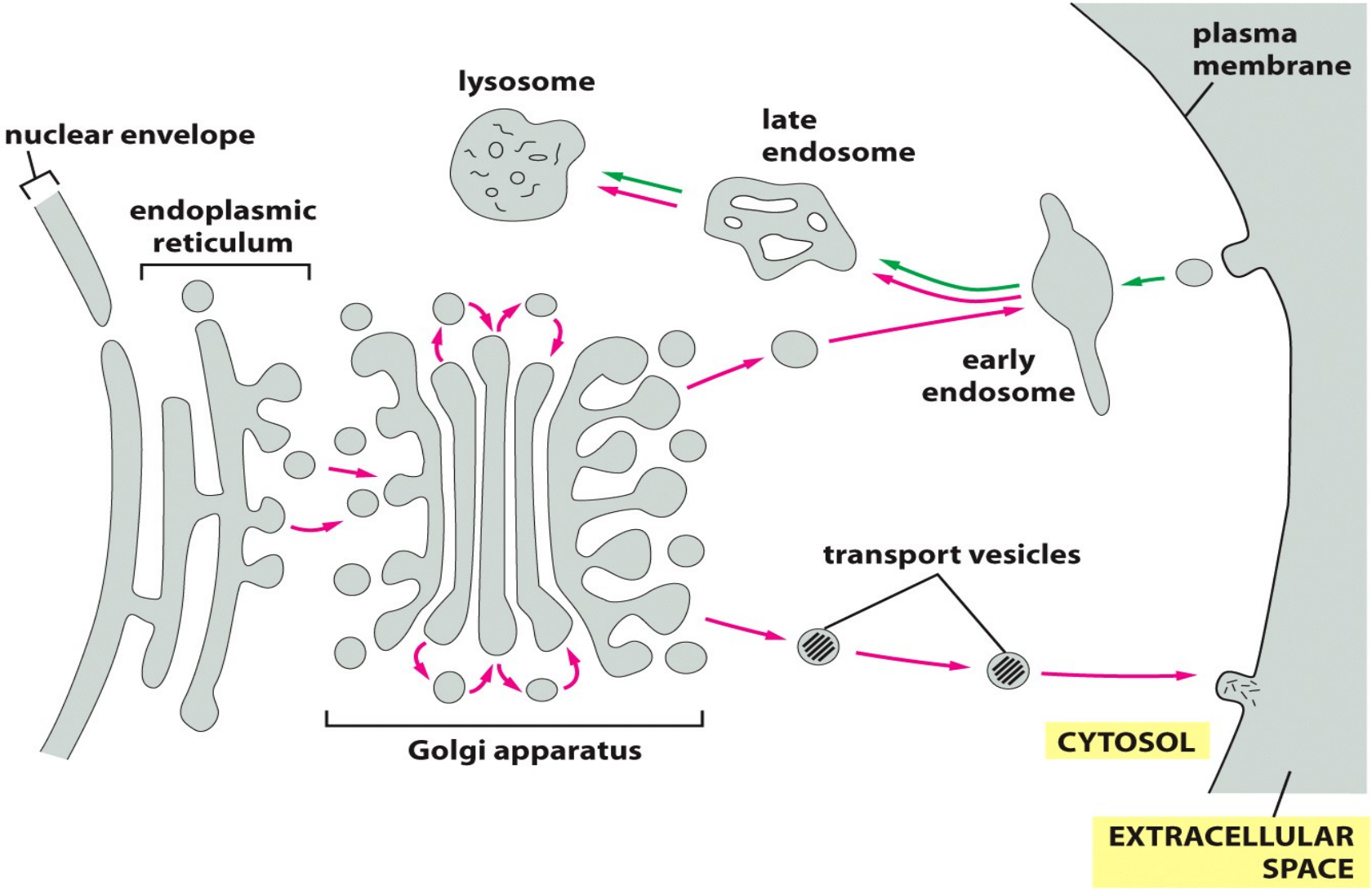
Transport mechanisms for proteins

- Gated transport
  - For example, between the cytosol and the nucleus via the *Nuclear pore complex*
- Transmembrane transport
  - Transport through protein translocators
  - Typically seen in cotranslational translocation, e.g. proteins destined for rER or Mitochondria
- Vesicular transport
  - Proteins can be moved only between compartments that are topologically equivalent

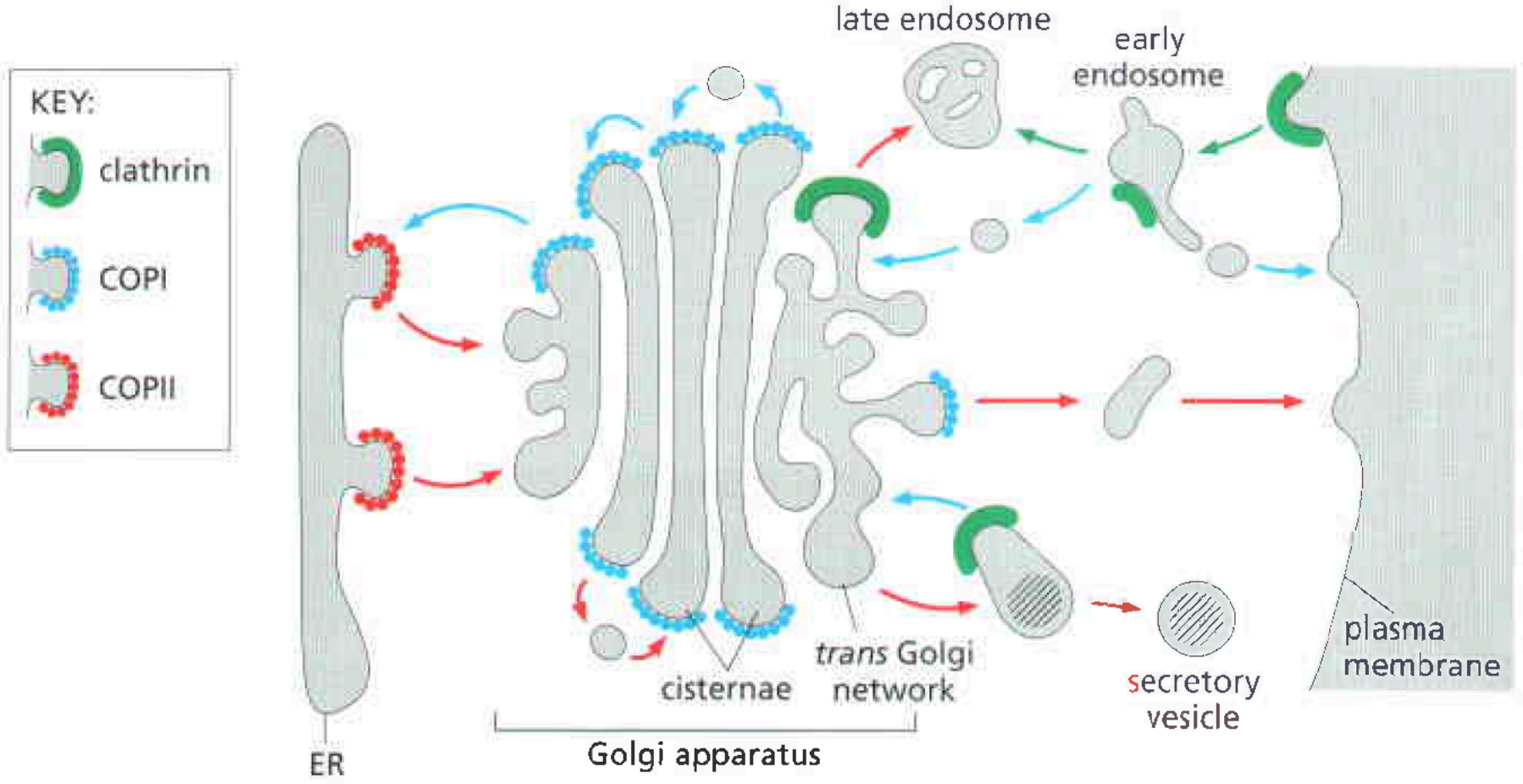
Topological relations of cellular organelles



