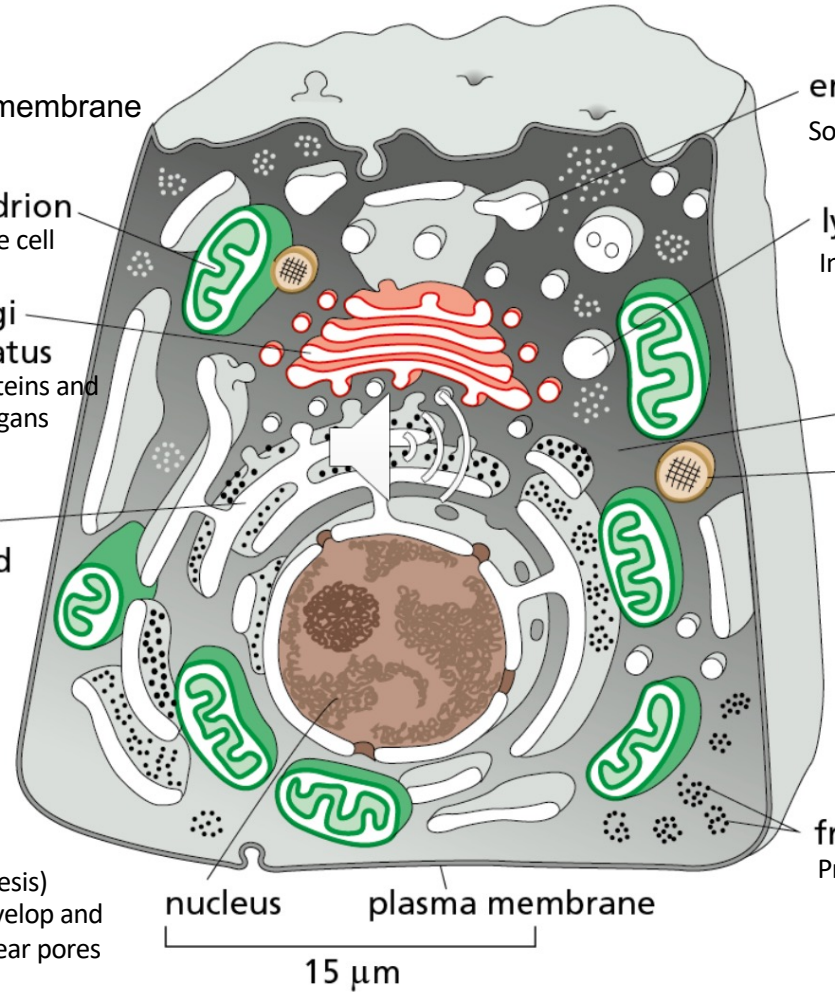
Aishe Sarshad
aishe.sarshad@gu.se

Institute of Biomedicine
Dept of Medical Biochemistry and Cell Biology

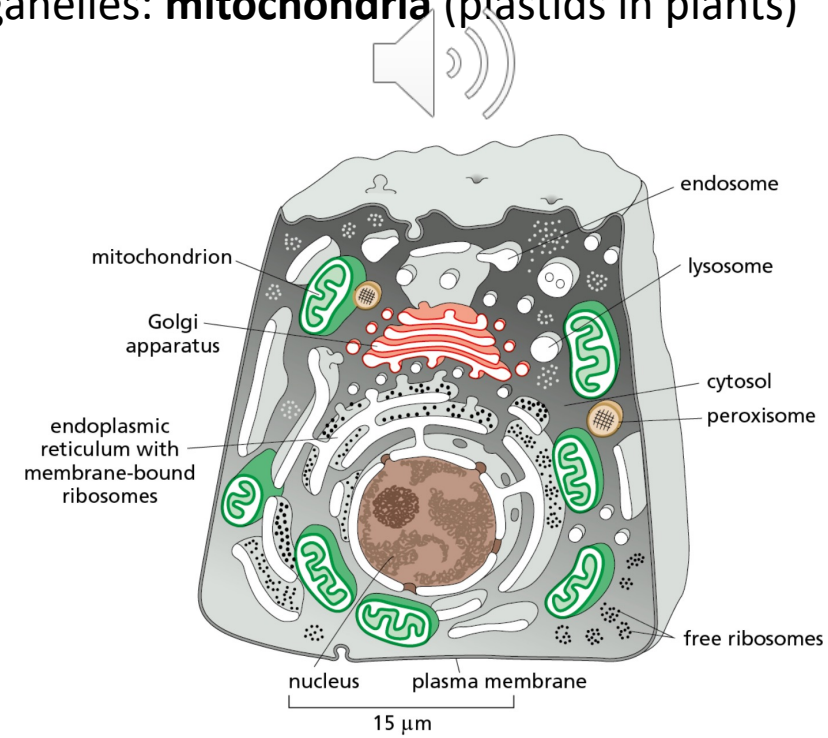
Protein Transport and the Secretory Pathway

1 The compartments of the cell



The major intracellular compartments

- 1) The **nucleus** and the **cytosol** connected by **NPC**
- 2) All the organelles in the secretory and endocytic pathways: **ER, Golgi apparatus, endosome, lysosome, transport vesicles and peroxisomes**
- 3) Endosymbiont-derived organelles: **mitochondria** (plastids in plants)

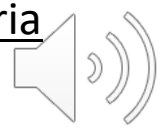


2 Protein transport

Proteins Can Move Between Compartments in 3 Different Ways

- 3 main ways for proteins to move from one compartment to another

1. Protein translocation:
   protein from **cytosol** to ER or mitochondria
   Usually uses a protein translocator

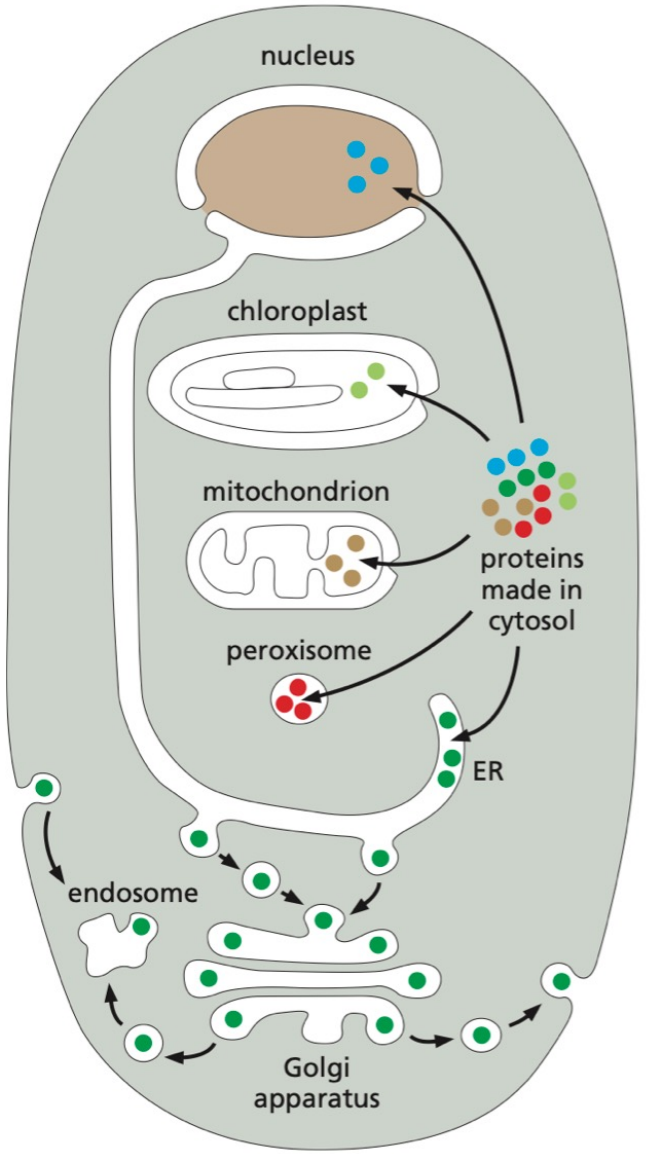


2. Gated transport
   protein and RNA move between **cytoplasm** to nucleus through NPC

3. Vesicular transport
   Vesicles which get loaded with cargo for transport
   Example transport between ER and golgi

3 mechanism of protein transport

Makromolekyler (proteiner) kan definieras att transporteras via tre olika generella processer



1 TRANSPORT THROUGH NUCLEAR PORES

Cytosol to nucleus through NPC
(active or passive transport)

TRANSPORT ACROSS MEMBRANES

Cytosol to ER, mitochondria, peroxisome via
Protein translocators and signaling sequences

3 TRANSPORT BY VESICLES

From ER to another membrane bound
compartment transport vesicles

Cell address

- Proteins need a **sorting signal** which is matched to a **sorting receptor** that mediates movement

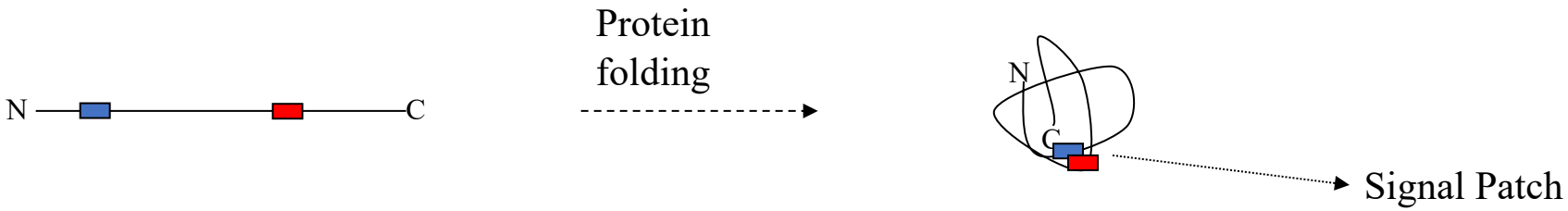
- Sorting signal is either

  A) a linear sequence of amino acids

  (protein translocation and found on N-terminus, they get removed by **signal peptidases** once the sorting is complete)

  B) a 3D arrangement of amino acids called **a signal patch**

  (linear and patch for nuclear/vesical transport)



- Each destination has a unique address tag

import into nucleus

– Pro – Pro – Lys – Lys – Lys – Arg – Lys – Val –

export from nucleus

– Met – Glu – Glu – Leu – Ser – Gln – Ala – Leu – Ala – Ser – Ser – Phe –

import into mitochondria

N – 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 plastids

N – Met – Val – Ala – Met – Ala – Met – Ala – S[illegible] – Leu – Gln – Ser – Ser – Met – Ser – Ser – Leu – Ser –

Leu – Ser – Ser – Asn – Ser – Phe – Leu – Gly – [illegible] – Leu – Ser – Pro – Ile – Thr – Leu – Ser – Pro –

Phe – Leu – Gln – Gly –

import into peroxisomes

– Ser – Lys – Leu – C

import into ER

N – Met – Met – Ser – Phe – Val – Ser – Leu – Leu – Leu – Val – Gly – Ile – Leu – Phe – Trp – Ala – Thr –

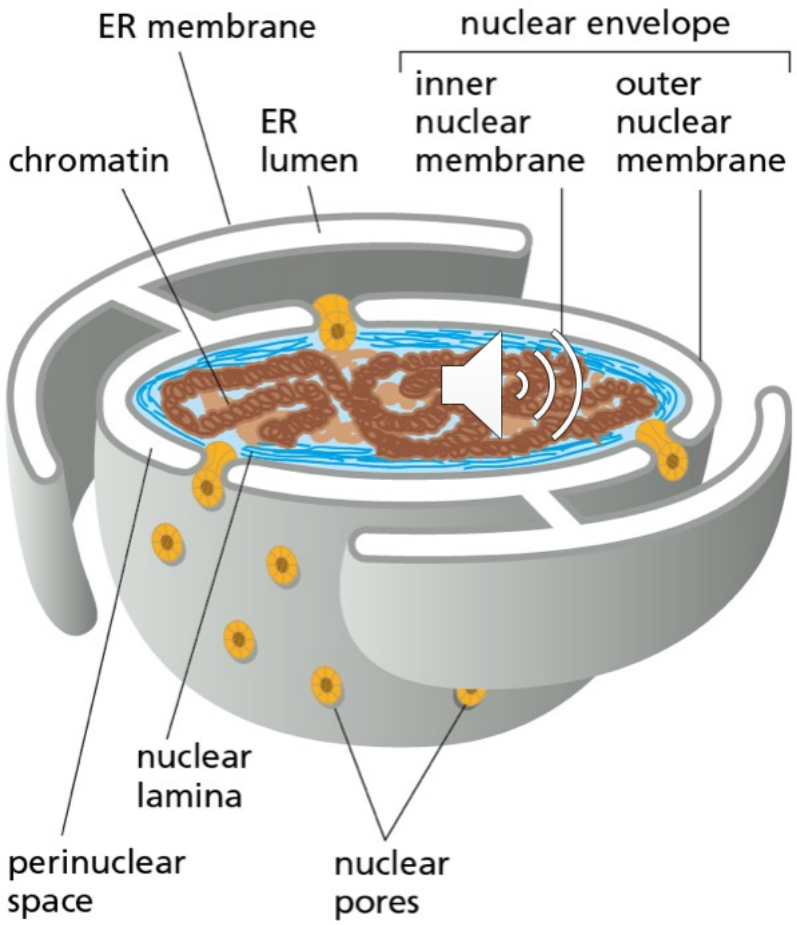
Glu – Ala – Glu – Gln – Leu – Thr – Lys – Cys – Glu – Val – Phe – Gln –

return to ER

– Lys – Asp – Glu – Leu – C

Figure 12–13 Examples of signal sequences that direct proteins to different intracellular locations.

