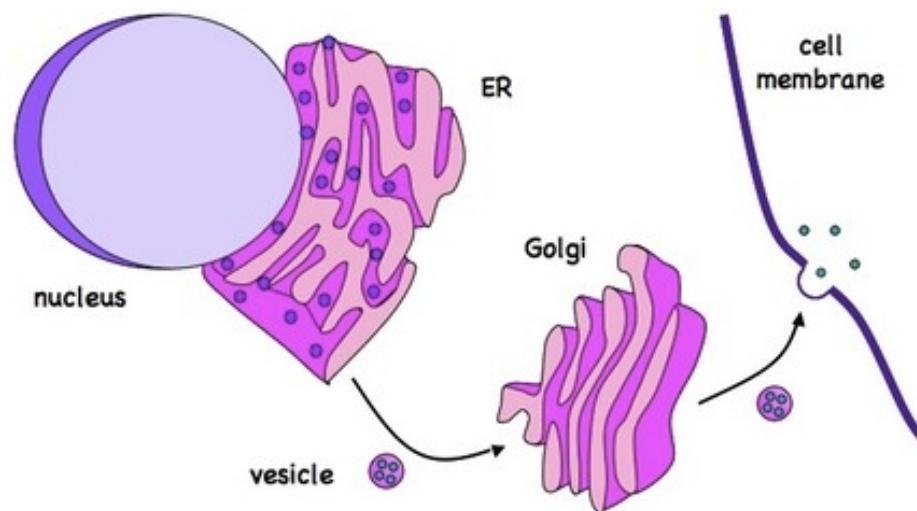


# Introduction to Molecular and Cellular Biology

## LECTURES 17-18:

### Vesicular transport



# LECTURES 17-18: VESICULAR TRANSPORT

- Introduction: basic concepts
- Molecular mechanisms and cell compartments diversity
- Transport ER => GA
- Transport GA => lysosomes
- Endocytosis
- Exocytosis



# NOBEL PRIZE 2013

for “discoveries of machinery regulating vesicle traffic, a major transport system in our cells.”

- James Rothman (protein machinery)
- Randy Schekman (responsible genes)
- Thomas Südhof (release signals)

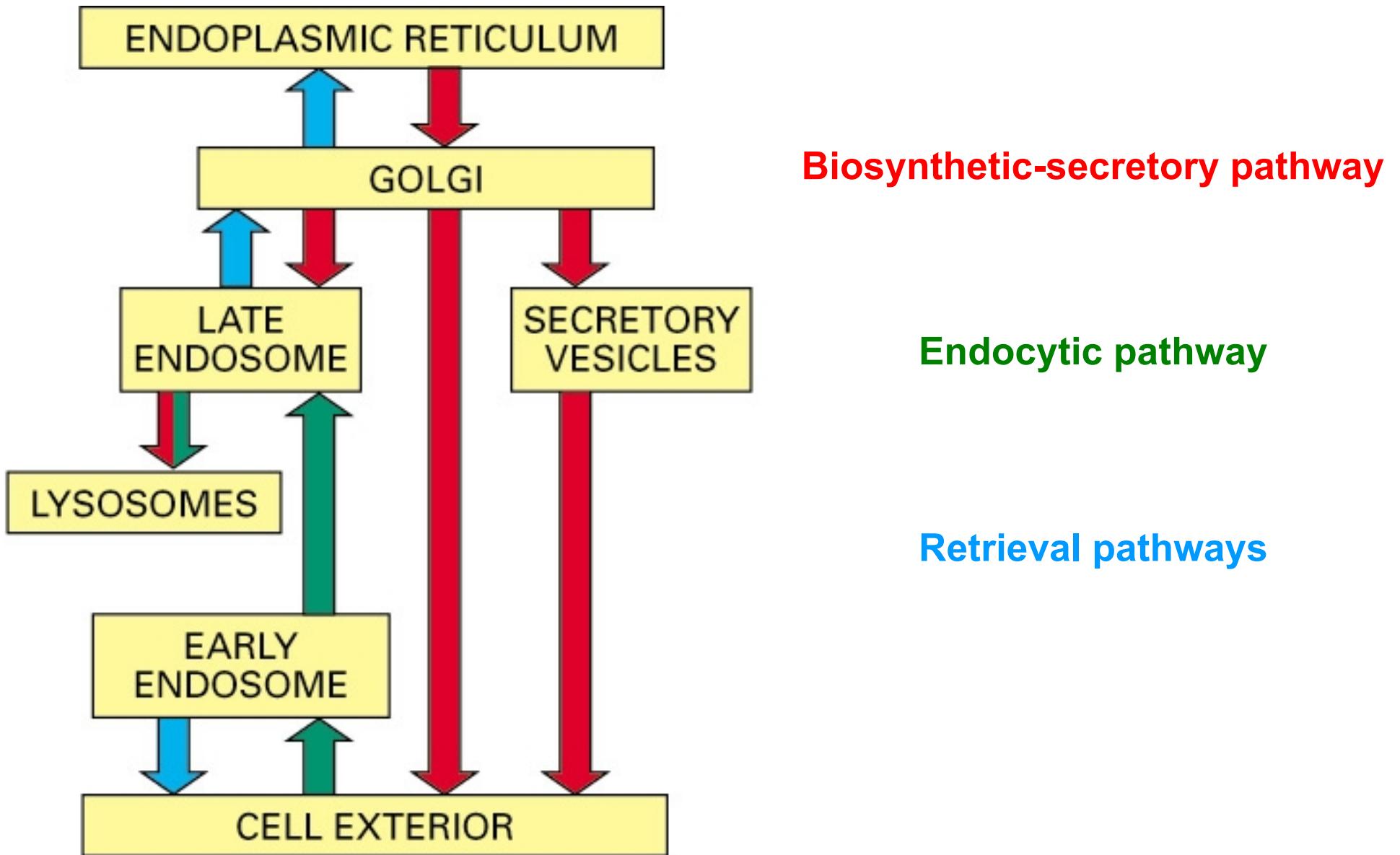


*How cells, which are factories producing molecules, organize a system to transport the molecules within cells and export them outside?*

# INTRODUCTION, BASIC CONCEPTS

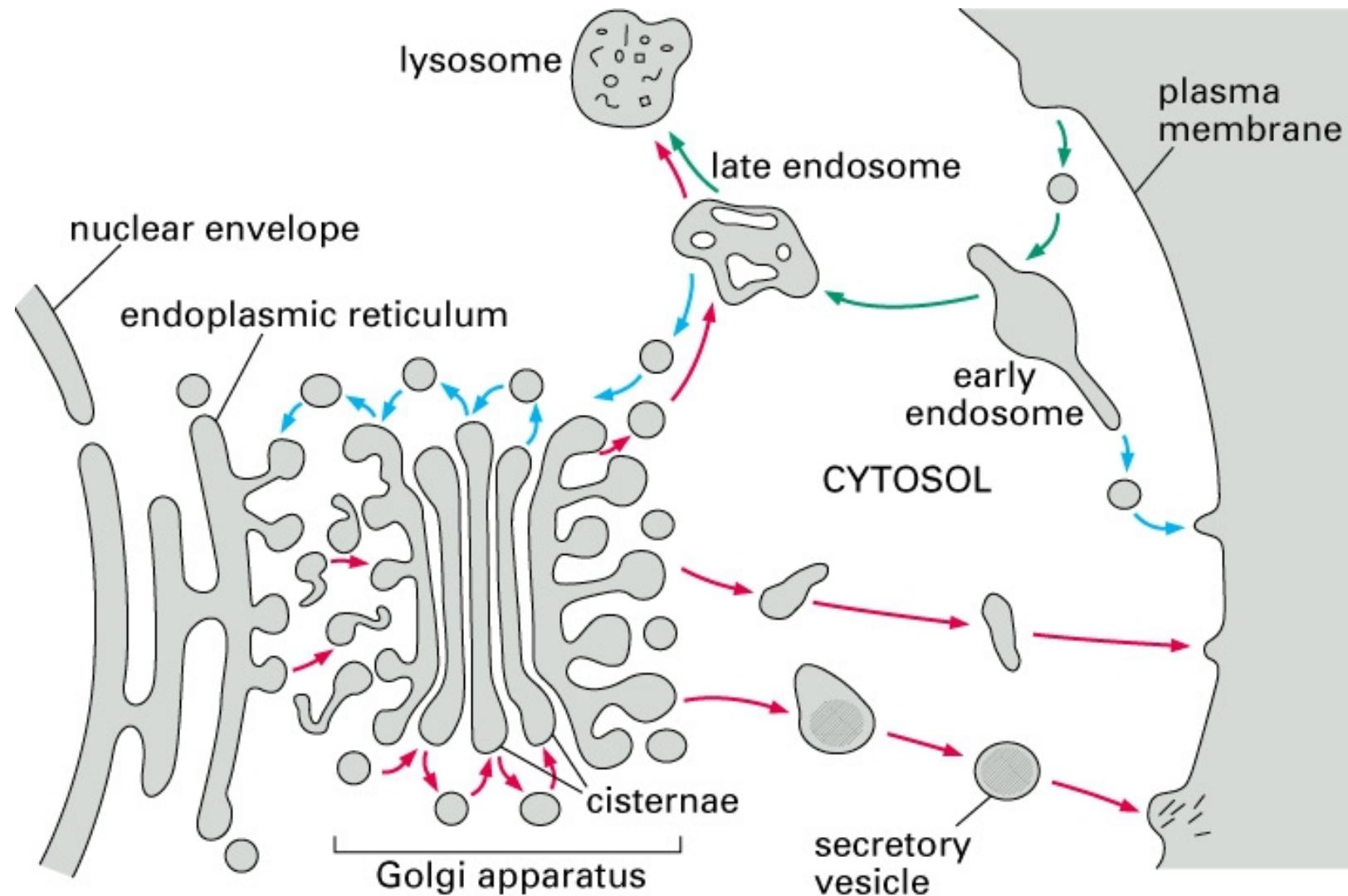
- **Prokaryotes:** communication and digestion are across plasma membrane.
- **Eukaryotes:** complex internal membrane system.
- **Endocytosis:** active transport of macromolecules into the cell.  
=> endocytic pathway
- **Exocytosis:** active transport of macromolecules out of the cell.  
=> biosynthetic-secretory pathway
- **Lumen:** interior space of membrane-enclosed compartment.
- **Transport packages = transport vesicles**
- **Cargo:** transported molecules

# INTRODUCTION: GENERAL SCHEME OF VESICULAR TRANSPORT



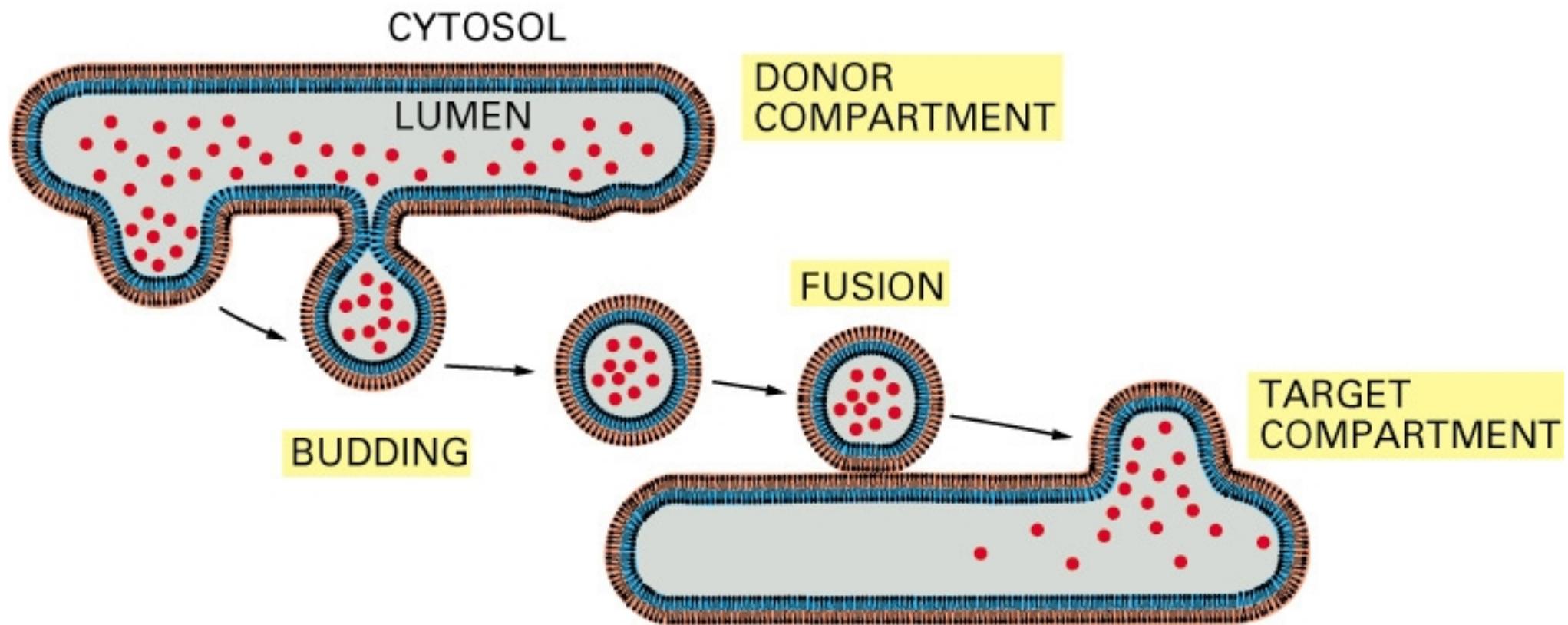
# INTRODUCTION: GENERAL SCHEME OF VESICULAR TRANSPORT

## ➤ Lumen topological equivalence



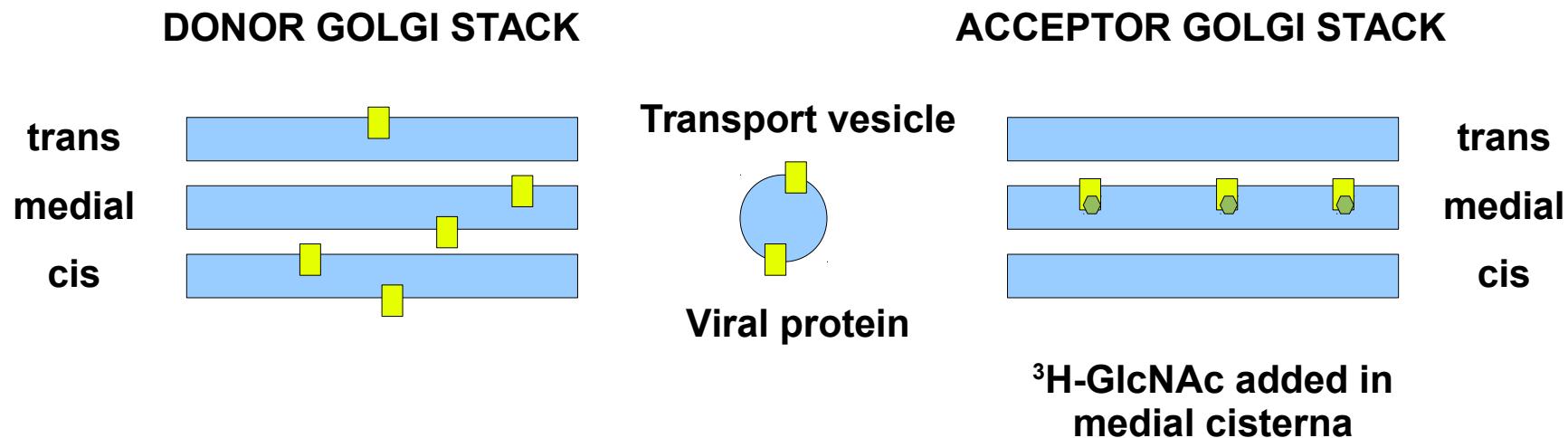
# INTRODUCTION: BUDDING AND FUSION

- Budding: separation of a vesicle from a donor compartment
- Fusion: docking of a vesicle to acceptor compartment