Vesicular transport across cell membrane



Vesicular trafficking: Coating pathways



Coating determines destination of Cargo

TYPE OF COATED VESICLE	COAT PROTEINS	ORIGIN	DESTINATION
Clathrin-coated	clathrin + adaptin 1	Golgi apparatus	lysosome (via endosomes)
Clathrin-coated	clathrin + adaptin 2	plasma membrane	endosomes
COP-coated*	COP proteins	ER Golgi cisterna Golgi apparatus	Golgi apparatus Golgi cisterna ER

* Coatomer protein complex

Phosphoinositides mark Organelles and Membrane Domains

- Inositol phospholipids are < 10% of total phospholipids
- Can undergo rapid phosphorylation/dephosphorylation to produce various types of phosphoinositides (PIPs)
- Interconversion is highly regulated and unique distribution within each organelle
- Coat proteins assembly is influenced by the distribution of the phosphoinositides

Intracellular locations of Phosphinositides

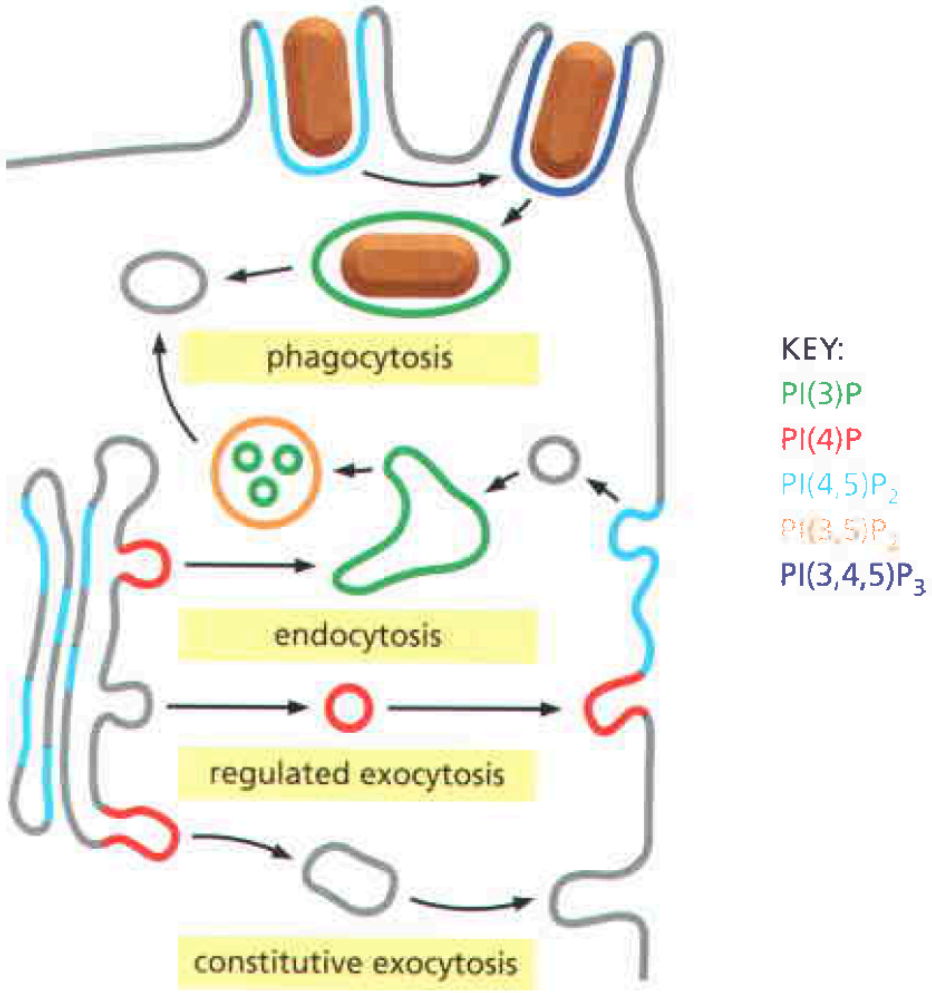


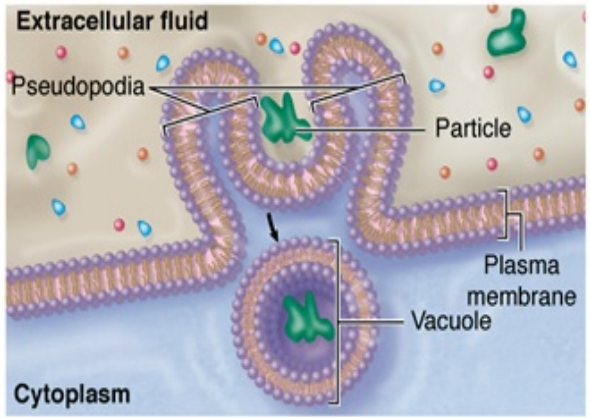
Figure 13-11 *Molecular Biology of the Cell* (© Garland Science 2008)

Rab proteins: Guides for vesicle targeting

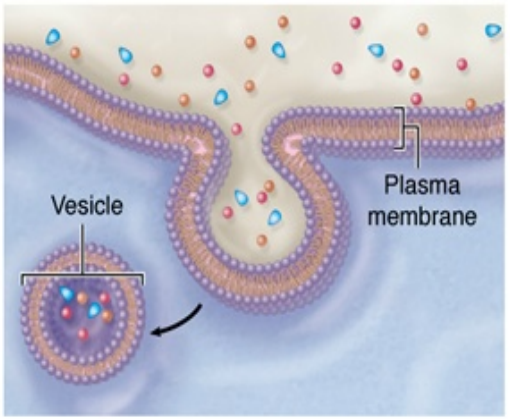
PROTEIN	ORGANELLE
Rab1	ER and Golgi complex
Rab2	*cis* Golgi network
Rab3A	synaptic vesicles, secretory granules
Rab4/Rab11	recycling endosomes
Rab5A	plasma membrane, clathrin-coated vesicles, early endosomes
Rab5C	early endosomes
Rab6	medial and *trans* Golgi cisternae
Rab7	late endosomes
Rab8	early endosomes
Rab9	late endosomes, *trans* Golgi network