1. Transport through nuclear pores (gated transport)



Gated transport goes through the Nuclear Pore Complex (NPC)

-small molecules (less than 5kD) freely pass through the NPC (passiv transport)

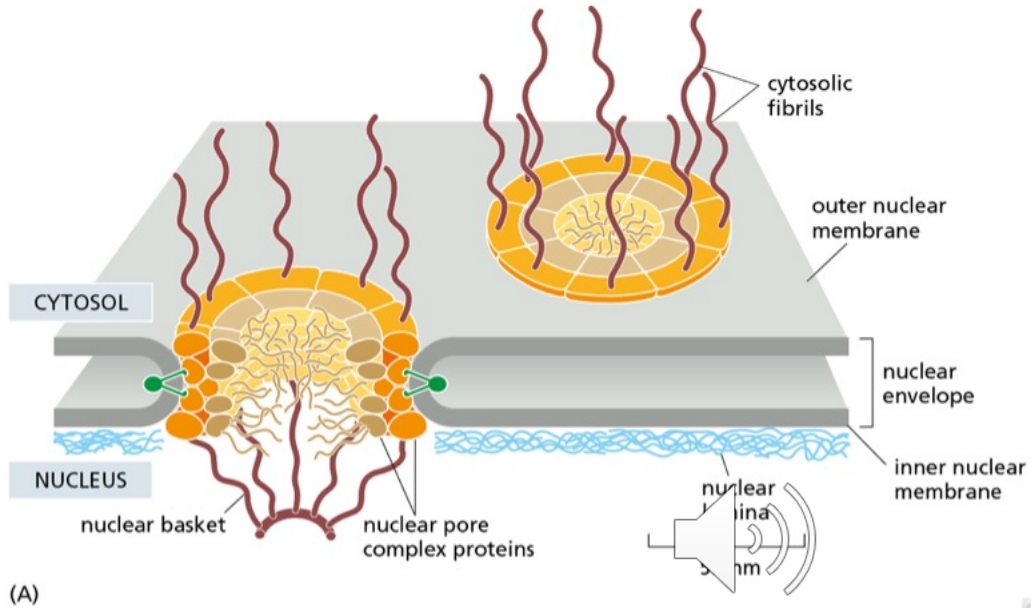
-larger molecules (up to 60kD) pass slowly through passive diffusion

-larger molecules (above 60 kD) have to activly be transported through the NPC (aktiv transport)

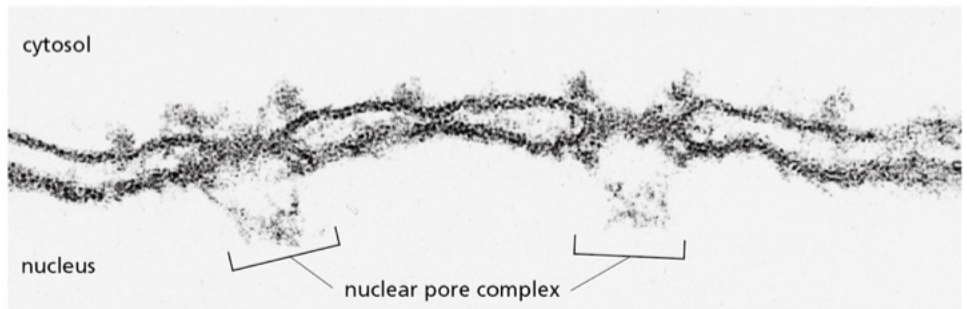
-Transport of makromolecules through the NPC is bidirectional meaning it goes both ways.

-Import of proteins such as transcription factors, histones etc (aktiv transport)

-Export of makromolecules like tRNA, mRNA and rRNA (aktiv transport)

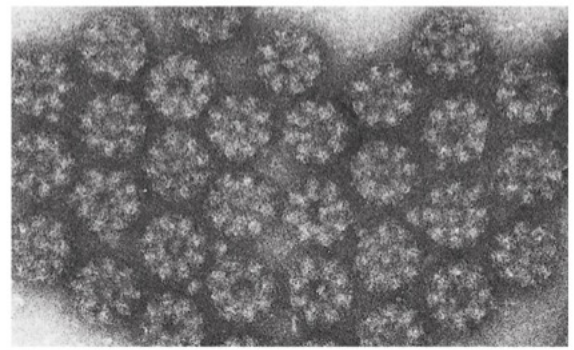


(A)



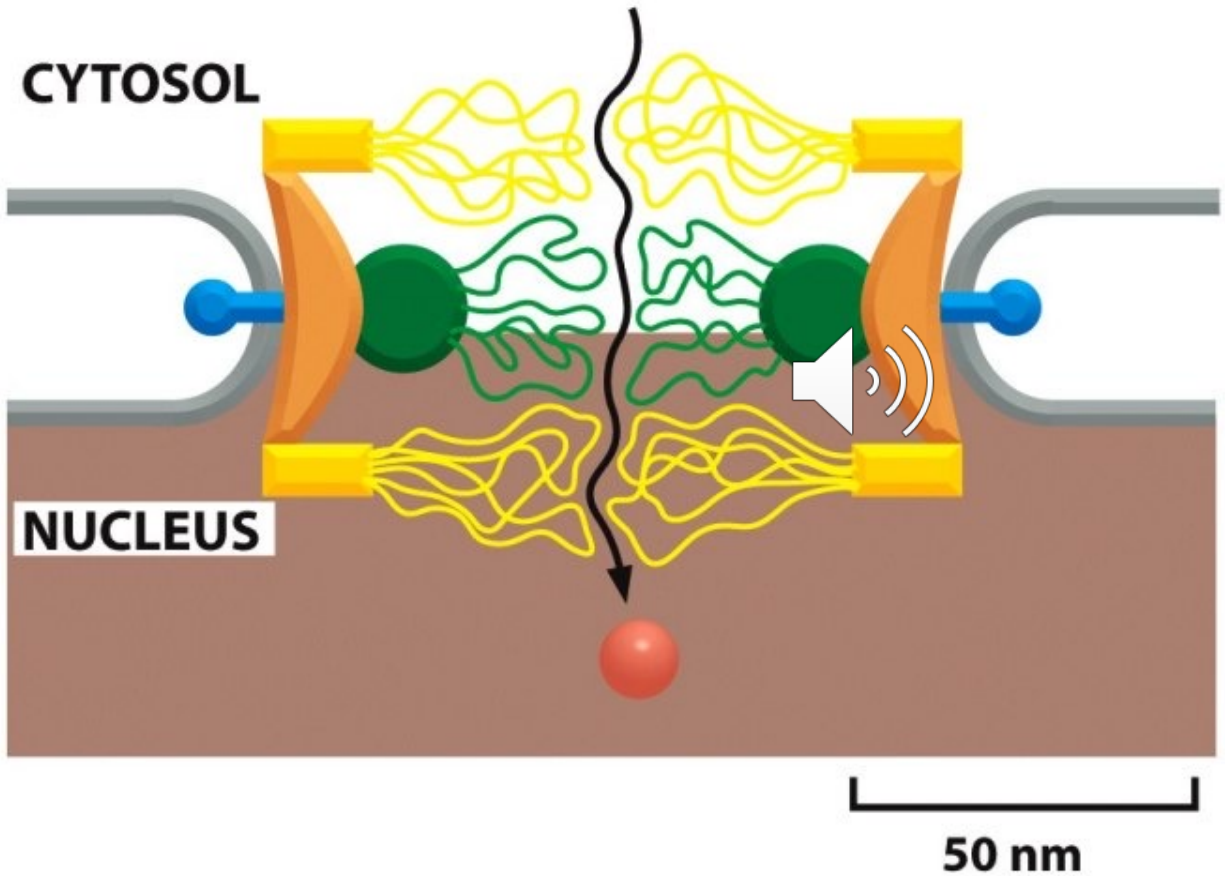
(B)

0.1 μm



(C)

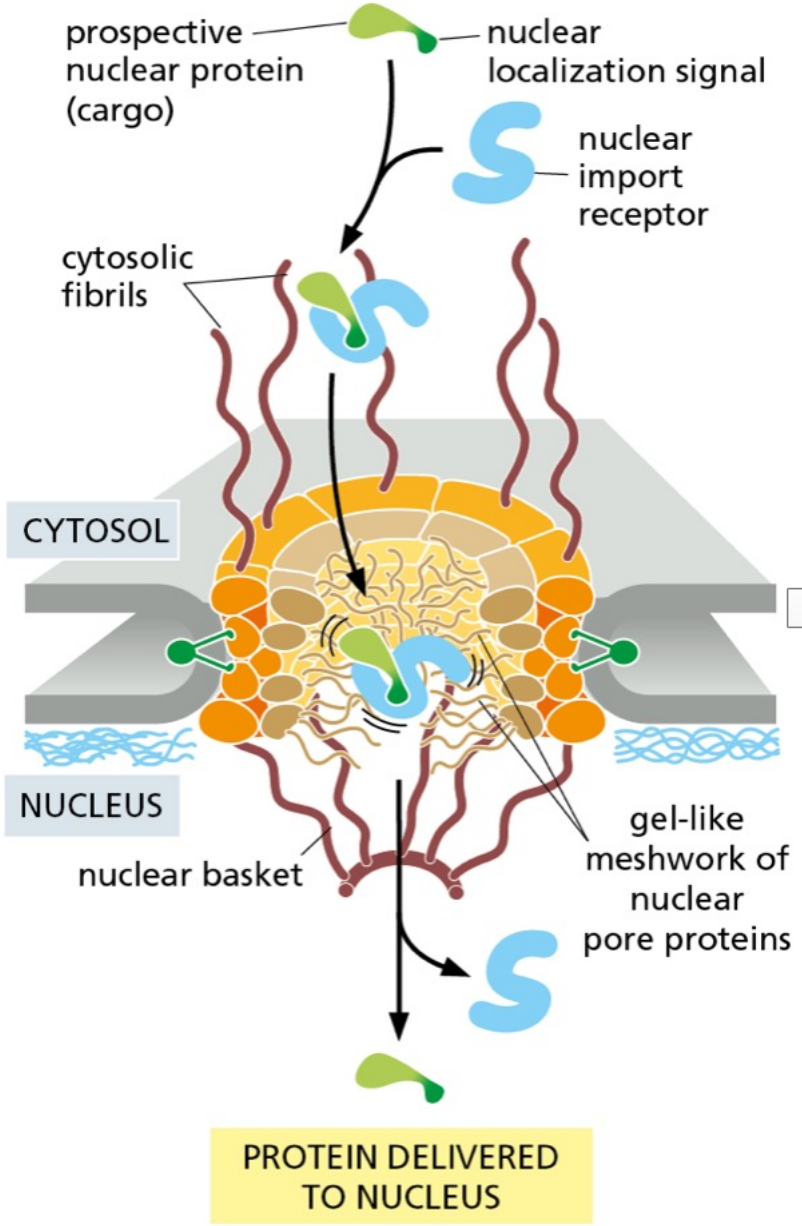
0.1 μm



size of molecules
that enter nucleus
by free diffusion

size of macromolecules
that enter nucleus
by active transport

Figure 12-10 Molecular Biology of the Cell 5/e (© Garland Science 2008)



1. To enter the nucleus, proteins must have a nuclear localization signal (NLS)

2. NLS is recognized by nuclear import receptors

3. Import receptors interact with cytoplasmic fibrils

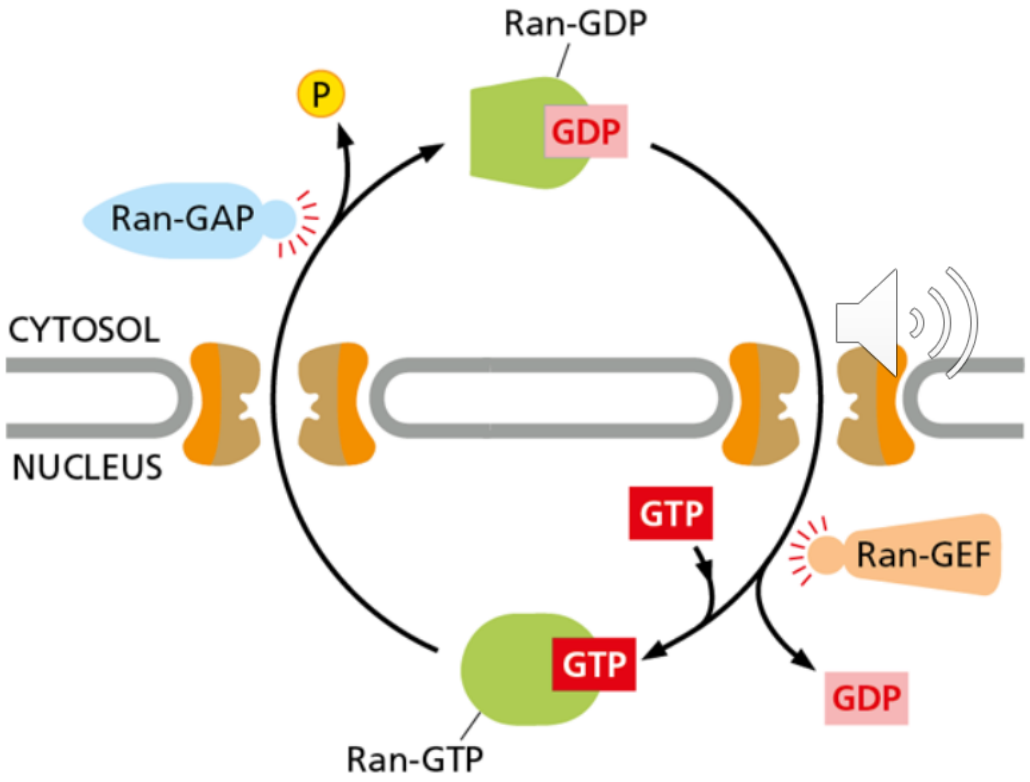
4. Nuclear import receptors opens a passage through the nuclear pore

5. The nuclear import receptors delivers the cargo to the nucleus and goes back into the cytoplasm

6. This processes requires energy!

Transport into the nucleus requires energy

Energy comes from hydrolysis of GTP



Active transport needs Ran protein

Rna can either be in complex with GDP (guanosine diphosphate) or GTP (guanin triphosphate)

In the cytoplasm there is more Ran-GDP

In the nucleus there is more Ran-GTP

When Ran-GDP enters the nucleus it will encounter Ran-GEF (guanosin exchange factor) which exchanges GDP to GTP