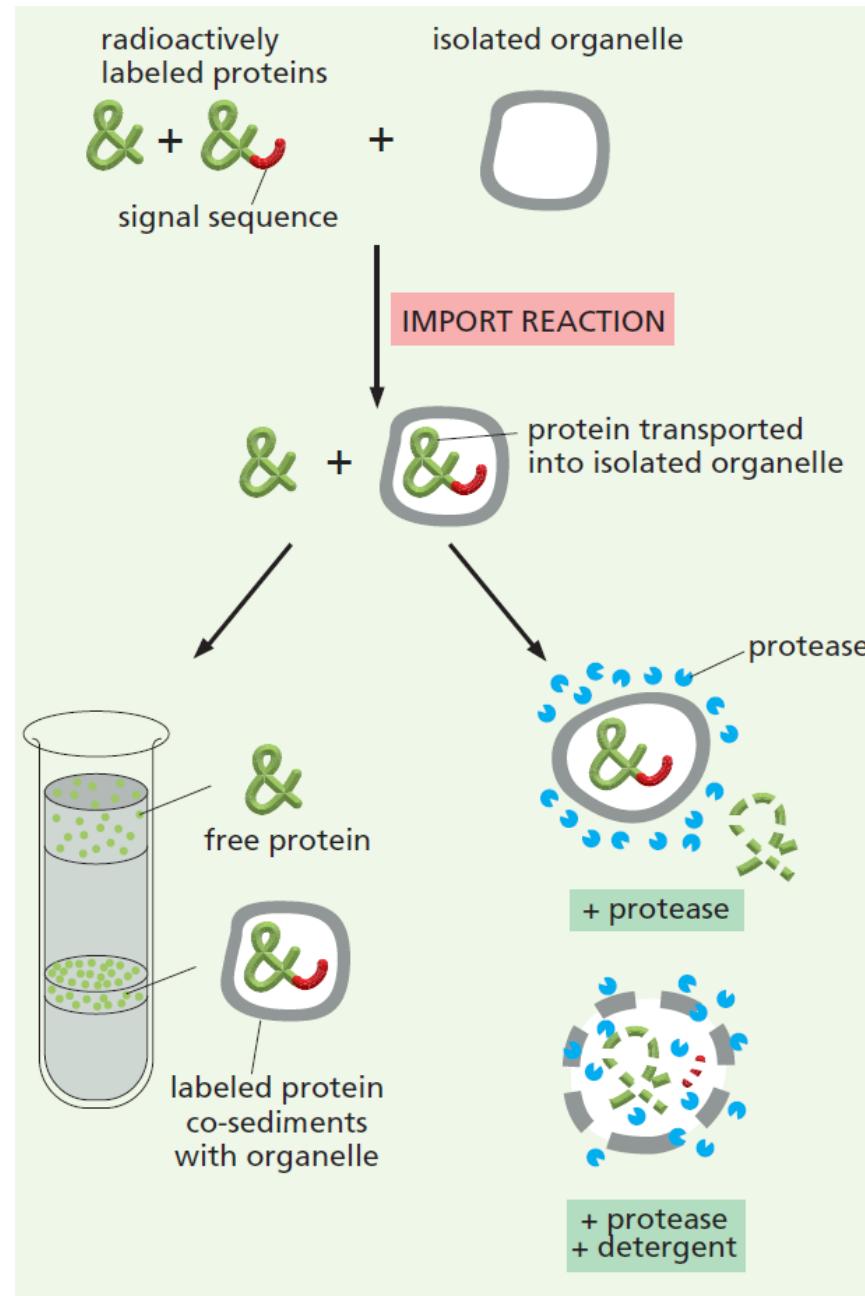


# HOW TO STUDY VESICULAR TRANSPORT: CELL-FREE SYSTEMS

- Donor and acceptor Golgi stacks
- + cytosole
- + ATP
- Donor Golgi stack lacks GlcNAc transferase
- Observation of the glycosylation state of a viral protein

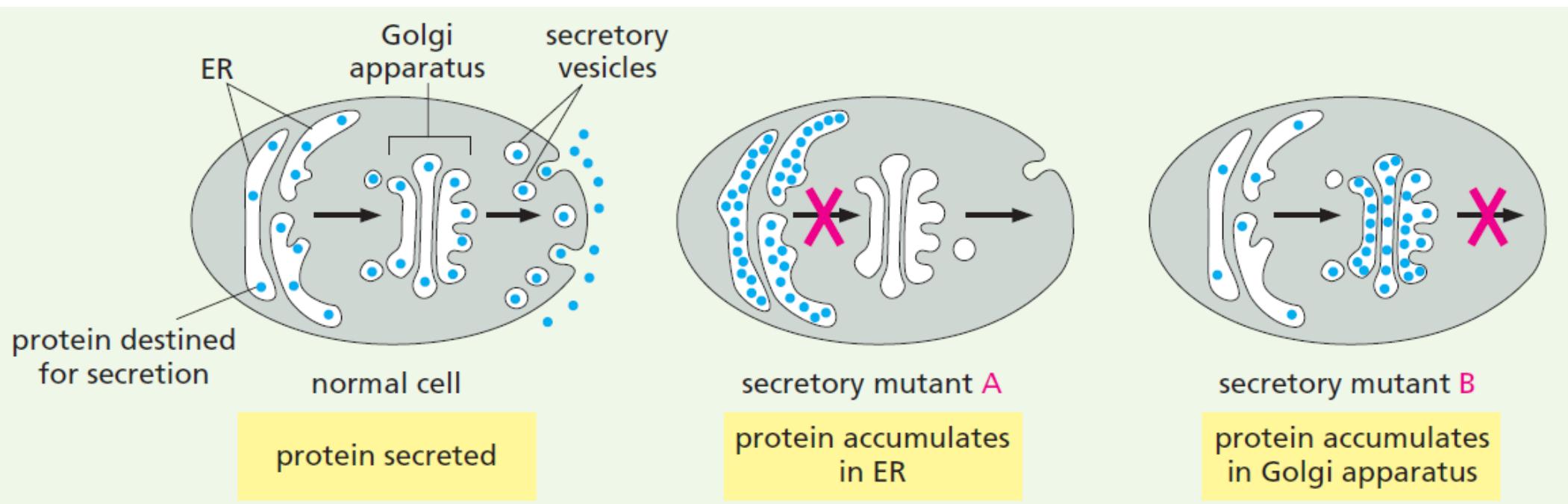


# HOW TO STUDY VESICULAR TRANSPORT: LABELLING

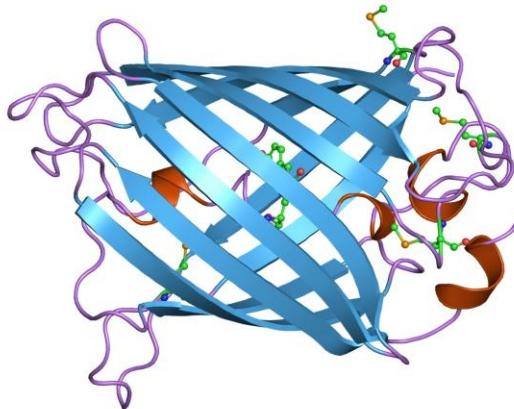


# HOW TO STUDY VESICULAR TRANSPORT: GENETIC APPROACHES

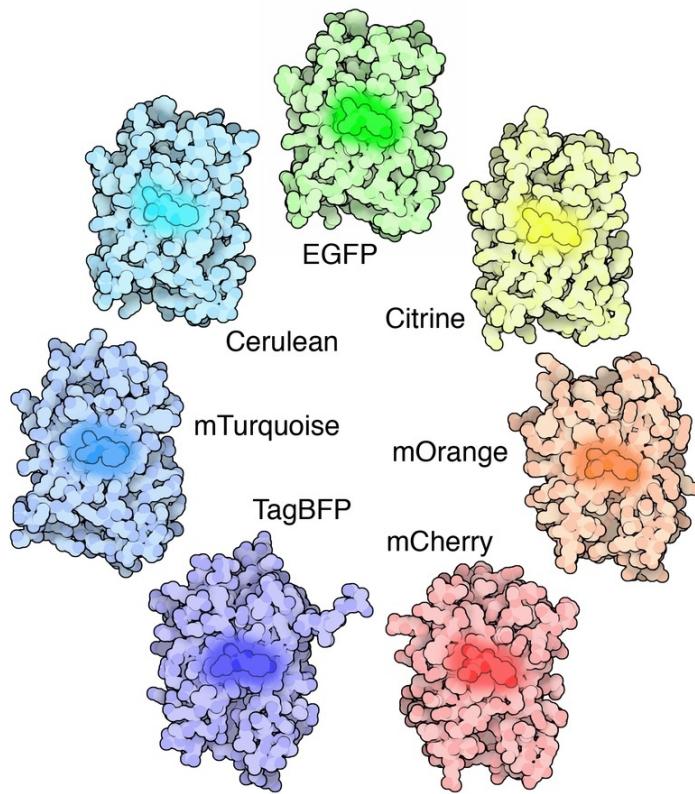
Mutations are made in the genes suspected of participation in vesicular transport.



# HOW TO STUDY VESICULAR TRANSPORT: GFP-FUSION

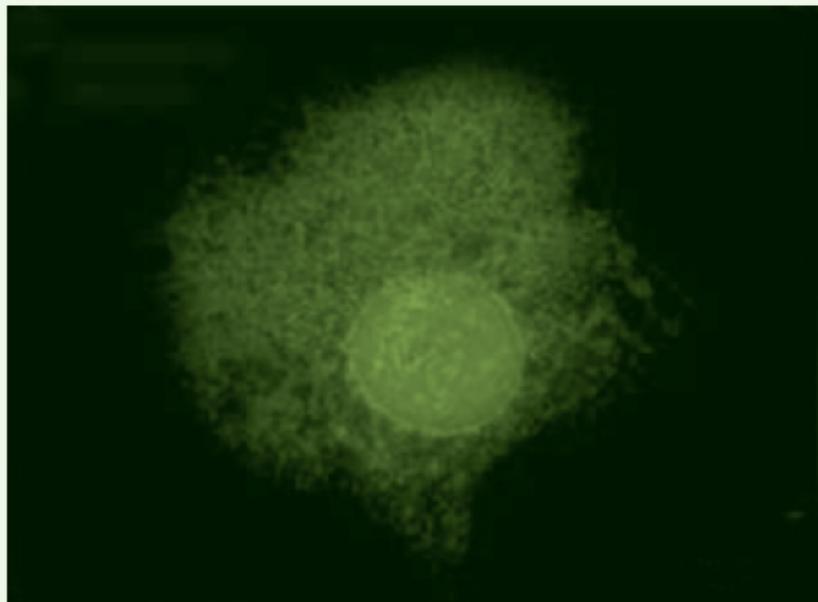


GFP: green fluorescent protein

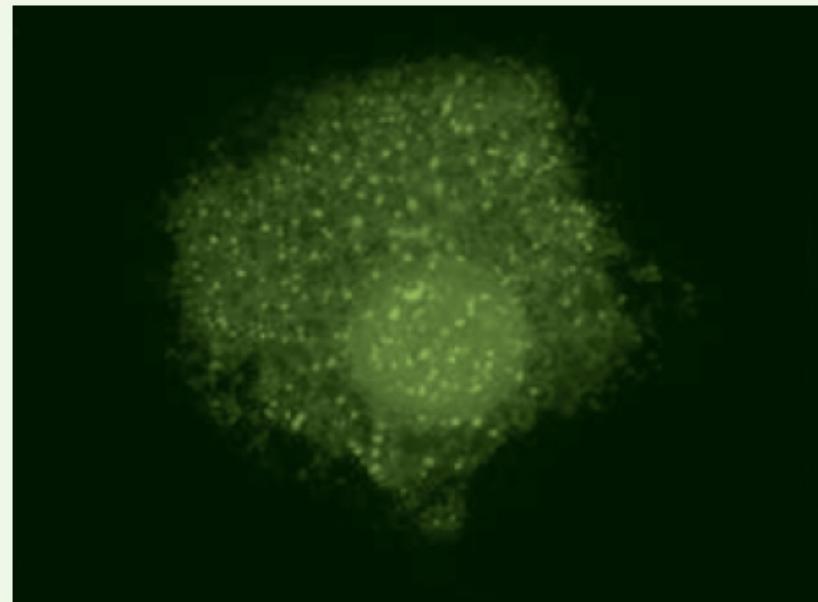


Organisms  
expressing FPs

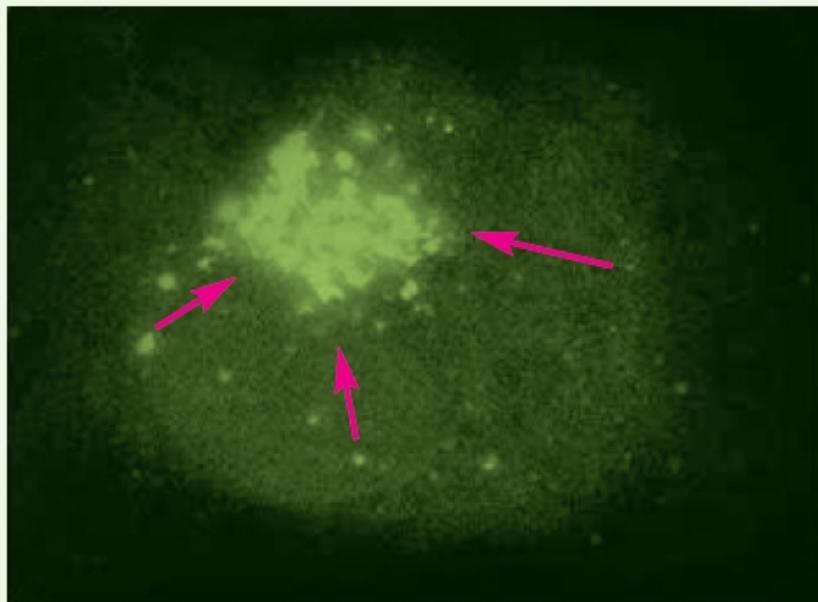
# HOW TO STUDY VESICULAR TRANSPORT: GFP-FUSION



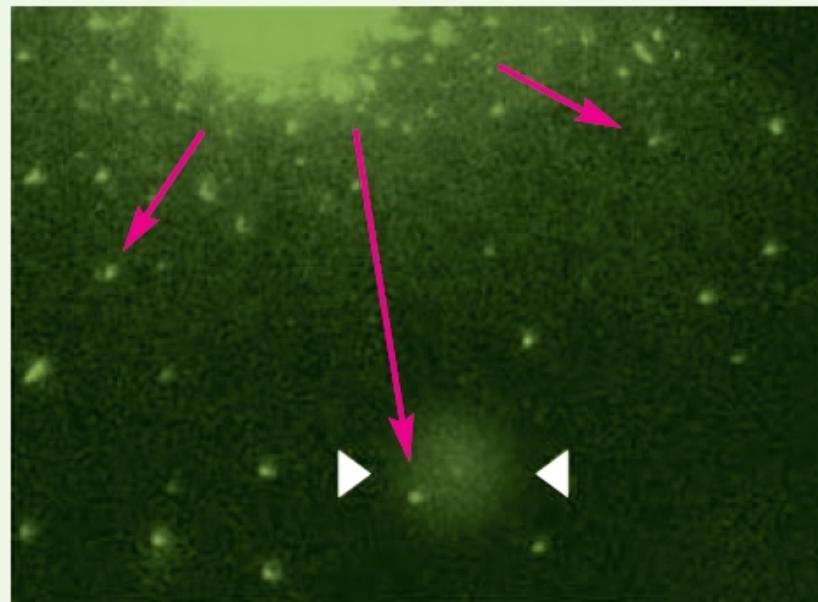
Synthesis hasn't start yet



ER



GA



Plasma membrane

# COMPARTMENTALIZATION

➤ Donor and acceptor compartments should be different

➤ Membrane markers:

- concentrations

- types

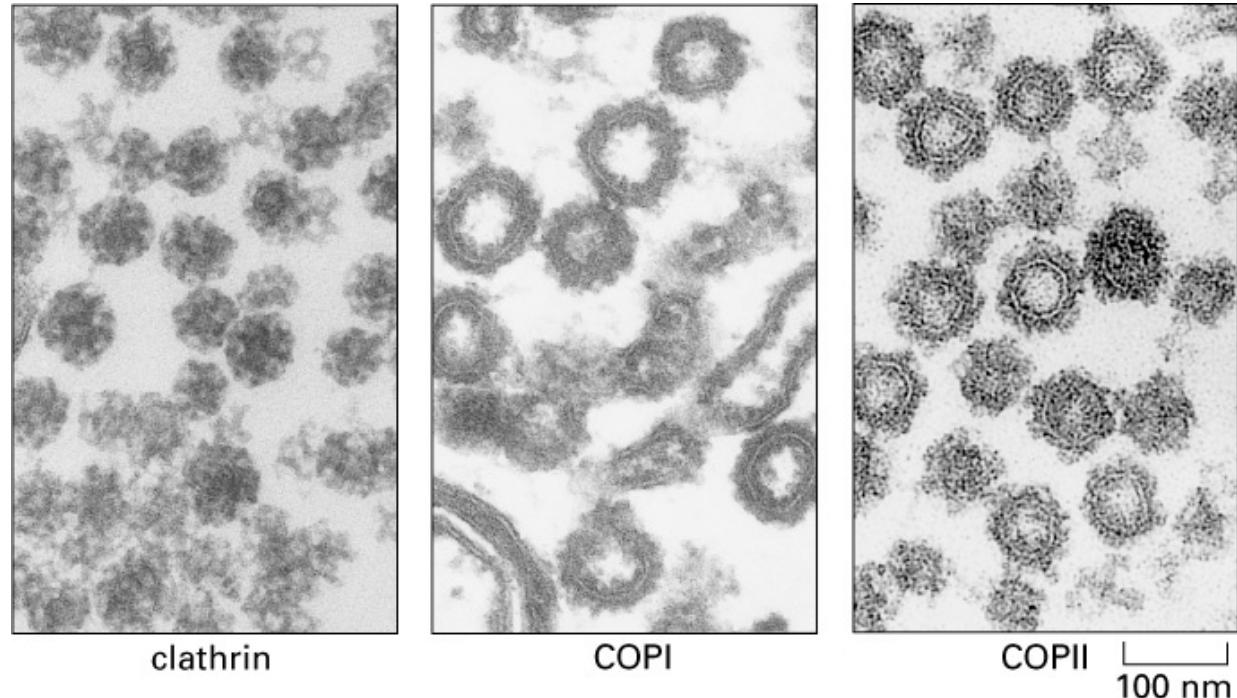
➤ Specific coating:

- concentration of cargo

- budding

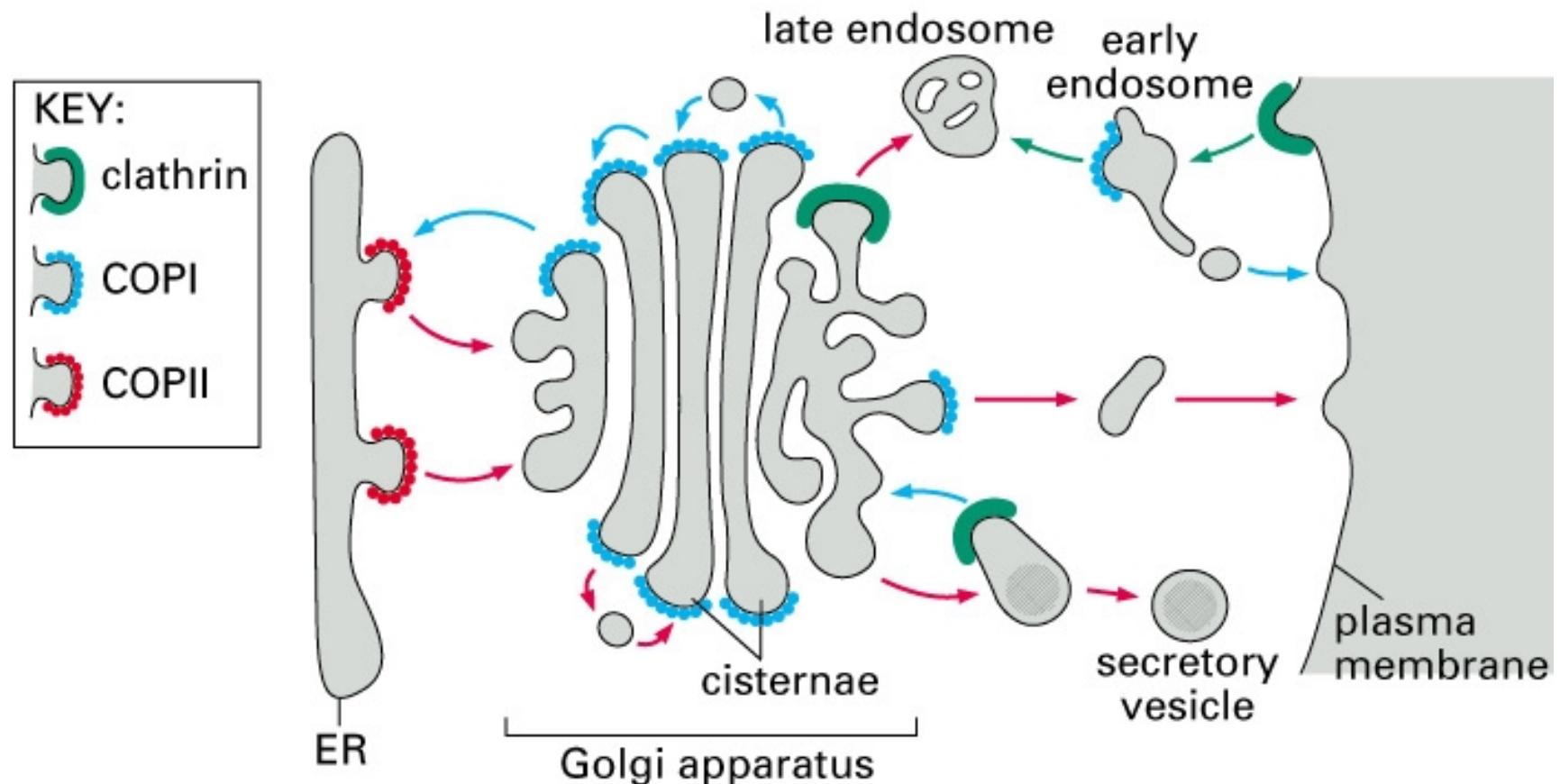
- types: clathrin, COPI, COPII

➤ Spheres and tubules, variable size



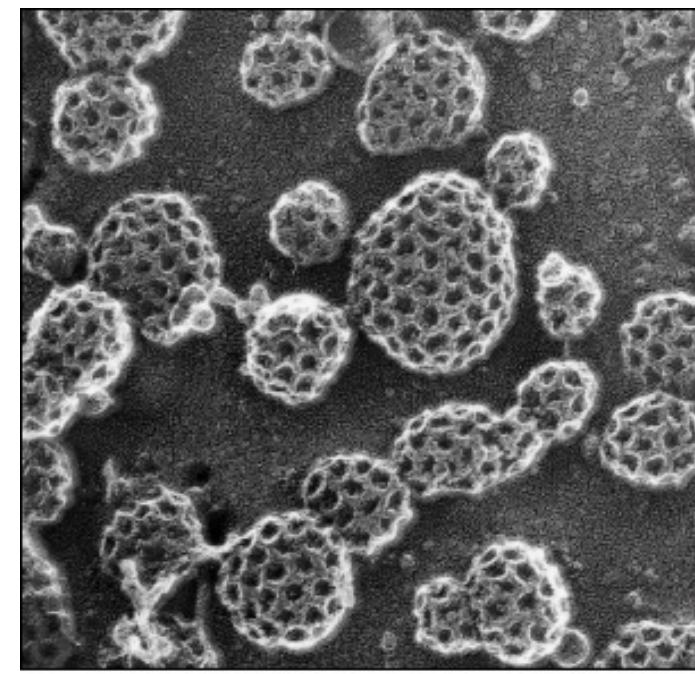
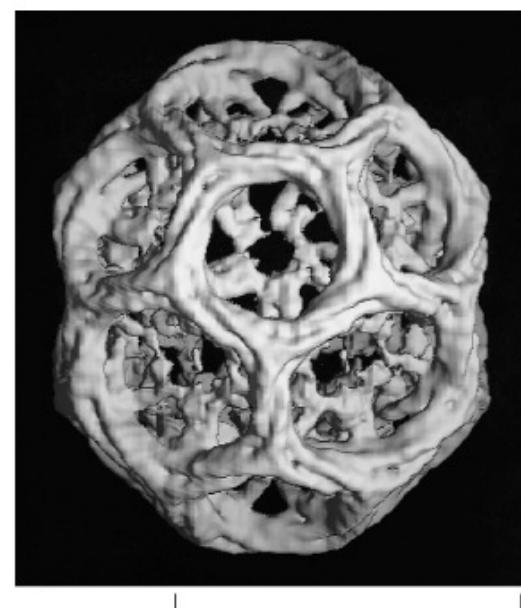
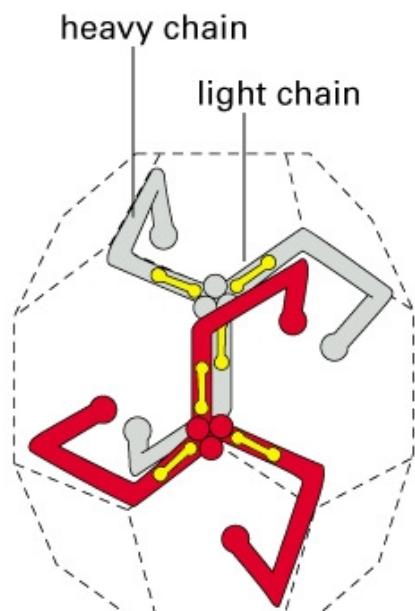
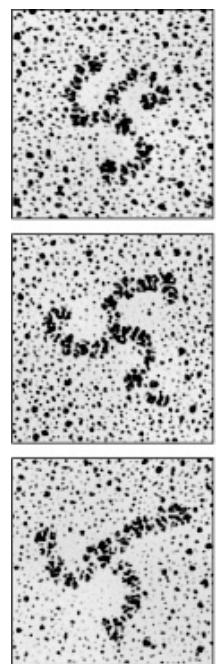
# COATING TYPES

- Clathrin: from GA, PM
- COPI: GA
- COPII: ER



# CLATHRIN COATING

- Clathrin: three large + three small chains = triskelion
- Self-assembly into hexagons and pentagons

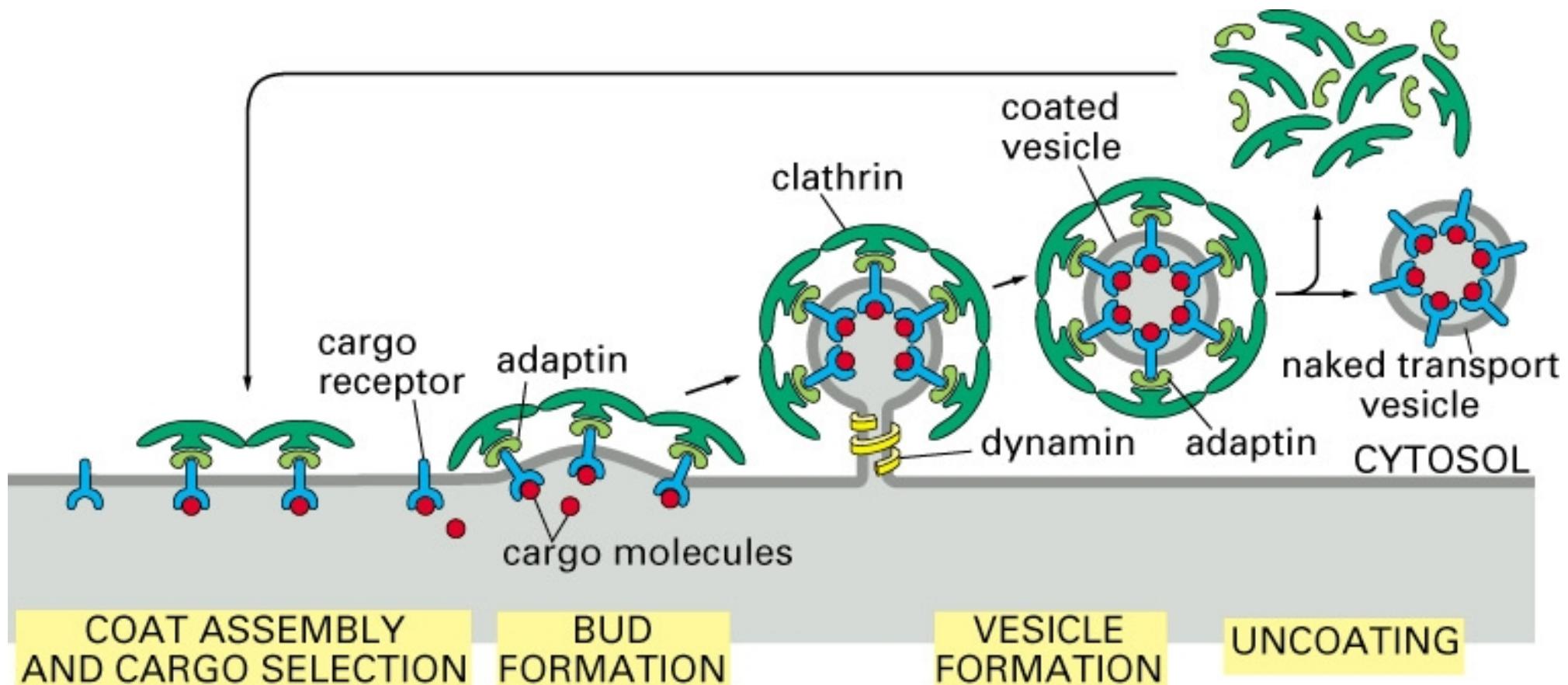


Clathrin vesicles

# CLATHRIN COATING

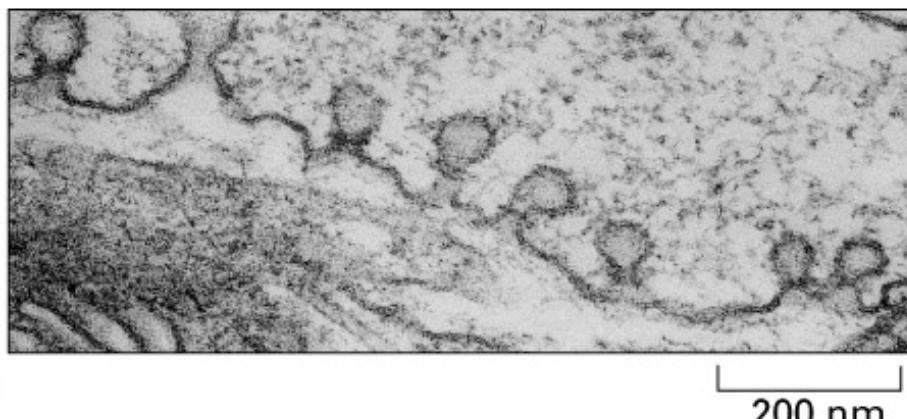
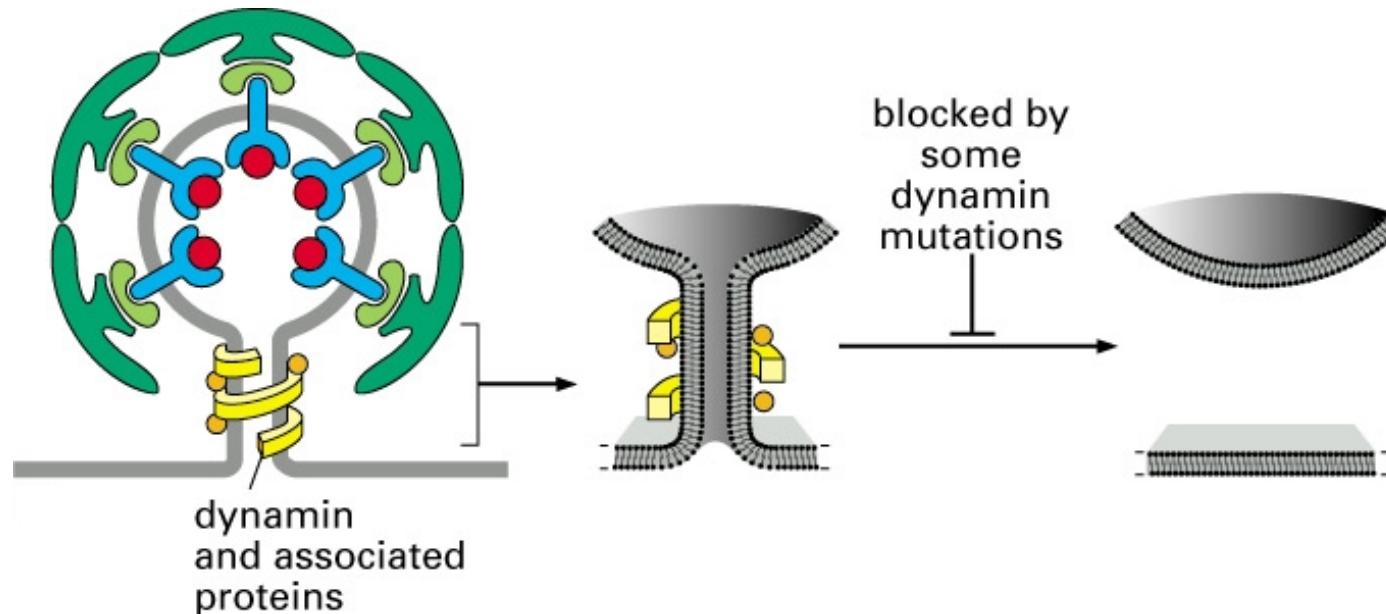
➤ Adaptein binds:

- clathrin coats
- auxiliary TM proteins
- cargo receptors



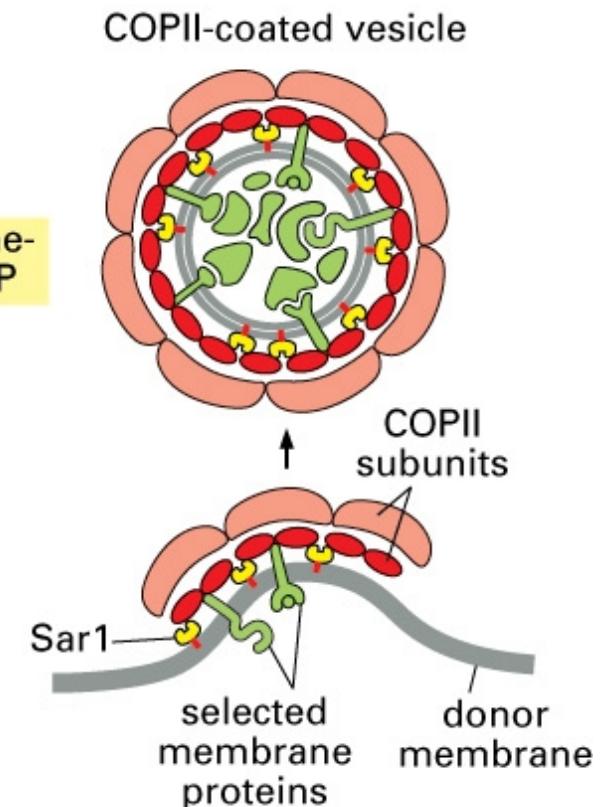
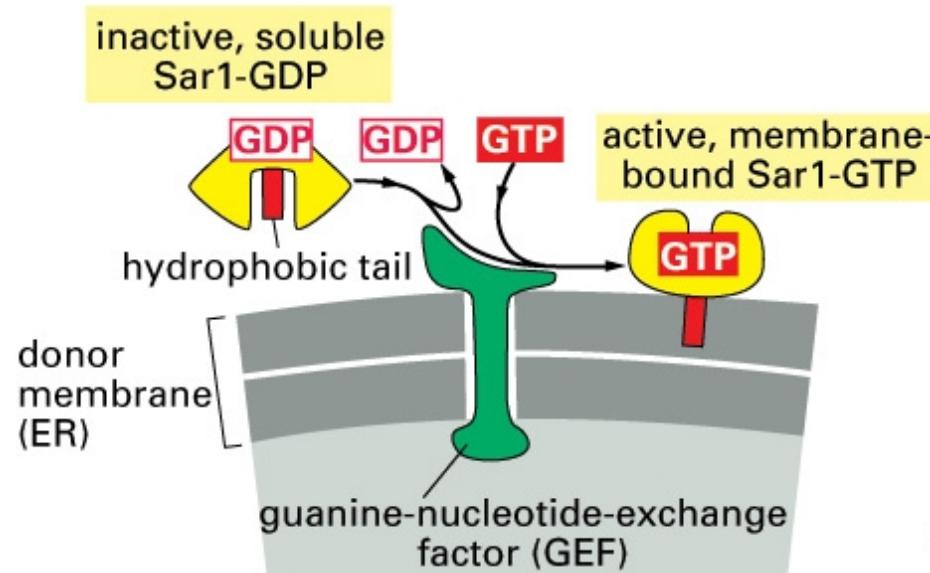
# CLATHRIN COATING

- Dynamin: GTPase performing pinching off
- Auxilin: uncoating ATPase
- Membrane composition => curvature, stiffness



# ROLE OF MONOMERIC GTPASES

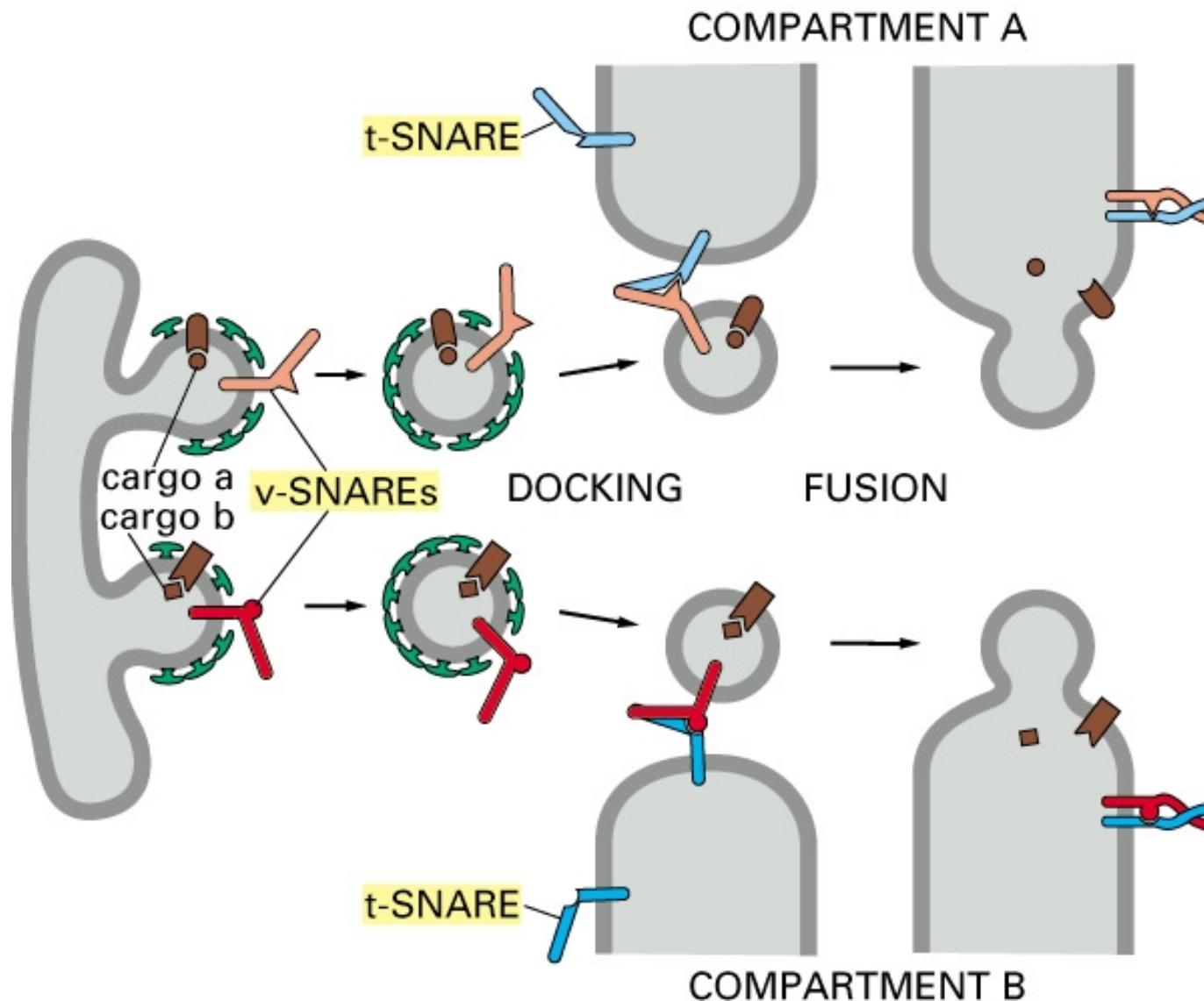
- GEF (guanine-nucleotide-exchange factor) activates:  $\text{GDP} \Rightarrow \text{GTP}$
- GAP (GTPase activating protein) activates:  $\text{GTP} \Rightarrow \text{GDP}$
- GTPases: monomeric and trimeric
- Coat-recruiting GTPases are in cytosole in inactive GDP-bound state:
  - ARF proteins (clathrin and COPI)
  - Sar1 protein (COPII)



- Protein-protein and protein-lipid interactions
- Coating proteins activate GTPases

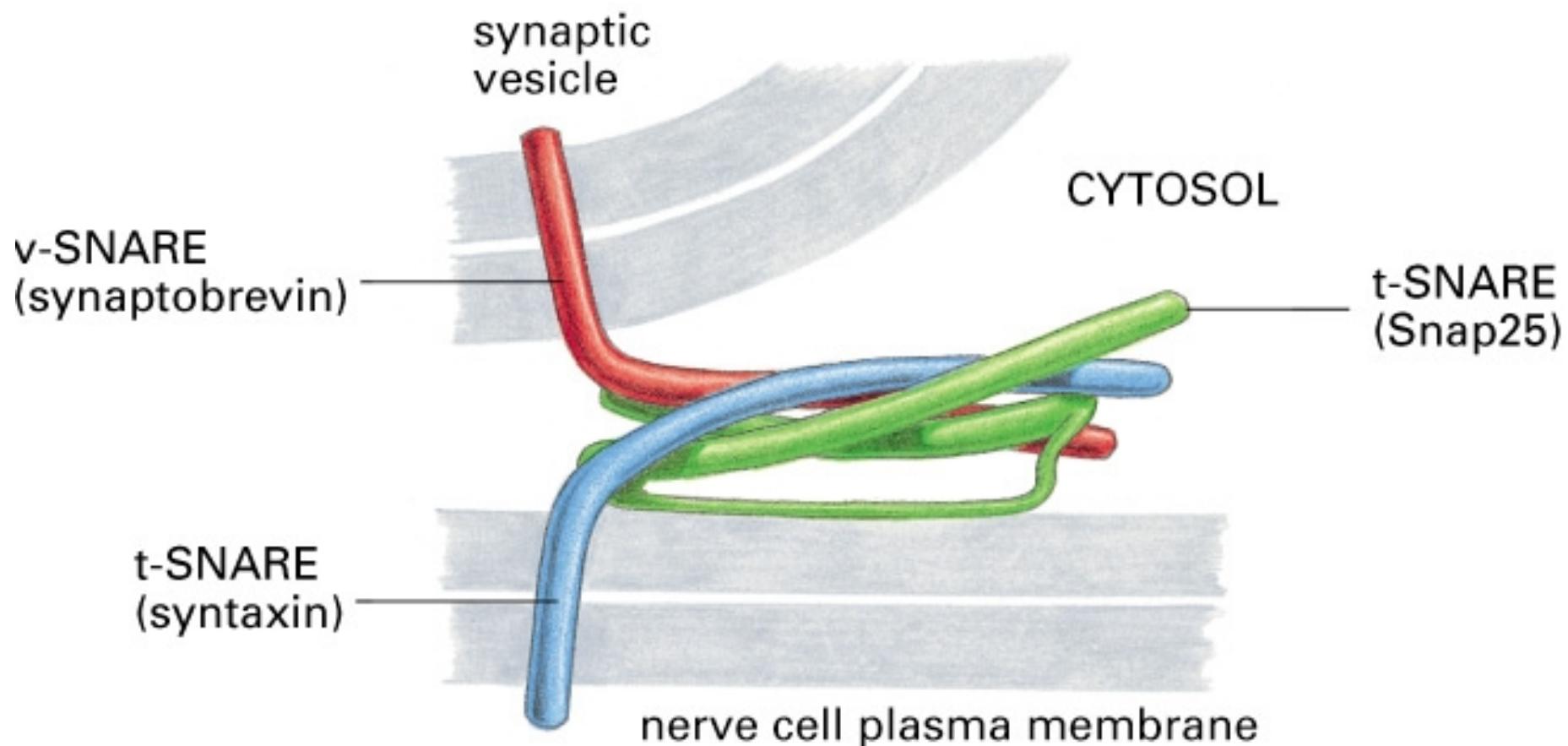
# TARGETING TO THE MEMBRANE

- SNAREs: tSNARE (target), vSNARE (vesicle)
- Rabs GTPases



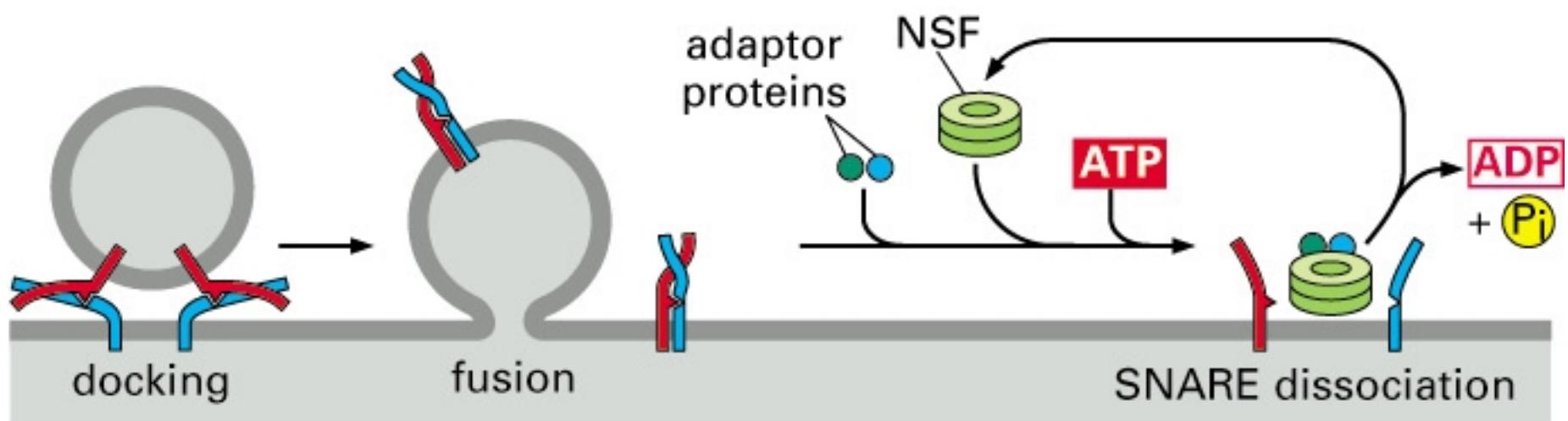
# SNARES

- ~ 20 different SNAREs, specific to organelles
- Trans-SNARE complexes formed by helical domains
- Tetanus: neurotoxins specific to SNAREs



# SNARES REACTIVATION

- NSF (N-ethylmaleimide sensitive fusion protein): ATPase, chaperon
- Adaptor proteins
- Specificity and control by reactivation



