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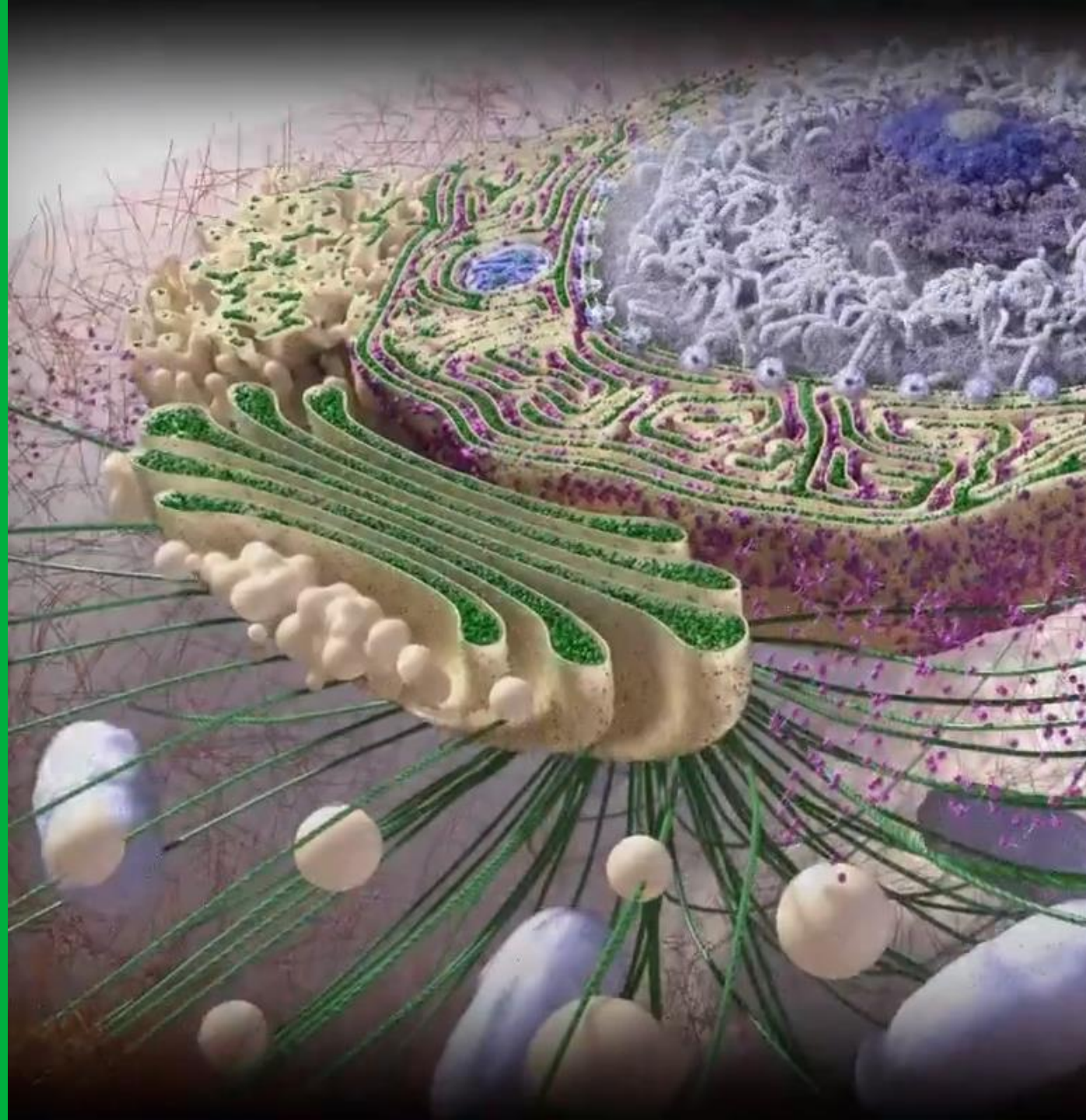
Intracellular transport

RNDr. Jan Škoda, Ph.D.
Department of Experimental Biology



Outline

- Important aspects of comparmentalization
- Protein sorting
- Protein translocation into nucleus, mitochondria/plastids, ER
- Vesicular transport
- Secretory and endocytic pathways
- Intercellular vesicular transport

Aspects of compartmentalization

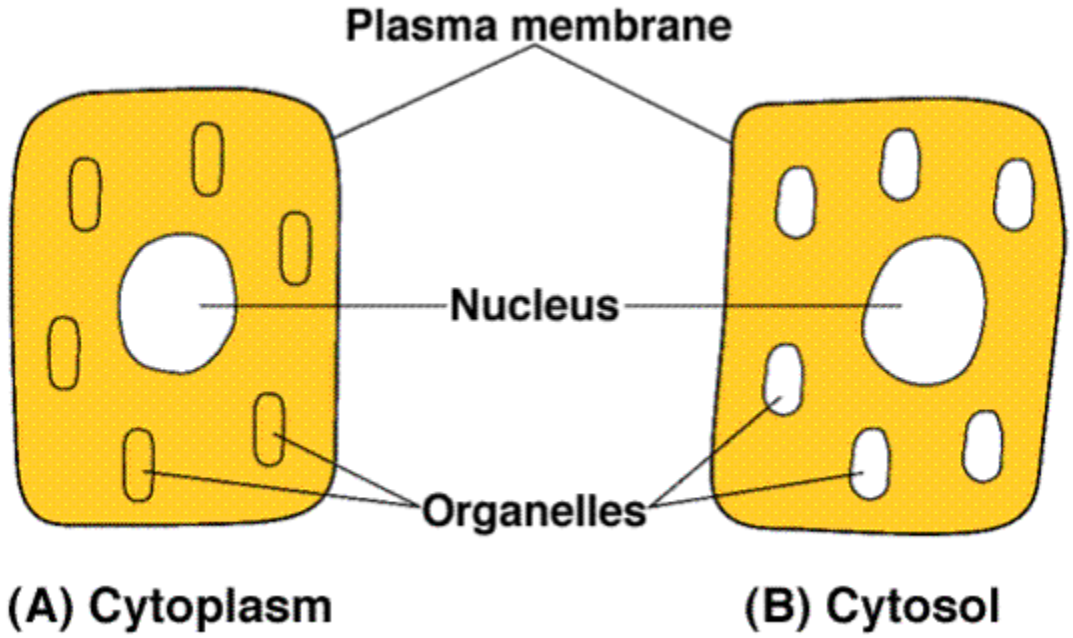
Cytosol vs. cytoplasm

Cytosol

- the fluid contained in the cell without membrane organelles

Cytoplasm

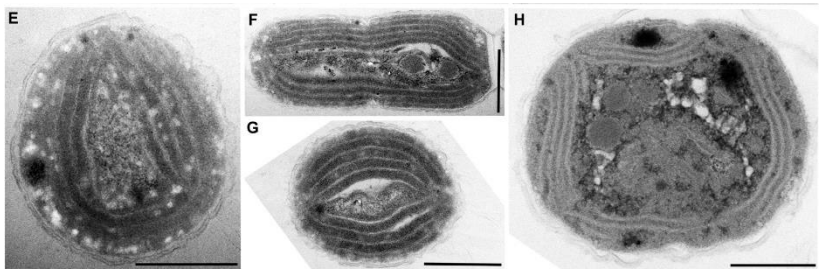
- Cytosol + membrane organelles without the nucleus



Prokaryotic vs eukaryotic cells

Prokaryotic cells

- Most cytosol only (including nucleoid)
- Cyanobacteria – thylakoids



Eukaryotic cells

- Plant cells – usually very limited cytosol (large vacuole)
- Cytoplasm full of membrane organelles

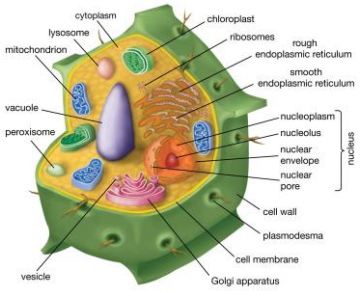
Compartmentalization: Pros & Cons

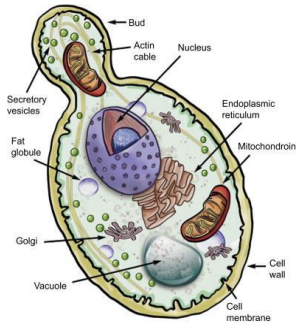
PROS

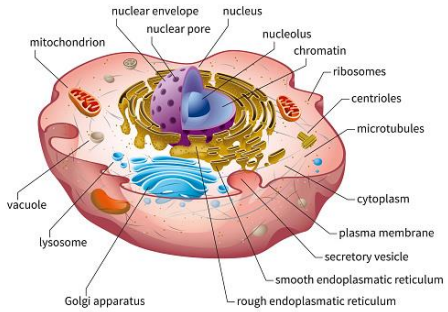
- **Separation** of biochemically incompatible processes or potentially harmful processes (degradation, oxidation)
- **Optimized efficiency** – chemically optimal environment (prevents substrate diffusion; different concentration of molecules, e.g., ions; pH)

CONS (REQUIREMENTS)

- **Coordination** of organelles: biosynthesis, anabolic and catabolic processes (signaling pathways)
- **Transport of molecules between organelles** (substrates, precursors, metabolites)
- **Precise sorting** of proteins and other molecules







Animal cell	Plant cell	Fungal cell
Plasma membrane	Plasma membrane	Plasma membrane
Glycocalyx	Cell wall	Cell wall
Nucleus	Nucleus	Nucleus
Endoplasmic reticulum	Endoplasmic reticulum	Endoplasmic reticulum
Golgi apparatus	Golgi apparatus	Golgi apparatus
Lysosomes	Vacuoles	Vacuoles
Peroxisomes	Peroxisomes	Peroxisomes
-	Glyoxysomes	Glyoxysomes
Mitochondria	Mitochondria	Mitochondria
-	Chloroplasts	-

Borders of the cell, interaction with its surrounding environment	*Catabolism*
Storage and expression of the genetic information, anabolism	*Energy metabolism, apoptosis*

Brief overview of cellular compartments and their function

- **Cytosol**: protein synthesis, signaling and metabolic pathways
- **Nucleus**: genome, DNA and RNA synthesis
- **Endoplasmic reticulum (ER)**: lipid synthesis, synthesis of secreted and integral membrane proteins, Ca^{2+} regulation
- **Golgi apparatus**: posttranslational modification of proteins, modifications of lipids, cargo sorting to the secretory pathway
- **Mitochondrion**: ATP synthesis (OXPHOS)
- **Chloroplasts**: ATP synthesis and carbon fixation (photosynthesis)
- **Lysosomes/vacuoles**: degradation of molecules / organelles (autophagy), turgor in plants/fungi
- **Peroxisomes, glyoxysomes**: oxidation of long chain fatty acids (β-oxidation), detoxification of various harmful compounds
- **Membrane vesicles**: transport of cargo between organelles/to PM, endosomes, exosomes…

Protein sorting

Protein sorting in eukaryotic cells

- **Protein synthesis occurs at ribosomes in the cytosol**
  - **Exceptions:** mitochondrial DNA- and chloroplast DNA-encoded proteins (own ribosomes)
  - (Nuclear translation under specific stress conditions?)

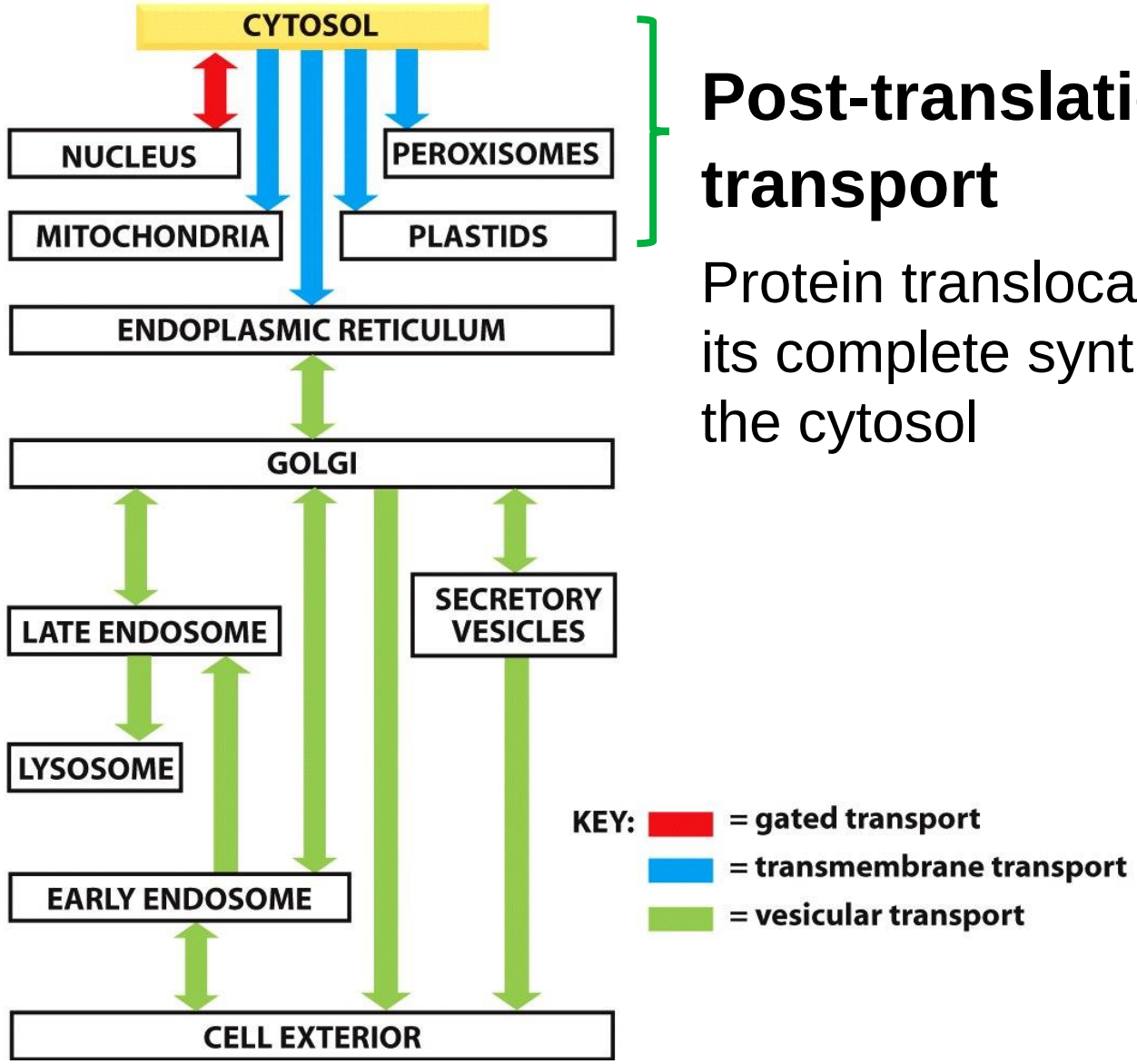
Sorting into specific compartments:

- **Gated transport**: cytosol–nucleus, through nuclear pore complexes
- **Transmembrane transport**: ER, mitochondria, chloroplasts, peroxisomes
- **Vesicular transport**: ER, Golgi apparatus, endosomes, lysosomes, secretory vesicles, plasma membrane, cell exterior

Overview of protein sorting

Co-translational transport

Protein translocated during synthesis at ER



Post-translational transport

Protein translocated after its complete synthesis in the cytosol

Targeting proteins into compartments

- **Signal sequence** (signal peptide)
  - 15-60 amino acids long sequence
  - Crucial for **targeting to specific organelles/compartments**
  - Different classes of signal sequences – organelle-specific

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^{-}

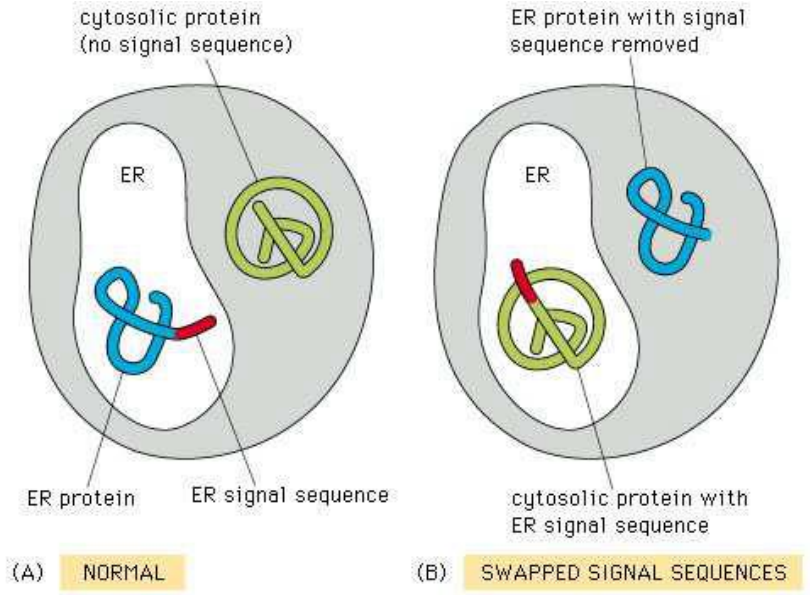
Some characteristic features of the different classes of signal sequences are highlighted in color. Where they are known to be important for the function of the signal sequence, positively charged amino acids are shown in *red* and negatively charged amino acids are shown in *green*. Similarly, important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

Targeting proteins into compartments

- **Signal sequence** (signal peptide)
  - Often at N-terminus: cleaved by **signal peptidase** once sorting is complete
  - Internal signal sequences (e.g., nuclear localization sequence)