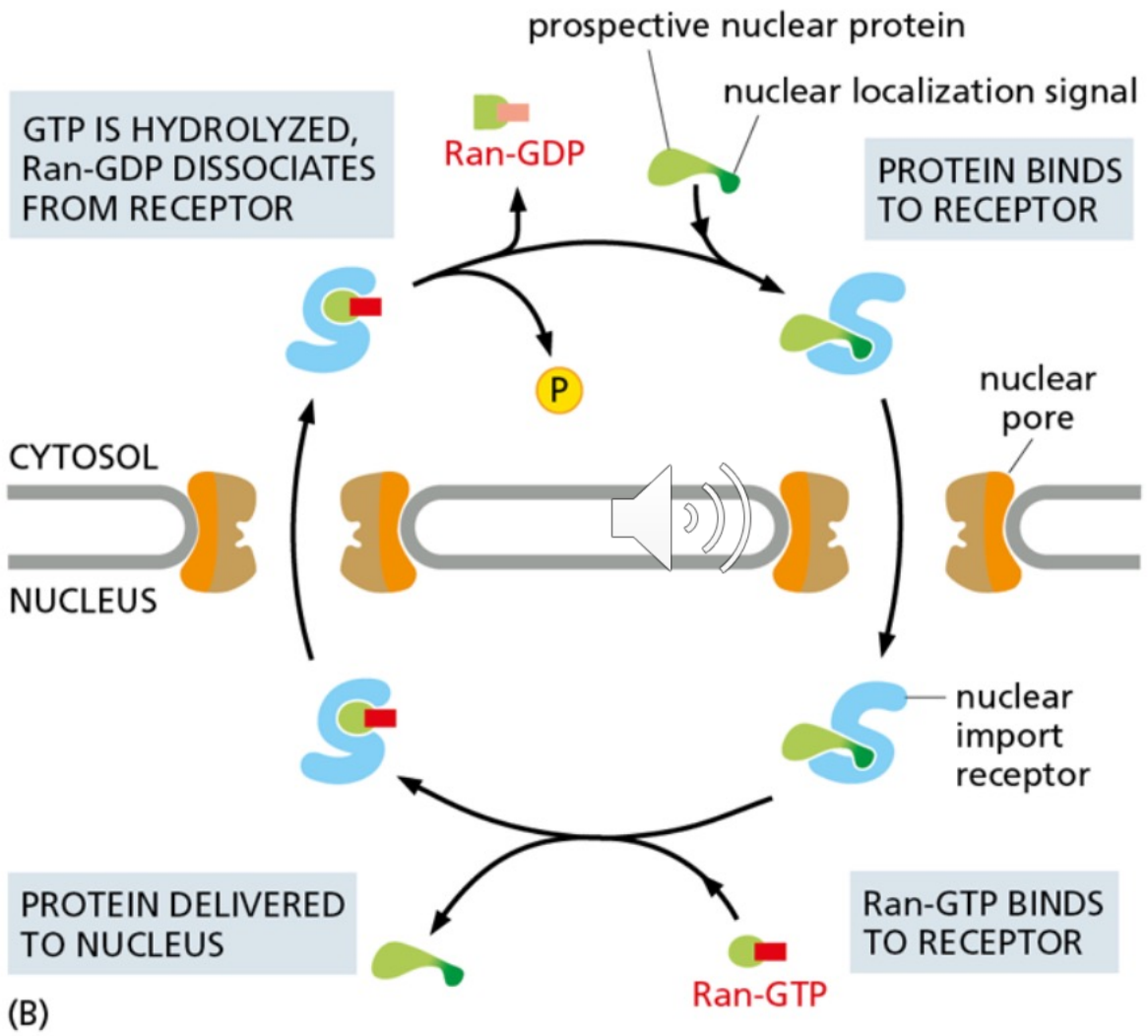
When Ran-GTP enter the cytoplasm it will encounter Ran-GAP which hydrolyses GTP to GDP

GDP and GTP are nucleotide important for energy conversion

More Ran-GDP in the cytoplasm

GTP to GDP

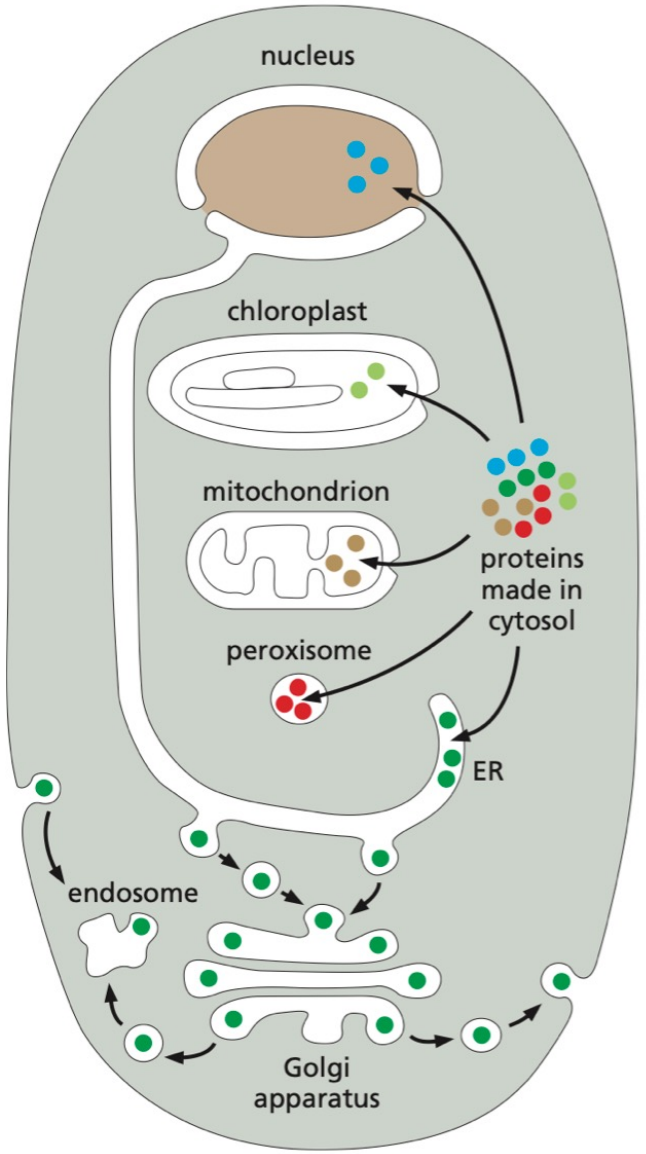
+ RAN-GAP



More Ran-GTP in the nucleus

3 mechanism of protein transport

Makromolekyler (proteiner) kan definieras att transporteras via tre olika generella processer



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From ER to another membrane bound
compartment transport vesicles

3 The Endoplasmic Reticulum (ER)

- Makes up more than 50% of the total membrane of a cell and 10% of total cell volume
- Expands inside the cytosol
- The inside of the ER is called **ER lumen**
- ER has a central function in synthesis of **lipids** and **proteins**
- ER lumen **stores Ca^{2+}** needed for cell signaling

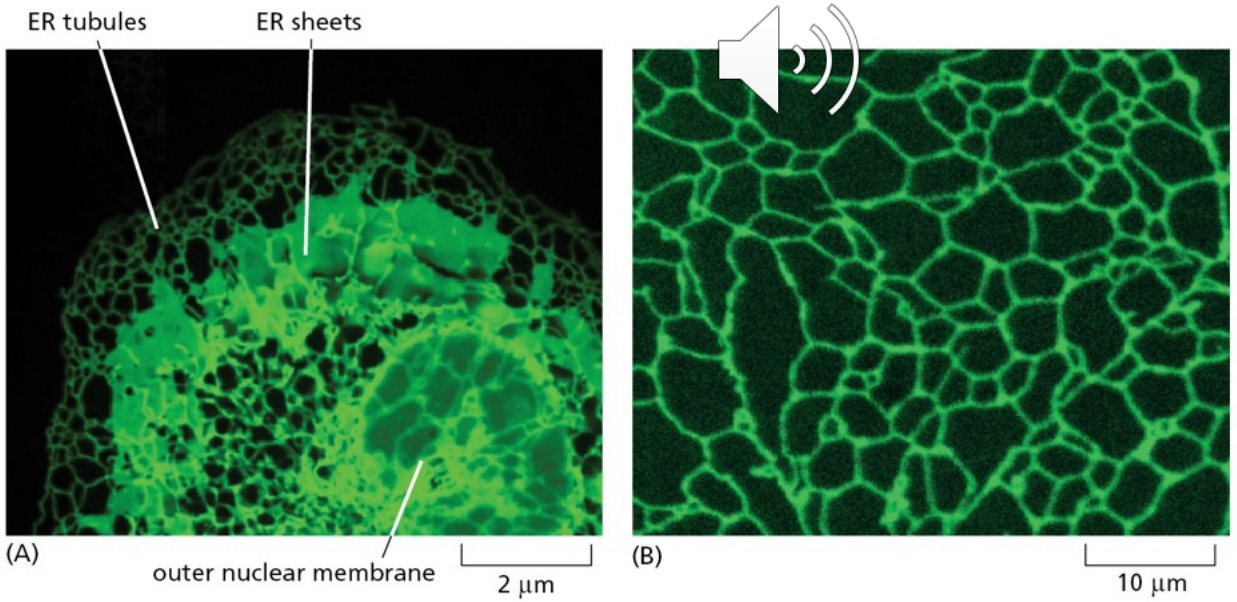
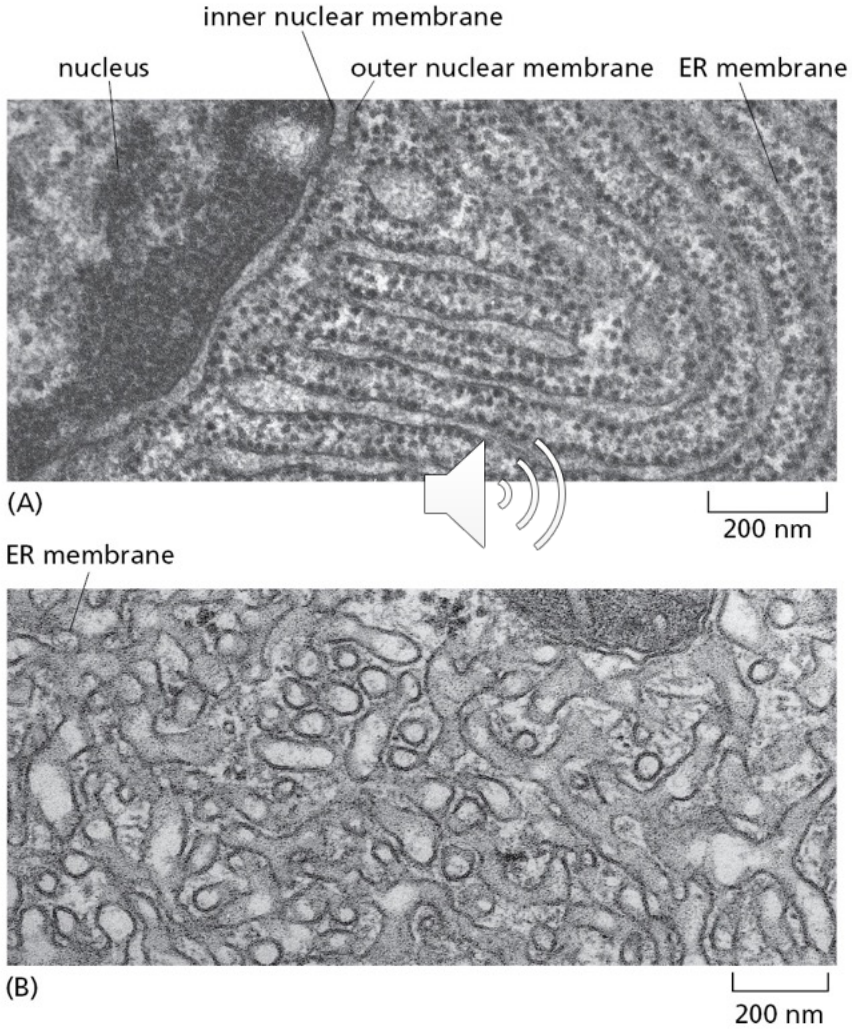


Figure 12–14 Fluorescence micrographs of the endoplasmic reticulum.

ER is structurally and functionally diverse

Rough ER –
protein synthesis

Smooth ER –
synthesis/metabolism of lipids



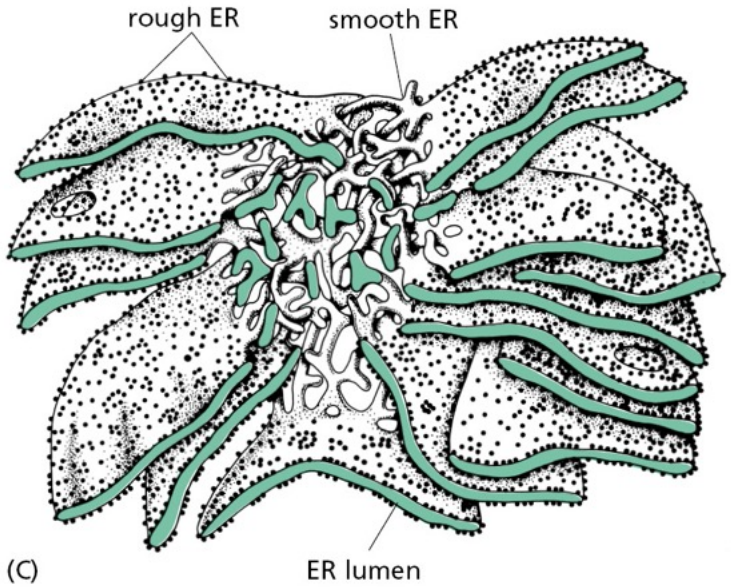


Figure 12–15 The rough and smooth ER.