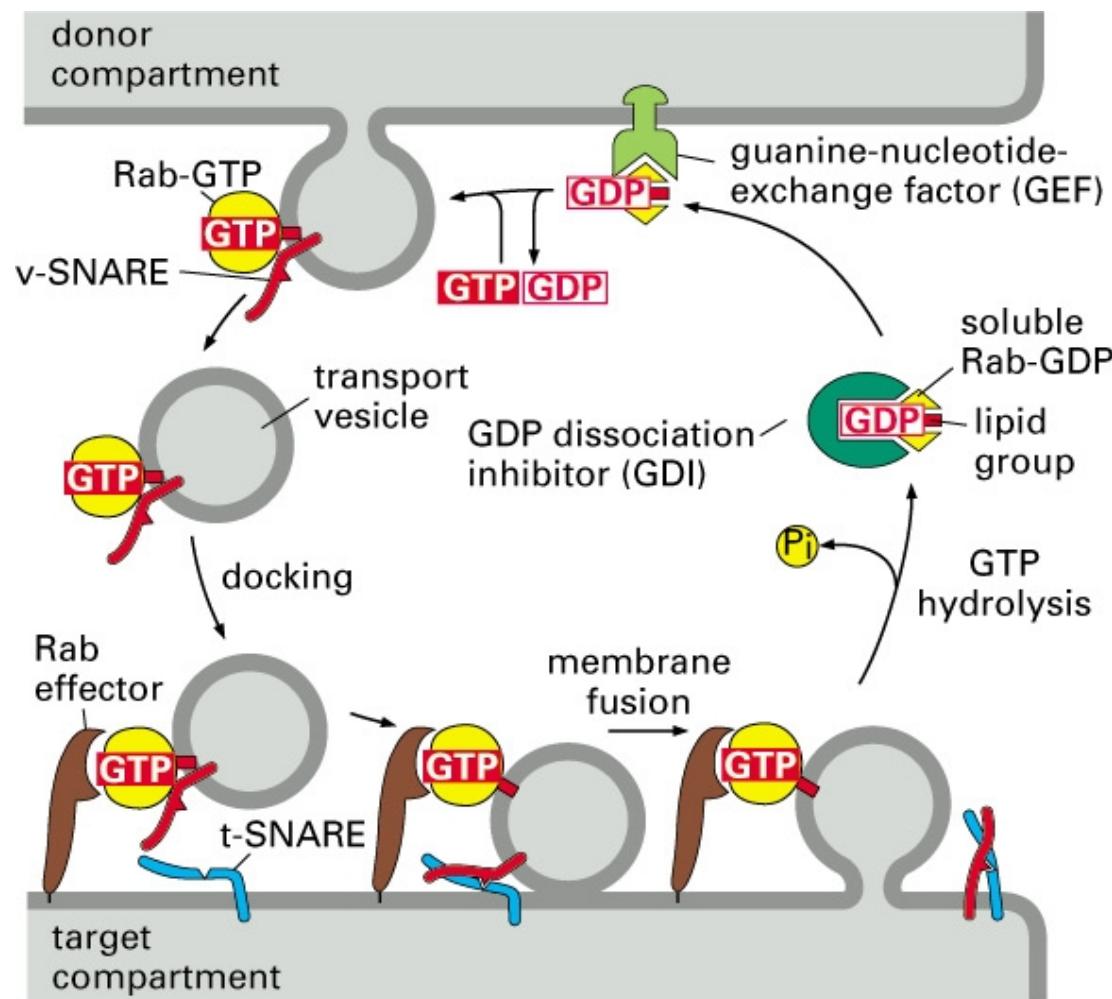
# RAB PROTEINS

- ~ 30 monomeric GTPases
- Function: specificity through docking and matching
- C-terminal sequence variability => specificity

PROTEIN	ORGANELLE
Rab1	ER and Golgi complex
Rab2	<i>cis</i> Golgi network
Rab3A	synaptic vesicles, secretory granules
Rab4	early endosomes
Rab5A	plasma membrane, clathrin-coated vesicles
Rab5C	early endosomes
Rab6	<i>medial</i> and <i>trans</i> Golgi cisternae
Rab7	late endosomes
Rab8	secretory vesicles (basolateral)
Rab9	late endosomes, <i>trans</i> Golgi network

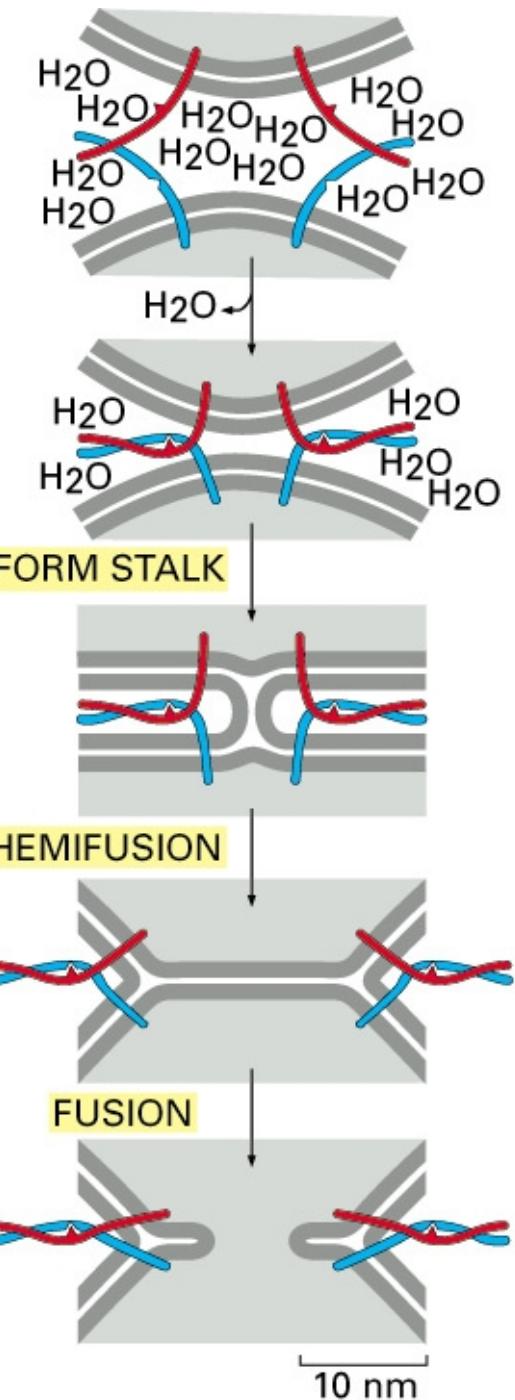
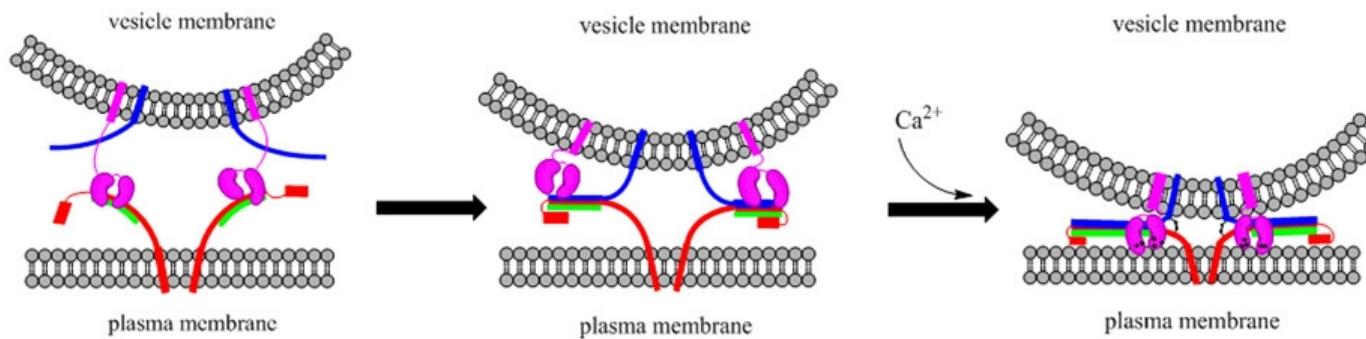
# RAB PROTEINS MECHANISM

- GDP-bound: inactive; GTP-bound: active
- Rab-effectors: highly variable auxiliary proteins
  - restriction of vesicle movements
  - stabilization of GTP-bound state



# DOCKING AND FUSION

- Docking: contact of two membranes
- Fusion: joining two lipid bilayers ( $\sim 1.5$  nm)
  - energetically unfavourable
  - triggered by signal (f.i. in exocytosis)
  - SNAREs play a key role
  - energy is obtained from the conformational change
  - zippering model

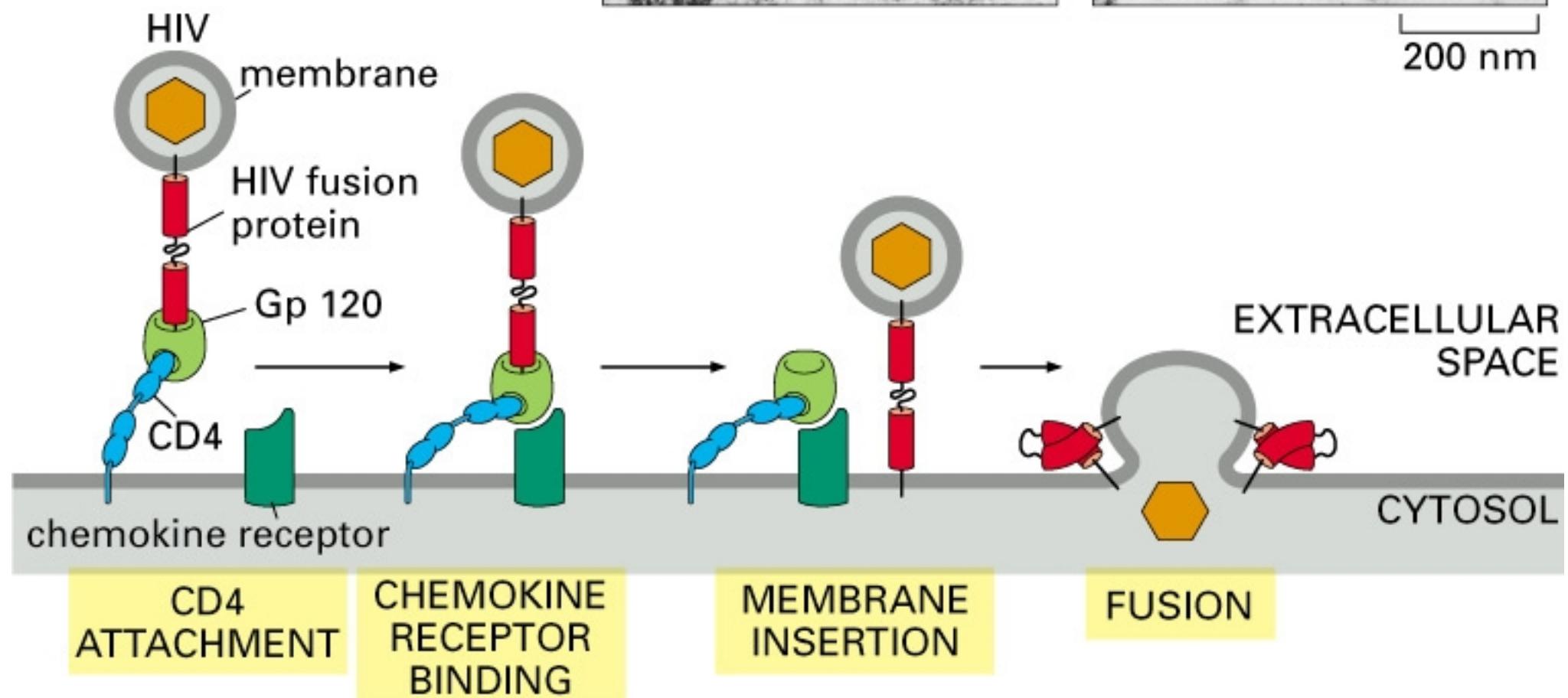
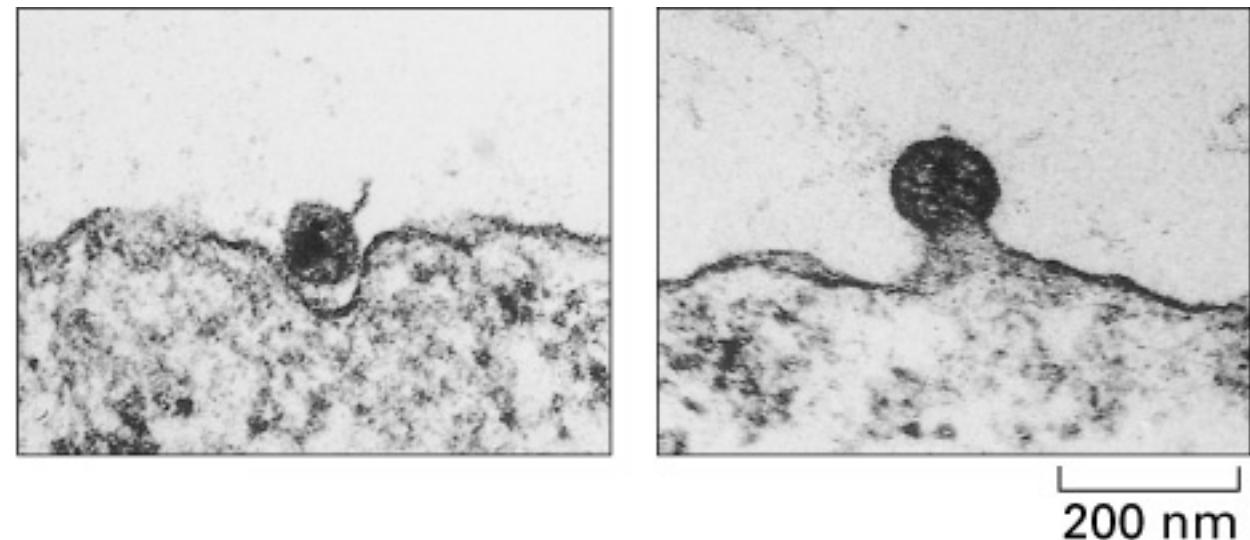