Table 13-1 *Molecular Biology of the Cell* (© Garland Science 2008)

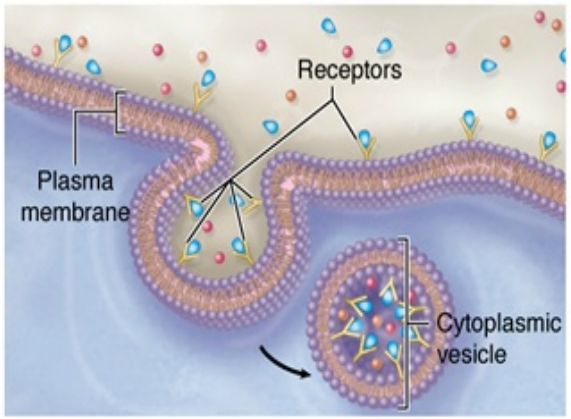
Endocytosis



a Phagocytosis



b Pinocytosis



c Receptor-mediated endocytosis

Functions:

Removal of cell debris

Microbial defense

Cell feeding

Decrease cell surface

Regulation of signaling

Uptake of important molecules (e.g. iron, special lipoproteins)

Stages of Endocytosis (electron microscope)

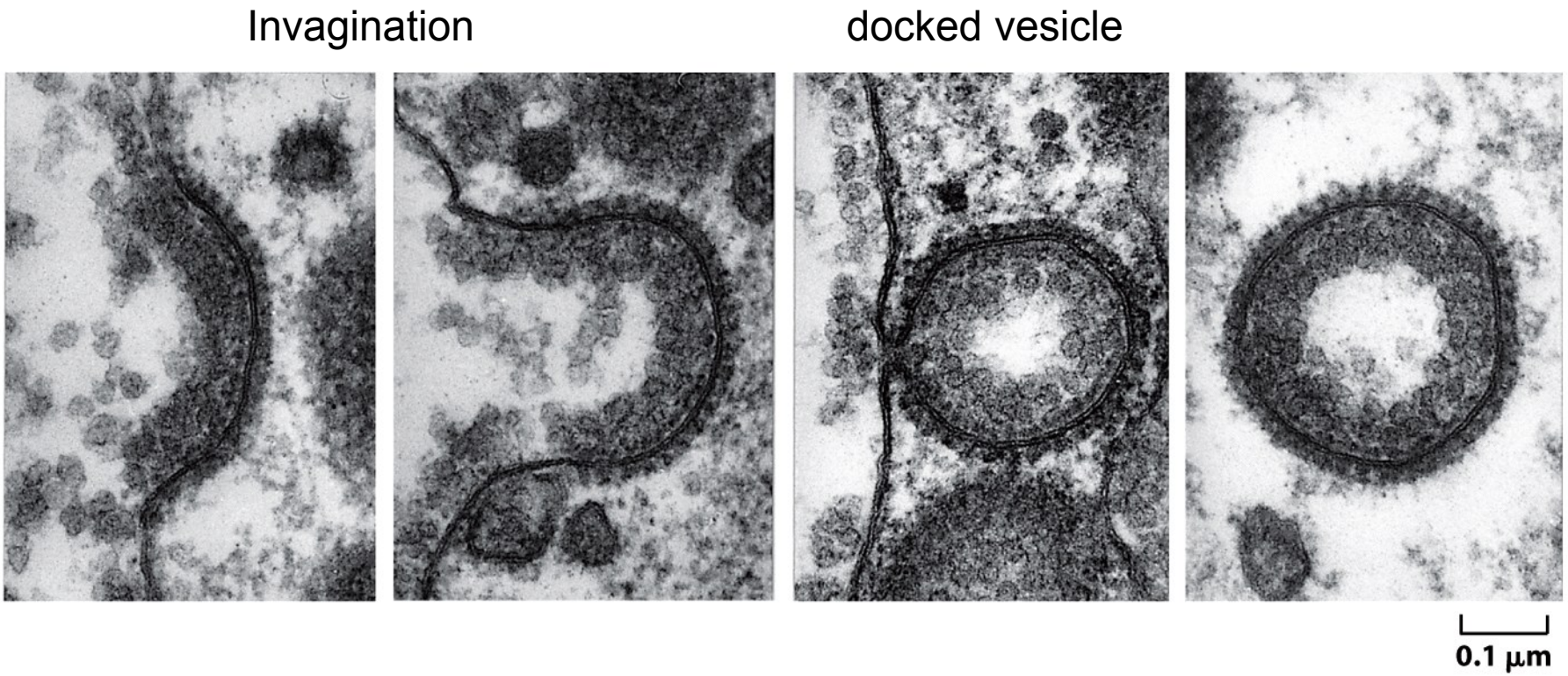
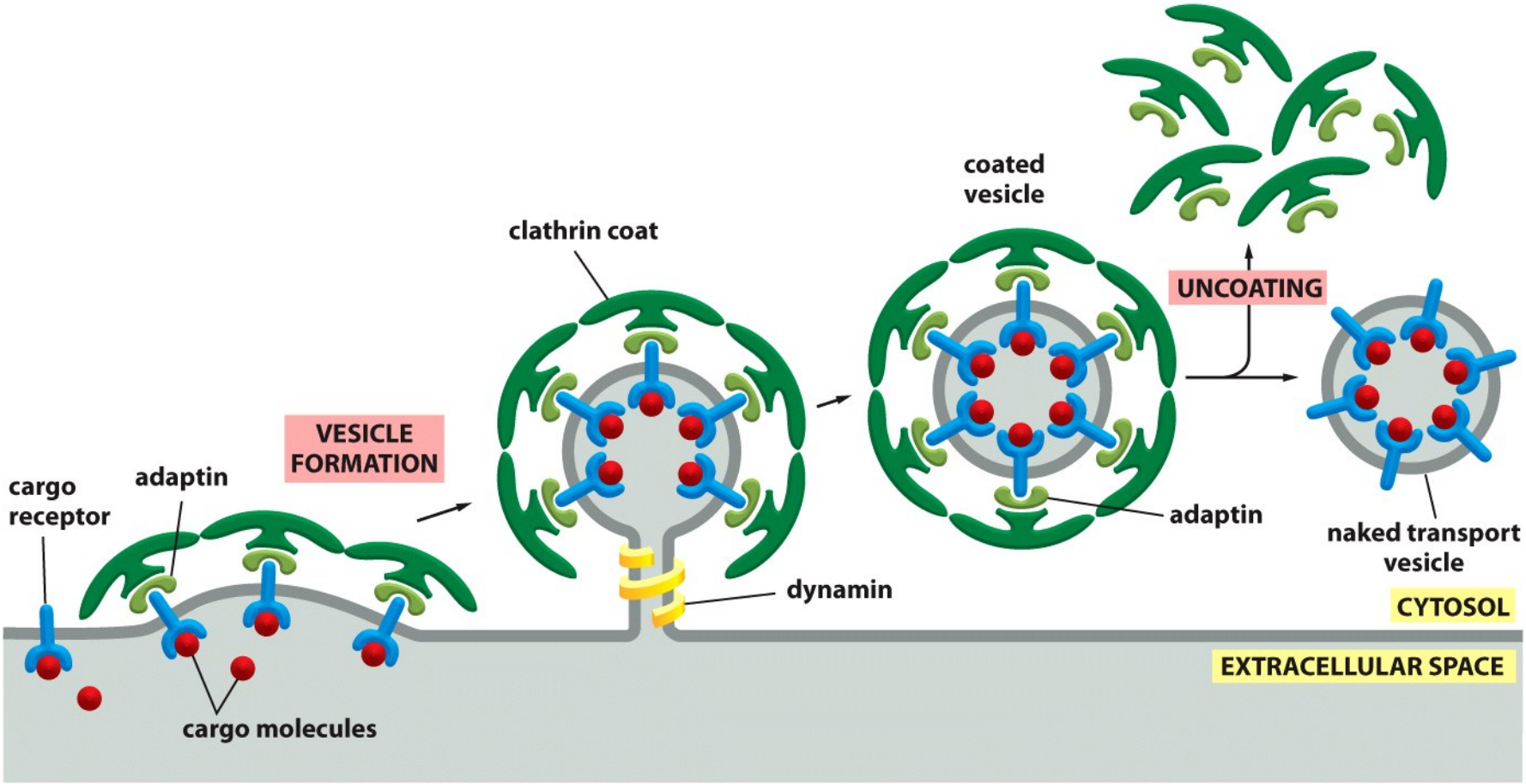