Main functions of ER

- Ratio of smooth and rough ER depends on the cell type aka the need the cell has

- Cells which secrete proteins have more rough ER
  - example pancreatic cells which secrete digestive enzyme,
  - or antibody secreting plasma cells

- Cells which produce hormones have more smooth ER
  - Example is liver cells which produce lipoprotein particles (carry lipids to other body sites)

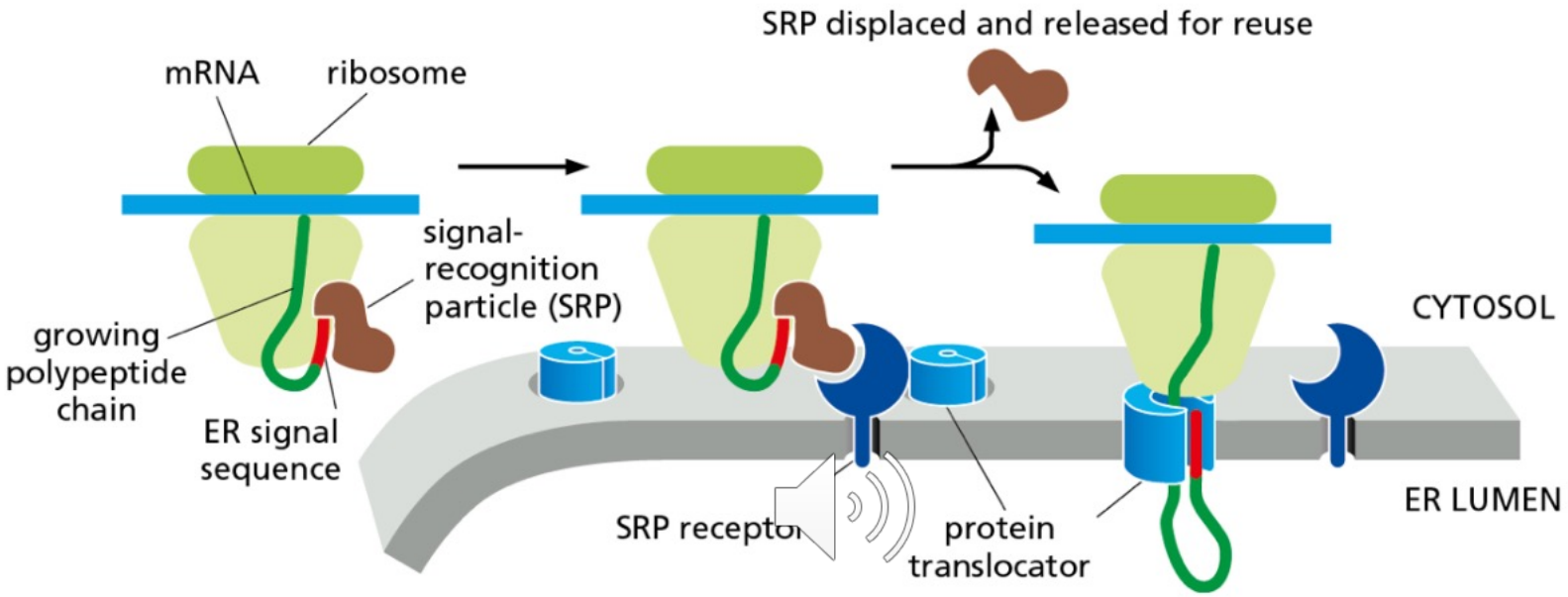
- Ca^{2+} release and uptake is done in the ER. This happens in response to extracellular signals.
  - Example is the ER in muscle cells called sarcoplasmic reticulum (SR).
  - There is release/uptake of Ca^{2+} by the SR that triggers muscle contraction/relaxation

Transport into the ER needs an ER signal sequence

Two types of proteins move into the ER:

1. **Transmembrane proteins** get embedded in the ER membrane
2. **Water-soluble proteins** are translocated through ER membrane to the ER lumen.
   Most move on to other destinations.

- The **ER signal** is recognized by and bound by the **signal recognition particle (SRP**).
- SRP also binds to the **SRP receptor** on the ER membrane



Proteins enter the ER before being completely synthesized

SRP recognized the ER translocation signal and binds to the SRP receptor.

When it binds to the receptor, SRP is displaced and the protein is threaded into the ER lumen with the help of protein translocator.

The signal recognition particle (SRP)

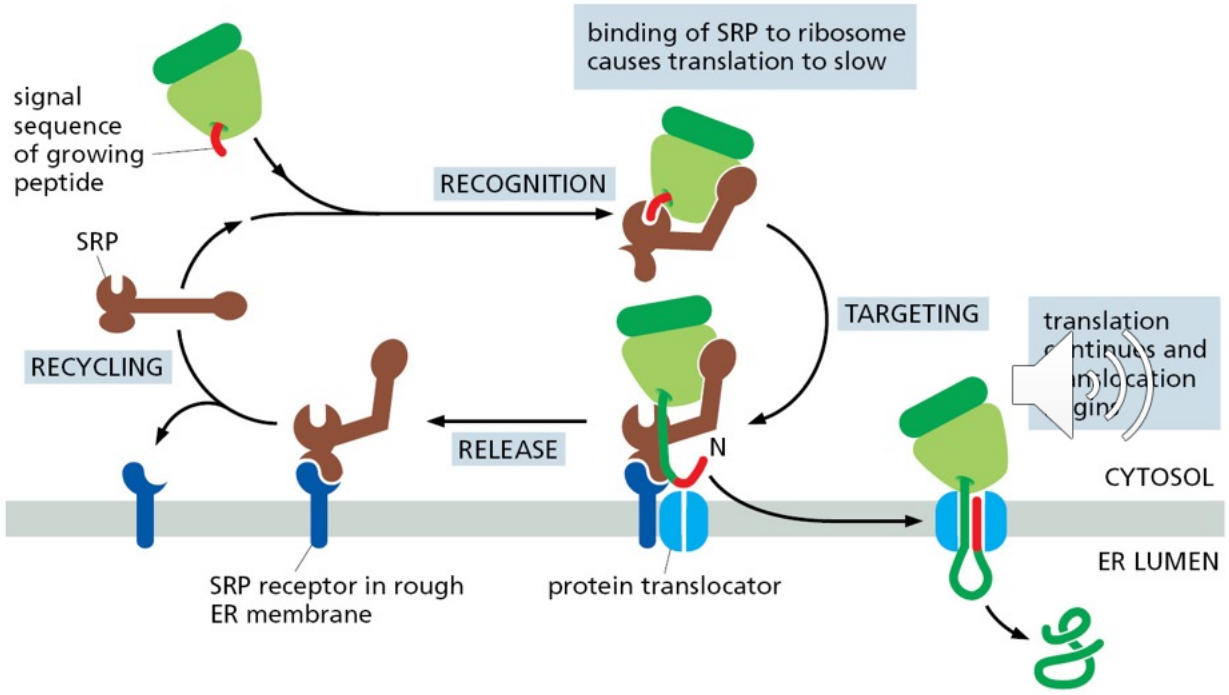


Figure 12–20 How ER signal sequences and SRP direct ribosomes to the ER membrane.

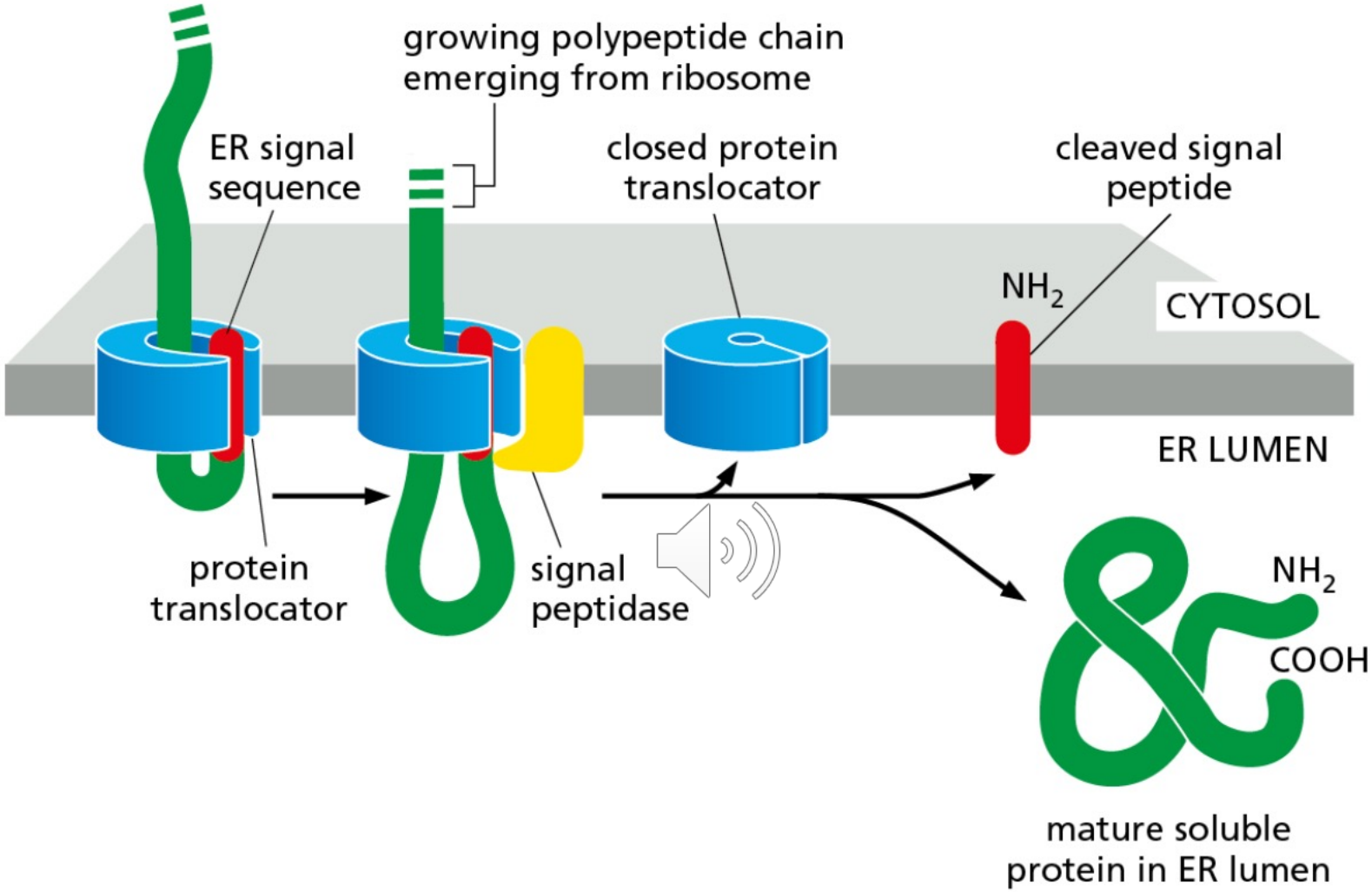
1. SRP binds to the ER signal sequence generated by the ribosome (**recognition**)

2. Binding of SRP to the ribosome causes translation to slow

3. A configuration change in SRP makes it a target to the SRP receptor (**targeting**)

4. The SRP receptor finds an available **protein translocator** to 'thread' the new protein through

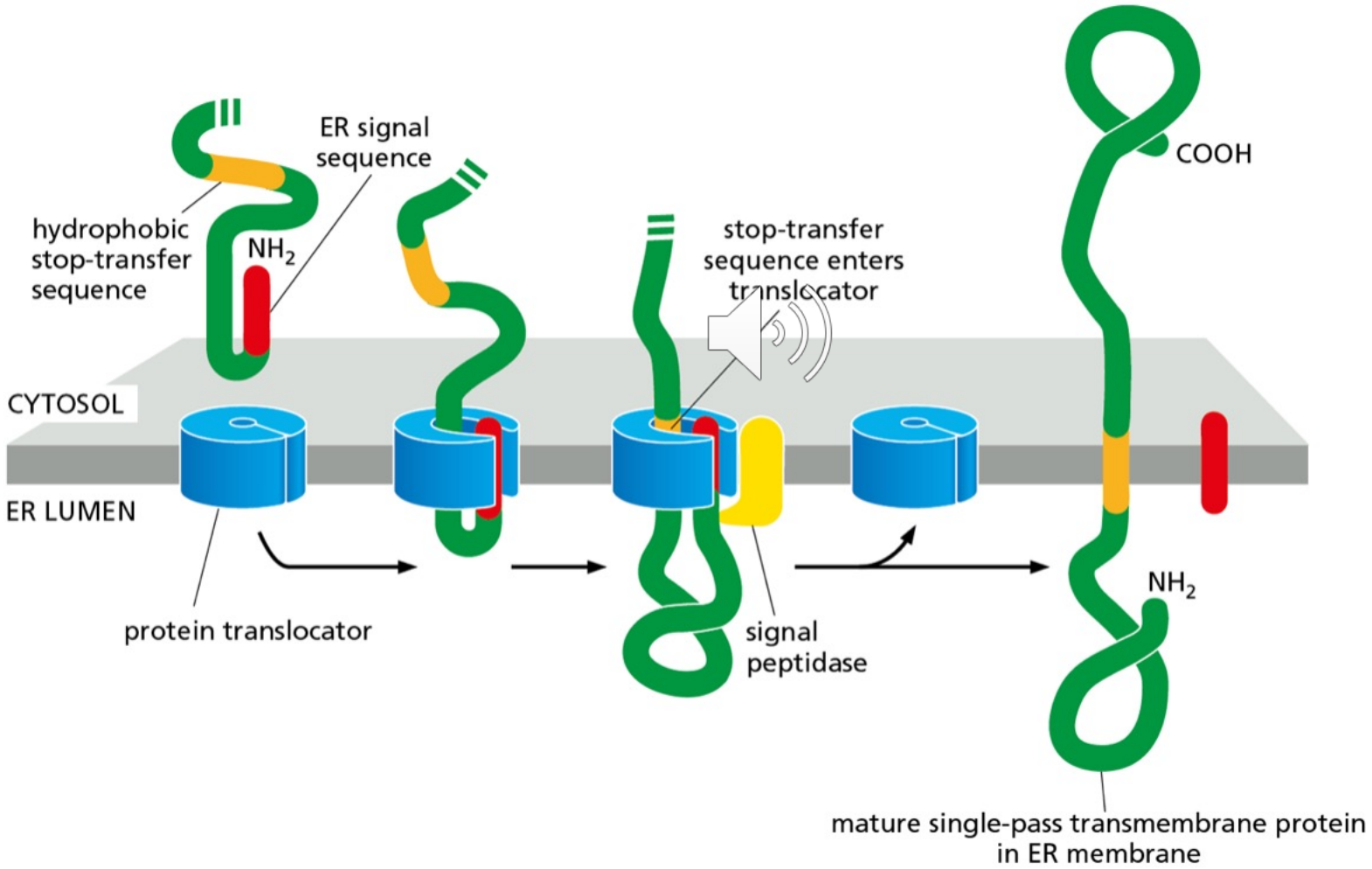
5. The SRP release the receptor and is recycled



-The signal sequence interacts with the translocator and also facilitates the opening of the protein translocator
-Signal sequence is removed by signal peptidase
-Mature protein is in the ER lumen

Some proteins made in the ER stay in the membrane

Transmembrane protein has a start-transfer sequence on the N-terminus which is recognized by translocator
The protein is ‘feed’ through until the stop-transfer sequence is recognized.