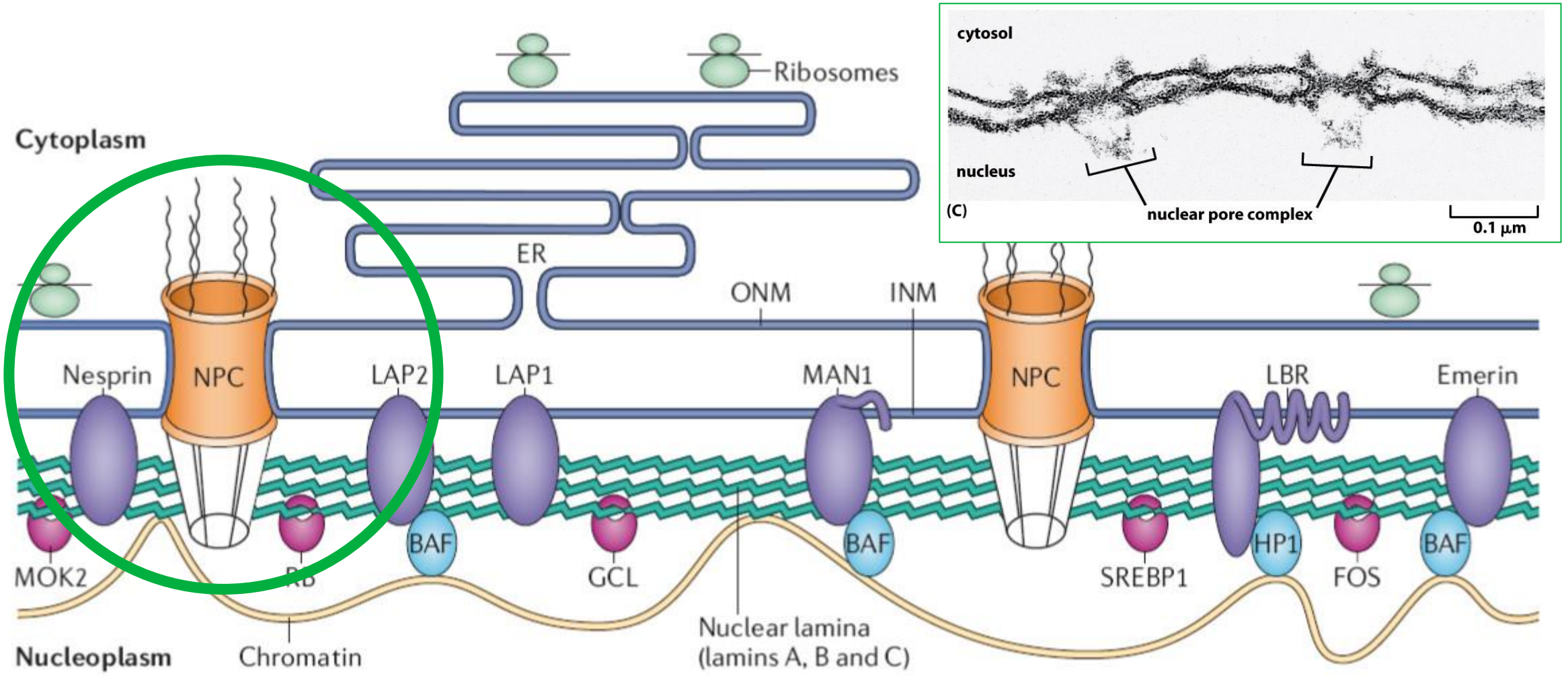
  - **Protein without a signal sequence remains in the cytosol**

  - Genetic engineering – introducing/manipulating the signal sequence: targeting proteins to specific compartments

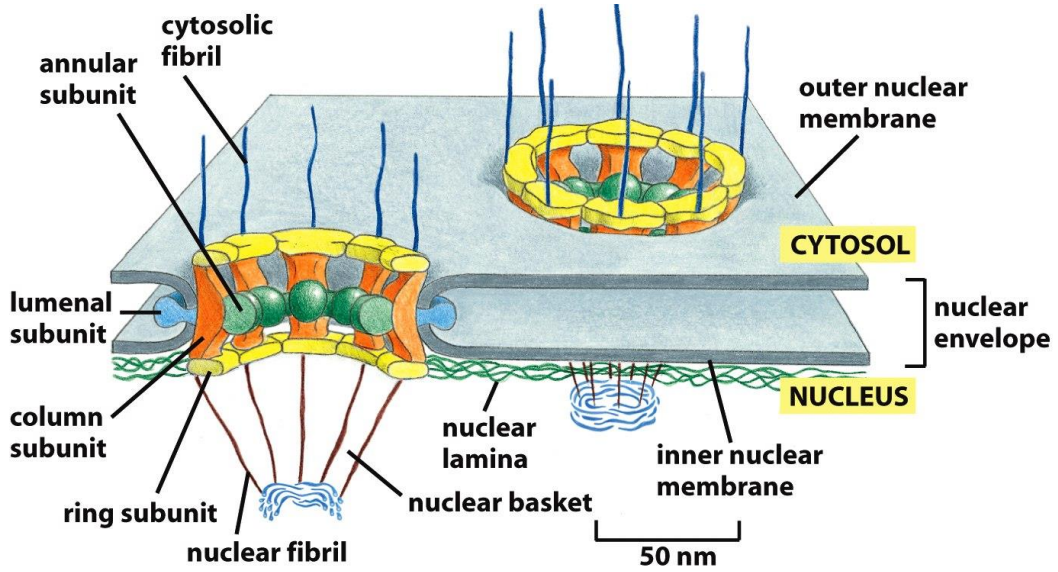


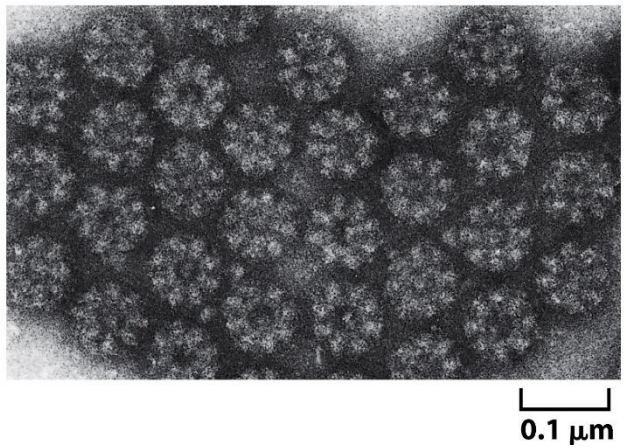
Protein translocation into nucleus

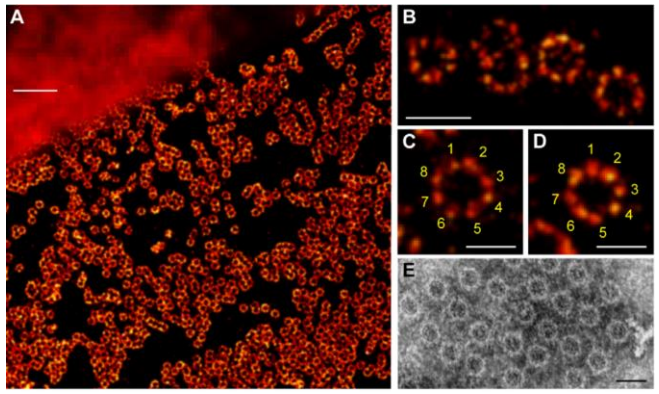
Gated transport: nucleus↔cytosol



Nuclear pore complexes (NPCs)



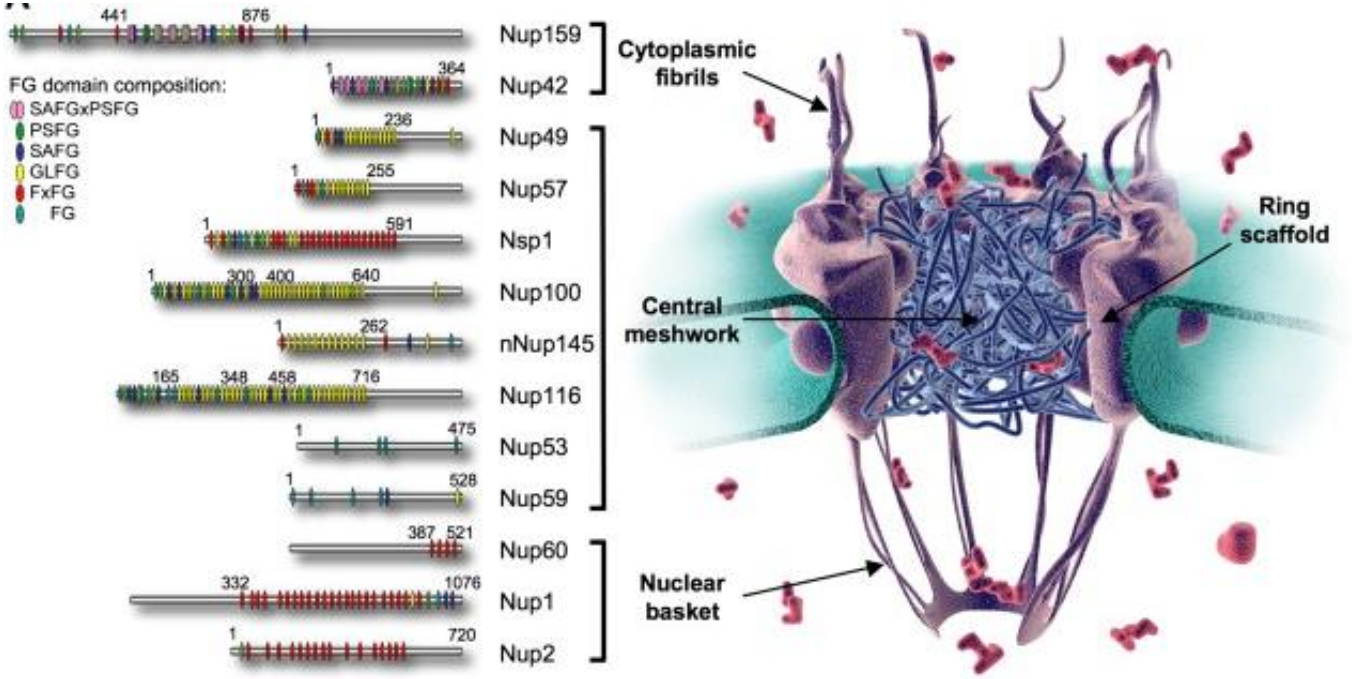




- **8-fold structure**, ~30 different proteins, **FG-repeats** (phenyalanin, glycin rich sequences)
- Small molecules (<5 kDa) diffuse freely
- **Macromolecules use nuclear import/export receptors – karyopherins**
  - Import: nuclear proteins, transcription factors, enzymes, etc.
  - Export: RNA, ribosomal subunits

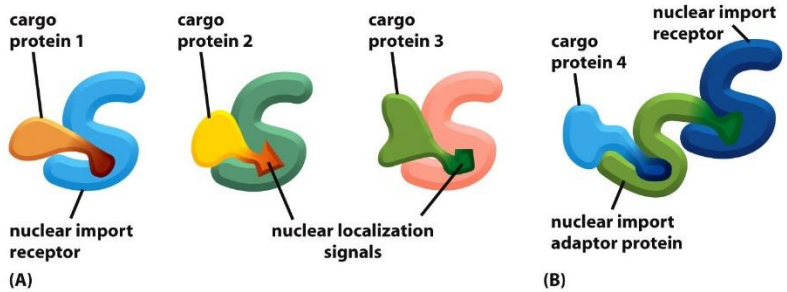
FG-repeats in NPC proteins

- Phenyalanin and glycin rich sequences
- Important for transport: binding of karyopherins



Import via nuclear pore complexes

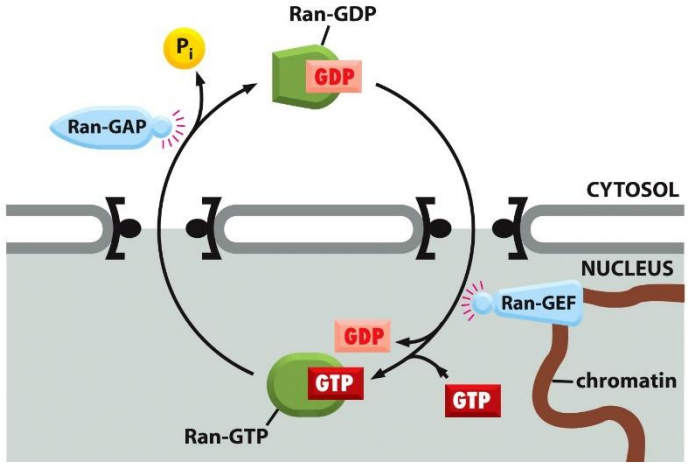
- Protein (large macromolecules) must contain **nuclear localization sequence (NLS)**
- **Importins –** karyopherins mediating nuclear import
  - **Bind NLS of the transported protein** (cargo protein)
  - **Bind to and move along FG-repeats of NPC proteins**



- **Importins transport cargo into the nucleus and return to cytosol**
- **Needs energy –** GTP hydrolysis by GTPase **Ran**

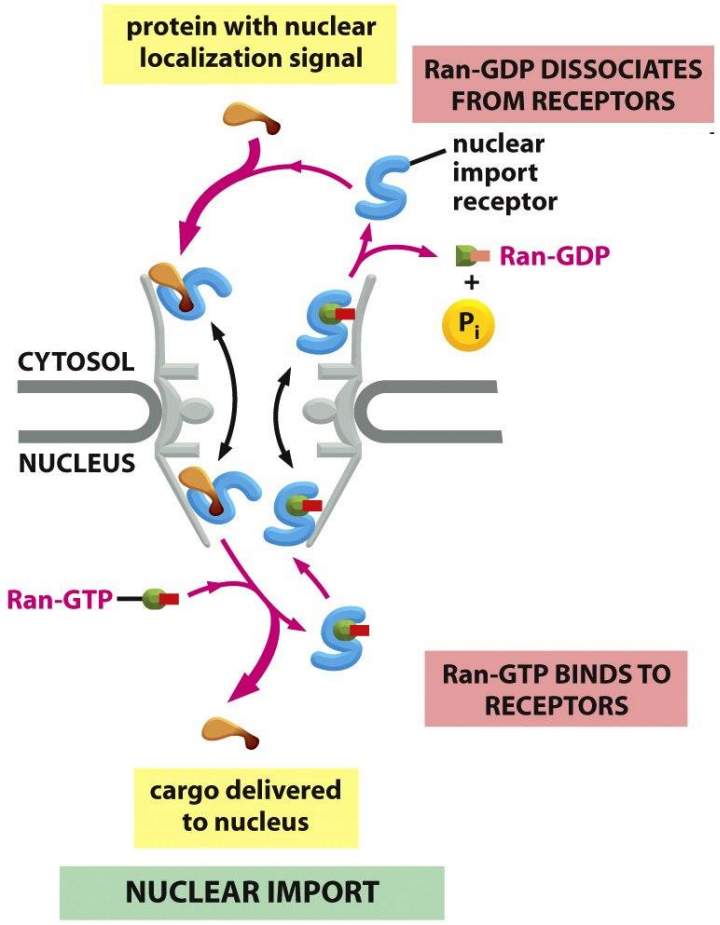
Ran-regulated transport through NPCs

- Ran GTPase in two states:
  Ran-GTP/Ran-GDP
- Ran-GTP/Ran-GDP regulated by:
  - Ran GTPase-activating protein – **Ran-GAP, cytosolic**
  - Ran guanine nucleotide exchange factor – **Ran-GEF, nuclear**



→ **Ran-GTP only in the nucleus**

→ **Cargo unloading**

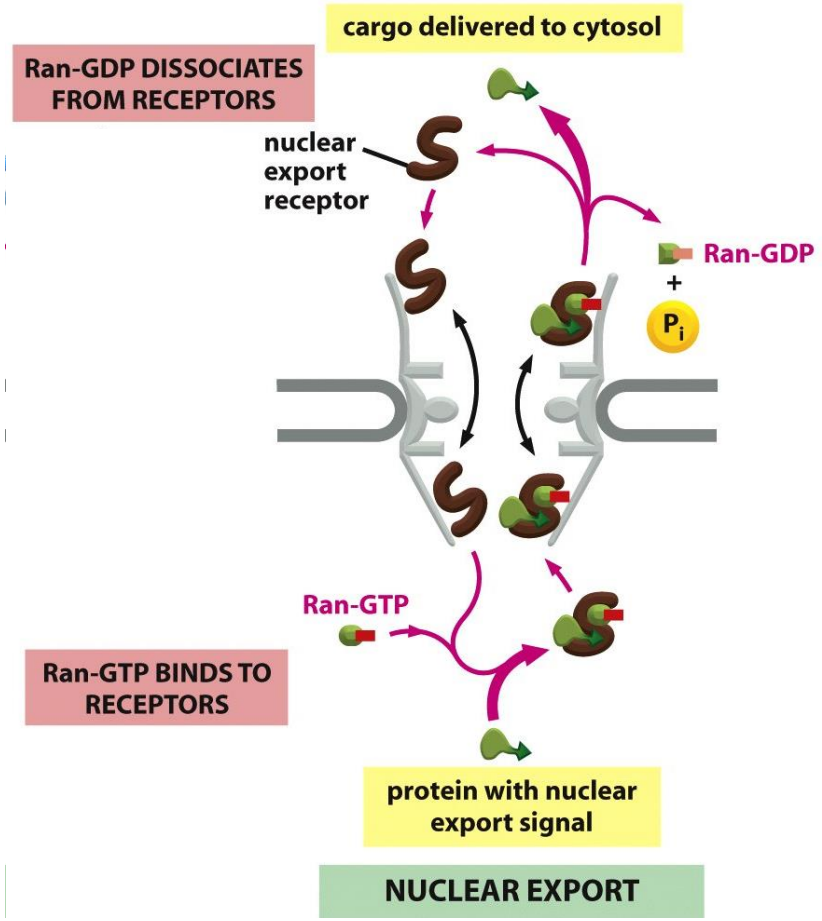


Nuclear export through NPCs

- rRNA, tRNA, mRNA transcribed in nucleus but active in the cytoplasm; +shuttling proteins

Reverse process to nuclear import:

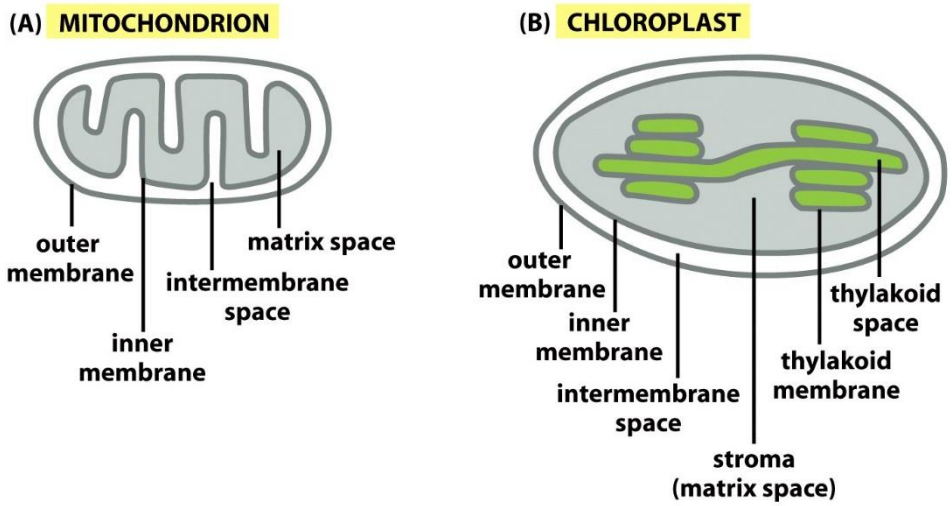
- Macromolecules must contain **nuclear export sequence (NES)**
- **Exportins** – karyopherins mediating nuclear export
- Needs energy for cargo release

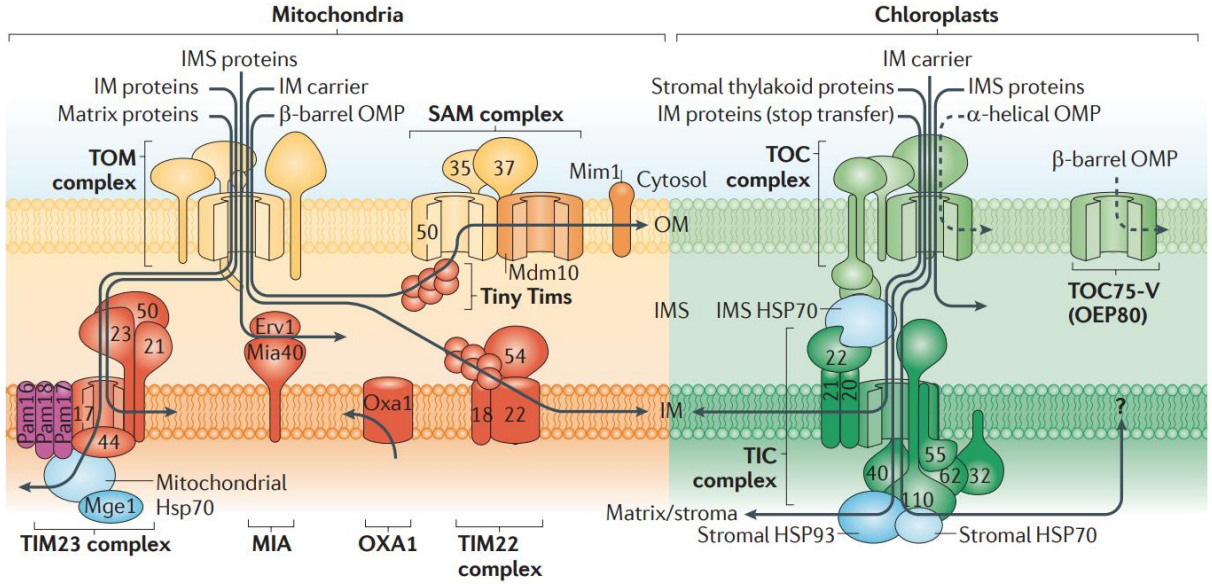


Protein translocation into mitochondria and plastids

Transport of proteins into mitochondria and chloroplasts

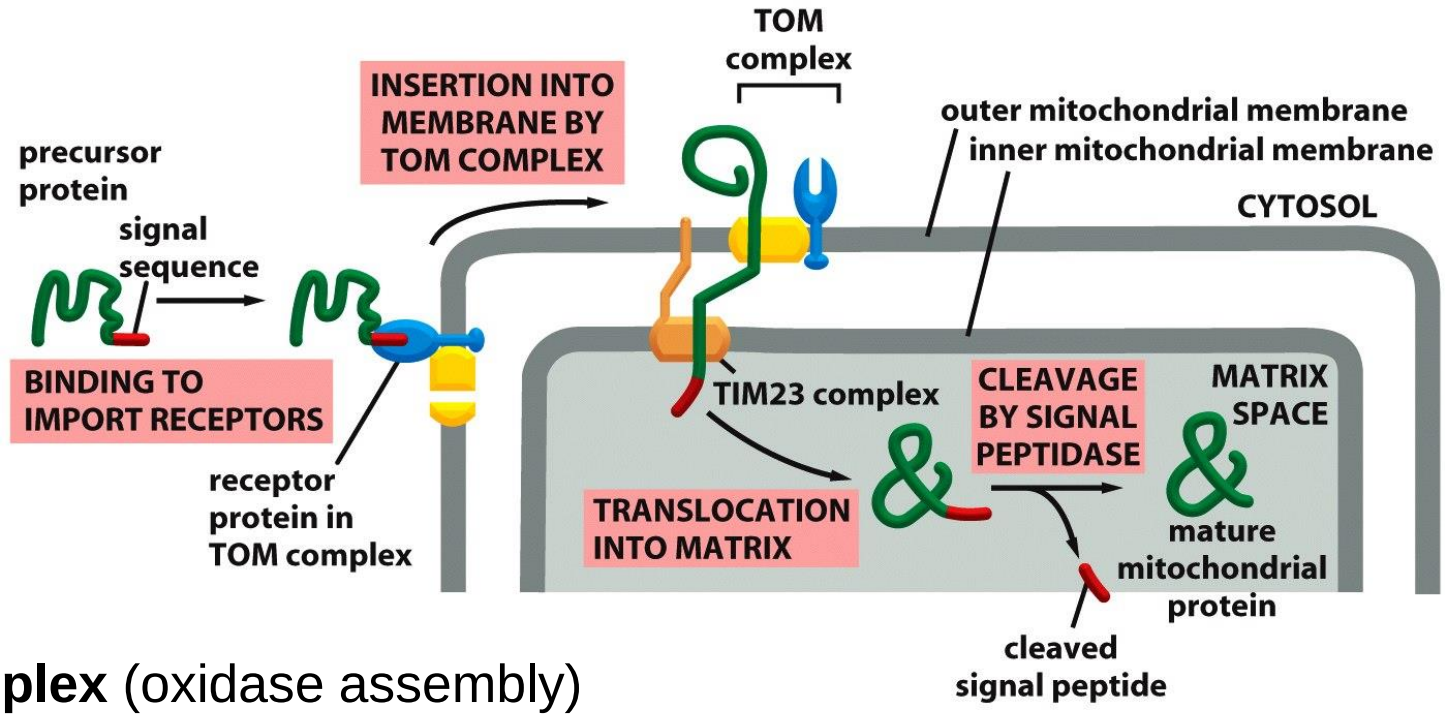
- **Protein translocators** at outer and inner membranes (+ thylakoid membrane in chloroplasts); **Signal sequence at N-terminus**



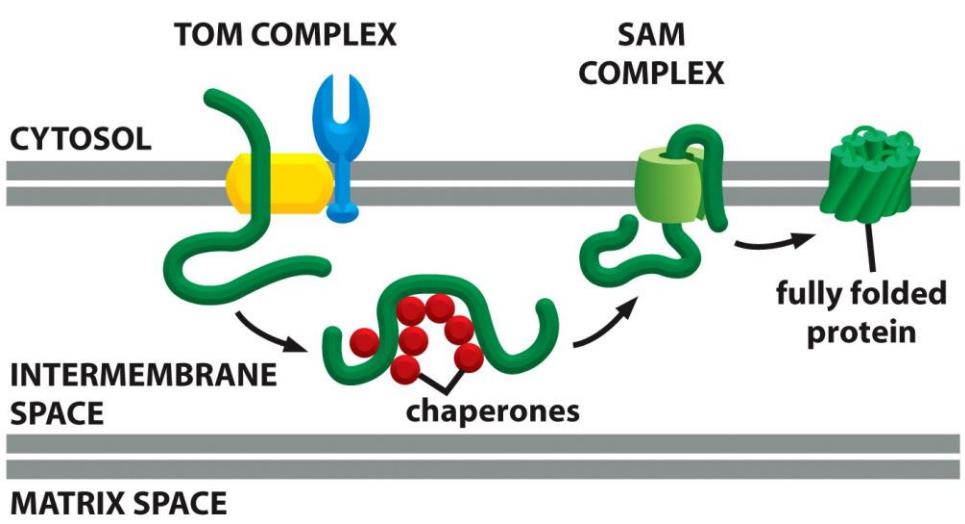


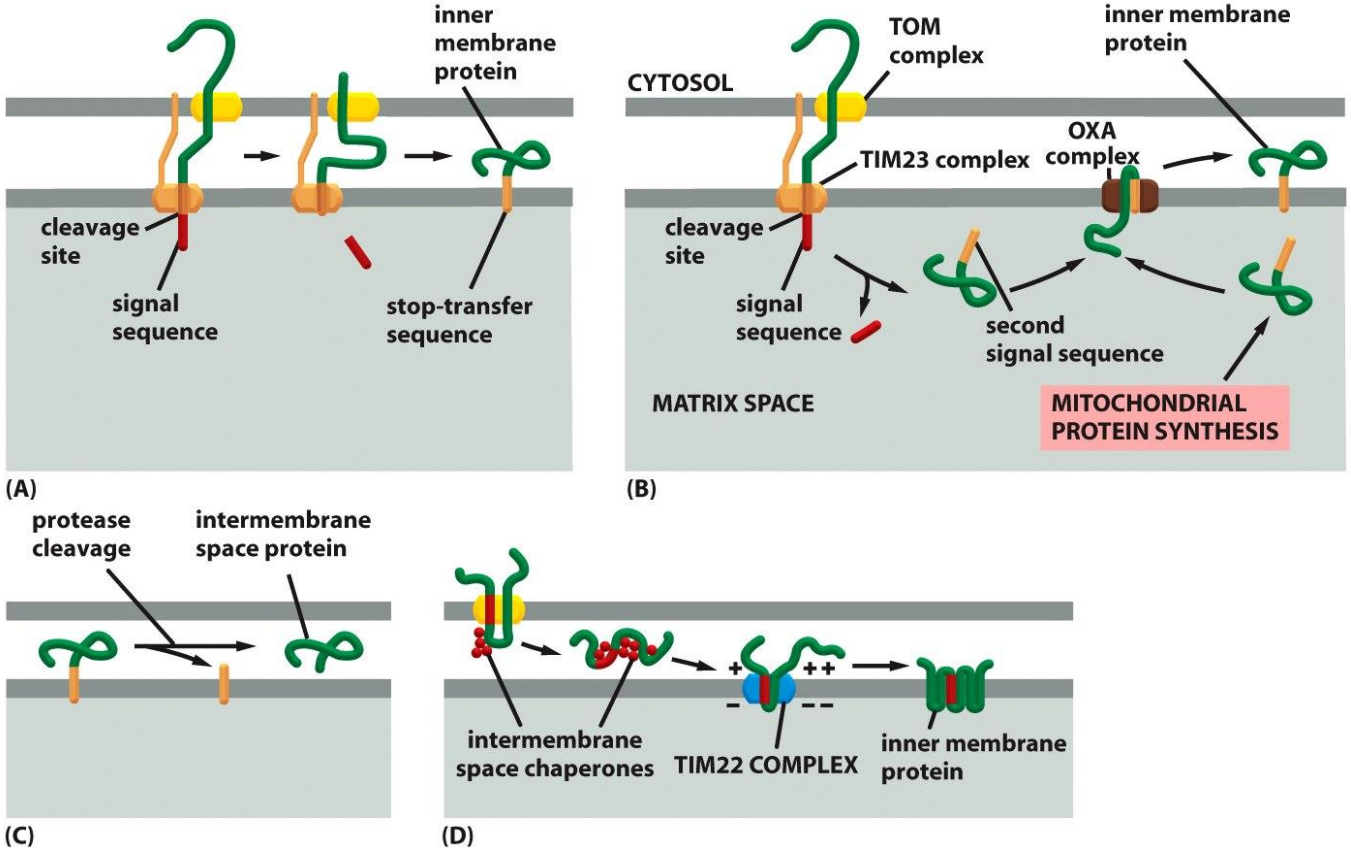
Protein import by mitochondria

- **Proteins transported as unfolded**
- Signal sequence is cleaved by **signal peptidase** after translocation
- Final conformation restored by **chaperons**
- **Insertion into the membrane**
  - Outer membrane: **SAM complex** (Sorting and assembly machinery)
  - Inner membrane: TIM or **OXA complex** (oxidase assembly)



Protein import by mitochondria





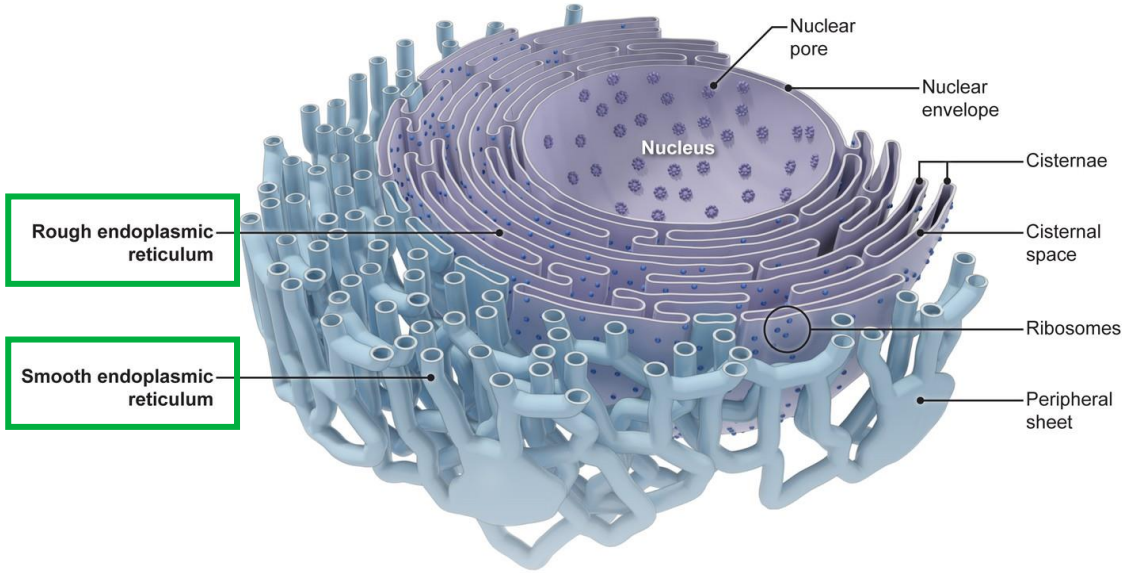
Protein translocation into ER

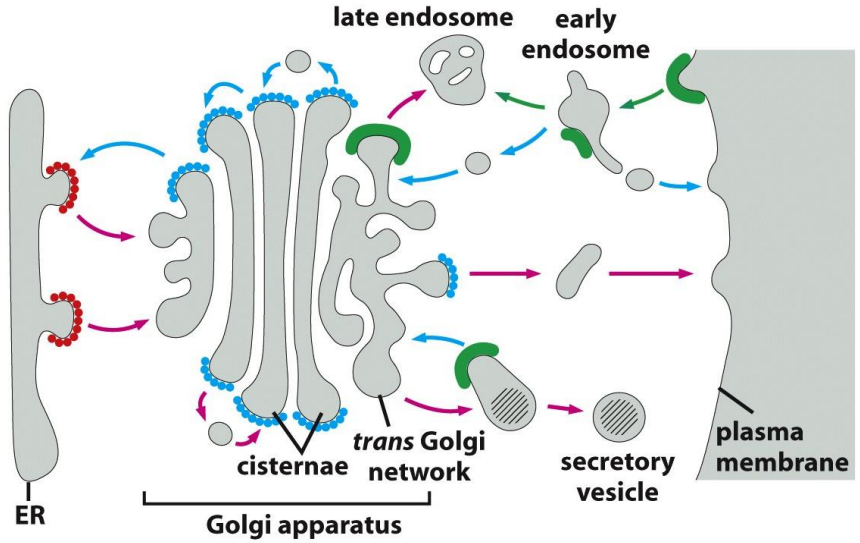
Endoplasmic reticulum

Rough ER

- **Ribosomes** at its surface: synthesis of secreted proteins and proteins for ER, Golgi, lysosomes, plasma membrane

- **Major hub of protein trafficking** (vesicular transport)





Protein translocation into ER

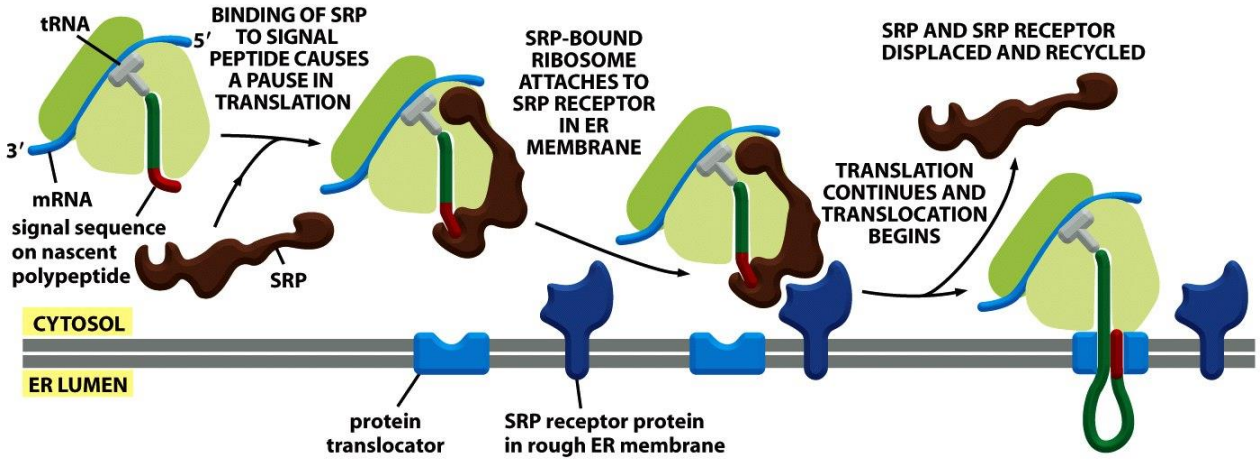
- **Co-translational translocation** – close ER–ribosome contact
- ER-specific **signal sequence** navigates attached ribosomes to ER