Figure 15-19a *Essential Cell Biology* (© Garland Science 2010)

Mechanism of endocytosis by clathrin-coated vesicles



Other types of mechanisms: caveolas, constitutive pinocytosis, macropinocytosis

Electron microscope image of clathrin-coated vesicles

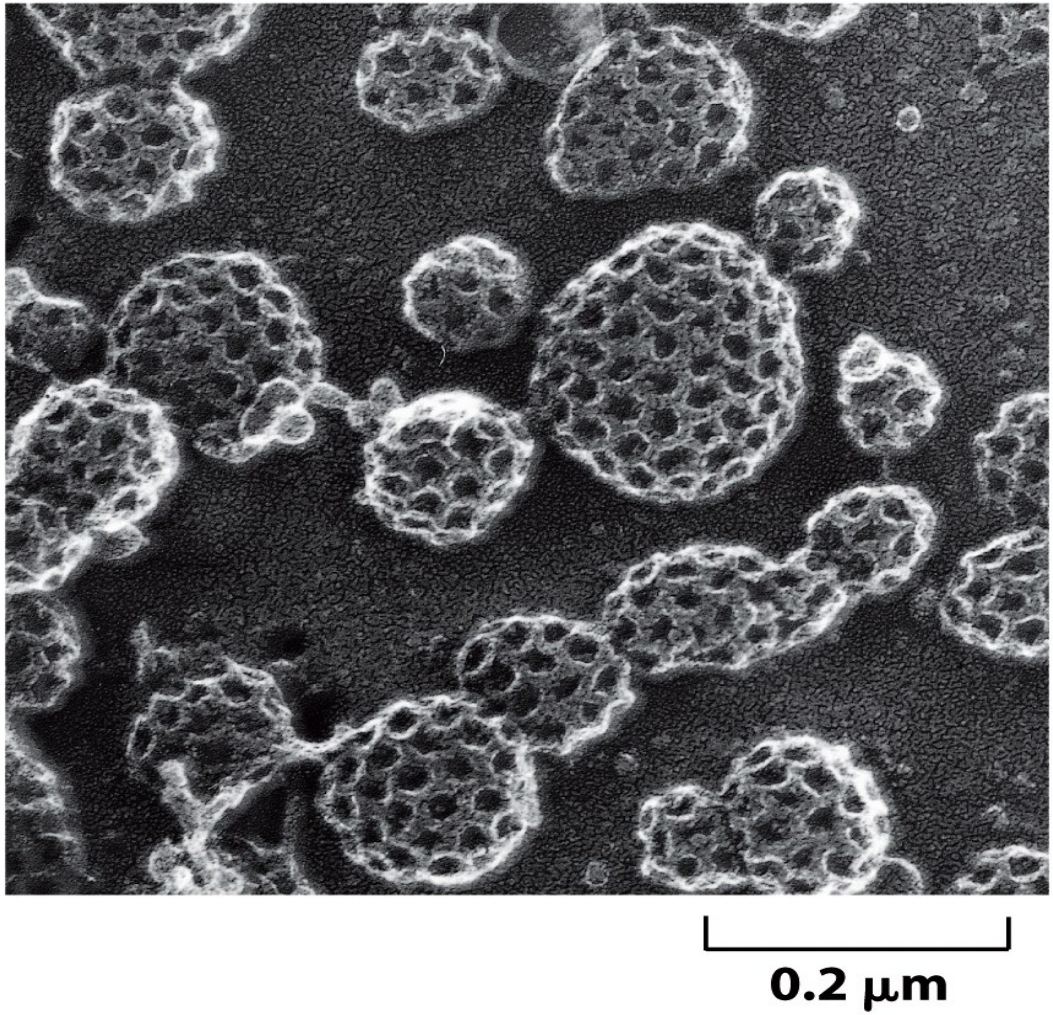


Figure 15-19b *Essential Cell Biology* (© Garland Science 2010)