# VIRAL FUSION

Similar mechanism to

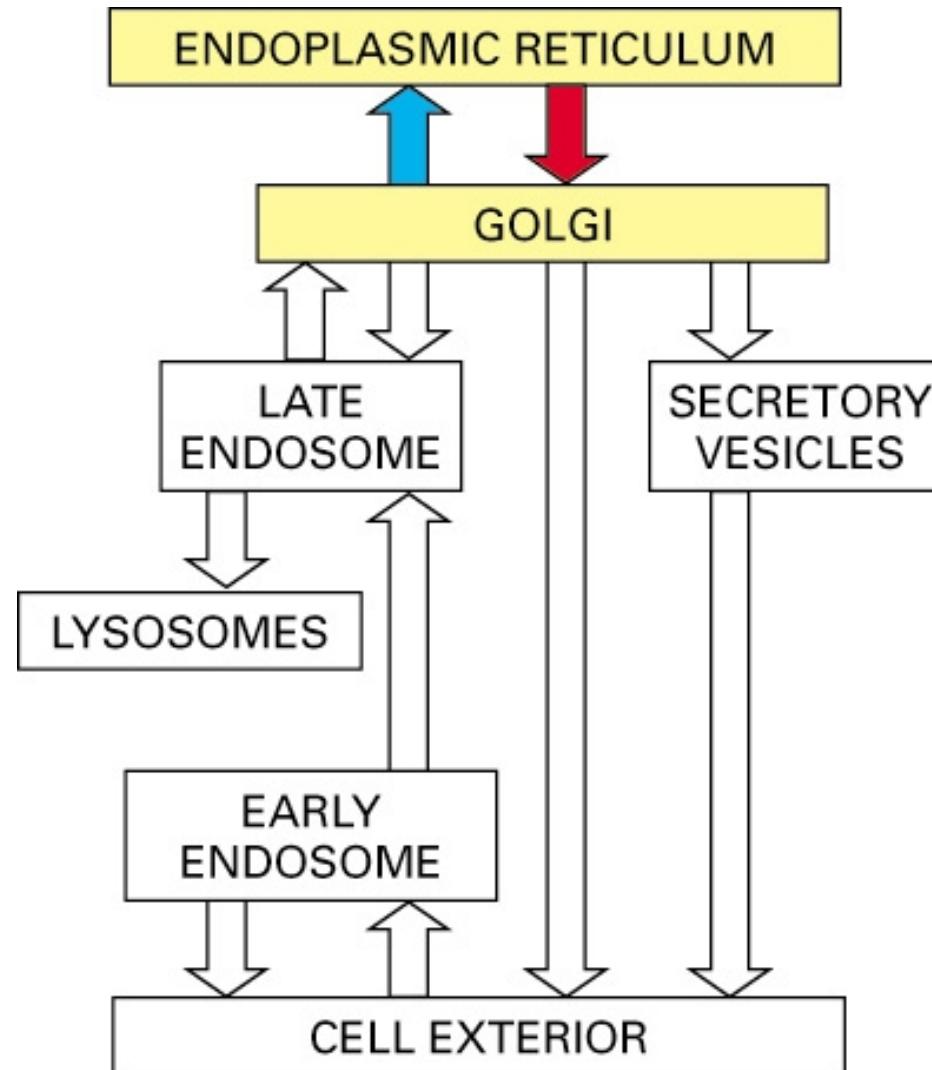
SNAREs: helical bundles

in fusion



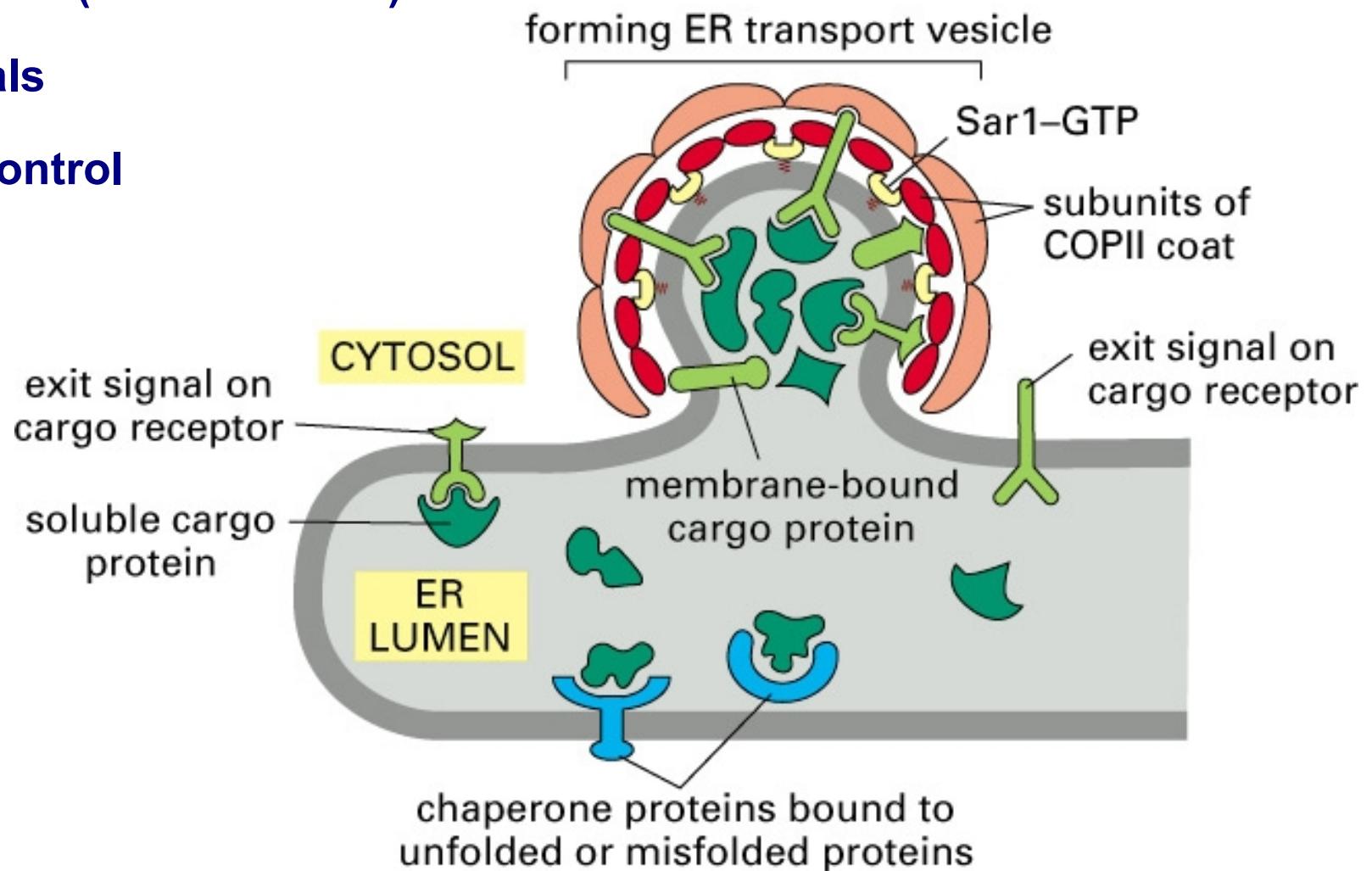
# TRANSPORT: ER <=> GA

Forward and retrieval transport, protein modifications => sorting



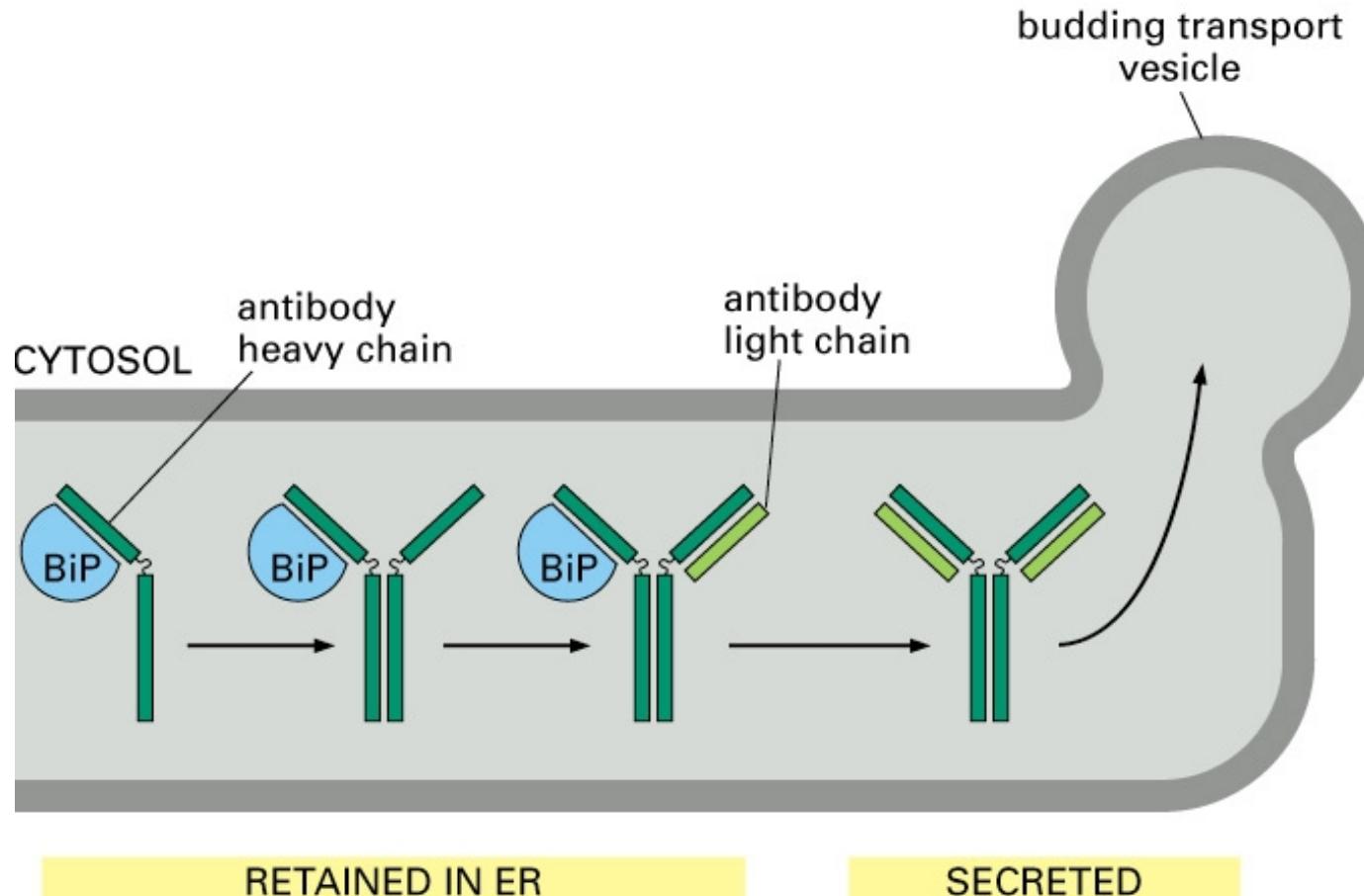
# COPII-COATING

- ~ 50nm vesicle
- ~200 membrane proteins
- ER exit sites (no ribosomes)
- Exit signals
- Folding control



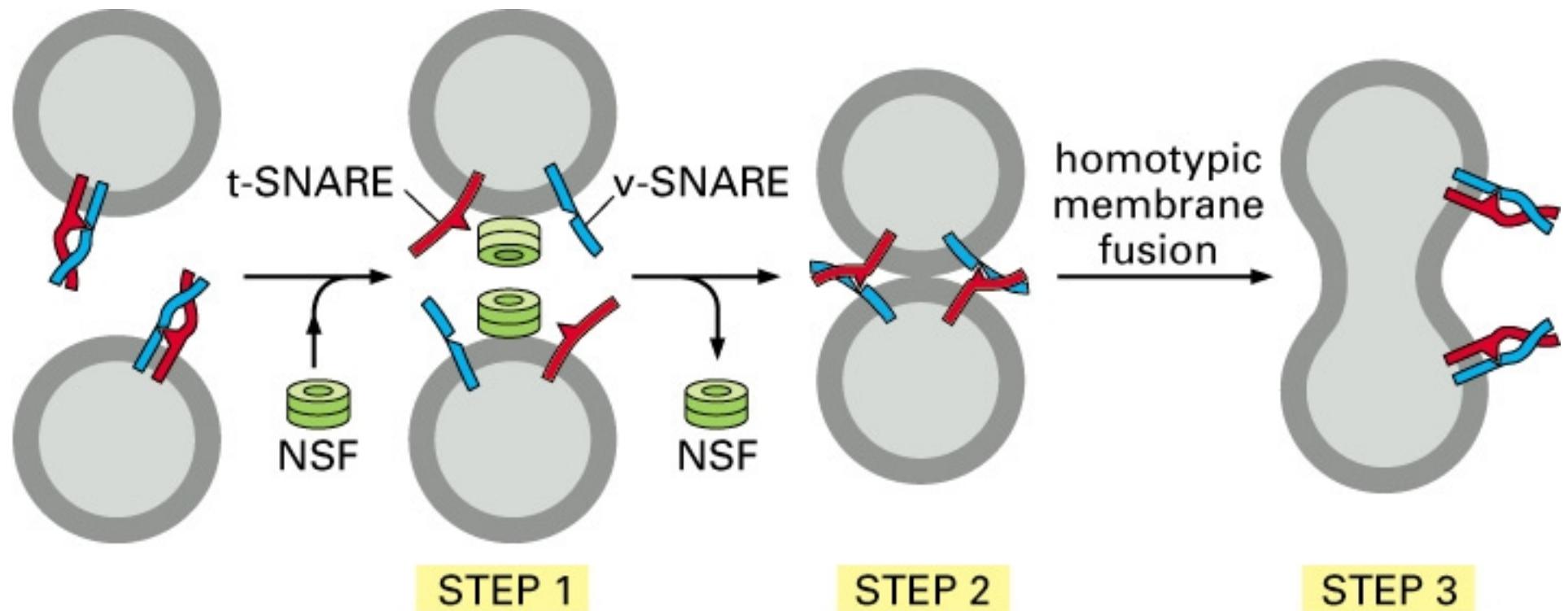
# RETENTION OF UNFOLDED PROTEINS

- BiP (binding immunoglobulin protein) and calnexin
- Exiting signals are covered
- About 90% of proteins are retained and degraded (T cell receptor)
- Cystic fibrosis: slightly misfolded Cl<sup>-</sup> transporter



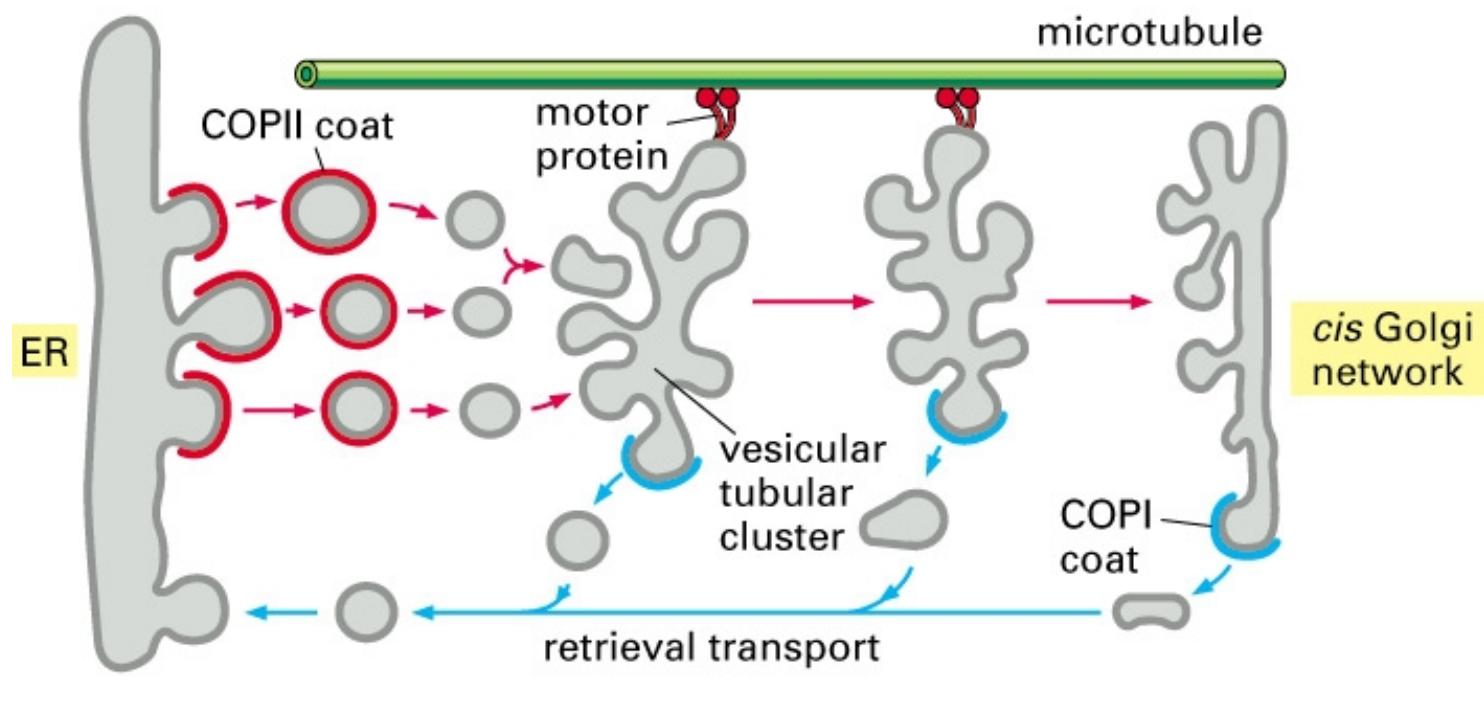
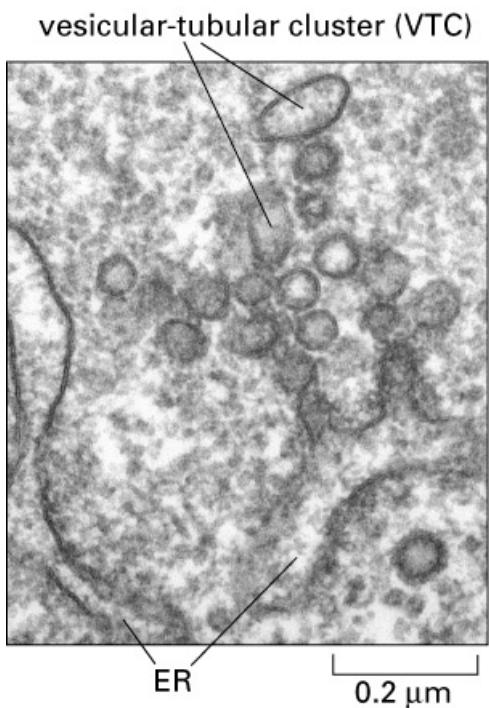
# FUSION OF SMALL VESICLES

- Homotypic: same compartments => same SNAREs
- Heterotypic: different compartments => different SNAREs
- Vesicular tubular clusters: ER-derived fused vesicles