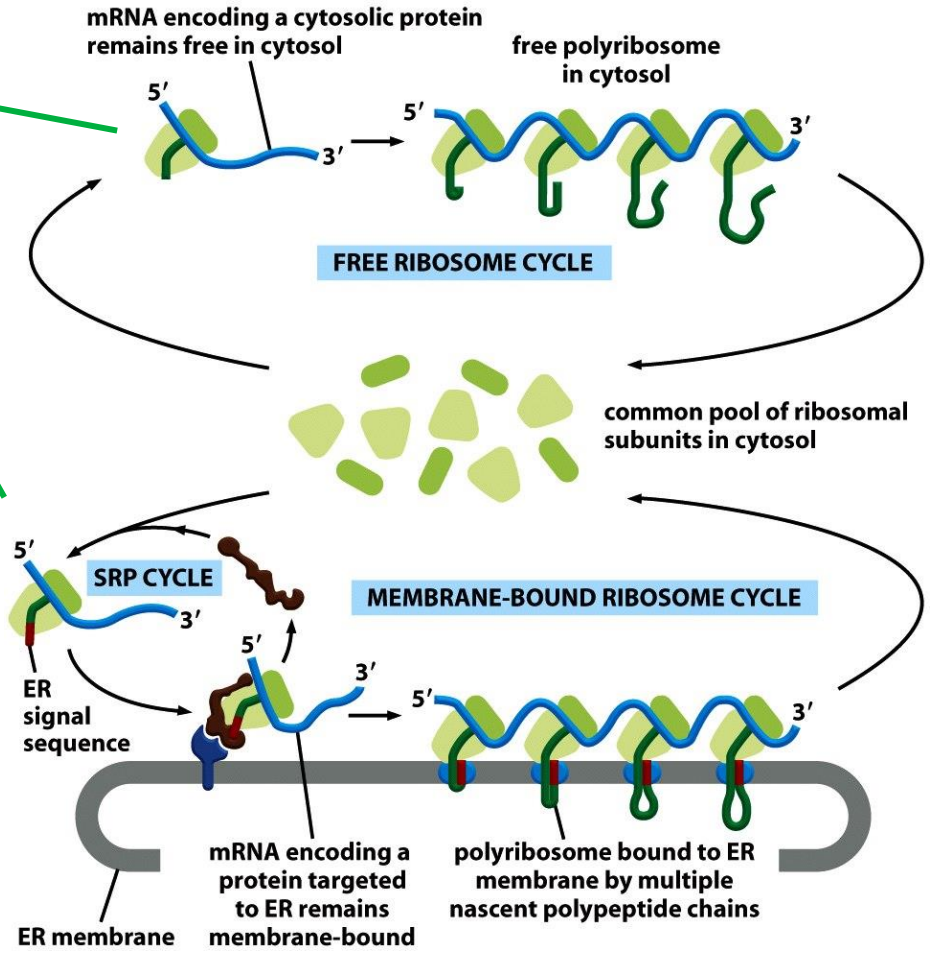
2 types of proteins:

- **Water-soluble proteins**
  - Fully translocated into the ER lumen; secretory proteins, proteins that function in the lumen of ER or other secretory pathway organelles
- **Transmembrane proteins**
  - Embedded in the membrane; some function in the ER membrane, most in other organelles, plasma membrane

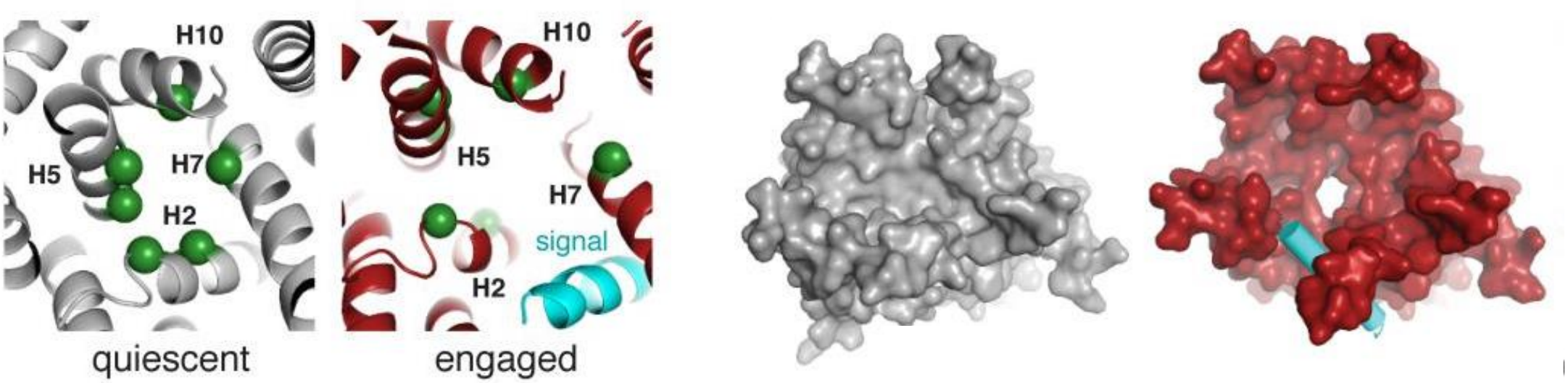
Rough ER – ribosomes directed by signal sequence

- ER and cytosolic ribosomes differ only by the protein they synthesize
- **SRP (signal-recognition particle)**
- **SRP receptor** in rough ER membrane



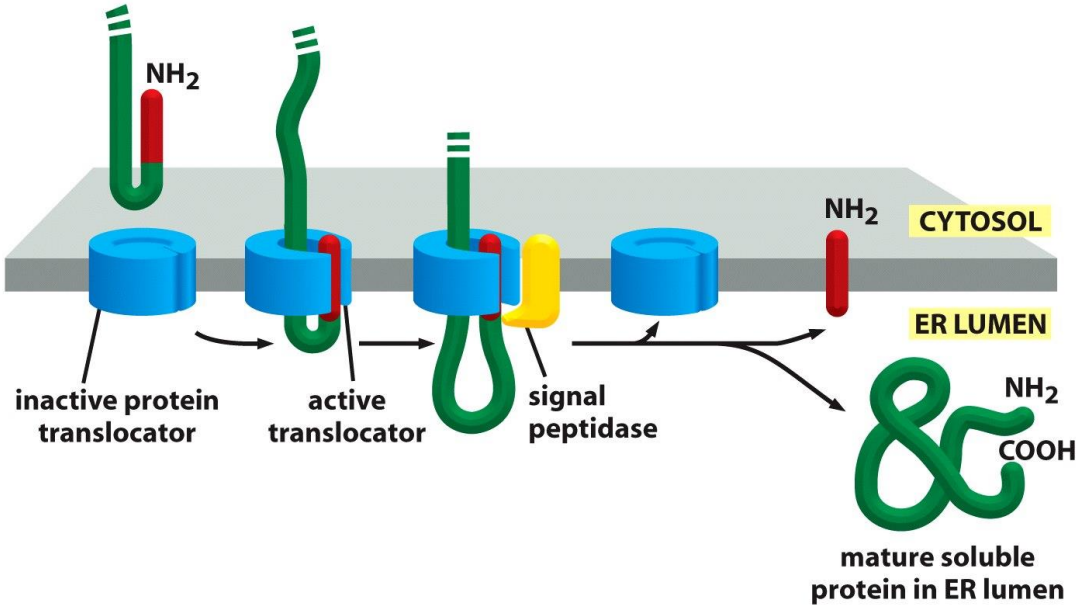


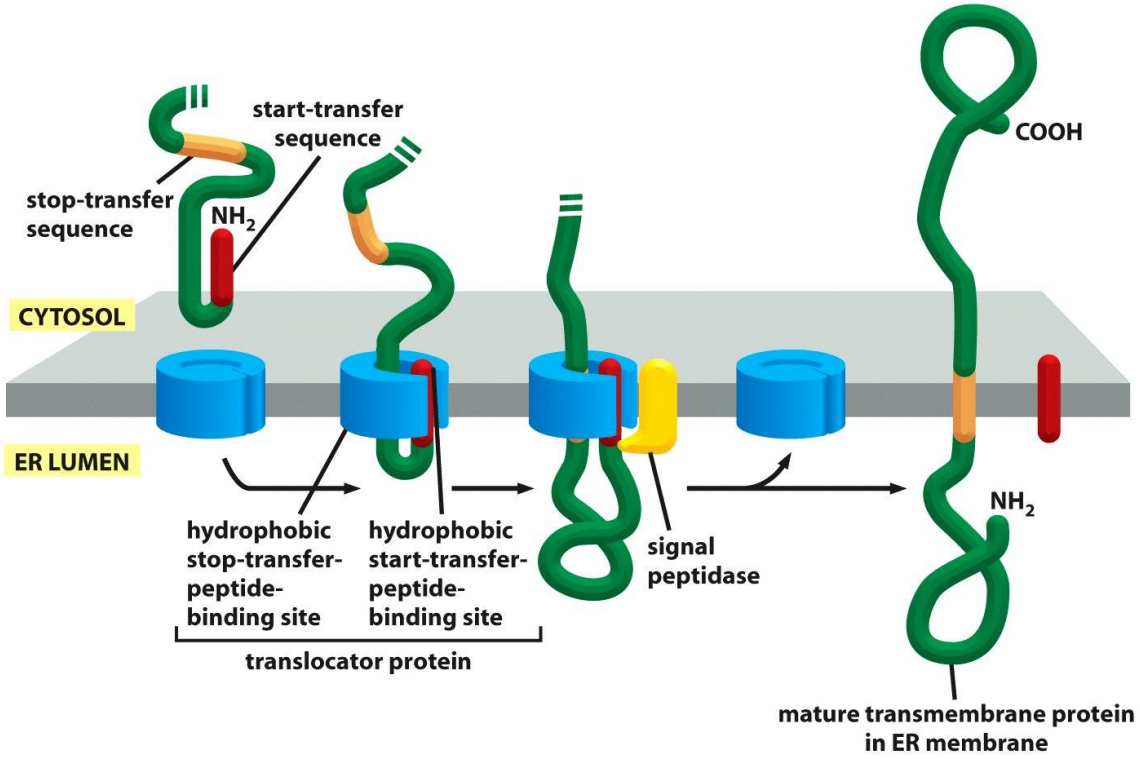
Binding of the signal sequence opens the translocator