Exocytosis: tethering, docking, and fusion

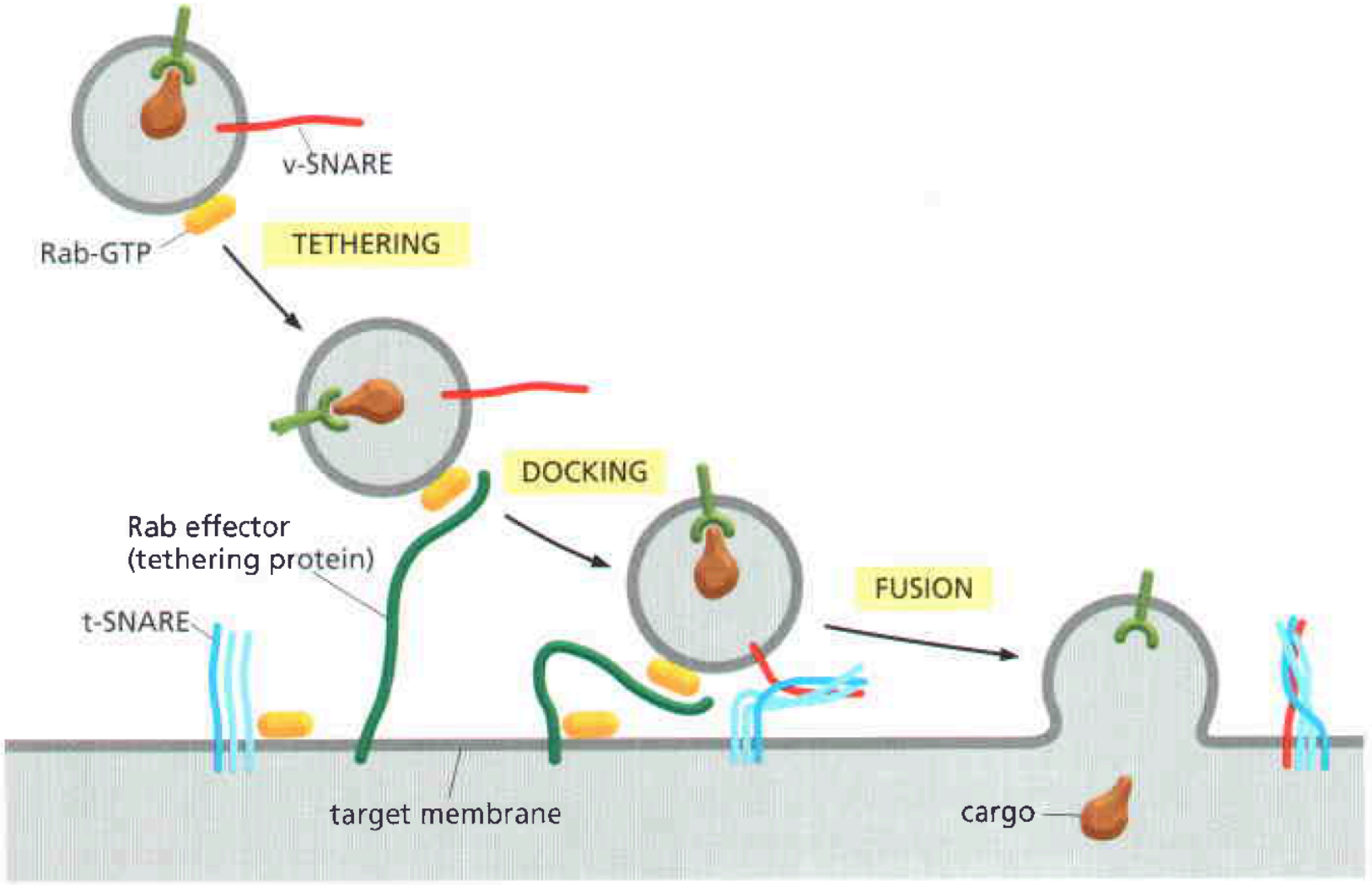


Figure 13-14 *Molecular Biology of the Cell* (© Garland Science 2008)