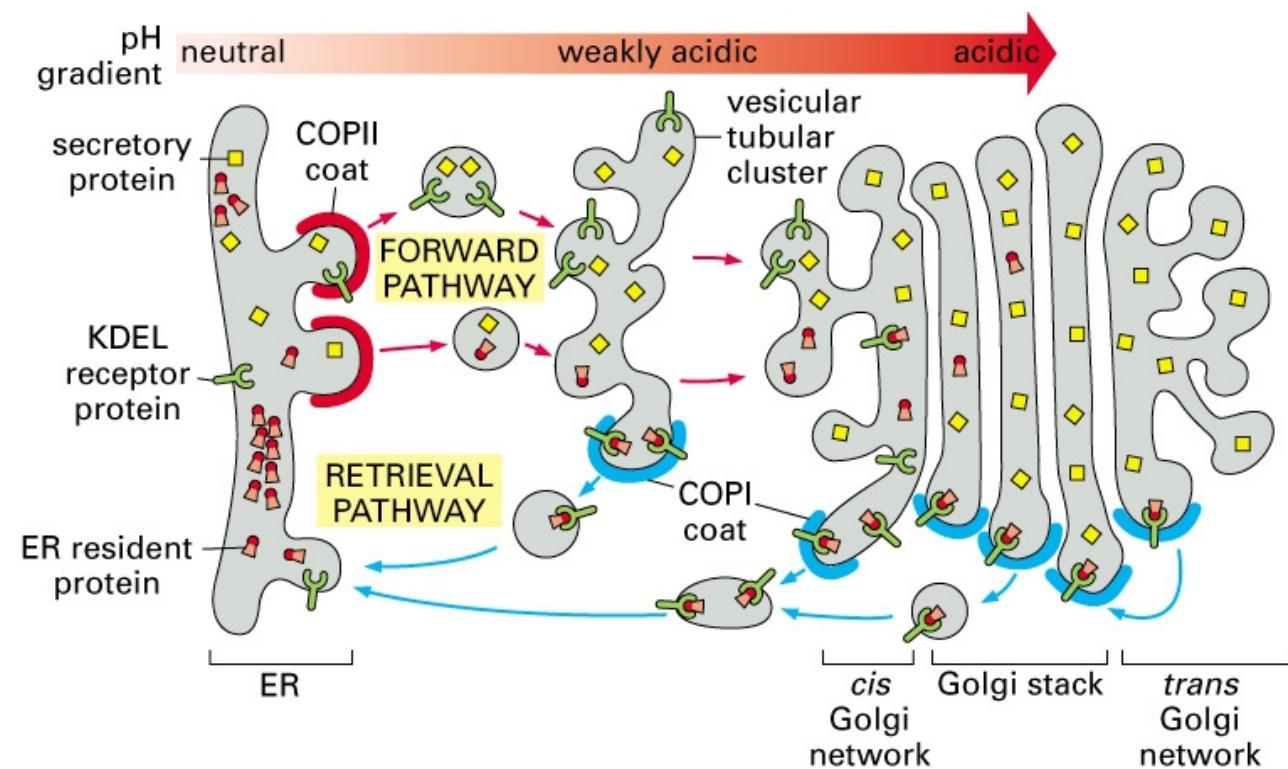
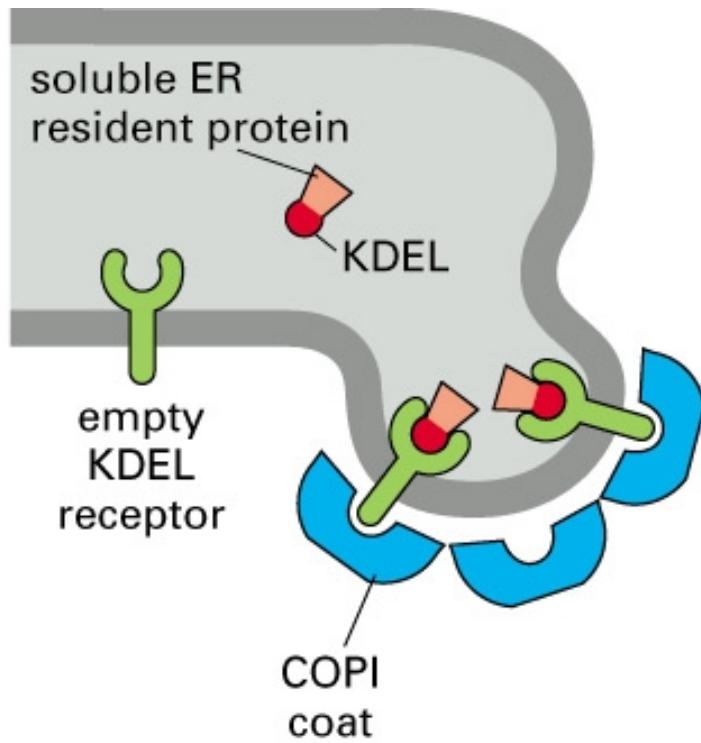
# VESICULAR TUBULAR CLUSTERS

Coating change specifies the direction of the transport.



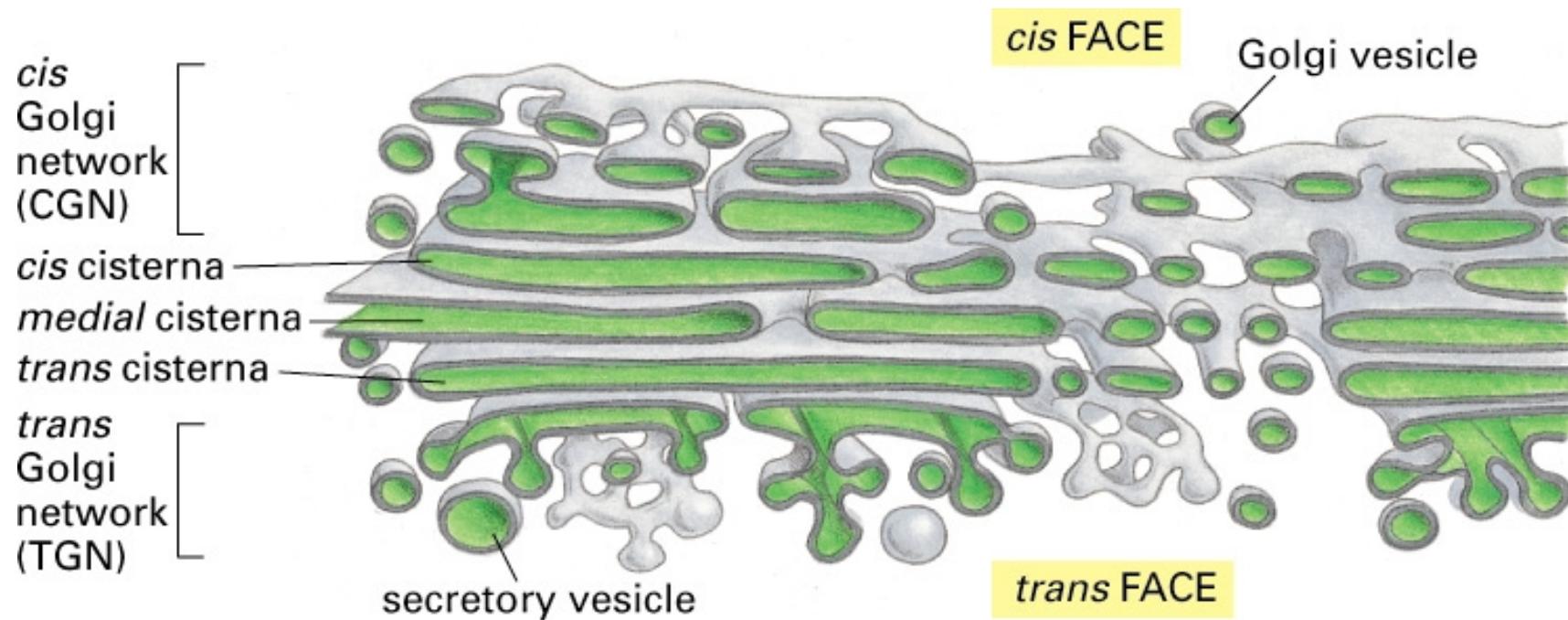
# RETRIEVAL PATHWAY

- ER retrieval sorting signals => COPI binding
- C-terminal KKXX motif for ER TM proteins
- C-terminal KDEL motif for ER soluble proteins, KDEL receptors:
  - removed by BiP => secretion; not removed => ER
  - KDEL receptors (different affinity in different compartments =  $f(pH)$ )

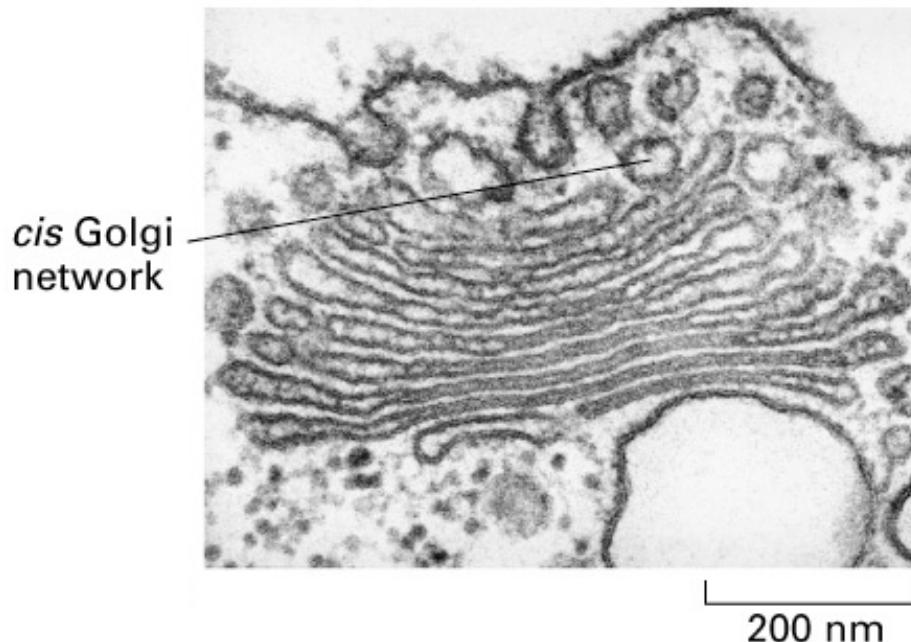
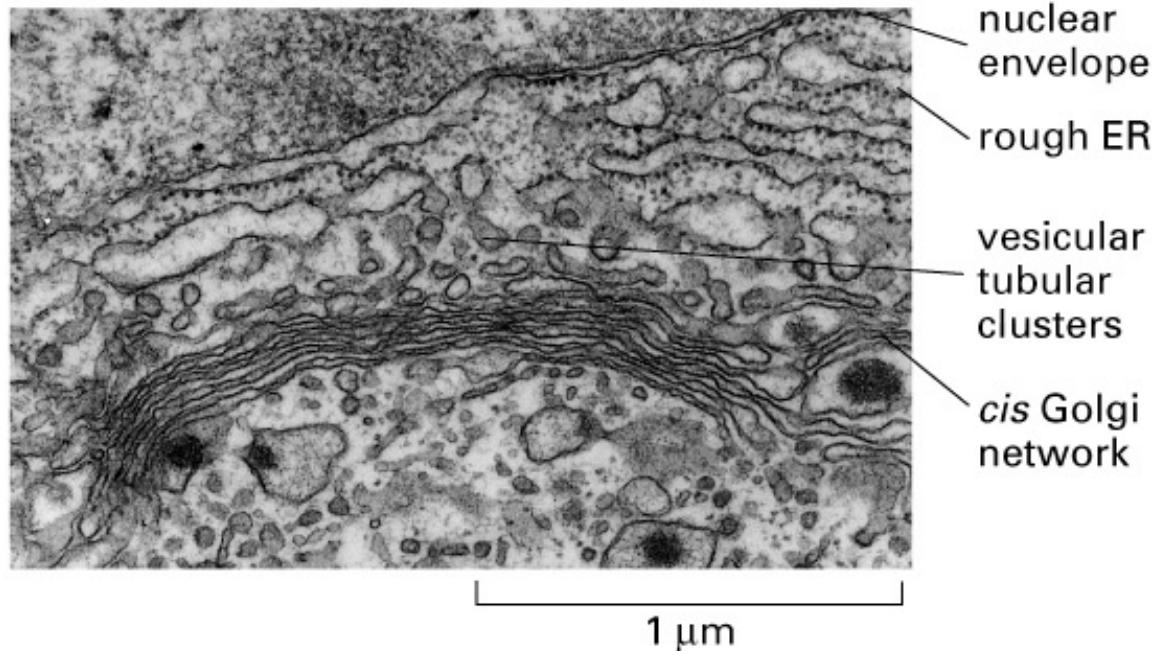


# FURTHER DETERMINATION OF COMPARTMENTALIZATION

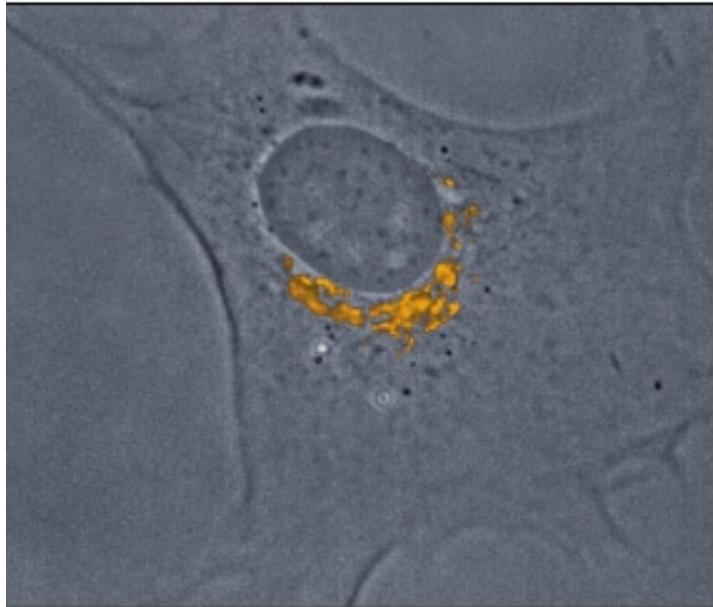
- Kin recognition: mechanism of retaining compartment-specific proteins: f.i. aggregation.
- TMD length (f.i. cholesterol in PM => thickness):
  - ER/GA: ~15aa
  - PM: ~20-25aa
- GA compartments



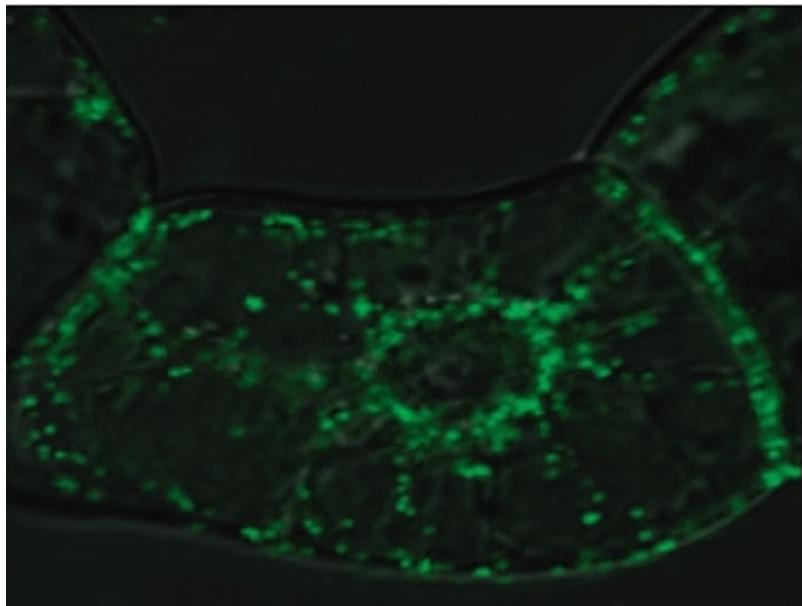
# GA STACKS



# GA STACKS



Polarization



GA enzymes in plants

secretion of mucus through apical surface

secretory vesicles containing mucus

Golgi apparatus

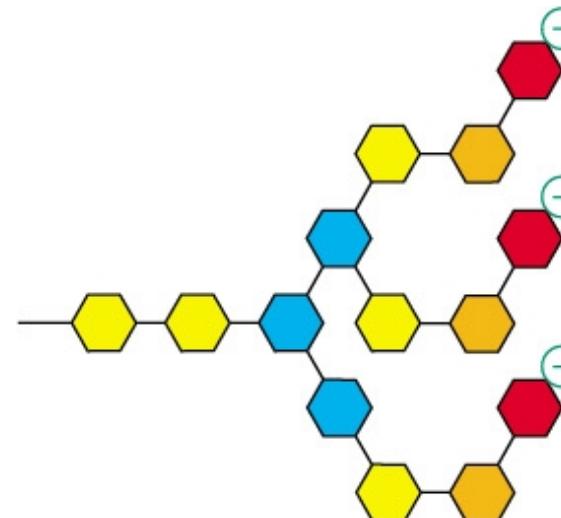
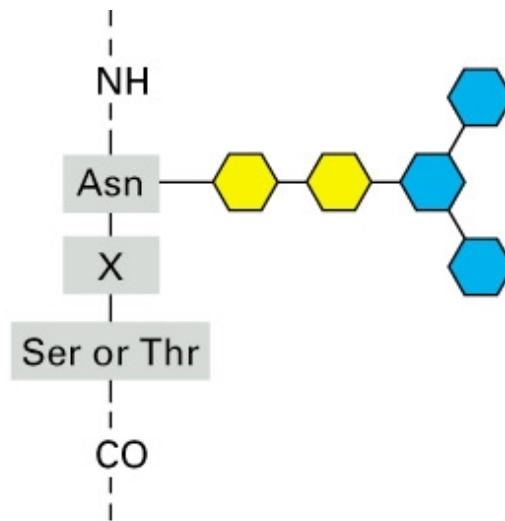
nucleus