– Growing polypeptide chain can be translocated through the membrane

Water-soluble vs. Transmembrane proteins

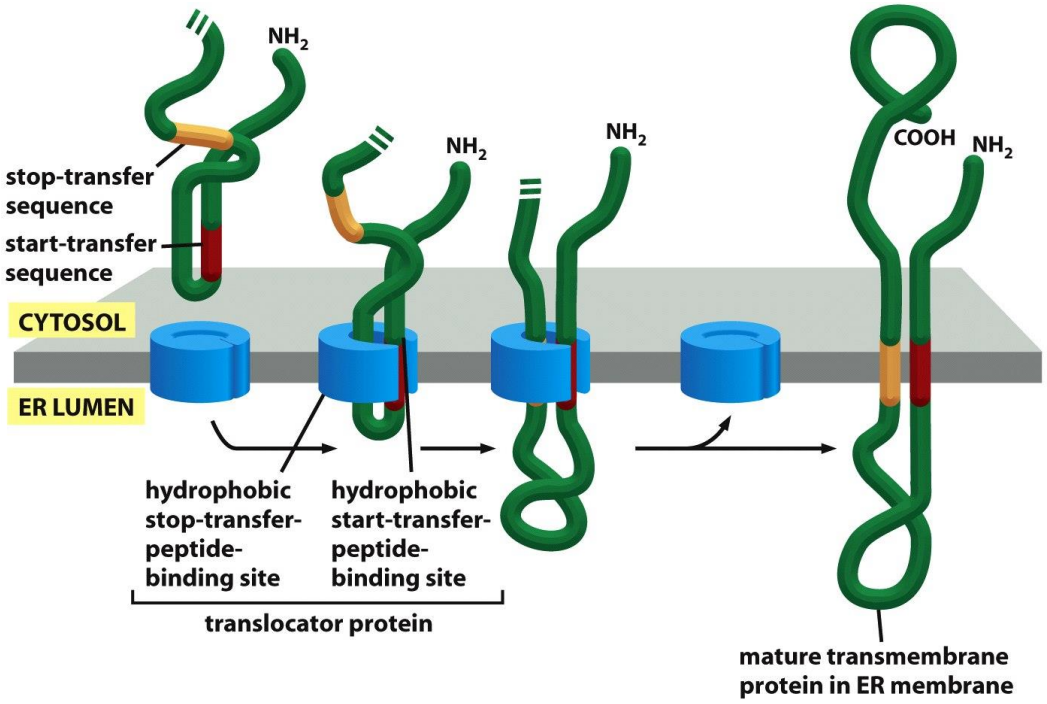


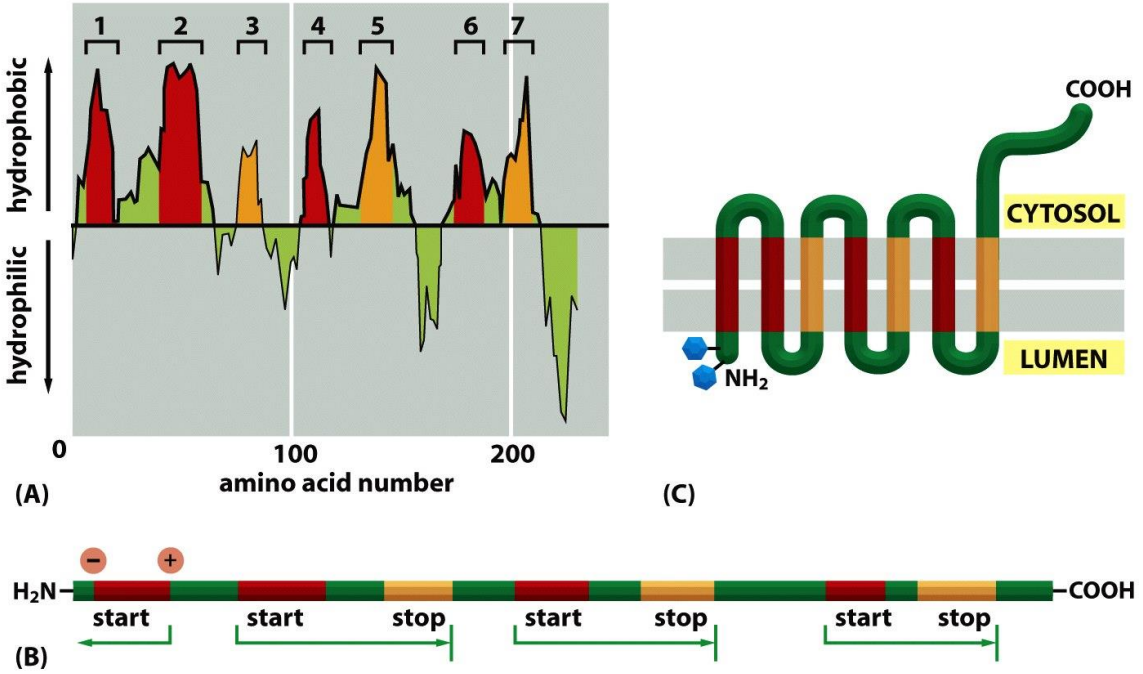


- Fully into the ER lumen
- Start- and stop-transfer signals

Multipass transmembrane proteins

- Position of start- and stop-transfer sequences defines protein integration



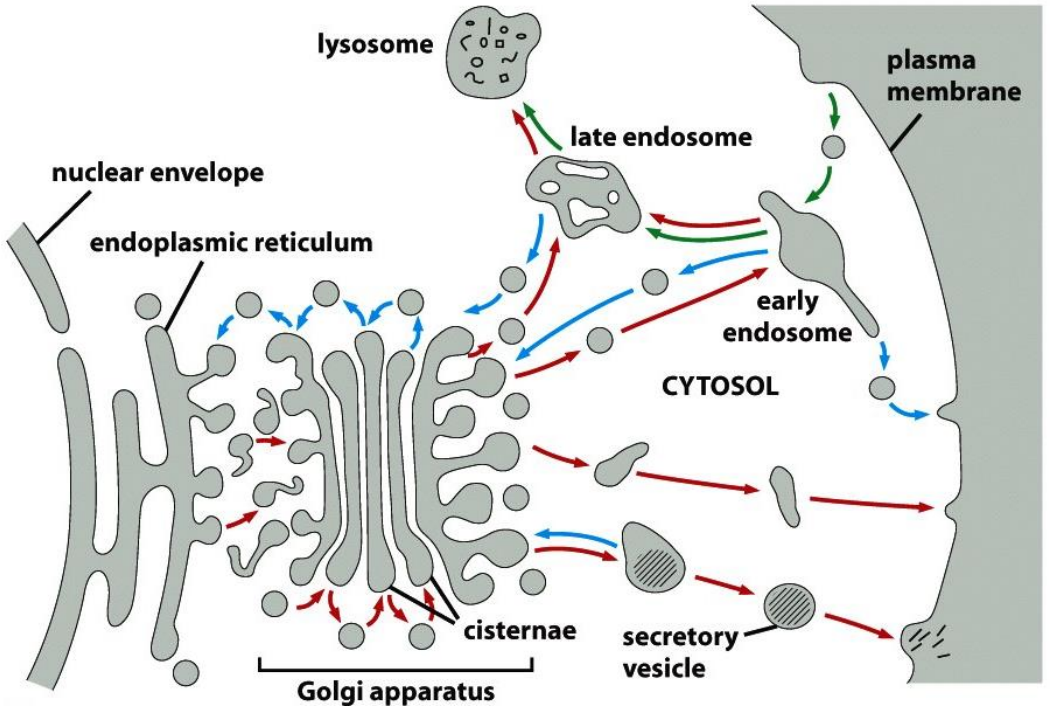


Vesicular transport

Vesicular transport

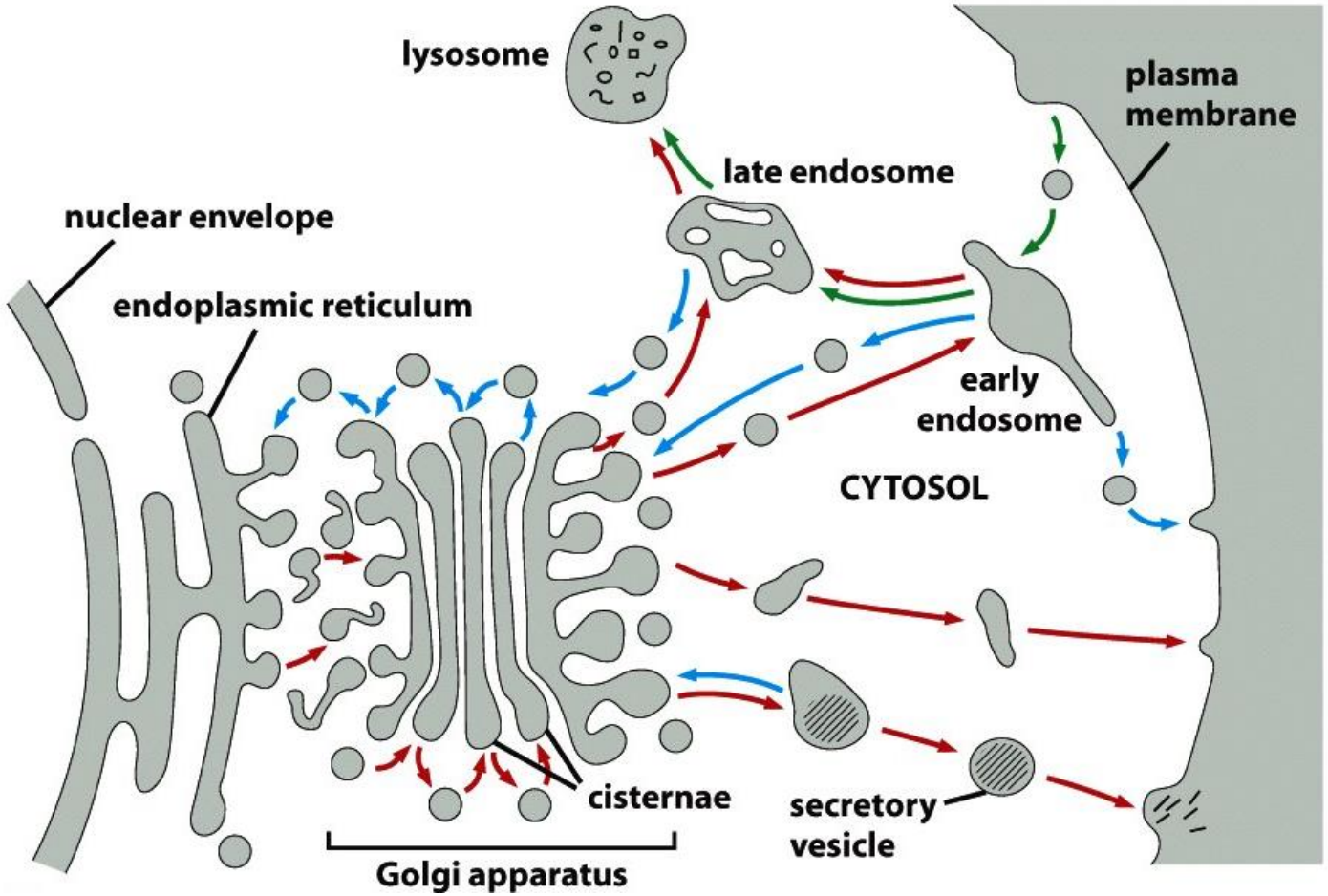
- **Bidirectional** transport among ER-Golgi-plasma membrane
- **Budding and fusion of membrane vesicles**
- Intracellular transport
- Intercellular communication

Anterograde trafficking →



← **Retrograde** trafficking

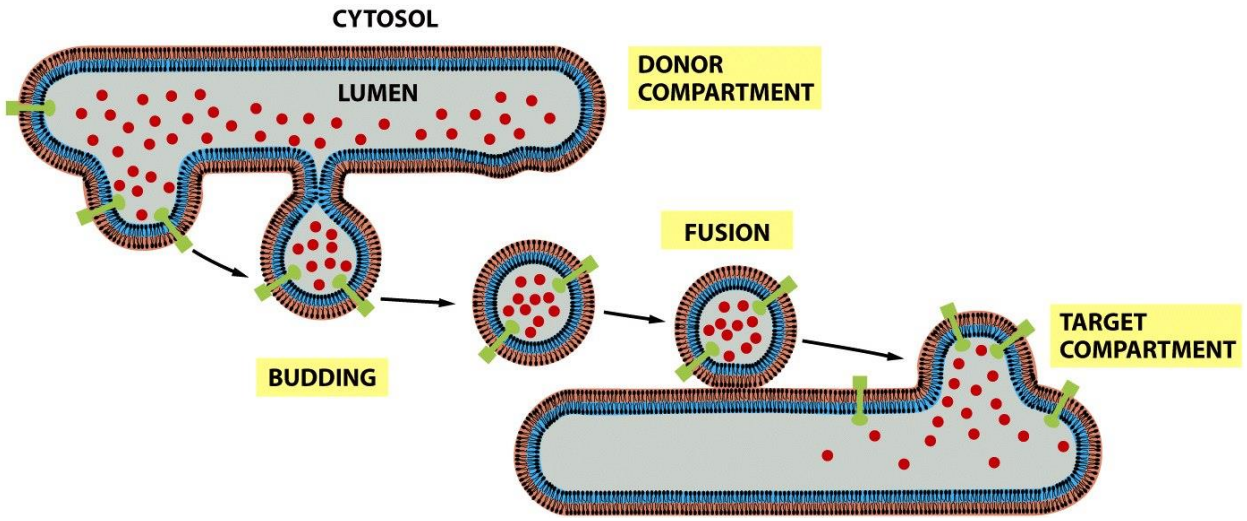
Vesicular road-map of the cell



- **Secretory pathway**
  - To plasma membrane and endosomes
- **Endocytic pathway**
  - From plasma membrane to endosomes and lysosomes
- **Retrieval pathway**
  - Returned/reused

Transport vesicles

- **Membrane-bound vesicles**
- Transport by diffusion (short distance; ER–Golgi) or **active transport along cytoskeleton** (long distances, directed transport)
- **Cargo** loading
  - **molecules from the lumen & membrane** of the donor organelles
  - **from outside the cell & plasma membrane (endocytosis)**
- e.g., membrane lipids and proteins from ER to plasma membrane



Vesicle budding

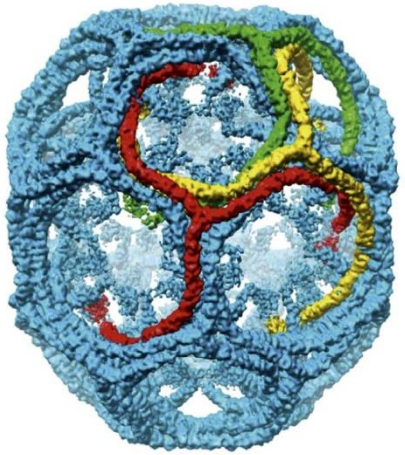
- **Driven by assembly of a protein coat** outside the forming vesicle
- Different protein complexes (**clathrin/adaptins**; **coatomers**)
  - Clathrin/adaptins – endocytosis, GA to endosomes/lysosomes
  - COPII (coat protein II) – ER to GA
  - COPI (coat protein I) – GA to ER
- The protein coat is **lost after the vesicle forms**

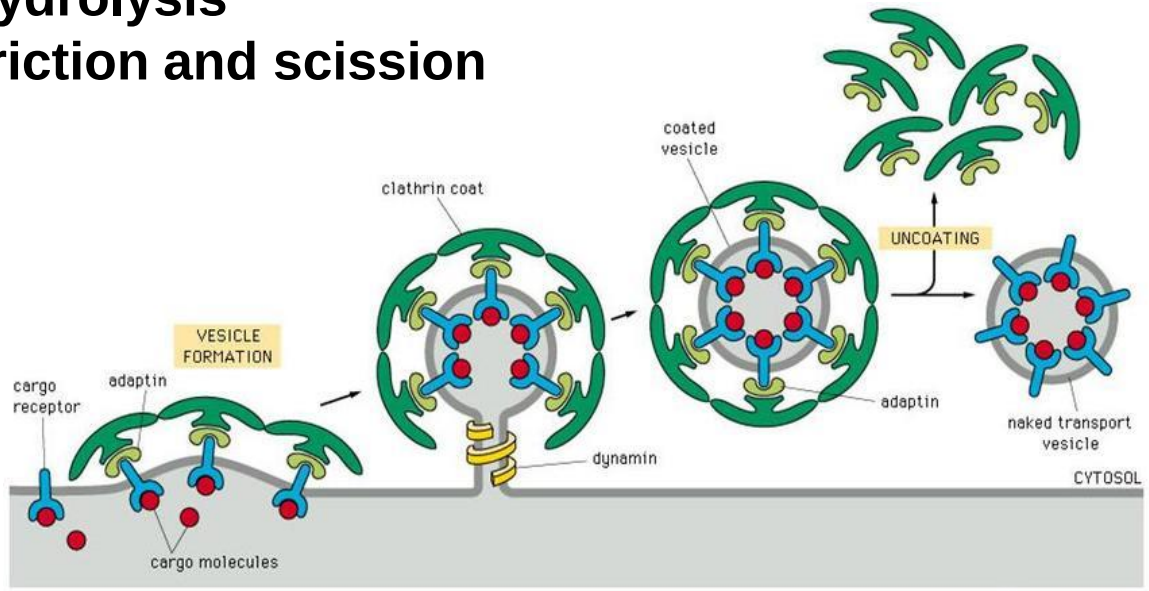
Main functions of protein coats:

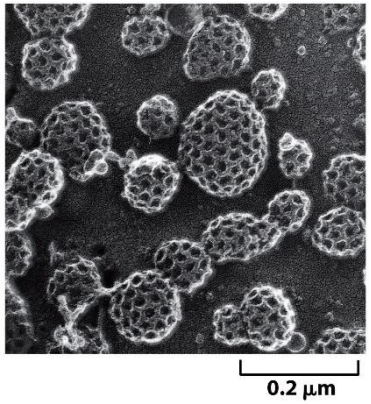
- **Formation of membranes into vesicles**
- **Cargo selection**

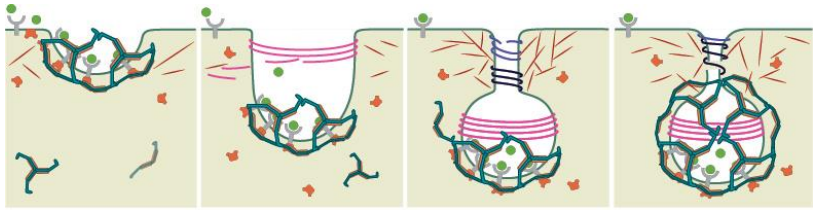
Clathrin-coated vesicle formation

- Protein coat: **network of clathrin triskelions**
- Specificity: **Adaptin** binds to **cargo receptors**
- **Dynamin rings**
  - **GTP-hydrolysis**
  - **Constriction and scission**

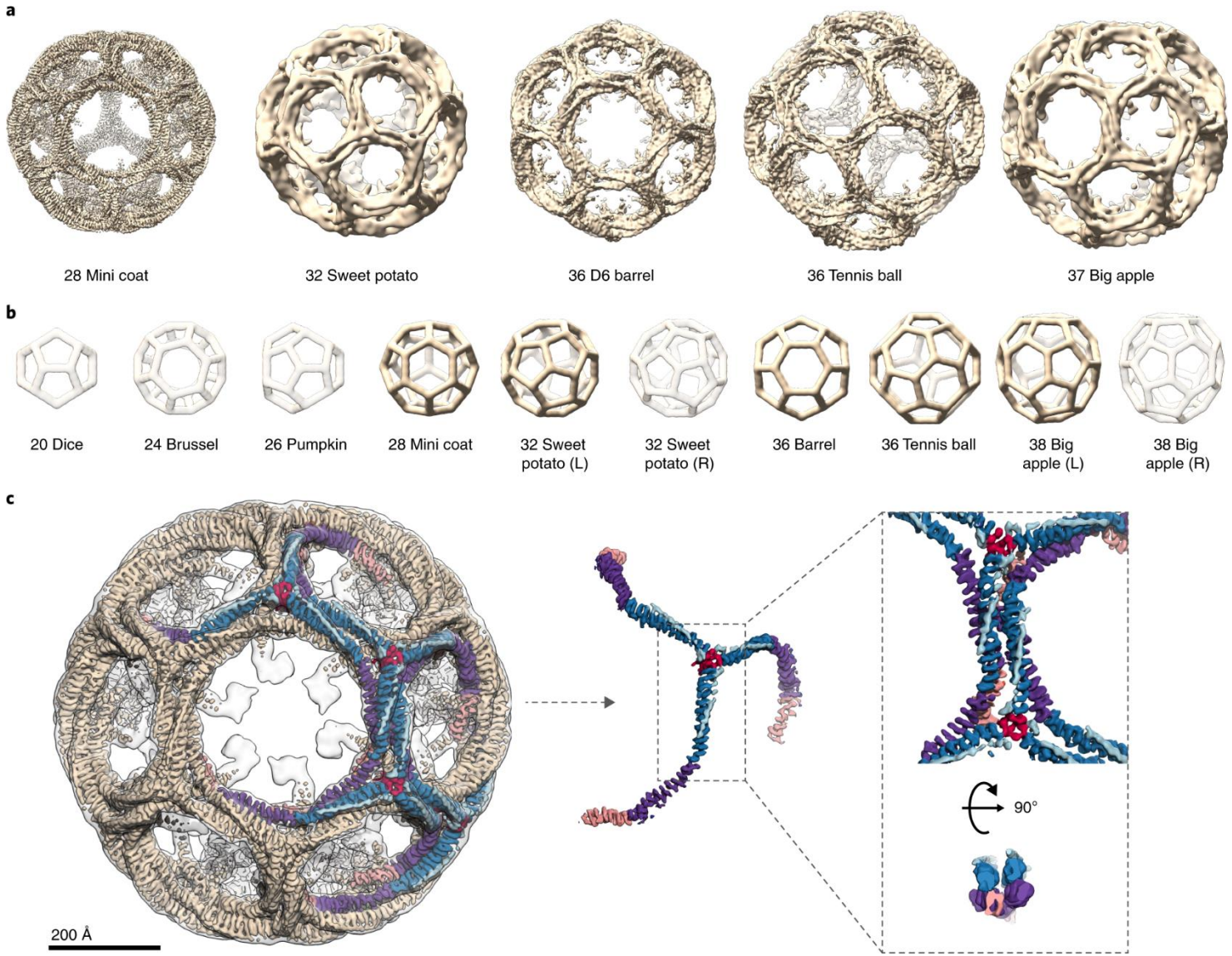






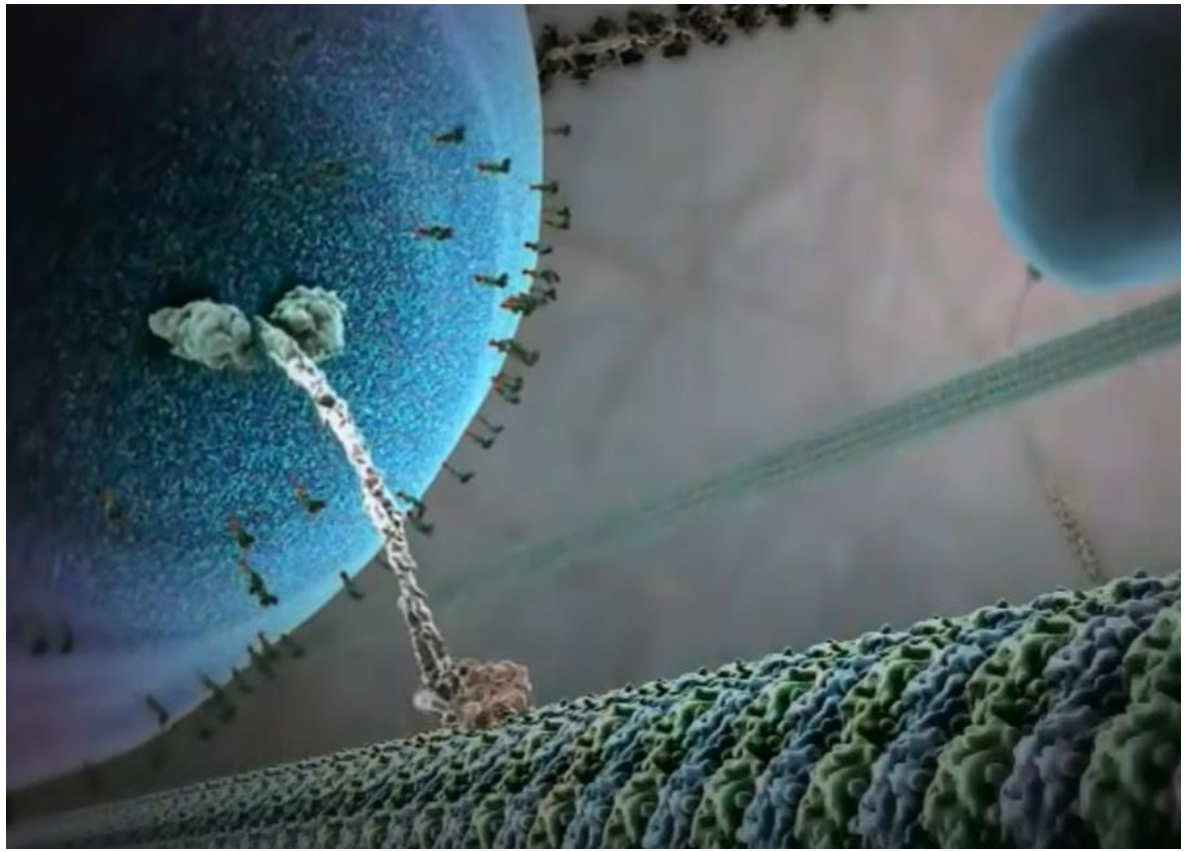


Gallery of clathrin cage architectures



Long distance – active transport

- **Movement along cytoskeleton**
  - **Microtubule-dependent transport**
  - Actin (microtubule-independent) transport
- **Motor proteins**