Mechanism of Vesicle Fusion

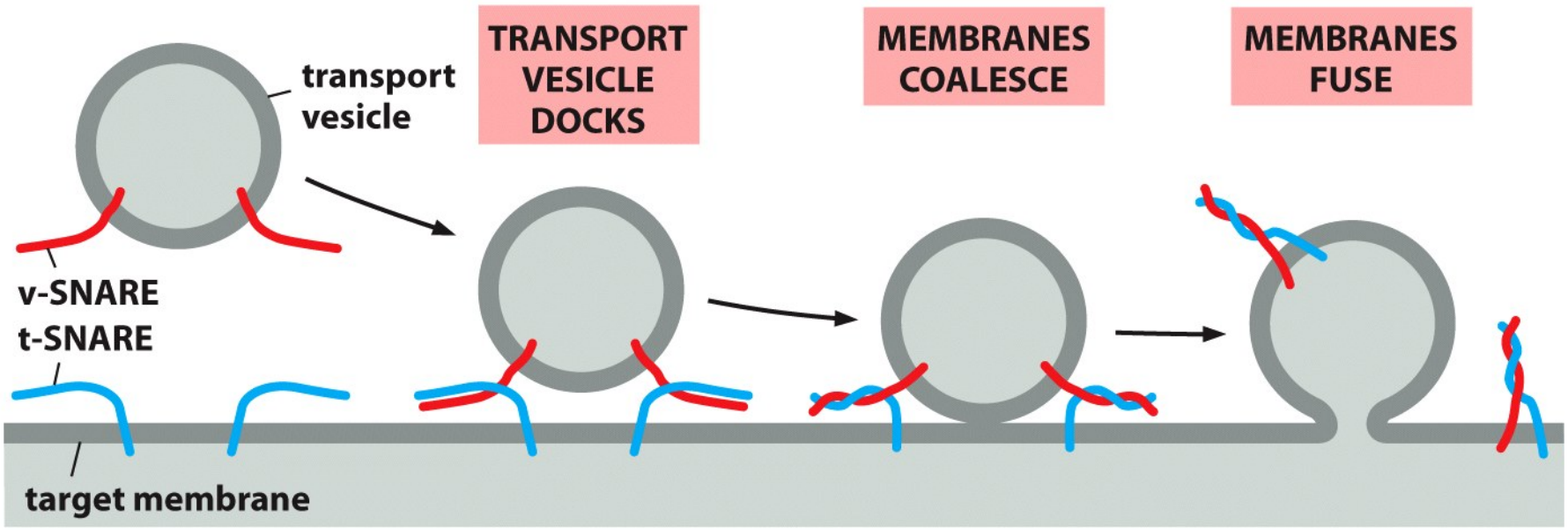
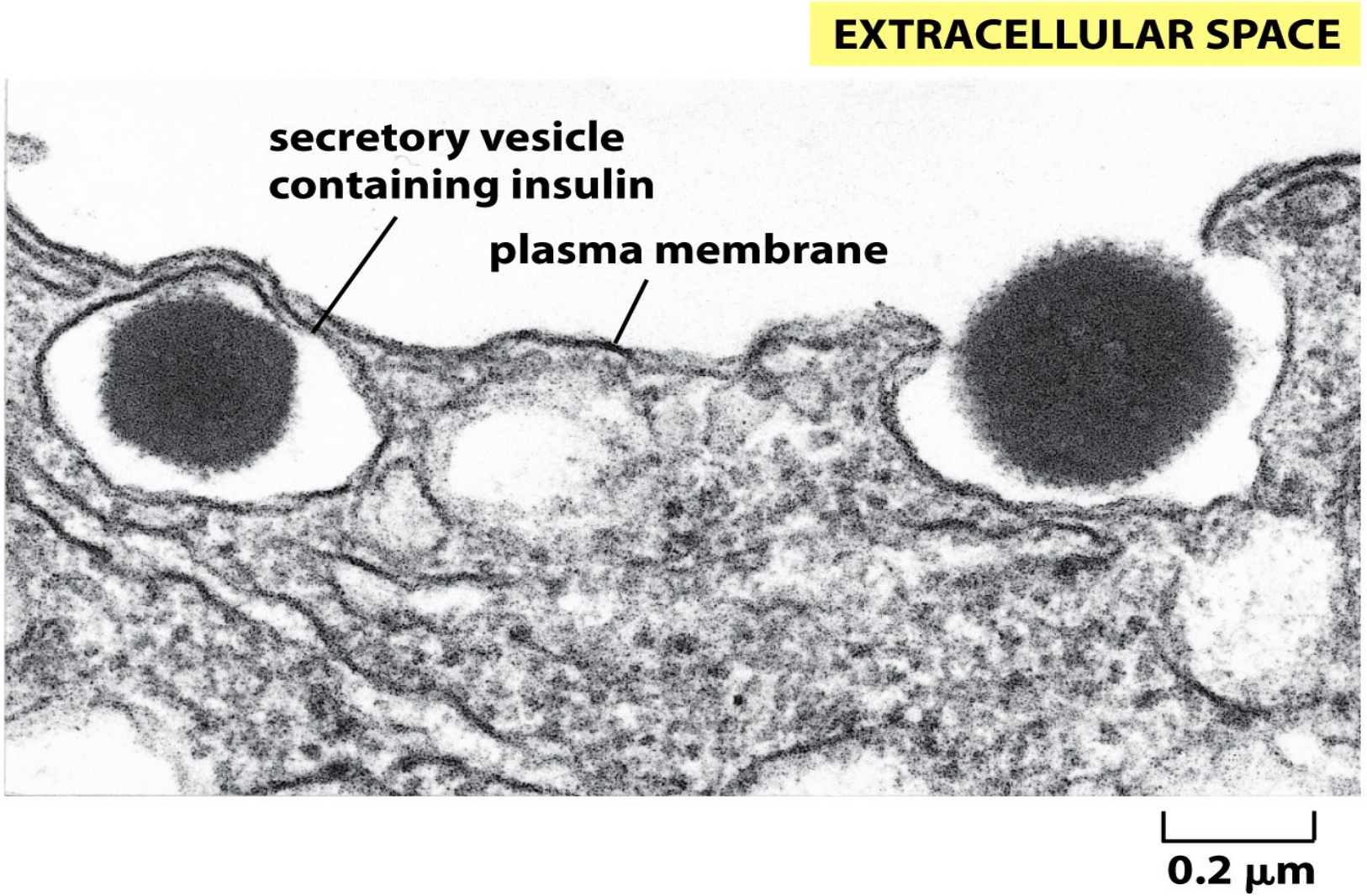
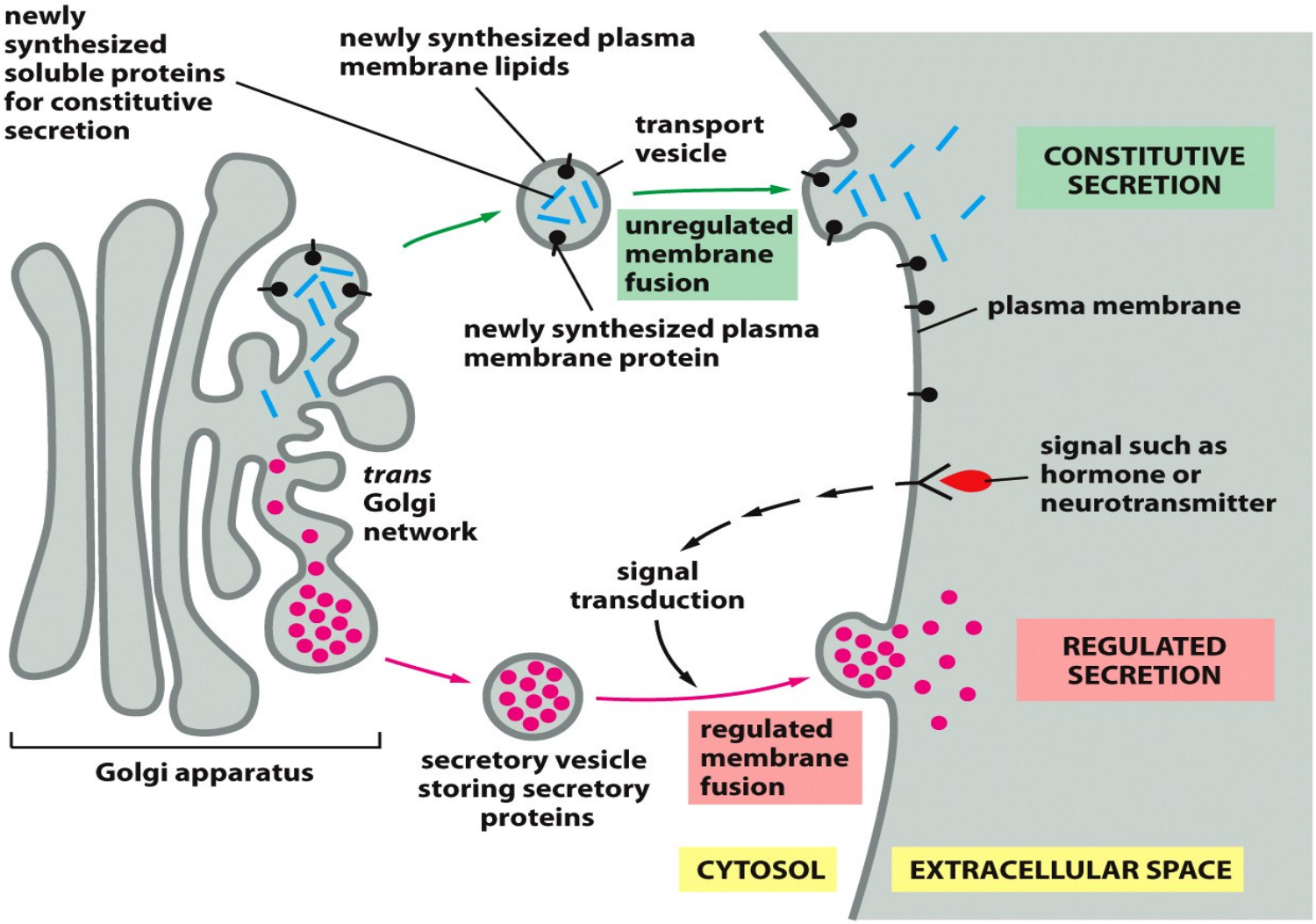


Figure 15-22 *Essential Cell Biology* (© Garland Science 2010)