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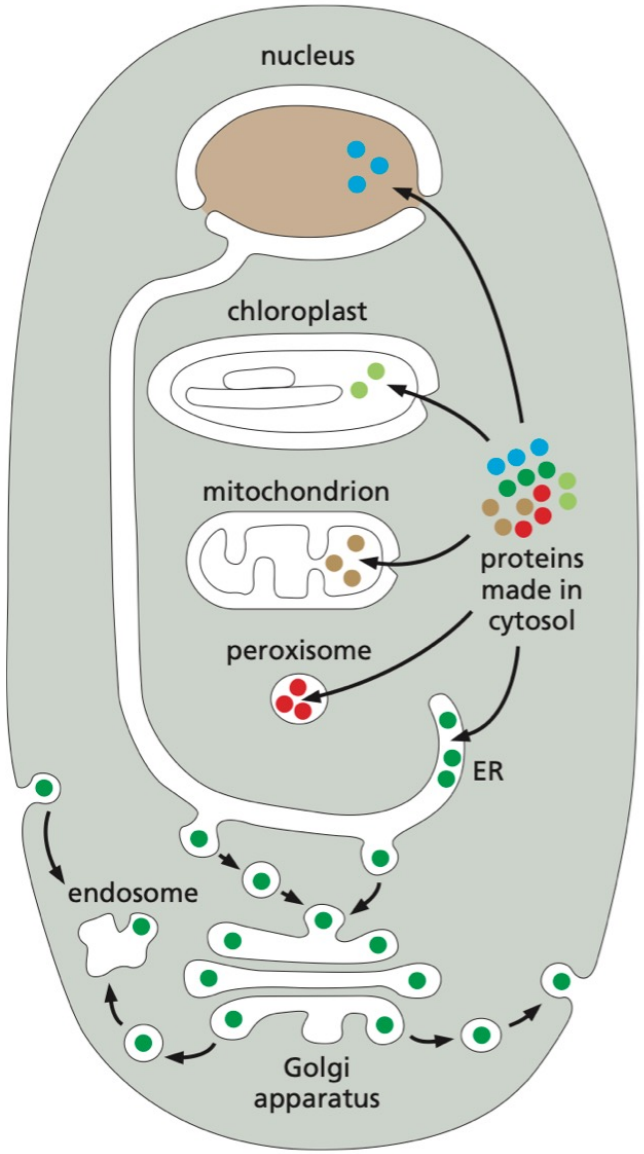

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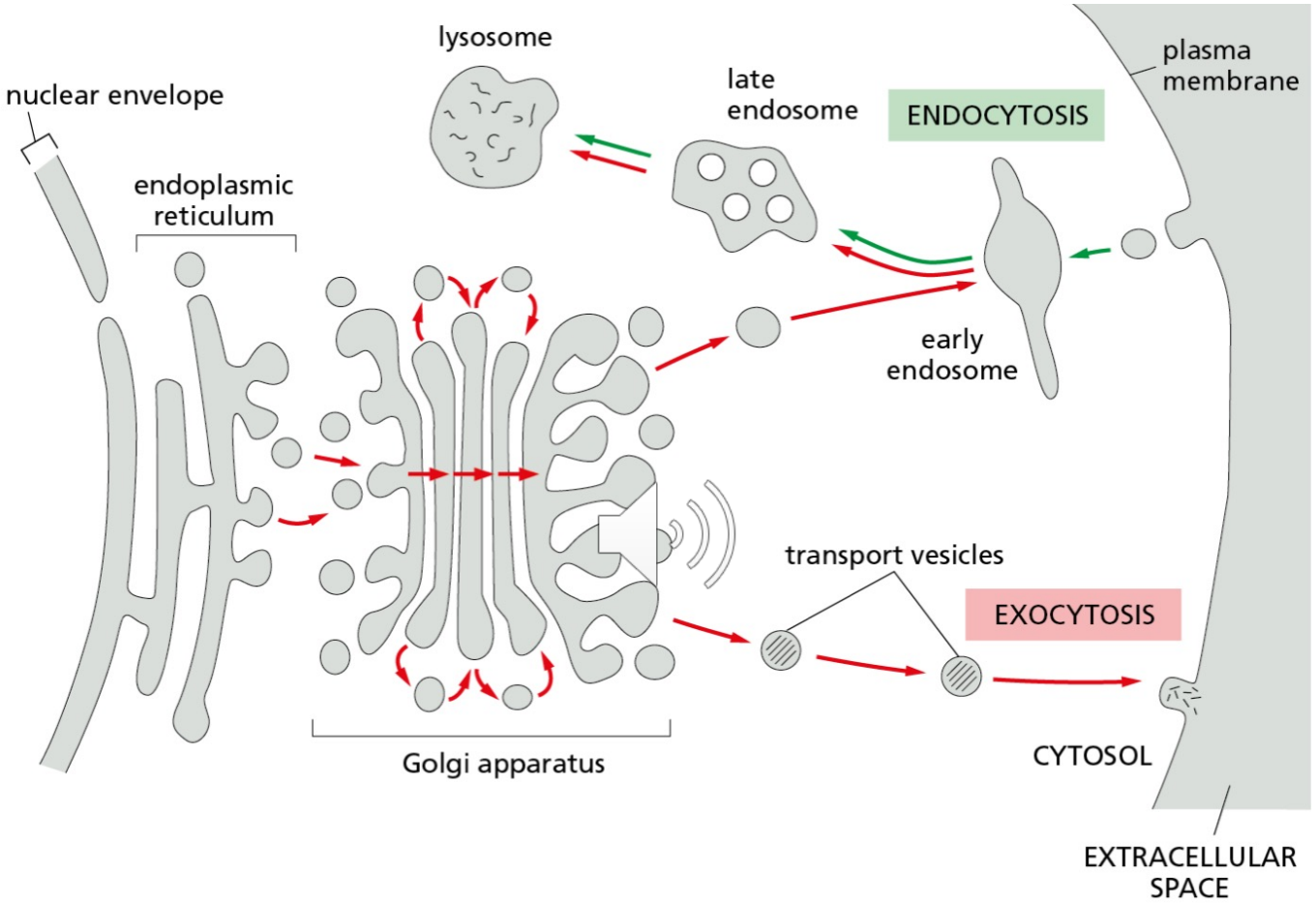
4 Vesicular Transport



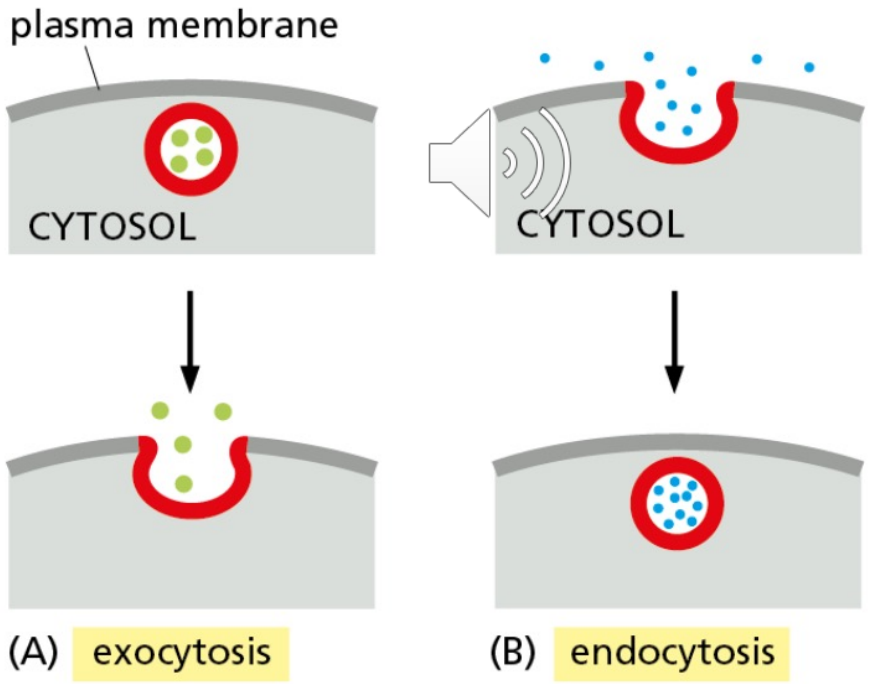
Synthesis of protein on ER membrane then pass through golgi to

a) the cell surface (exocytosis) or

b) from the golgi vesicles pass via endosomes to lysosomes for degradation (endocytosis)

Exocytosis - proteins and molecules are secreted out of cell

Endocytosis – molecules from outside the cells are imported in to the cell



Clatherin: from Golgi apparatus to endosome and from plasma membrane to Golgi via endosomes.

COPI: from Golgi cisternae and the ER

COPII : vesicles from ER to Golgi

Retromer: retrieval pathway from endosome to Golgi

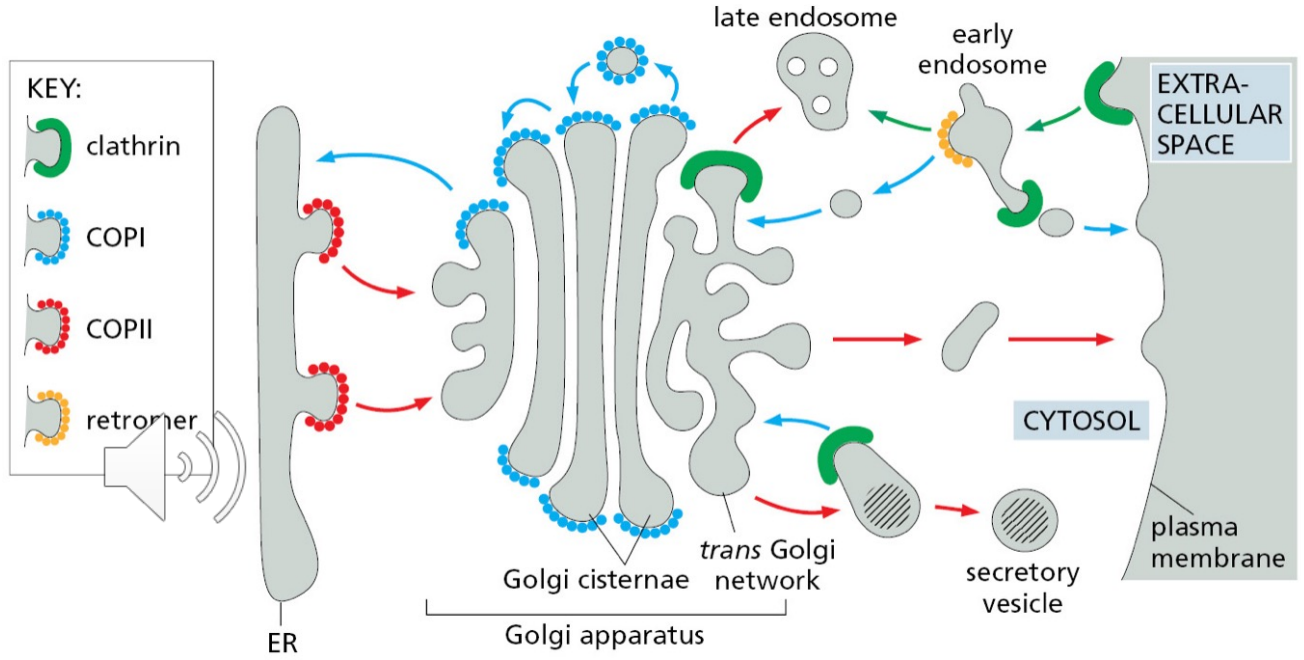
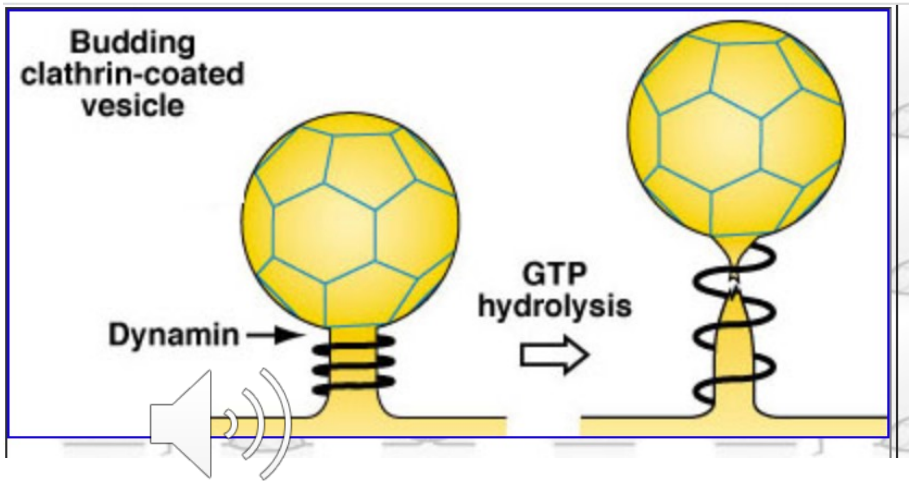
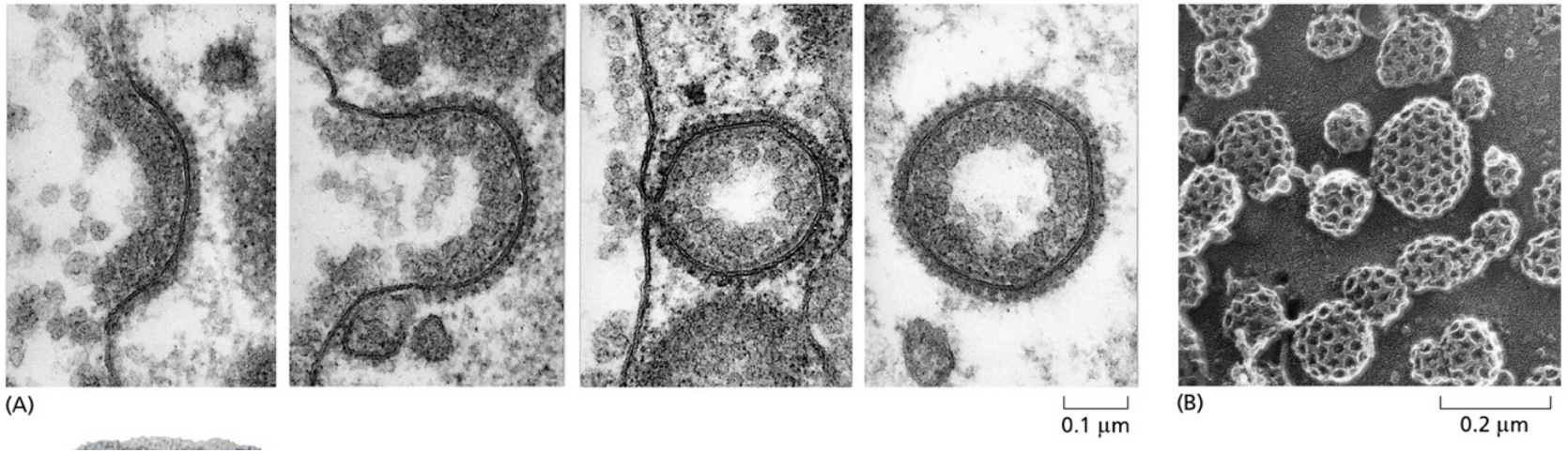


Figure 13–5 Use of different coats for different steps in vesicle traffic.