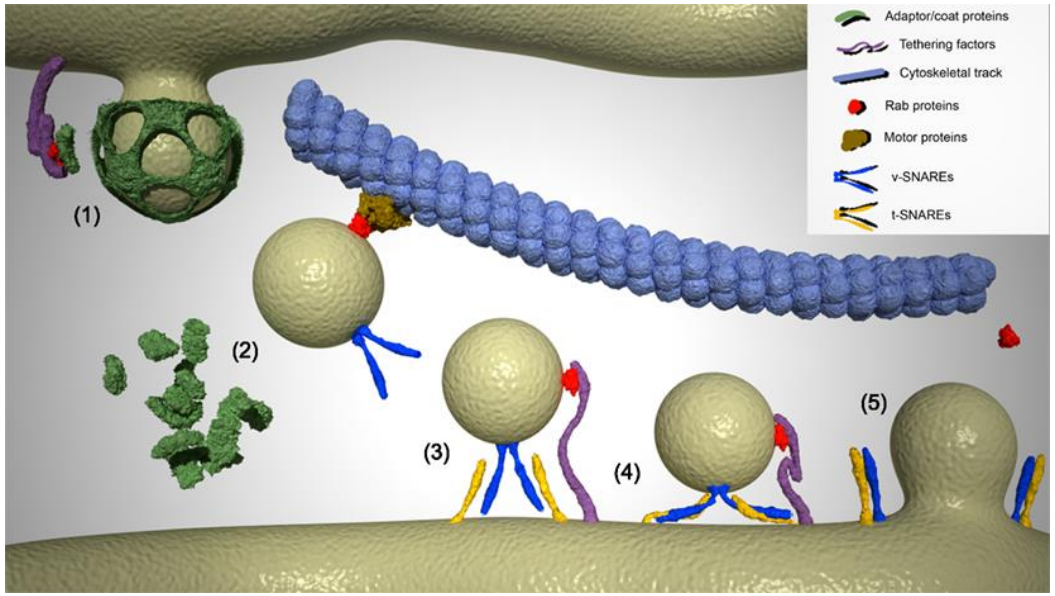


https://youtu.be/y-uuk4Pr2i8

Target recognition

Vesicle tethering

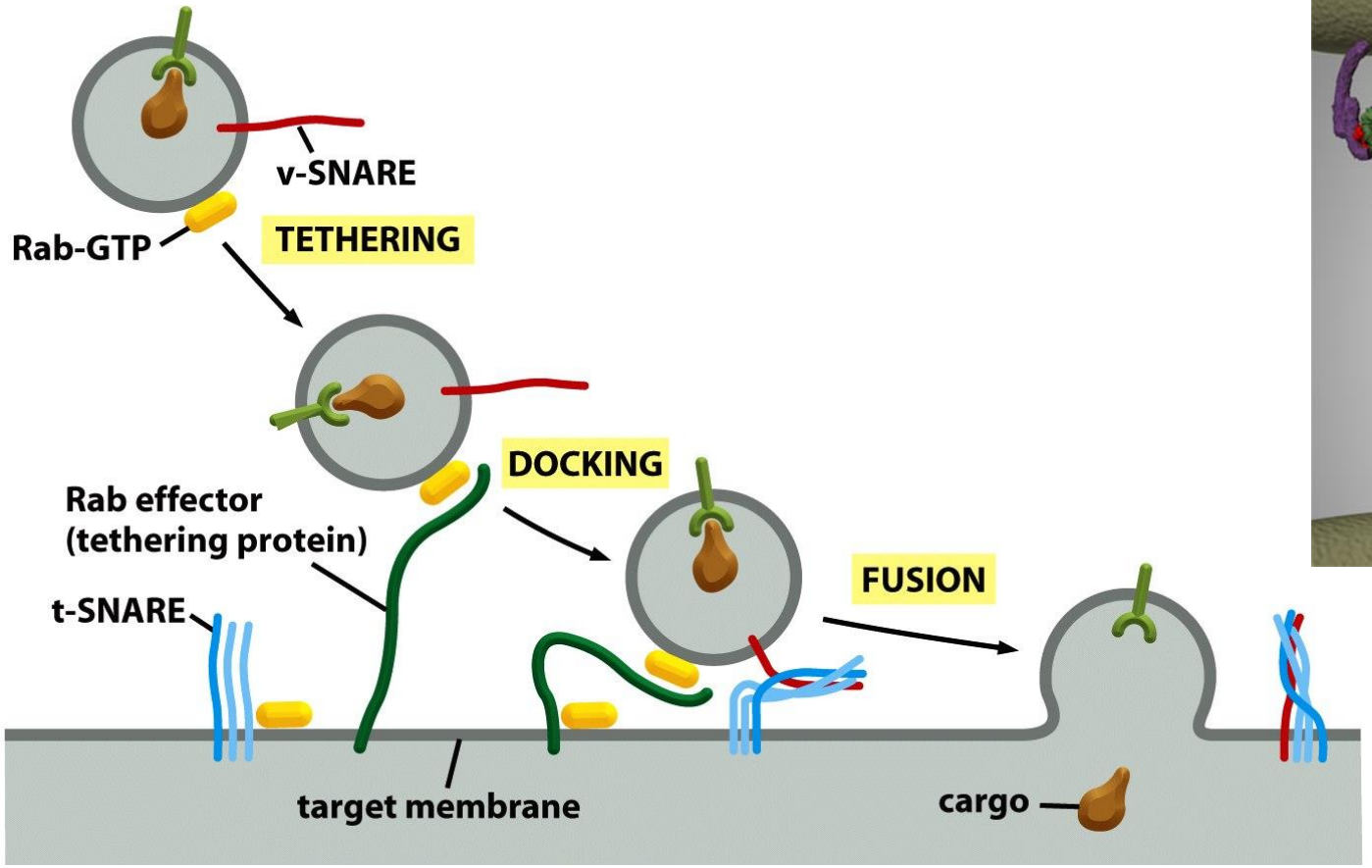
- **Rab proteins** – small G proteins
  - **GTPase activity** (Rab-GEF: GDP→ GTP)
  - GTP form anchored to the membrane
  - Binds to **tethering factors** (Rab effectors) at the target membrane

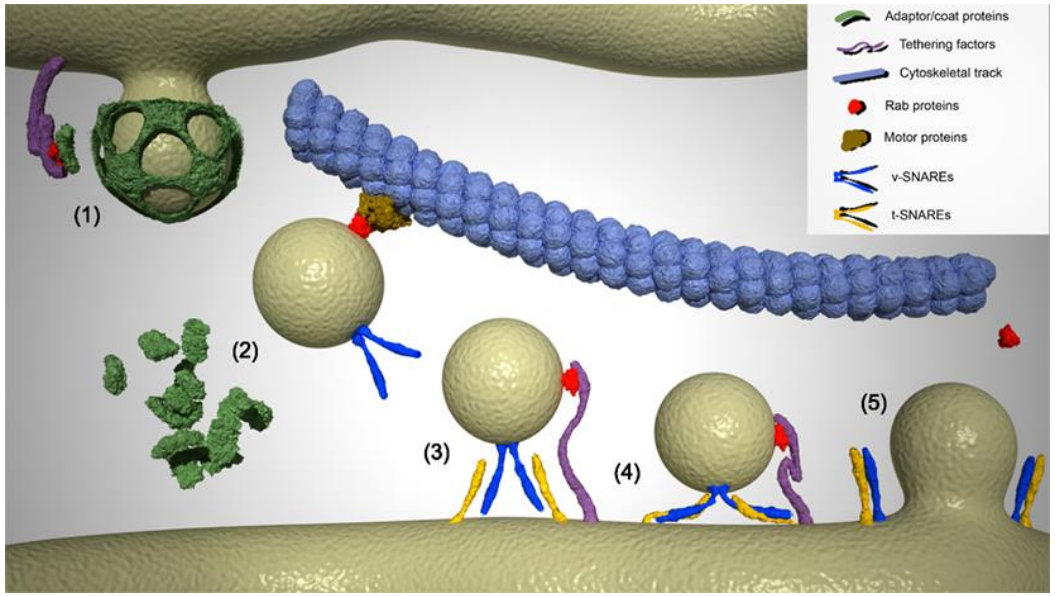


Vesicle docking

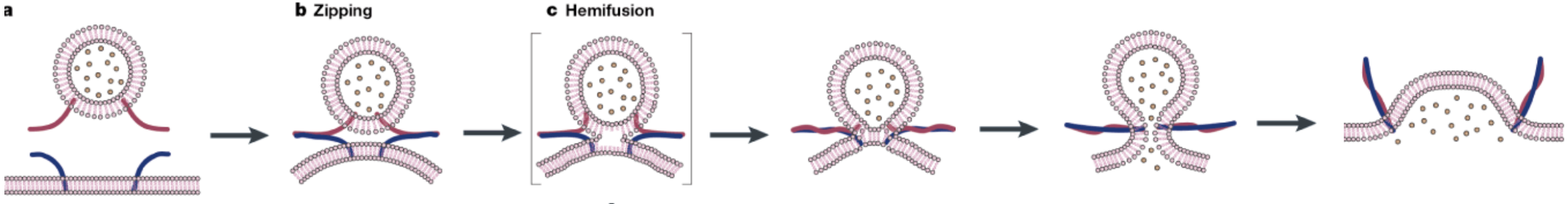
- **SNARE**
  - Interaction of **v-SNARE** (vesicle) & **t-SNARE** (target)
  - Affect target specificity by interacting with tethering factors

Target recognition: Rab proteins – SNAREs – fusion

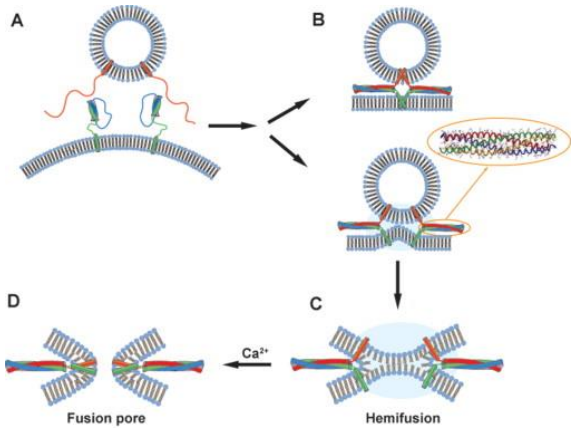


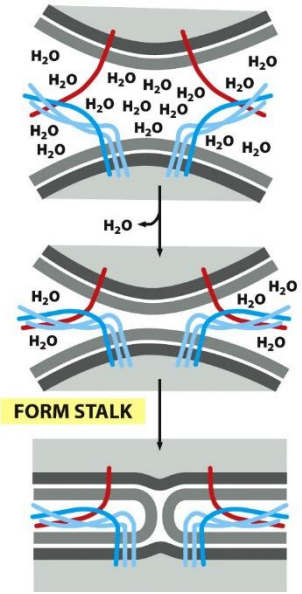


Membrane fusion



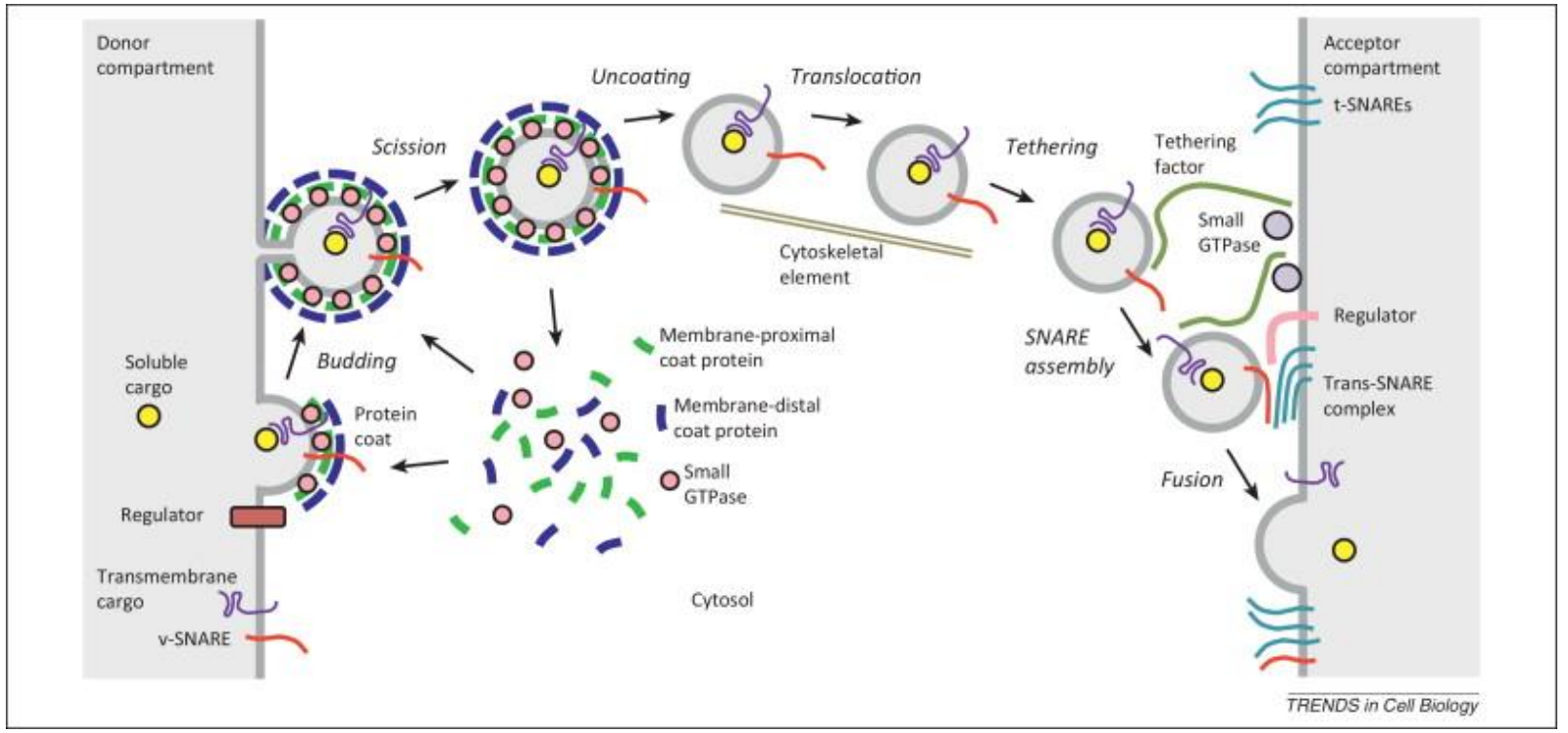
- **SNAREs mediate tight connection** between the fusing membranes
- Expels water molecules from the hydrophilic surface
- Ca^{2+} generally promotes fusion completion





Complex machinery – understanding still limited

Vesicular transport in brief



Secretory and endocytic pathways

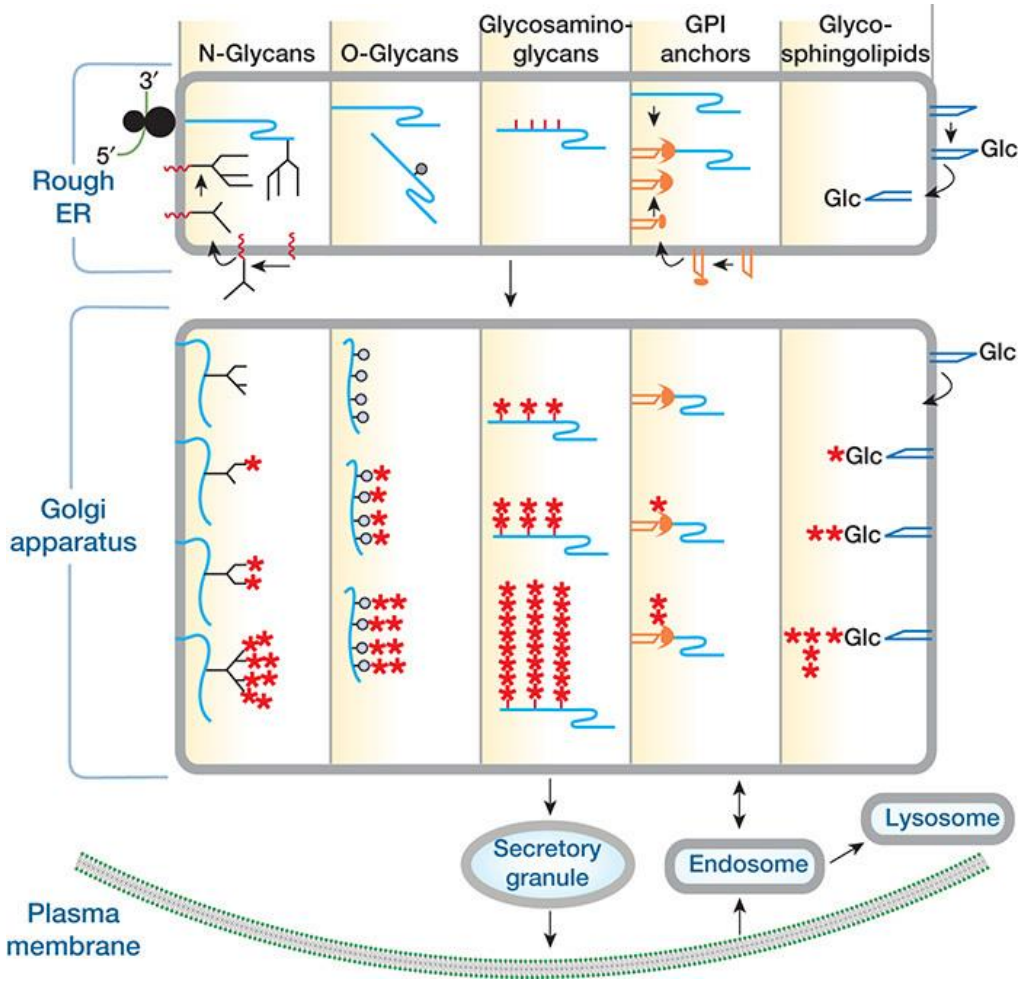
Secretory pathway

- Luminal cargo: **secreted proteins, lipids, saccharides**
- Membrane proteins, lipids/glycolipids: **insertion into plasma membrane** or **function in the vesicles**