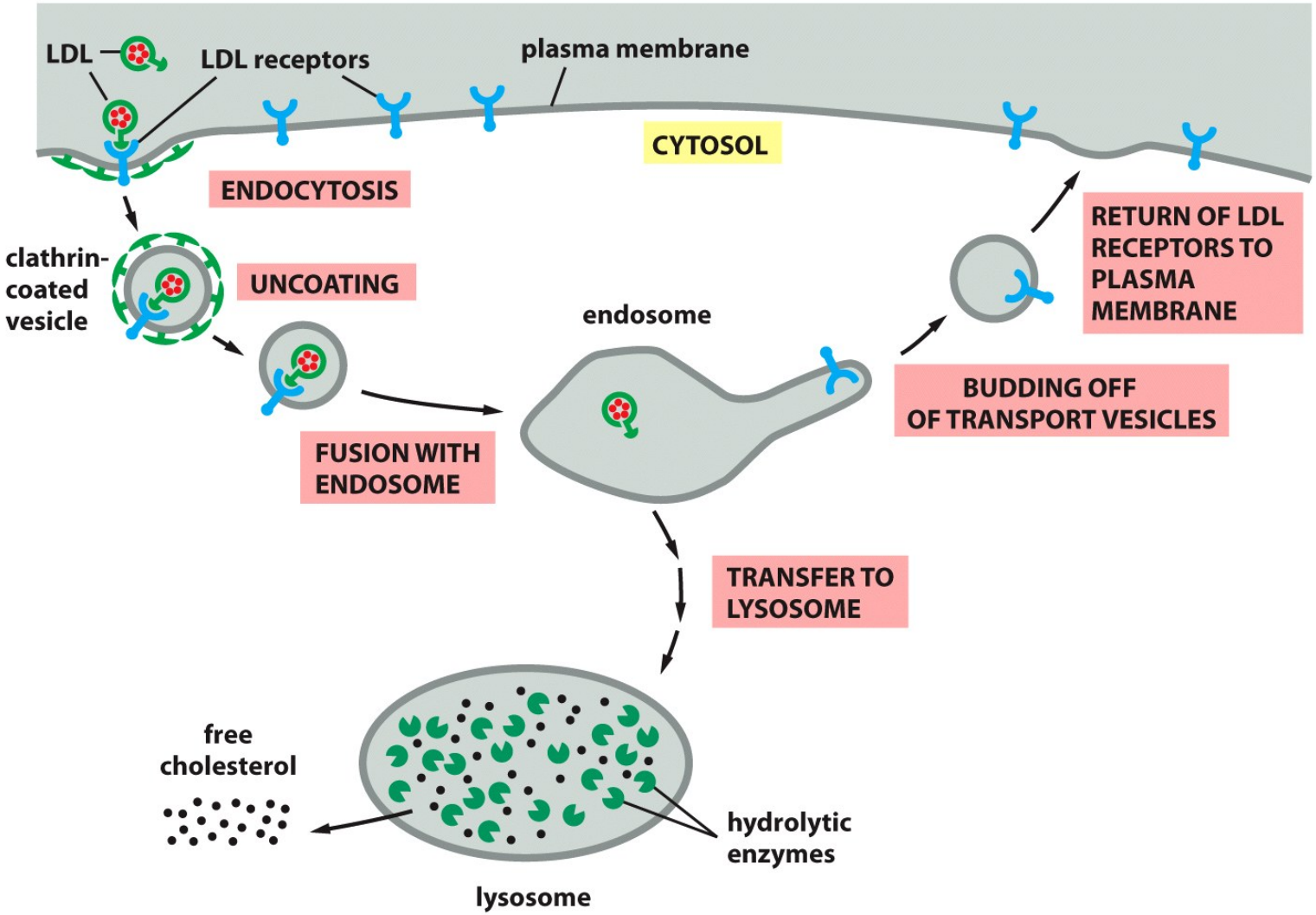
Insulin secretion (seen under electron microscopy)



Constitutive versus Regulated Exocytosis



Recycling of membrane components



Apoptosis = "Falling off"

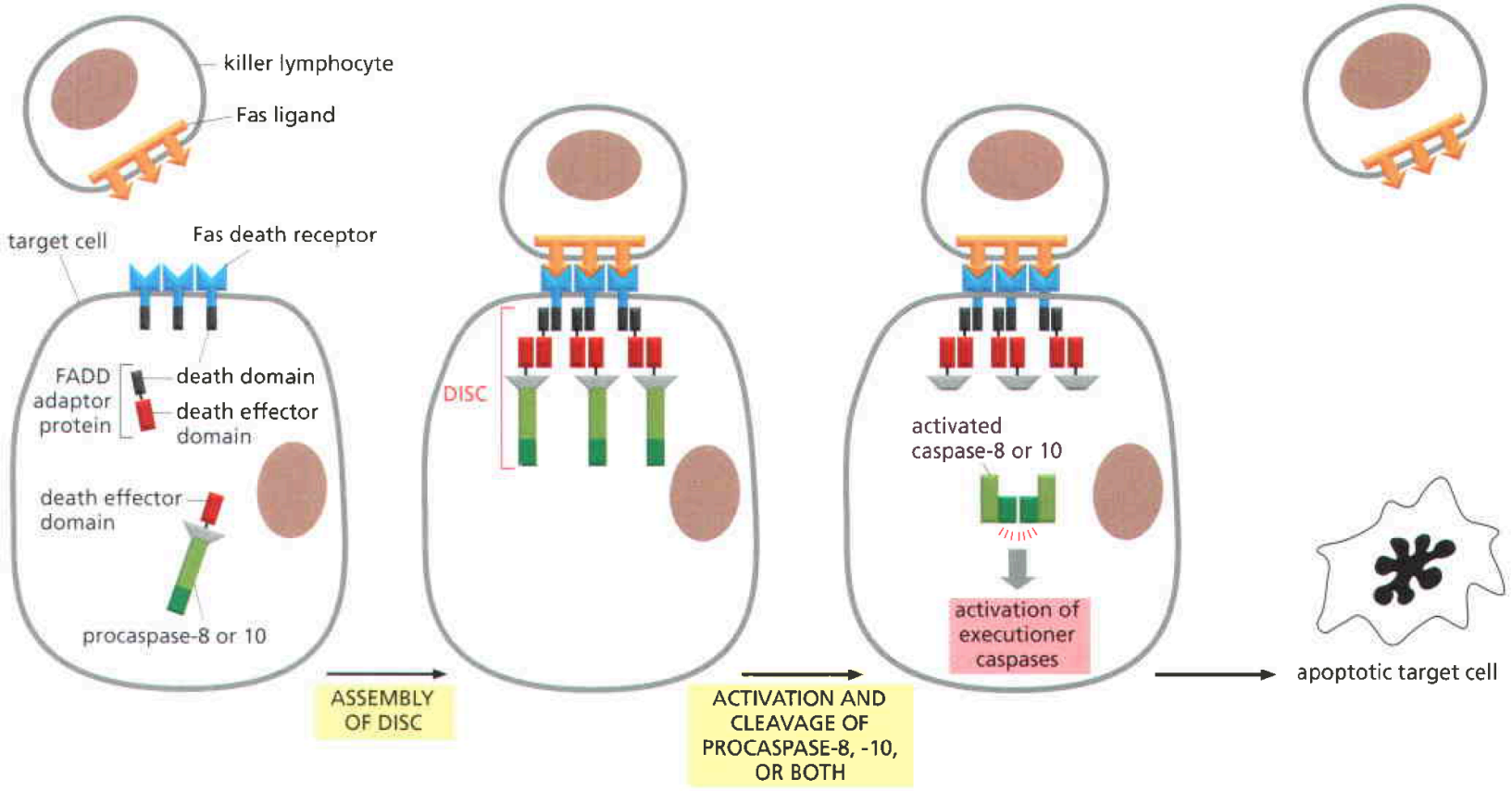
- Most common form of programmed cell death
- Cell death without an inflammatory response
  - Contrary to cell necrosis – massive inflammatory response
- Apoptotic cells undergo characteristic changes
  - Cleavage of chromosome into distinct fragments
  - Flip of Cytosolic *Phosphatidylserine* to the extracellular side = anti-inflammatory "eat me" signal
  - Relocation of *cytochrome c* from Mitochondria to Cytosol