Clathrin coated vesicles bud from Golgi to endosomes with the help of Dynamin (this processes uses GTP hydrolysis) .

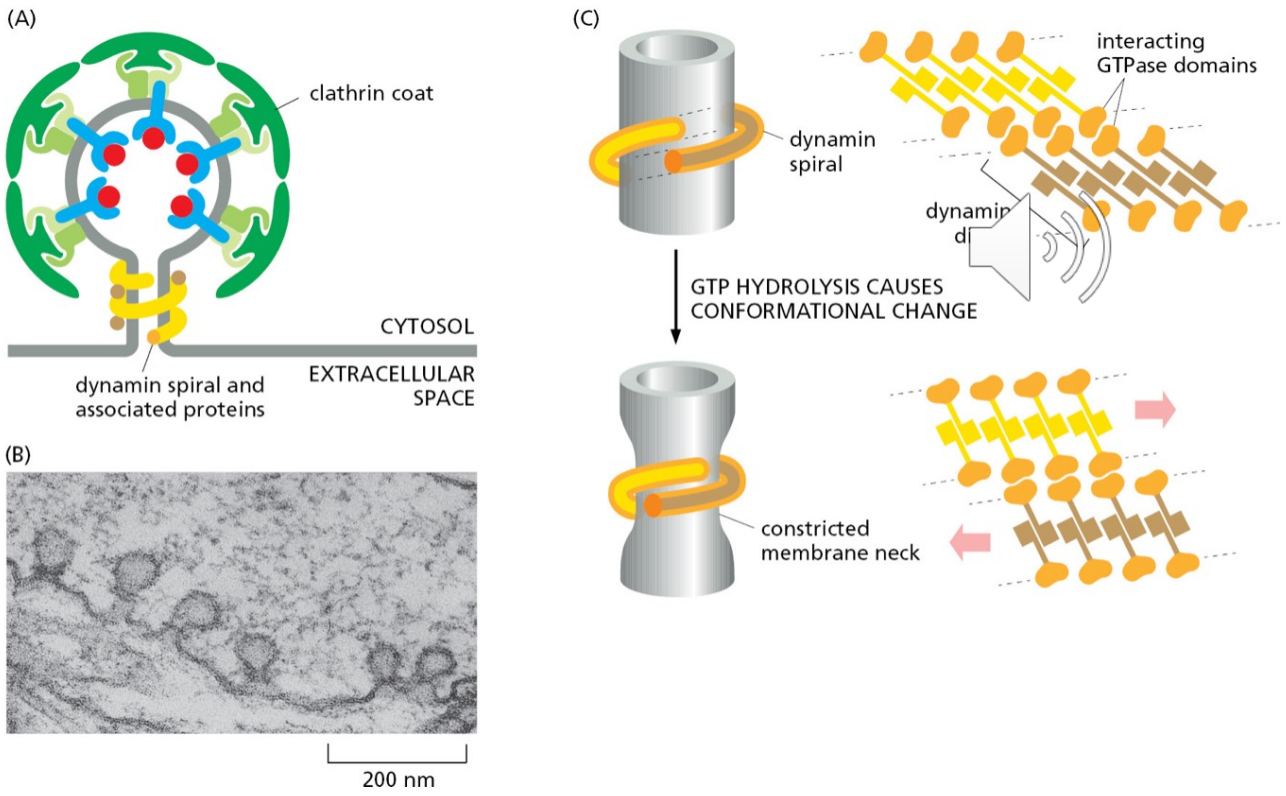
Clathrin also helps to form the vesicle bubble





Dynamin

- **Dynamin** assembles at the base of the vesicle and helps pinch it off
- Once the vesicle is released then it quickly loses its Clatherin coat



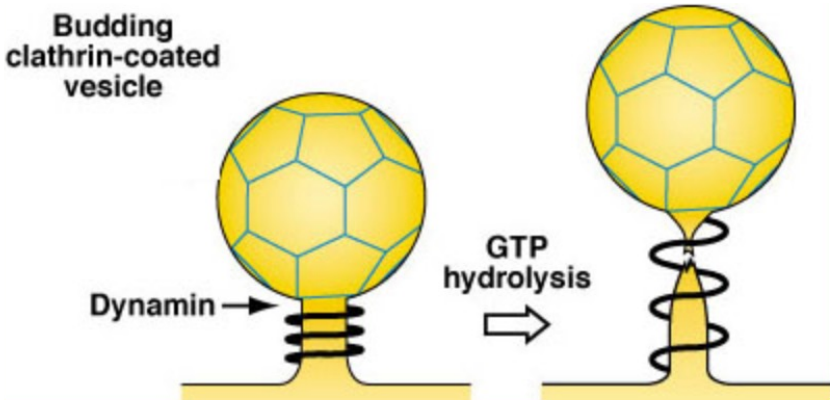


Figure 13–14 **The role of dynamin in pinching off clathrin-coated vesicles.**

Adaptor Proteins Select Cargo into Clathrin-coated Vesicles

- Clathrin do not select the specific cargo (**decides destination**)
- **Adaptins**, also a coat protein, select the cargo. There are different adaptins

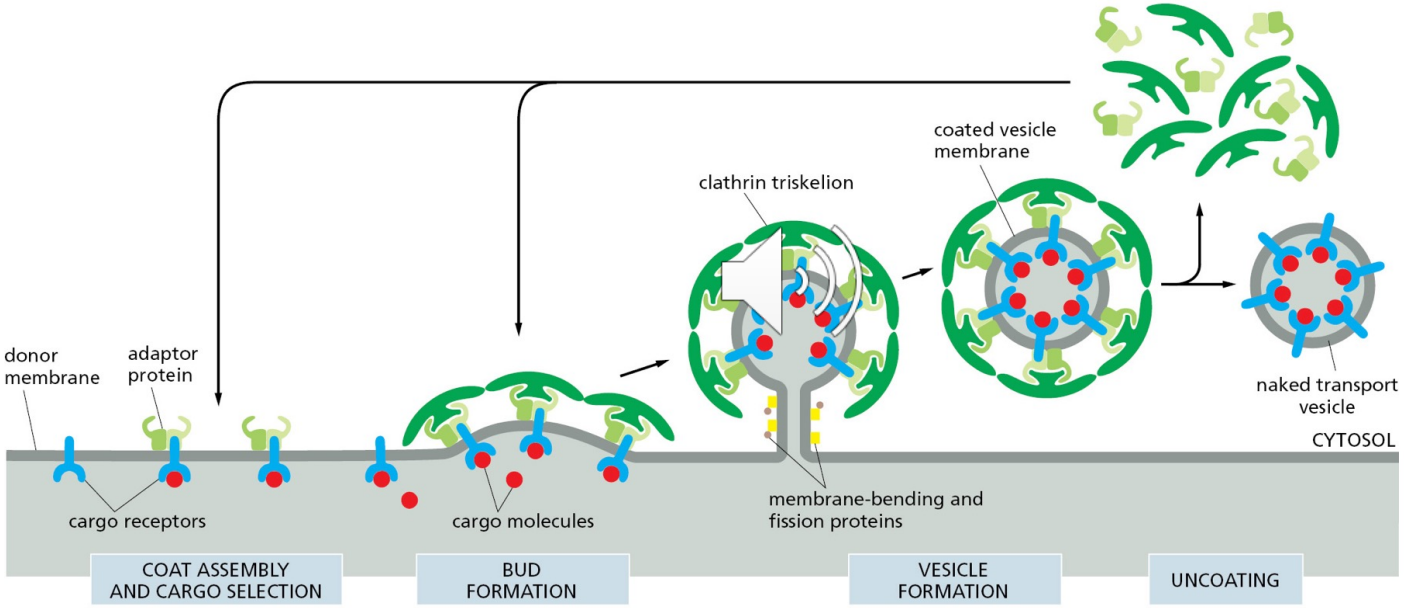


Figure 13–8 The assembly and disassembly of a clathrin coat. The

1. **Adaptins** bind **cargo receptor** and mediate binding to **clathrin**
2. Formation of vesicle and budding
3. Removal of clathrin and interaction with vesicle and destination

Vesicle targeting

- The vesicle needs to find its correct location
- Specificity is ensured by surface makers on the vesicle
- Two types of markers act sequentially to ensure the specificity of vesicle targeting

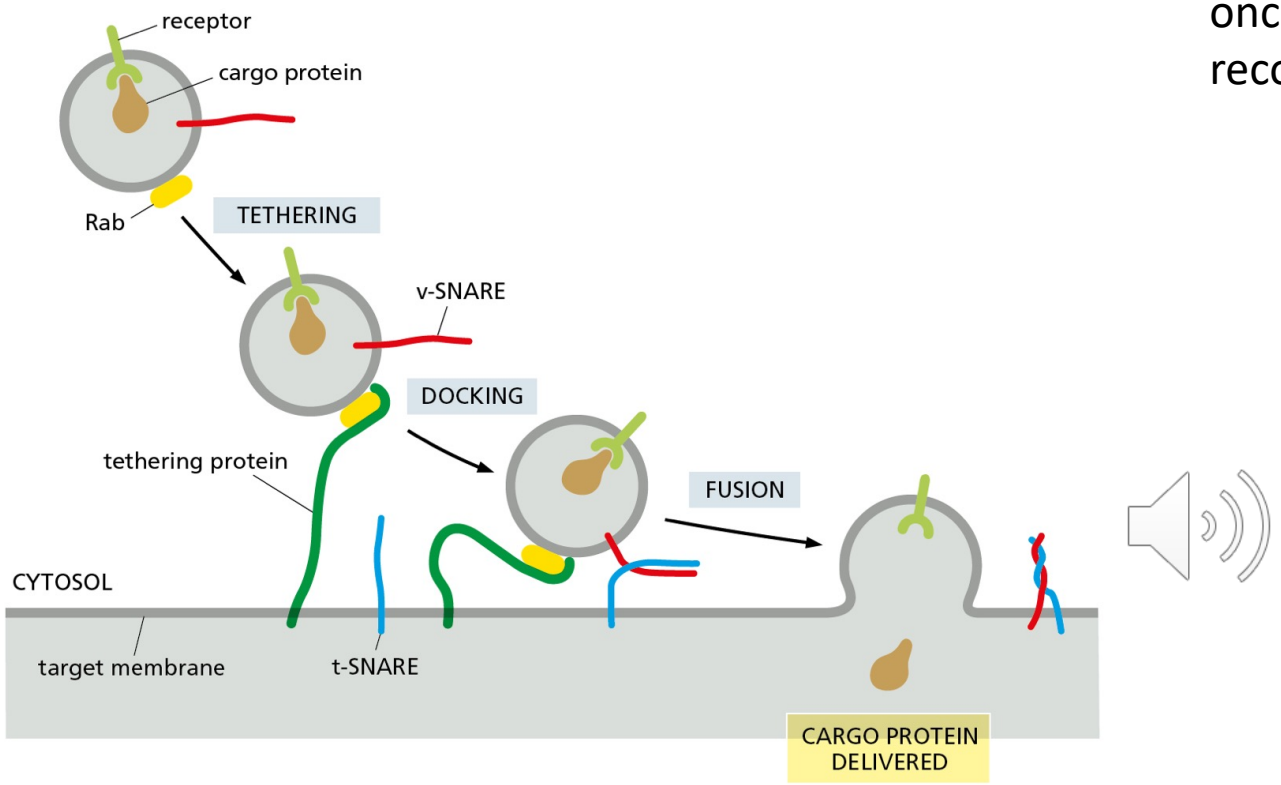
1) **Rab protein**
   (direct the vesicle to specific spots on the correct target membrane)
2) **SNARE proteins.**
   (enables fusion with lipid bilayer)

TABLE 13–1 Subcellular Locations of Some Rab Proteins

Protein	Organelle
Rab1	ER and Golgi complex
Rab2	*cis* Golgi network
Rab3A	Synaptic vesicles, secretory vesicles
Rab4/Rab11	Recycling endosomes
Rab5	Early endosomes, plasma membrane, clathrin-coated vesicles
Rab6	Medial and *trans* Golgi cisternae
Rab7	Late endosomes
Rab8	Cilia
Rab9	Late endosomes, *trans* Golgi network

Vesicle docking

once the vesicle reaches target it must be recognized at destination site and docked

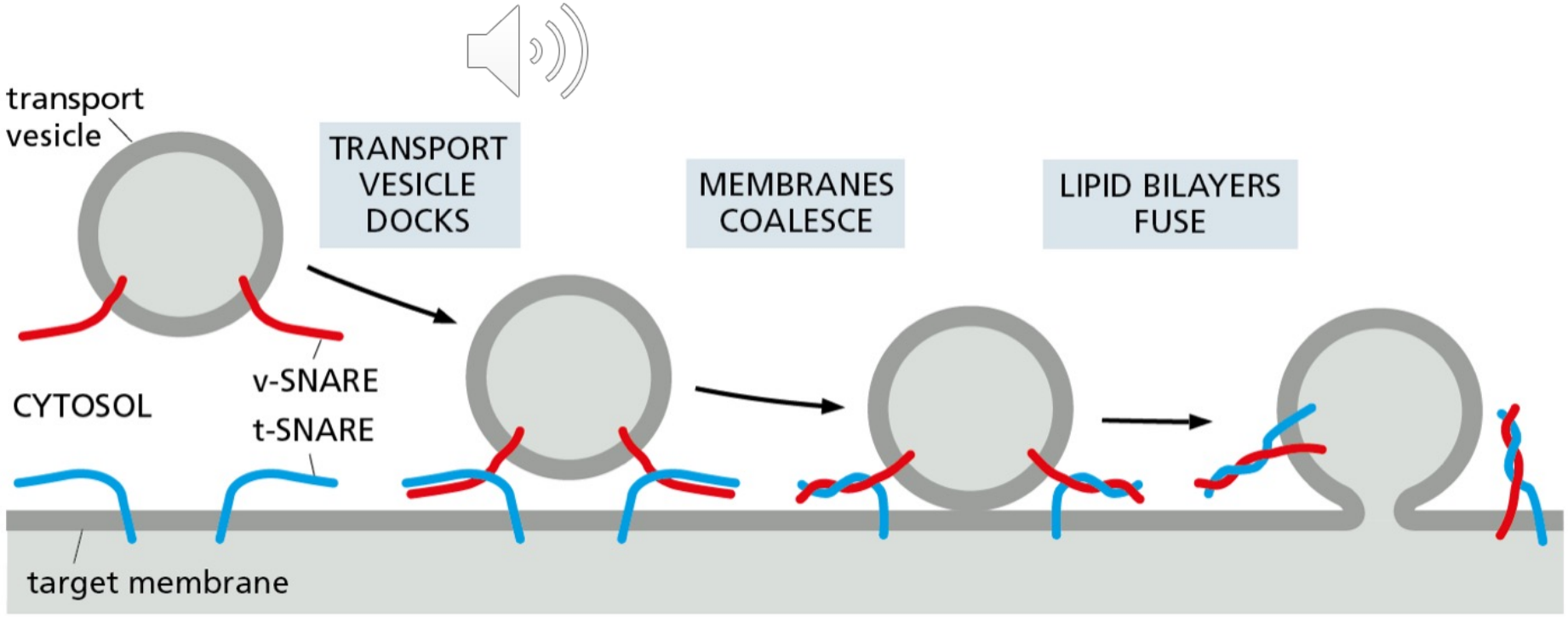


Rab proteins (there are many types) on the surface of vesicle is recognition by **tethering proteins** which captures a vesicle by grabbing hold of its Rab protein