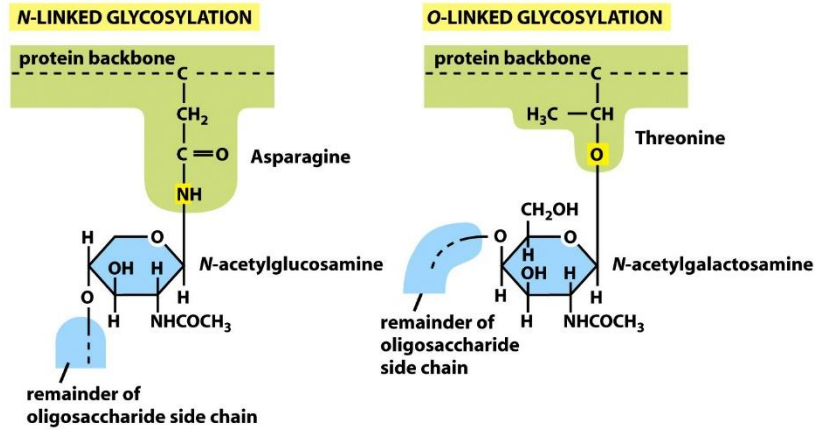
Gradual modifications (maturation) of proteins, lipids

- **ER – resident luminal enzymes**
  - e.g., disulfide bonds between cysteines (stability of secreted proteins)
  - **Protein N-glycosylation** (90% glycoproteins) – addition of precursor oligosaccharide in ER
- **Golgi – membrane bound enzymes**
  - Oligosaccharides processing
  - **Protein glycosylation: O-glycosylation and further N-glycosylation**

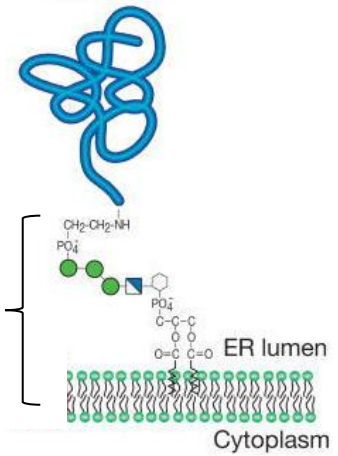
Glycoconjugation



- **N-glycans**
- **O-glycans**



- Glycosylphosphatidylinositol (GPI) anchors



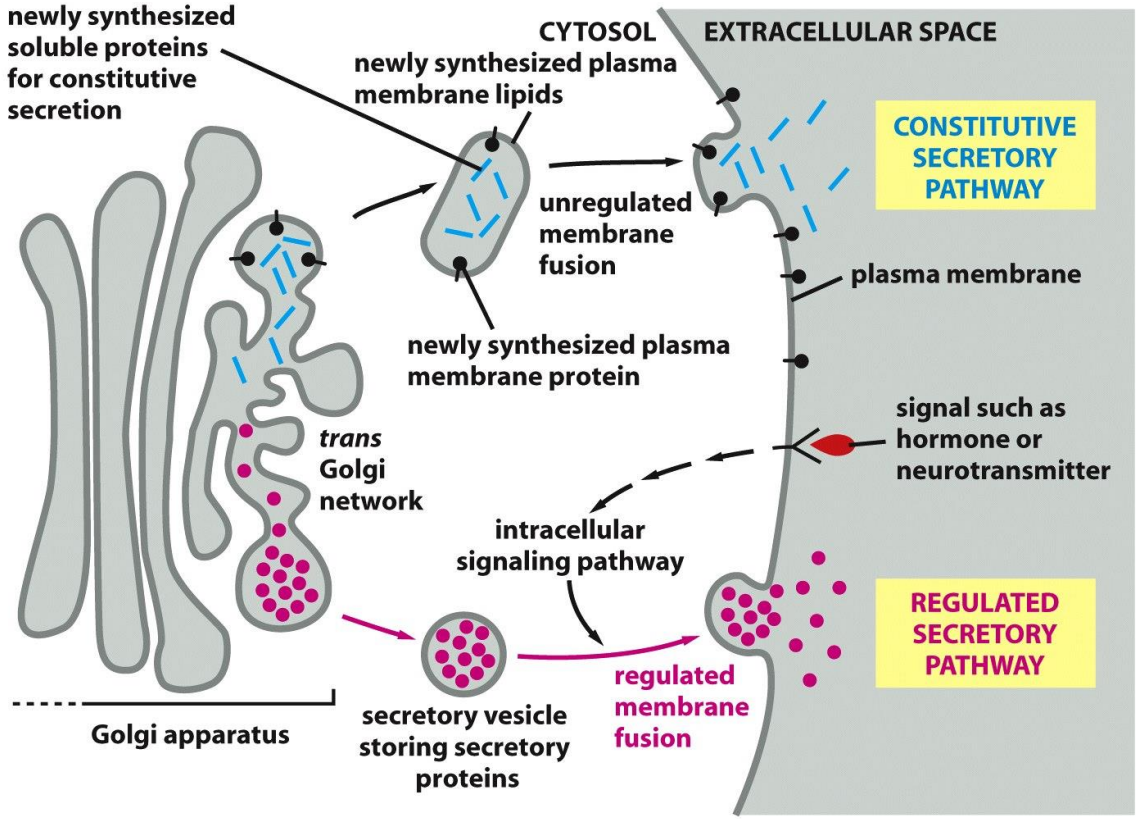
Exocytosis / Secretory pathway

Constitutive

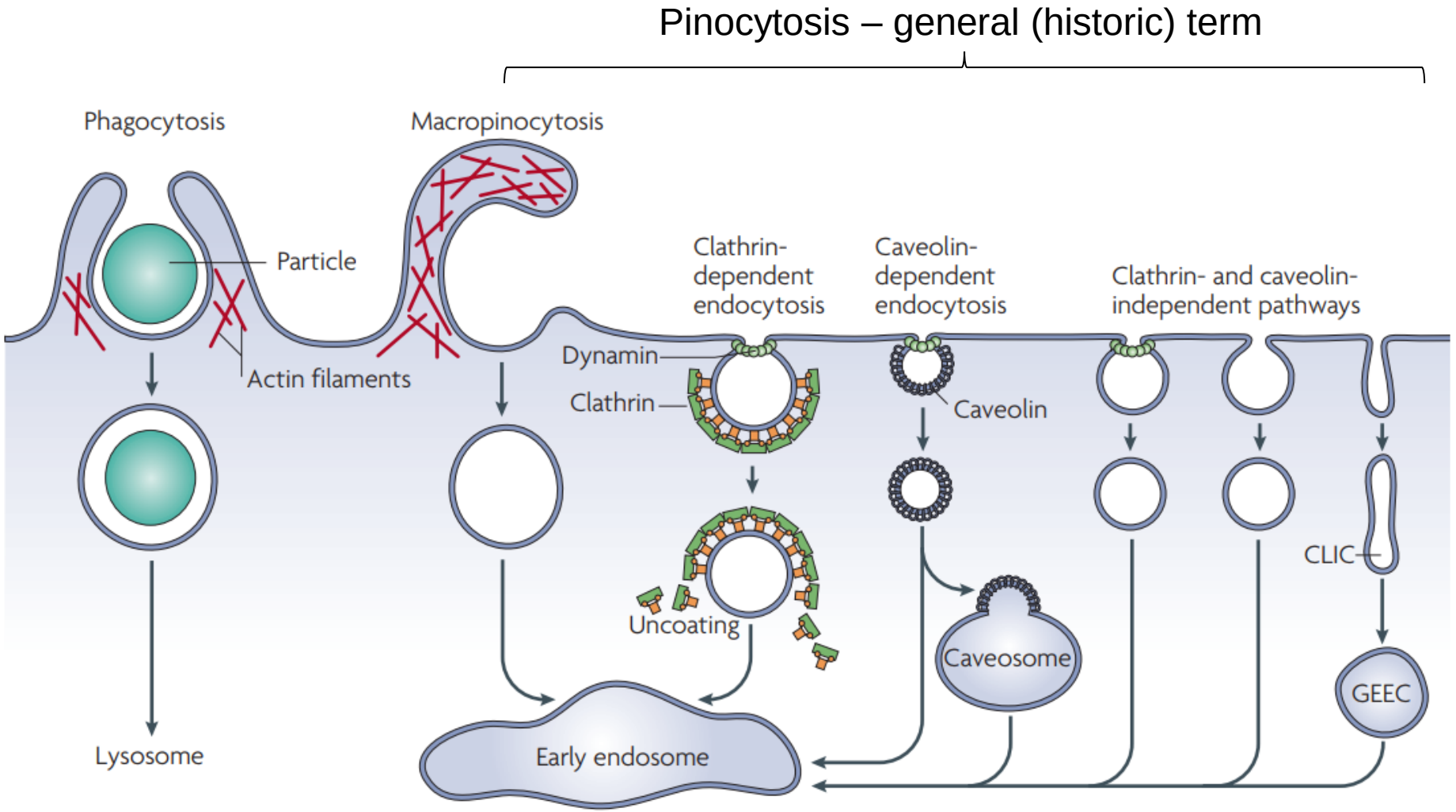
- **Unregulated by stimuli**
- Operates continuously in all cells

Regulated

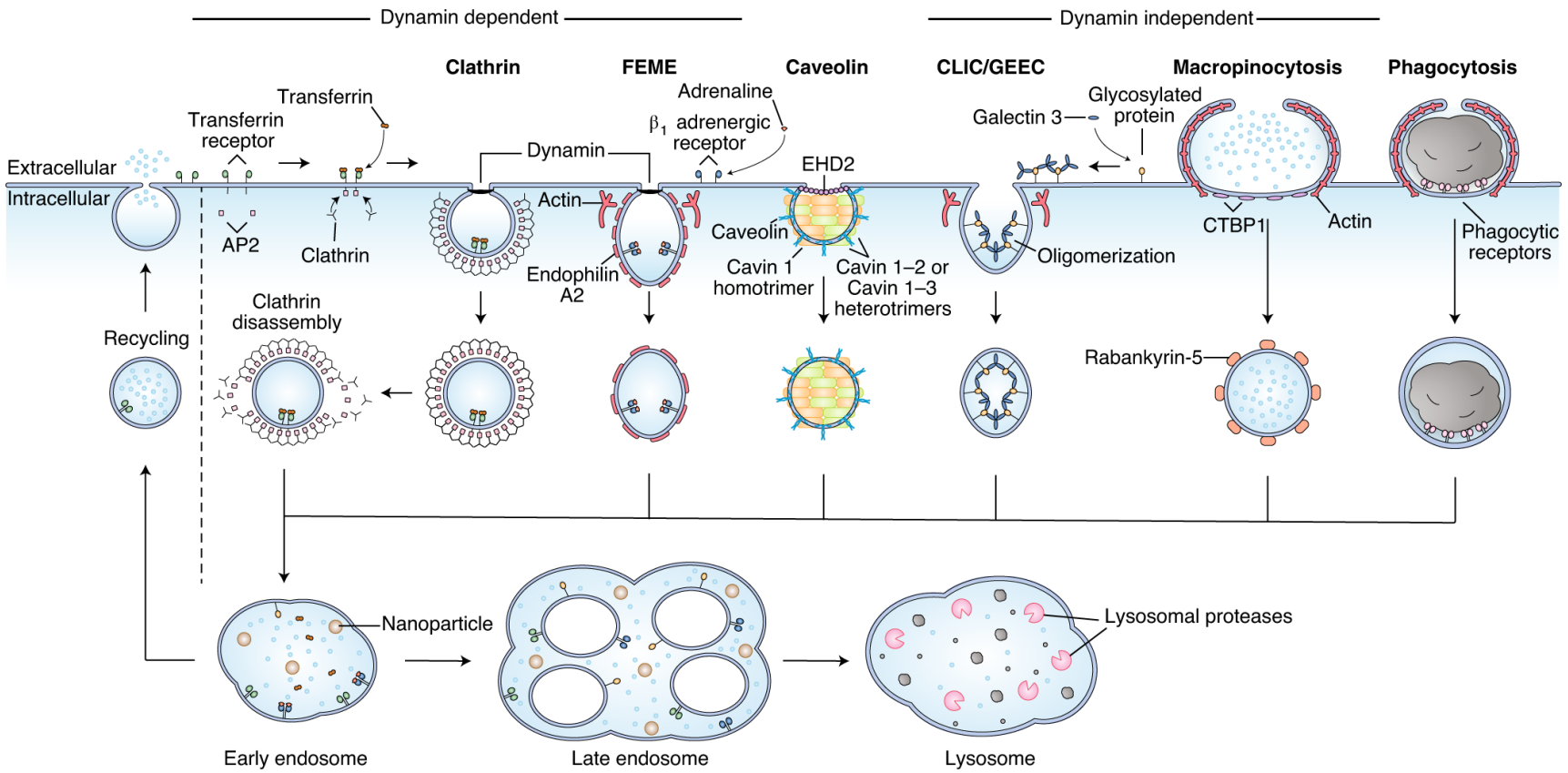
- **“On demand” secretion** of hormones, neurotransmitters, digestive enzymes in **specialized cells**
- **Vesicles wait near plasma membrane**
- **Signal** triggers intracellular pathway – often ↑Ca^{2+} – membrane fusion



Endocytic pathway – 5 major mechanisms



Endocytosis – much more complex classification

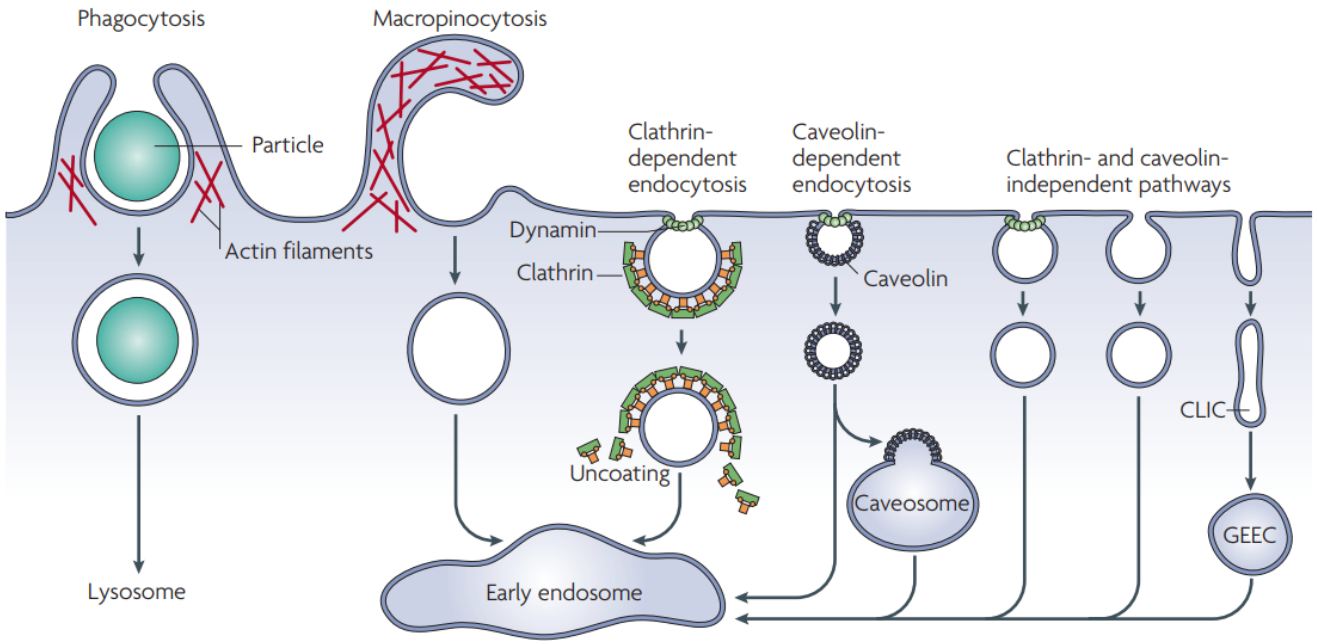


– Reading for interested students

Endocytosis – major differences

Clathrin-dependent & caveolin-dependent

- Dynamin-dependent
- Vesicles <= 100 nm



- **Phagocytosis & macropinocytosis**
- Actin-dependent, dynamin-independent
- Vesicles > 200 nm (> 500 nm for phagocytosis)