Apoptotic intracellular machinery: Caspases

- Proteases with C – Cysteine in their active site that cleave target proteins at Aspartic acid residues (C – ASP ases)
  - Not all are involved in apoptosis
  - Some mediate inflammatory and immune responses
- Mediate a proteolytic cascade – irreversible process
  - Initiator caspases
  - Executioner caspases
    - Cleave *Nuclear lamins* leading to the breakdown of the nuclear membrane
    - Activate an endonuclease -> leading to the characteristic DNA fragmentation
    - Also breakdown the cytoskeleton and cell-cell adhesion proteins
- Activated by multiple pathways

Extrinsic receptor-ligand pathway

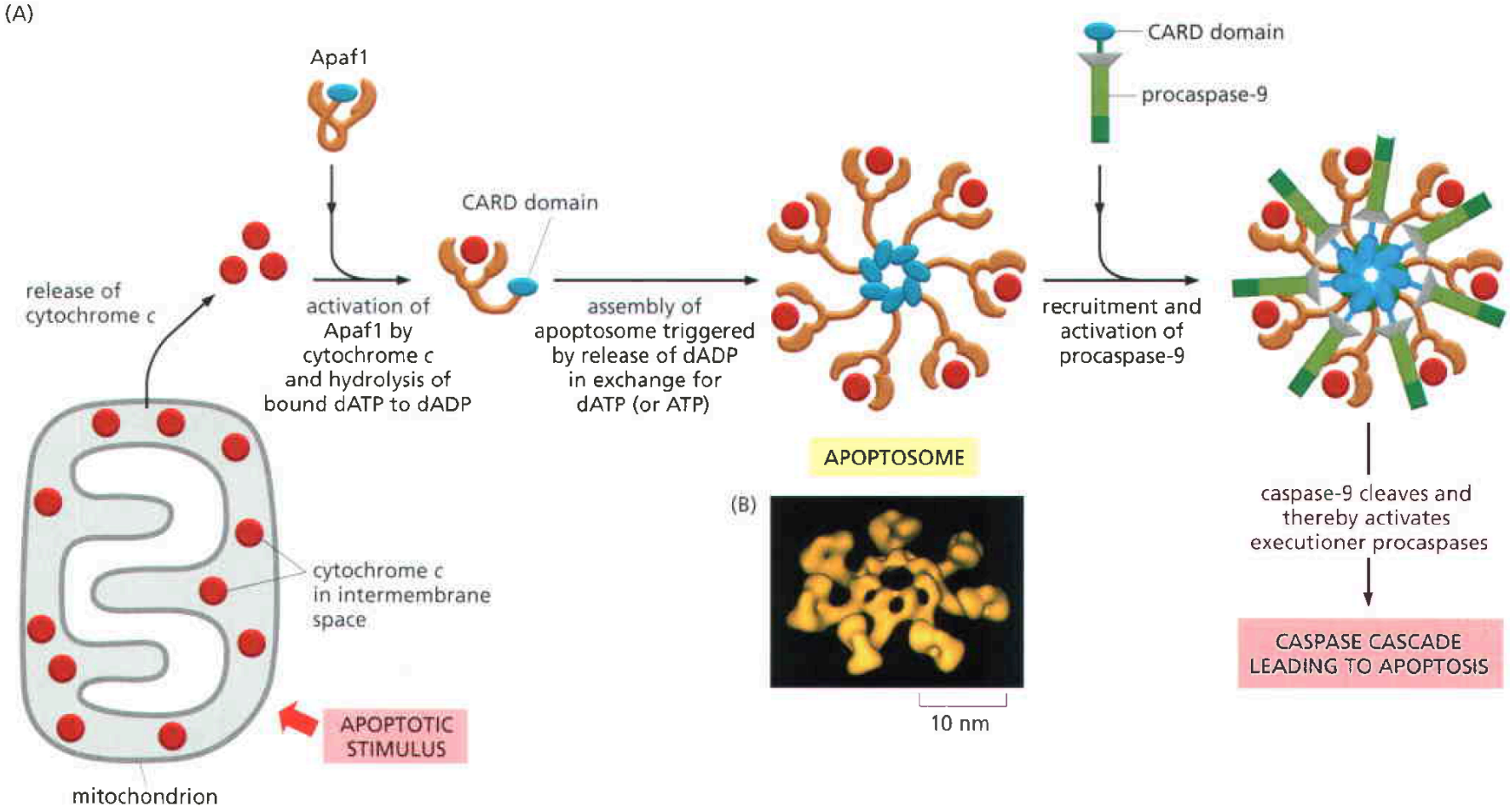
- E.g. Negative selection of T-cells



Intrinsic Mitochondrial pathway

- Irreversible cellular injury/DNA damage/decreased hormonal stimulation
- Membrane injury leads to inactivation of *anti-apoptotic* Bcl2 proteins (**Bcl2** and **Bcl-X_L**), which are involved in stabilization of the Mitochondrial membrane
- Additionally, *pro-apoptotic* Bcl2 proteins (**Bax** and **Bak**) are activated which destabilize the Mitochondrial membrane
- Disruption of Mitochondrial membrane leads to relocation of *cytochrome c* into the cytosol
- *Cytochrome c* can activate Caspases (procaspase 8 and 9)
- Caspase-8 and Caspase-9 activate the executioner caspases (Caspase-3 and Caspase-6)
- Proteolytic cascade leading to Apoptosis

Intrinsic pathway



Cytotoxic $CD8^+$ T-cell pathway

- MHC-I – Antigen complex is recognized by CD8+ T-cells
- T-cells secrete Perforins -> that create pores in the Target cell
- T-cells also secrete Granzyme
- Granzyme enters Target cells through the Perforin pores and activates host cell's Caspase machinery

DysregulatedApoptosis

- Evasion of apoptosis by cancer cells
- Failure of apoptosis of neurons in the first year of life
- Failure of canalization of the Gut (Anal atresia)
- Autoimmune diseases

Thank you for your attention