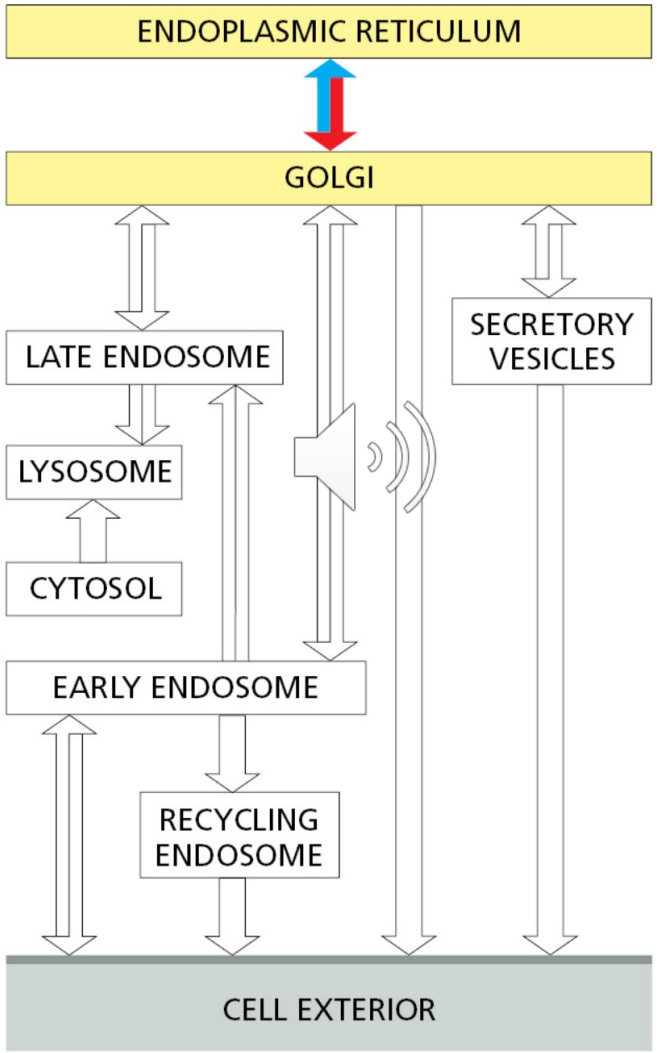
Then, **SNAREs** on the vesicle (v-SNAREs) interact with SNAREs on the target membrane (t-SNAREs). Docking is complete

SNAREs Mediate Membrane Fusion

- SNAREs mediate membrane fusion
- 35 different SNAREs, each associated with a different organelle
- **v-SNARE** (on vesicle) and **t-SNARES** (on destination) act together called a *trans-SNARE complex*
- Membrane fusion requires bringing the lipid bilayers of two membranes to within 1.5 nm of each other so that they can merge. When the membranes are in such close proximity, lipids can flow from one bilayer to the other.
- Water gets displaced

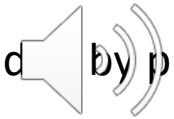


Road map of vesicular transport



Exit from ER is controlled to ensure protein quality

- Proteins stay in ER if they have **ER retention** signal
- Most proteins leave ER but they have be **folded properly**
- Chaperon proteins help to fold misfolded proteins
- If they don’t succeed, the misfolded protein is degraded by proteosome in the cytoplasm



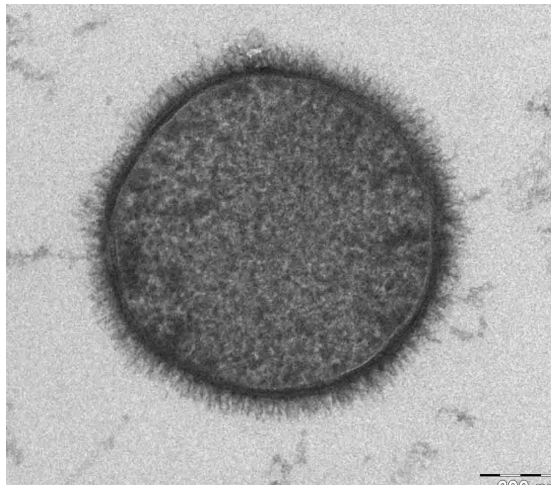
Most proteins are modified in the ER

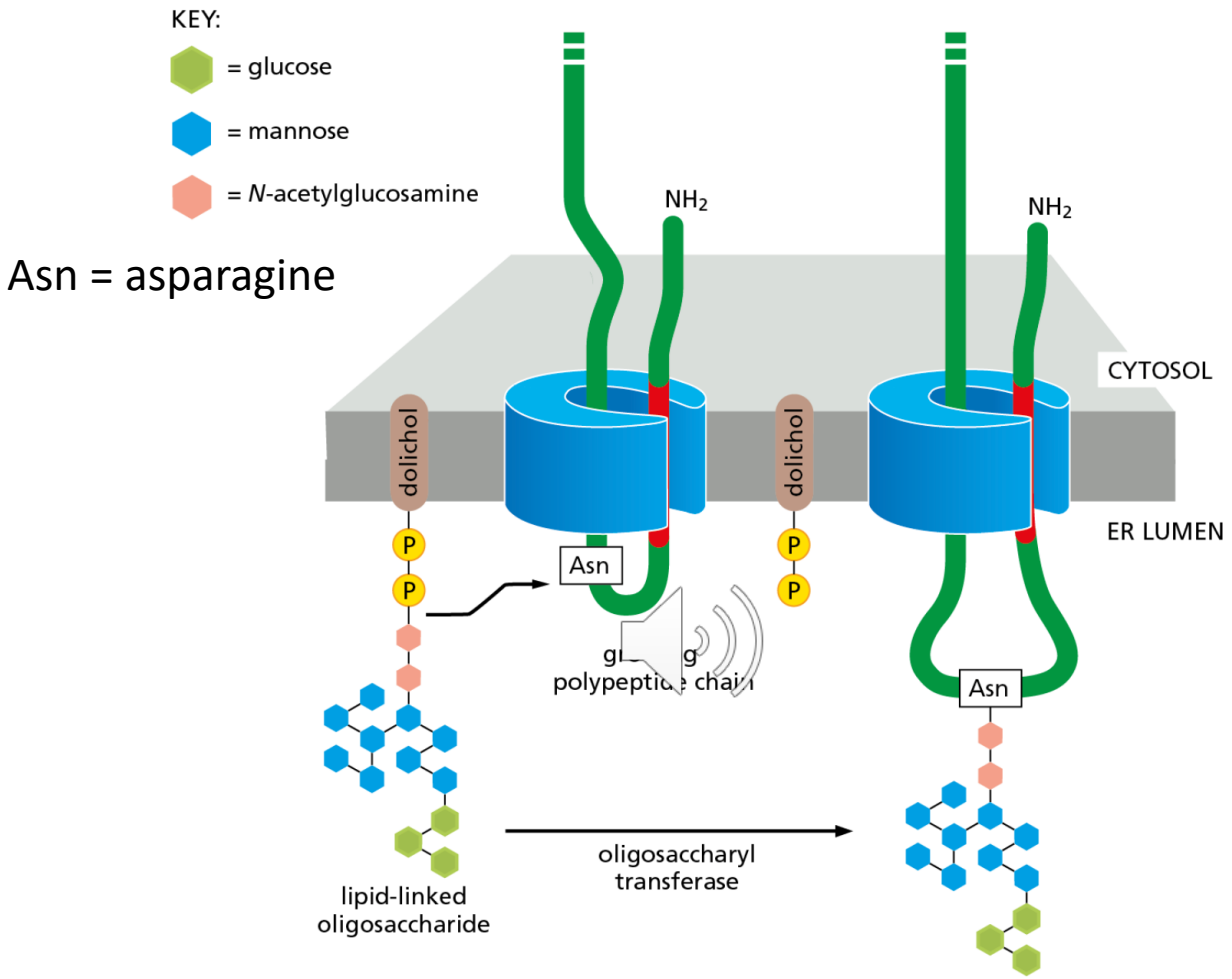
many proteins are **glycosylated** (addition of sugars or oligosaccharides)
Proteins are glycosylated to: protect from degradation, help with correct folding, transport

Glycosylating enzymes are found in the ER but not in the cytoplasm

glycocalax

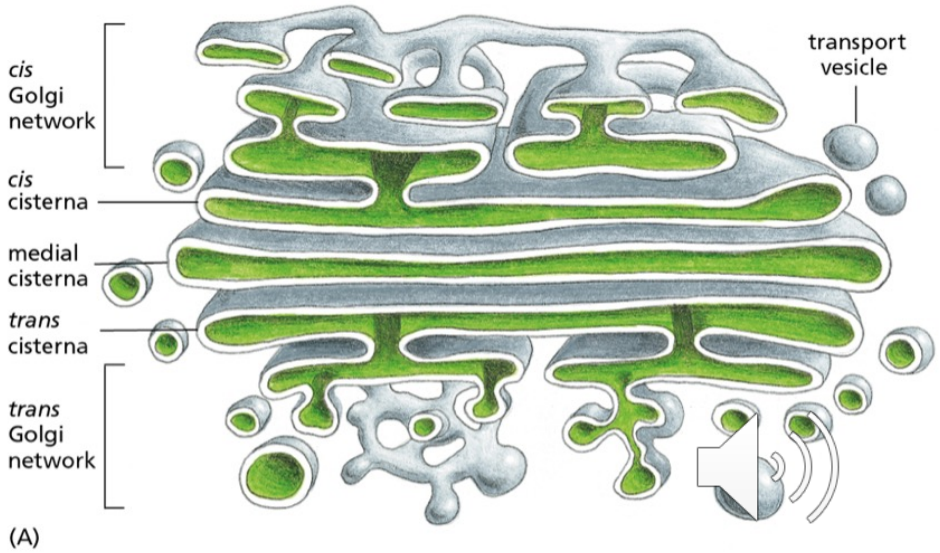
Helps cells recognize each other

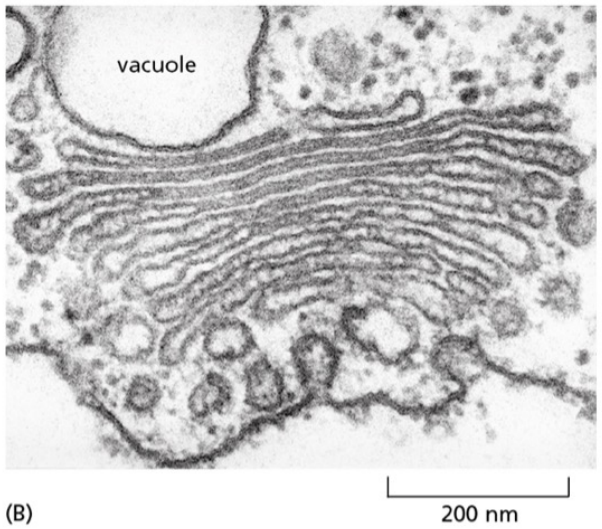




- 14 sugars are added simultaneously to a protein with glycosylation site
- The oligosaccharide is added to dolichol, a lipid, in ER membrane
- Then its added to the protein at an Asn site (asparagine)
- Process is catalyzed by oligosccharyl transferase (only found in ER lumen)
- This whole process is called **N-linked** glycosylation

Proteins are further modified and sorted in the Golgi





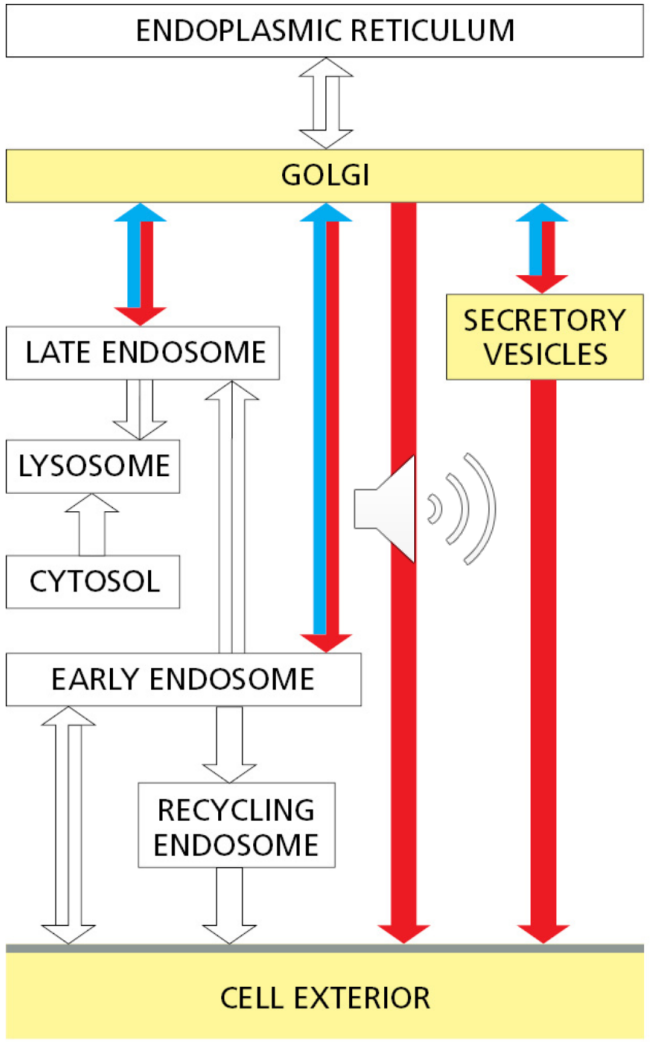

(C)

Cis – entry, closest to ER
Trans – exit, closes to plasma membrane

Proteins travel in cisterna by
(1) Transport vesicles that bud from one cisterna and fuse with another
(2) By maturation process cisterna migrate through golgi stacks.