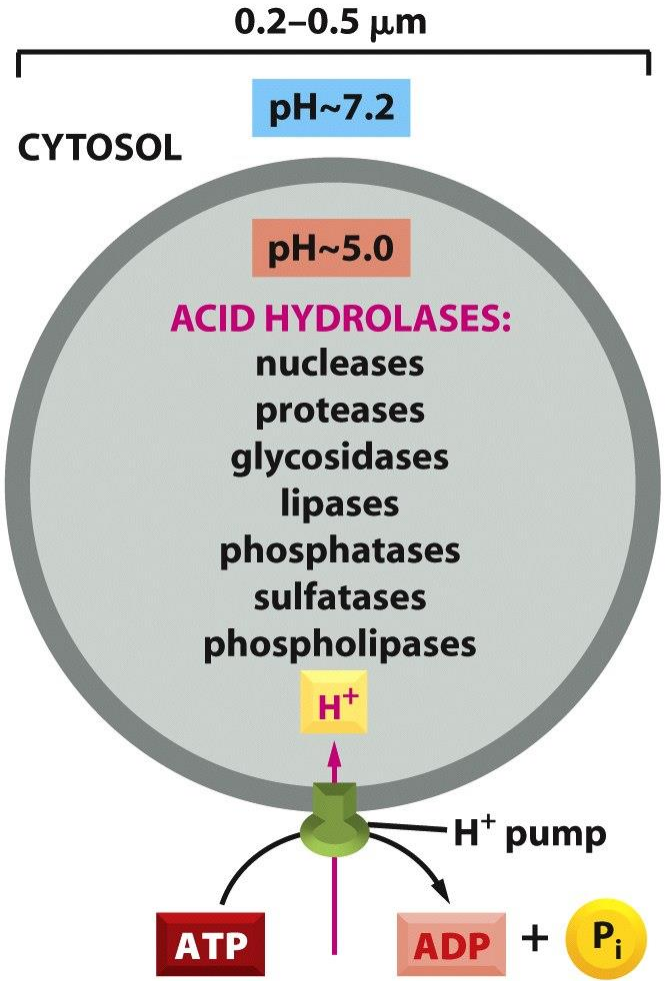
Phagocytosis

- Recognition of self and non-self – **specific receptors** on the surface
- Conserved among *vertebrates* and invertebrates
- **Defense against infections (immune system)** – e.g., *macrophages* or hemocytes
- **Food uptake** – protists

- Actin-mediated formation of pseudopodia
- Large particle enclosed in the **phagosome** (vesicle) – **fusion with (maturation into) lysosome**

Lysosomes

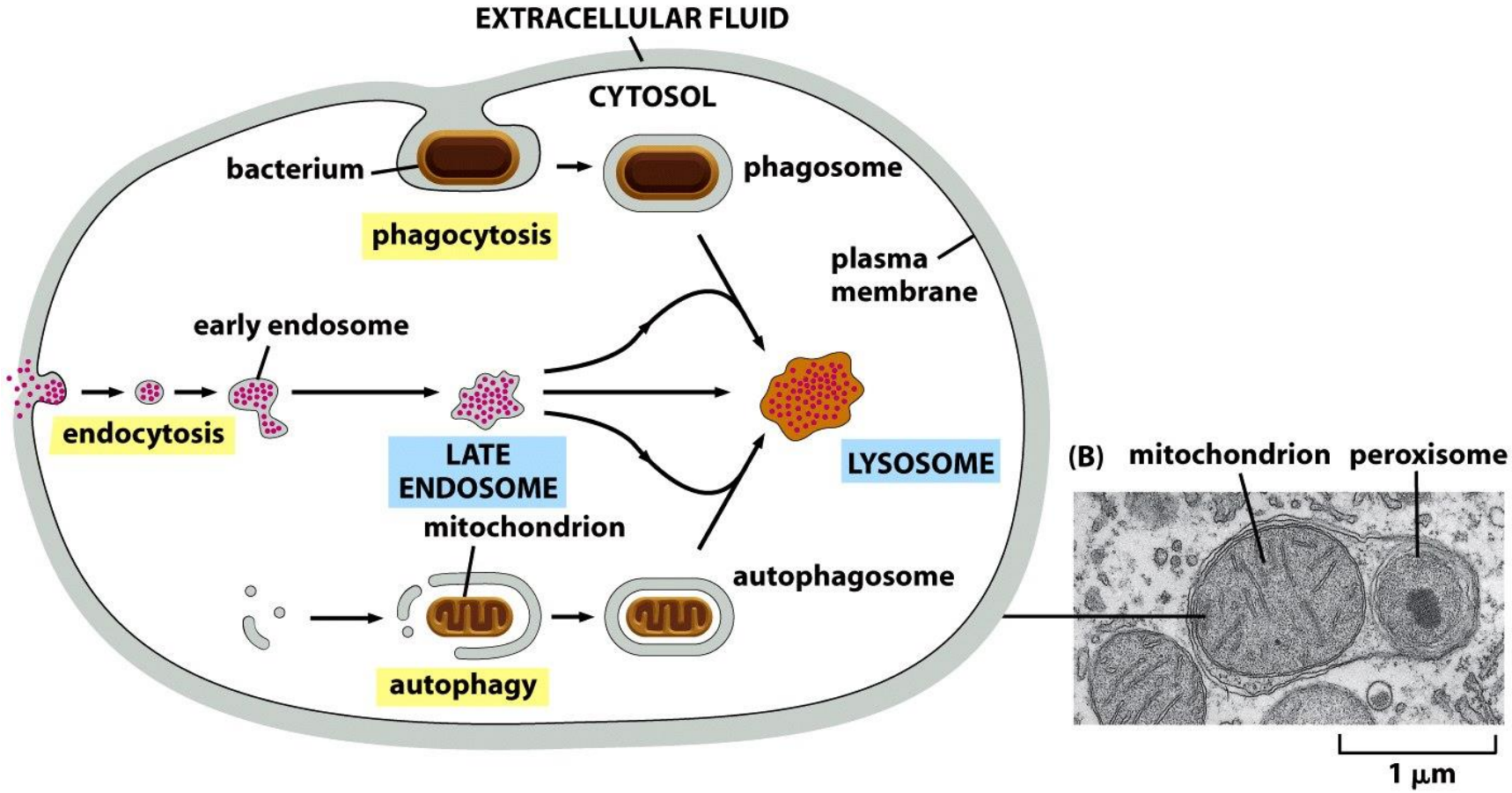
- Highly **acidic pH ~ 5** maintained by **V-ATPase proton pumps**
- Important for **acidic hydrolases** (~50 different)
  - Not active in the cytosol due to pH
  - Heavily glycosylated membrane proteins are protected from hydrolases



Fuse with and degrade cargo of

- **Endosomes** (general endocytic pathway)
- **Phagosomes** (phagocytosis)
- **Autophagosomes** (autophagy – self degradation)

Pathways leading to degradation in lysosomes



Intercellular vesicular transport

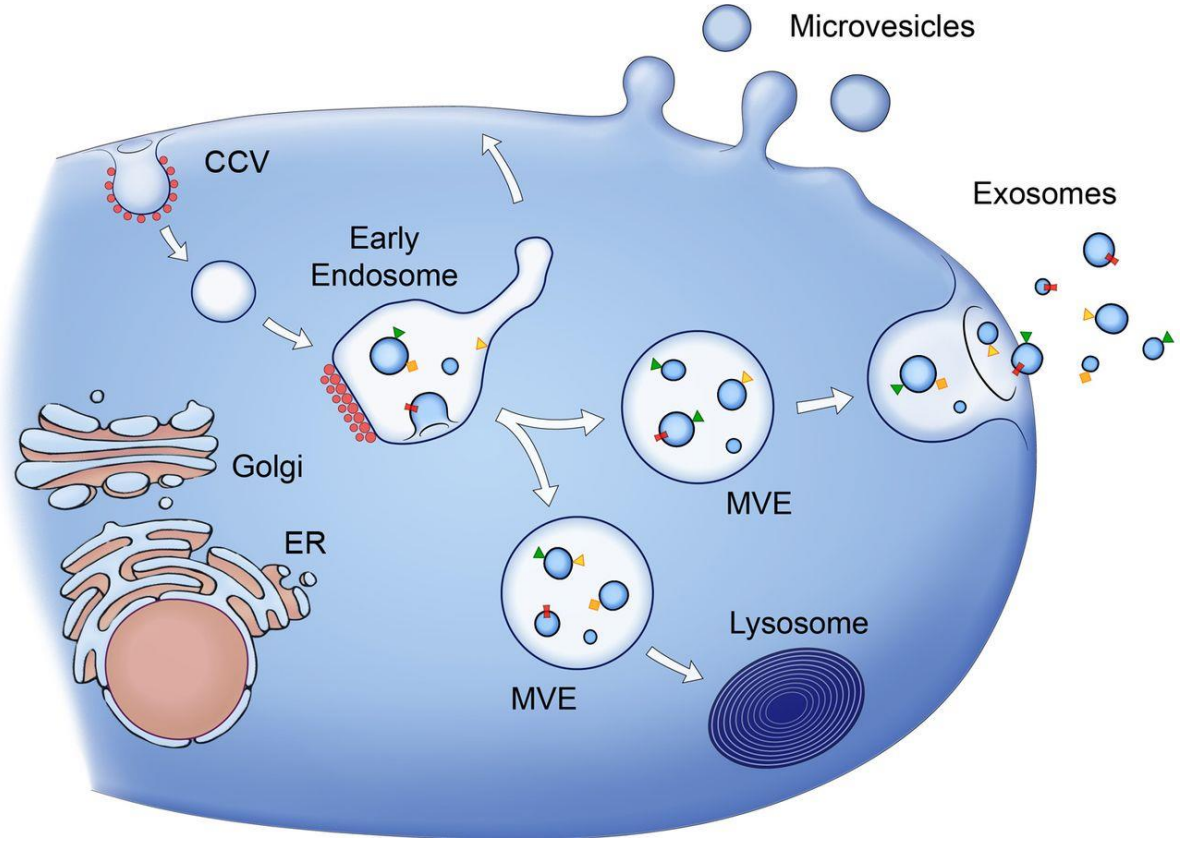
Intercellular vesicular transport

Exosomes

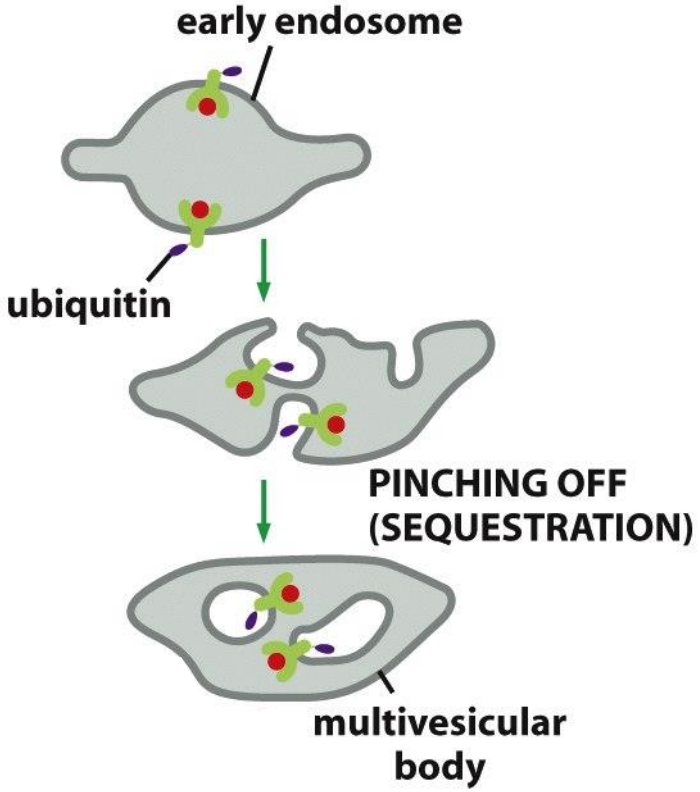
- Derived from **multivesicular bodies** (MVB or MVE)
- Generally smaller: 30-100 nm

Microvesicles

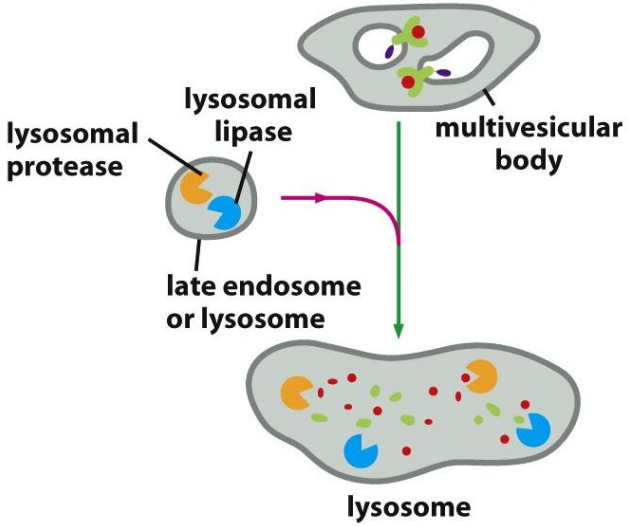
- **Plasma membrane budding** and fission
- Larger: 100-1000 nm

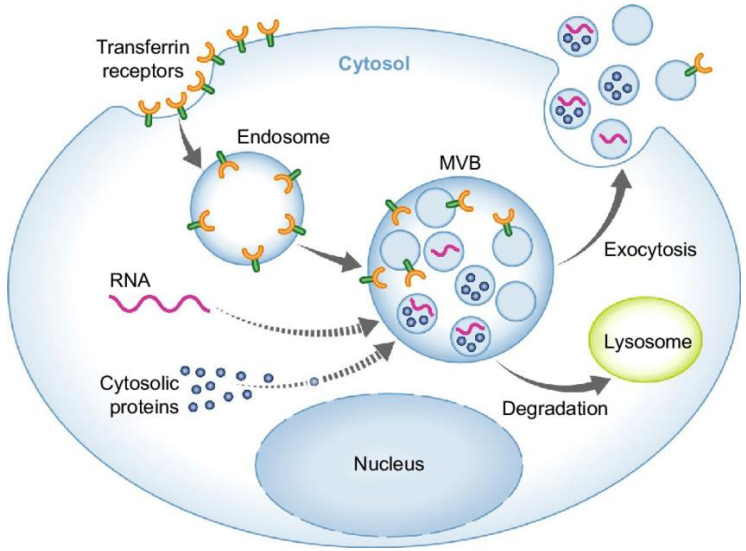


Formation of multivesicular bodies

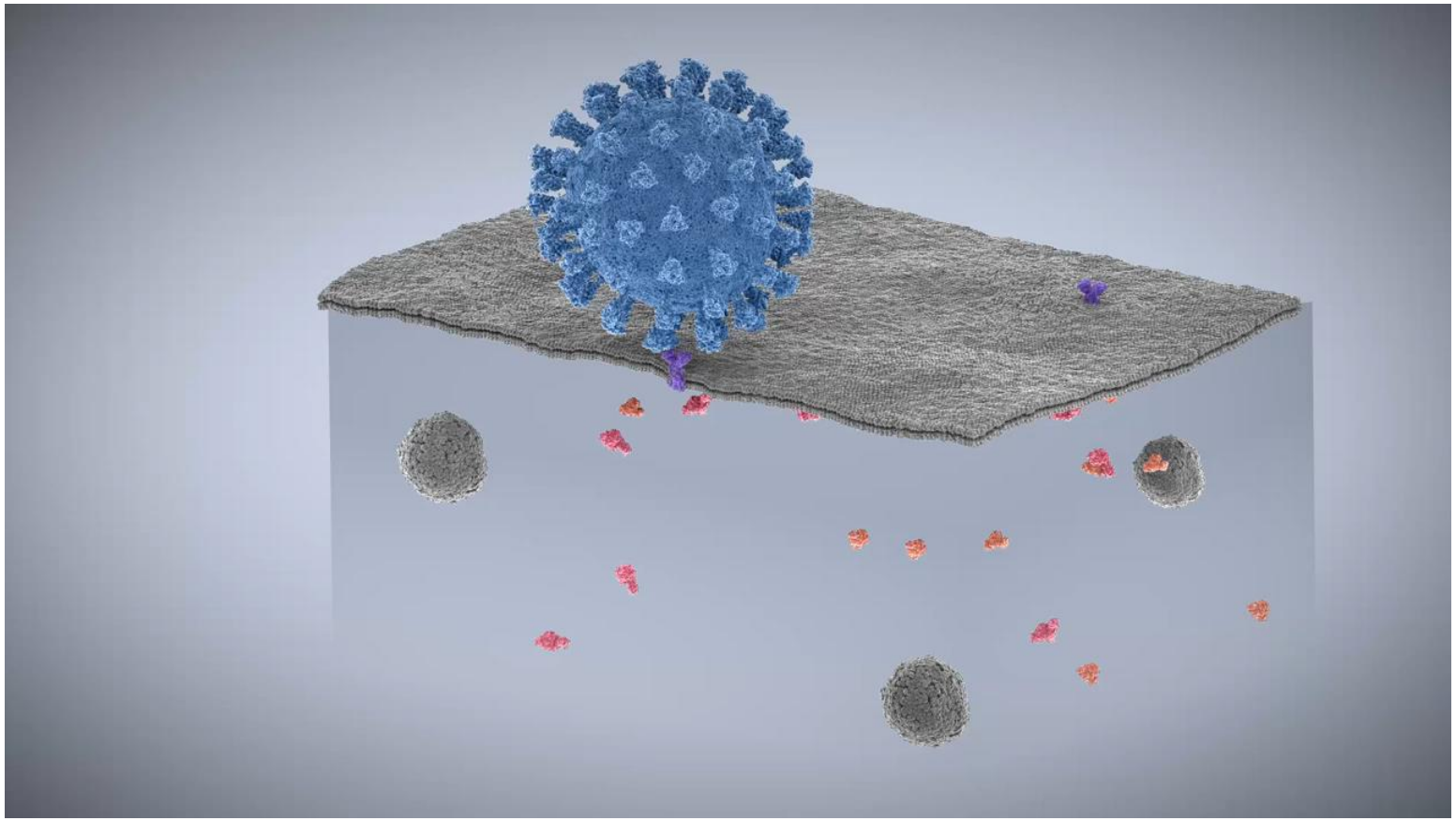


- **Rerouted to plasma membrane:** exosomes
- **Fusion with lysosome:** degradation of membrane proteins and lipids





SARS-COV-2 clathrin-mediated endocytosis



- Viral S protein binds to ACE2 receptor on the cell surface