Proteins are further modified in Golgi by adding more sugars

Transport from the Golgi apparatus



- 3 paths of delivery from the Golgi
  1) To lysosome via the endosome
  2) secretory vesicle
  3) To plasma membrane

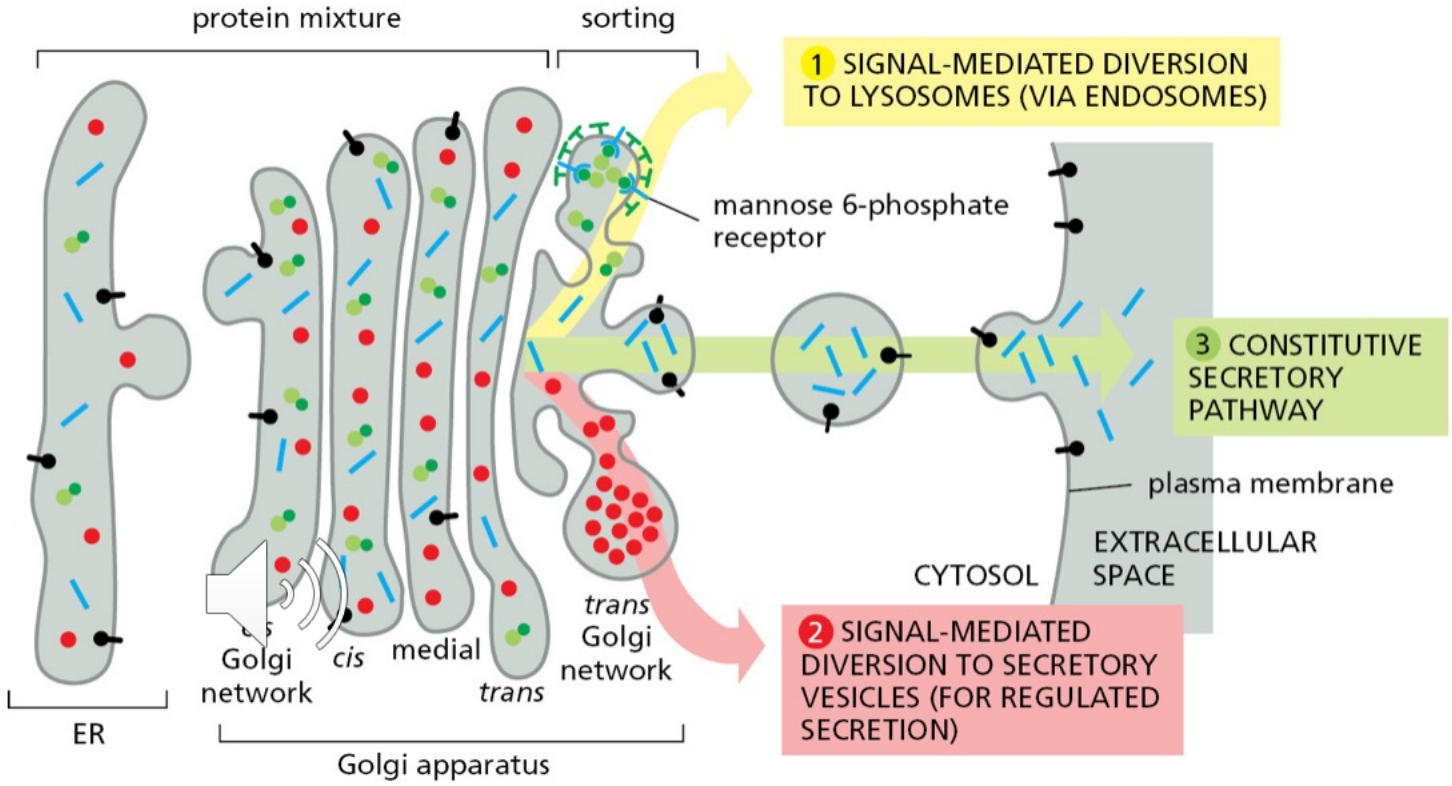
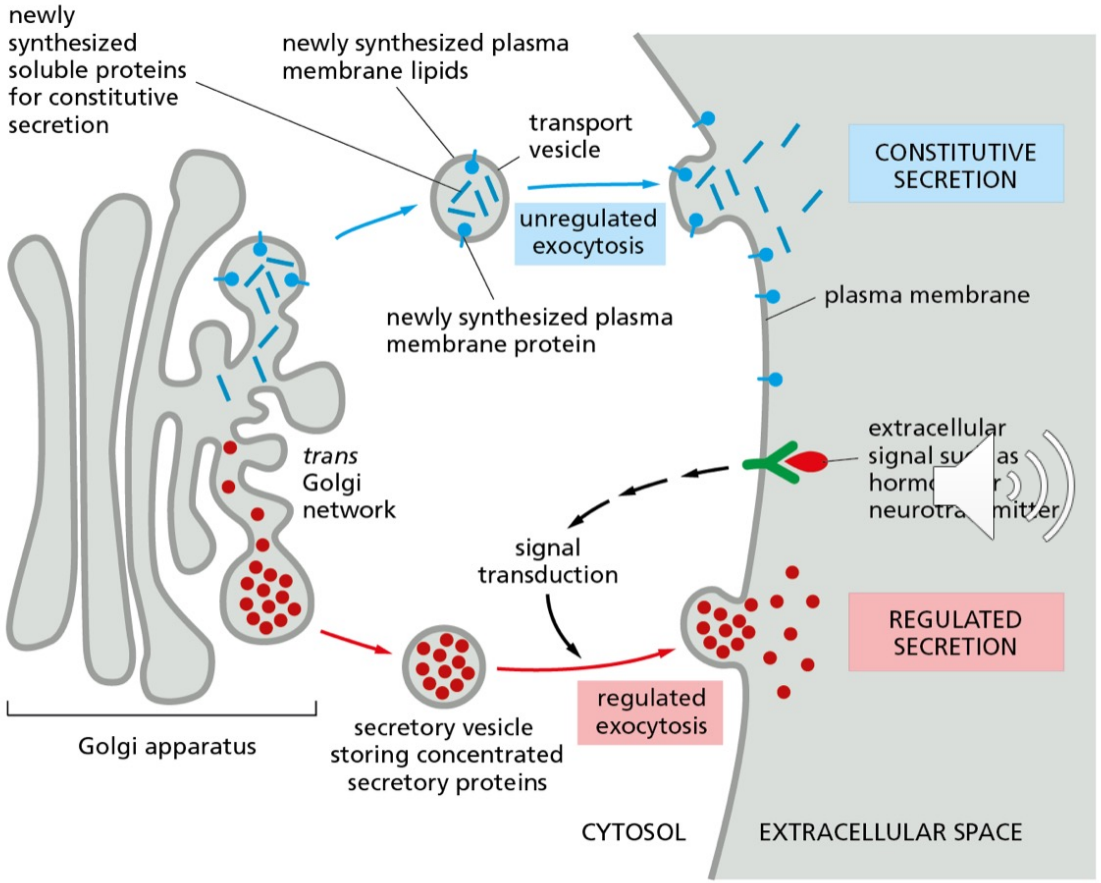


Figure 13–38 The three best-understood pathways of protein sorting in the *trans* Golgi network. (1) Proteins with the mannose 6-phosphate (M6P) marker



Trans Golgi pathway

Proteins aggregated due to surface proteins.

Aggregation causes increased protein concentration.

When signal from outside the cell comes in, then the secretion occurs

Secretory proteins are released by exocytosis

Constitutive Exocytosis pathway supplies the plasma membrane with newly made lipids and proteins.

Plasma membrane needs new material in order to divide.

Constitutive exocytosis also **secrets** proteins to the outside of the cells

Works in all cells

Regulatory Exocytosis pathway – only active in secreting cells.
Ex are hormone, mucus release **in secretory vesicles.**

Proteins in secretory vesicles form **aggregates** and are stored in vesicles until needed. I.e., signal from outside cell informs when to secrete material.

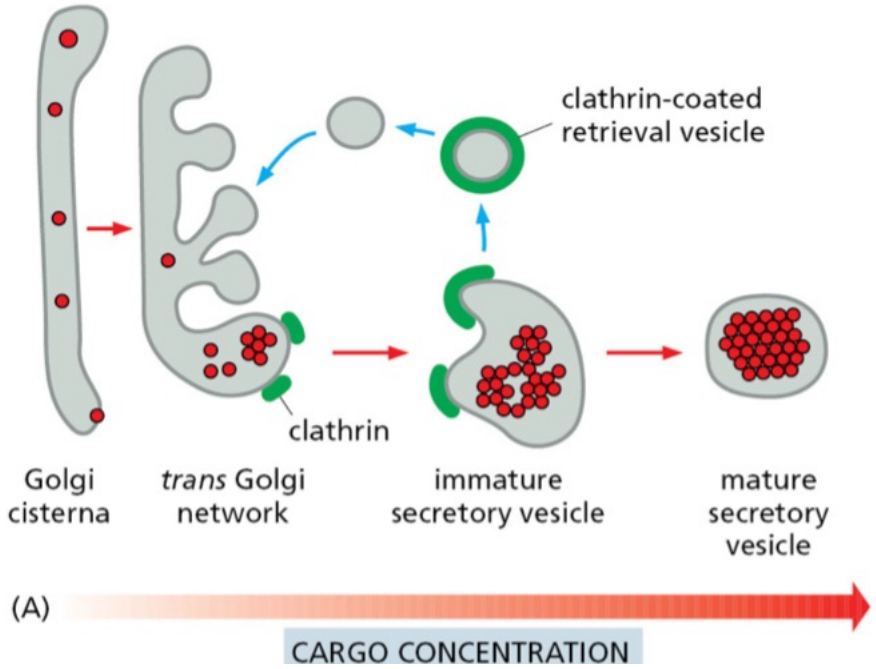
Protein aggregation also increases protein concentration

Secretory Vesicles Bud from the *Trans* Golgi Network

- Cells that are specialized for secreting some of their products rapidly and on demand, concentrate and store these products in **secretory vesicles.**
- Release the vesicle content to cell exterior by **exocytosis**
- Hormones, enzymes, histamines

- As new secretory vesicles are formed they are called *immature secretory vesicles*. Part of the Vesicle goes back to the Golgi to recycle Golgi Material back the Golgi but also to increase the Concentration of secreted material

- Immature secretory vesicles also fuse with one another



Release of insulin in pancreatic cells

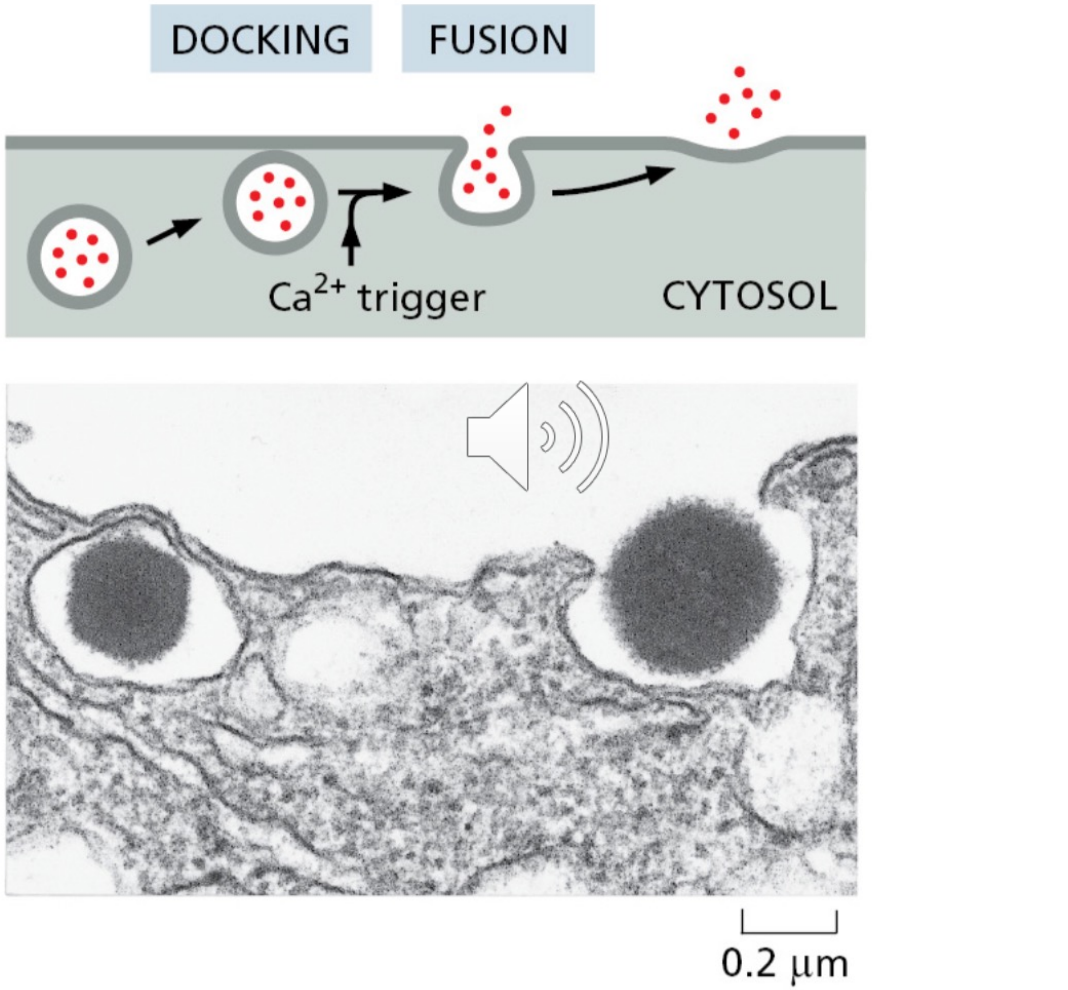


Figure 13–43 Exocytosis of secretory vesicles. The process is