10  $\mu$ m

Goblet cell of small intestine

# ER/GA: OLIGOSACCHARIDES PROCESSING

- $NX[S,T] \Rightarrow N\text{-oligomerization in ER}$
- $GA \Rightarrow$  complex or high-mannose oligosaccharides



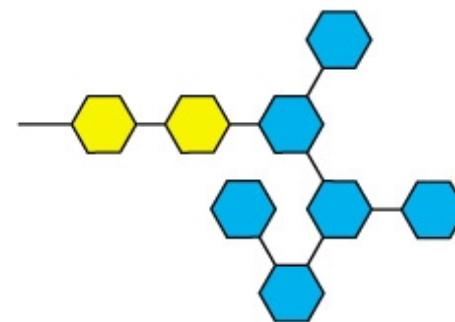
## KEY

= *N*-acetylglucosamine (GlcNAc)

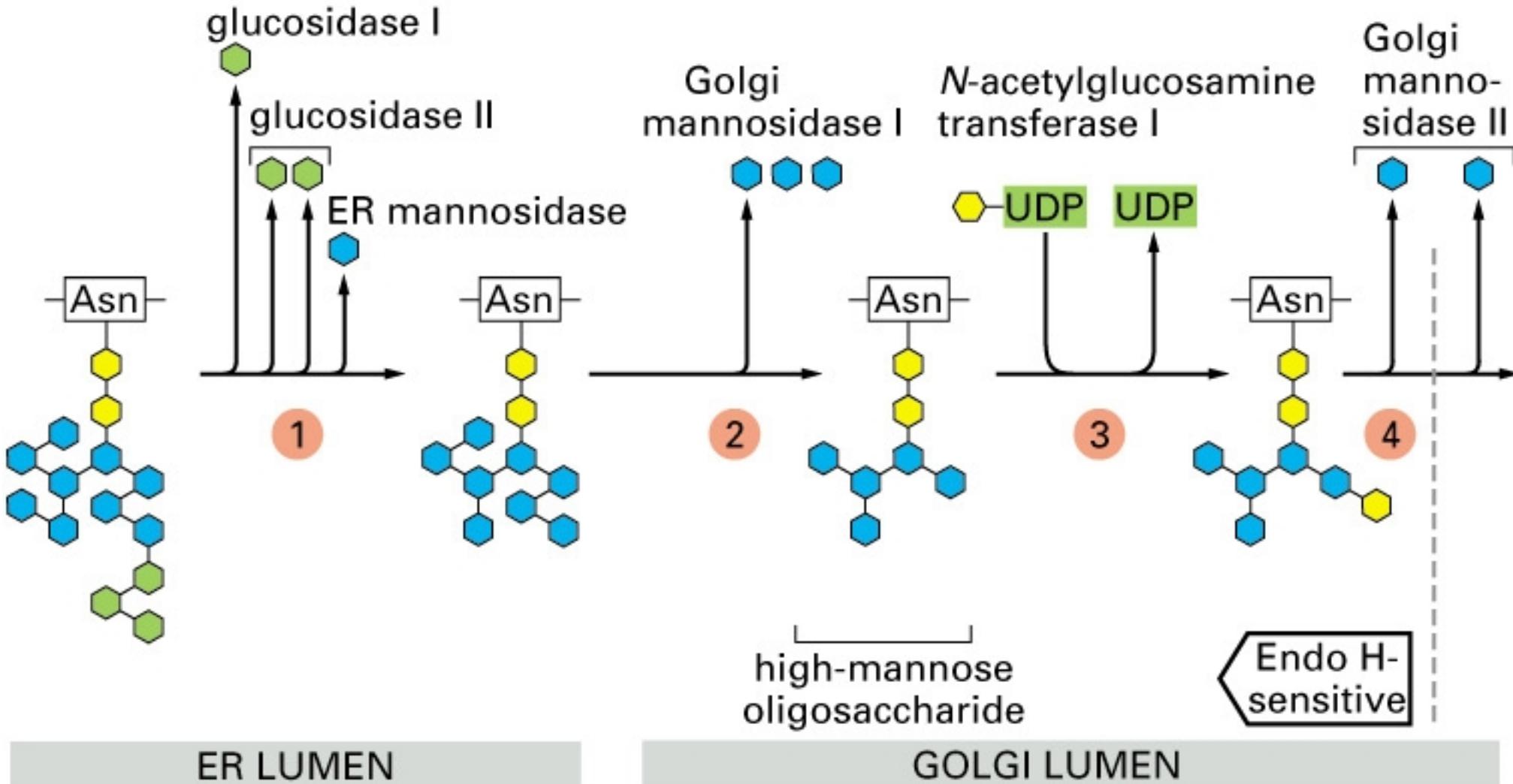
= mannose (Man)

= galactose (Gal)

= *N*-acetylneurameric acid (sialic acid, or NANA)



# ER/GA: OLIGOSACCHARIDES PROCESSING

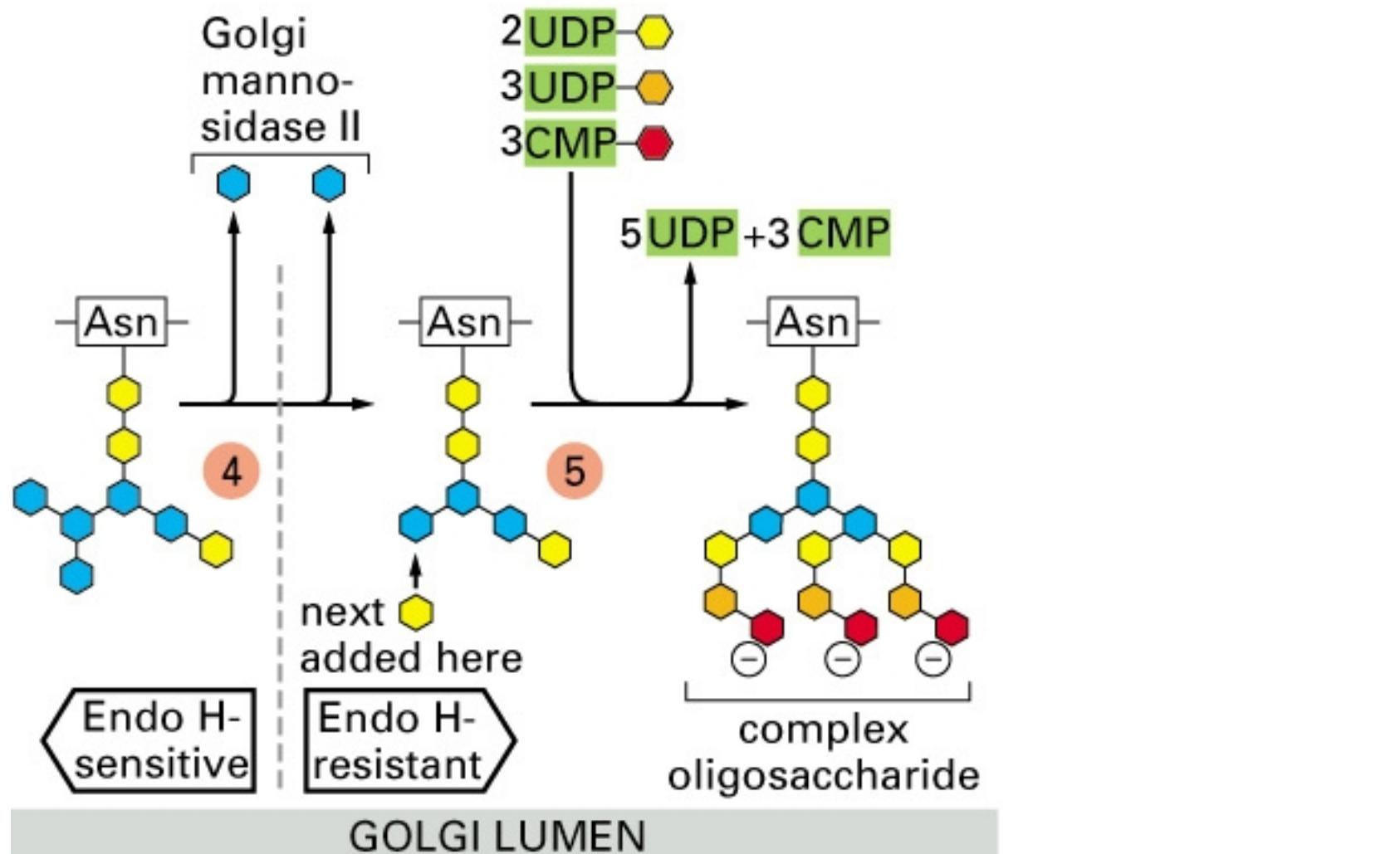


KEY:

- Yellow hexagon = *N*-acetylglucosamine (GlcNAc)
- Blue hexagon = mannose (Man)
- Green hexagon = glucose (Glc)

- Yellow circle = galactose (Gal)
- Red hexagon with a minus sign = *N*-acetylneurameric acid (sialic acid, or NANA)

# ER/GA: OLIGOSACCHARIDES PROCESSING



KEY:

Yellow hexagon = *N*-acetylglucosamine (GlcNAc)

Blue hexagon = mannose (Man)

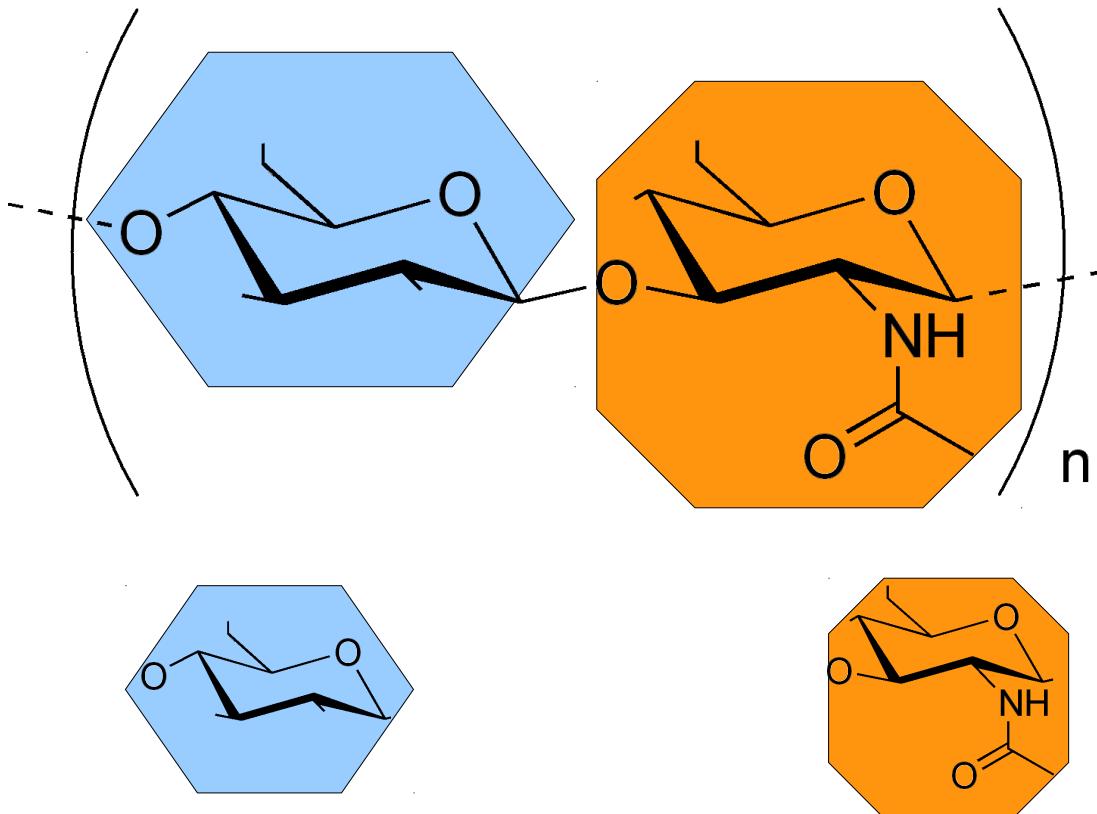
Green hexagon = glucose (Glc)

Yellow hexagon = galactose (Gal)

Red hexagon with minus sign = *N*-acetylneurameric acid (sialic acid, or NANA)

# O-GLYCOSYLATION

- GA: O-glycosylation (Tyr, Thr, Ser) => proteoglycans
- Glycosaminoglycans



Hexose/Hexuronic acid:

- GlcU
- IdoU
- Gal
- Sulfated derivatives

Hexosamine:

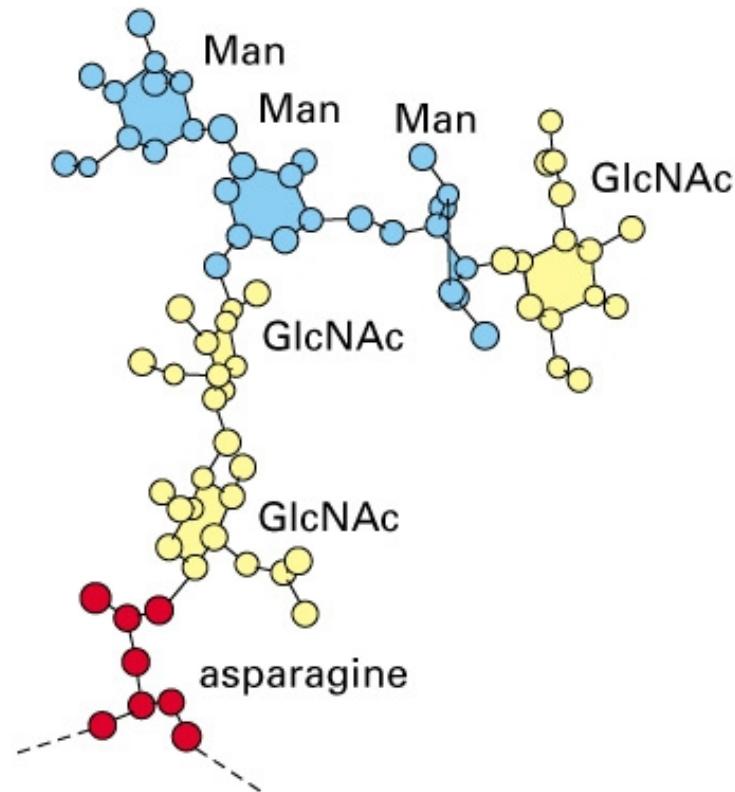
- GlcNAc
- GalNAc
- Sulfated derivatives

GAGs:

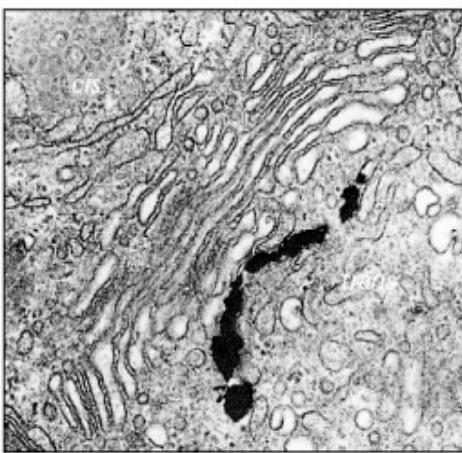
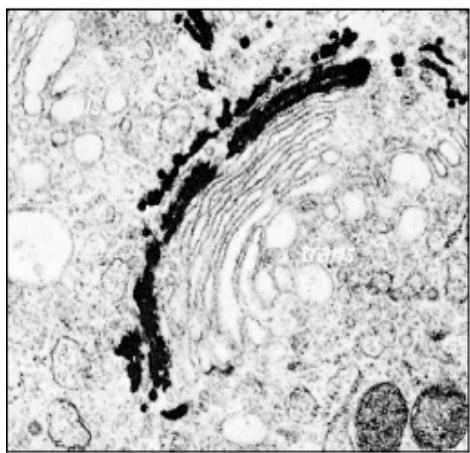
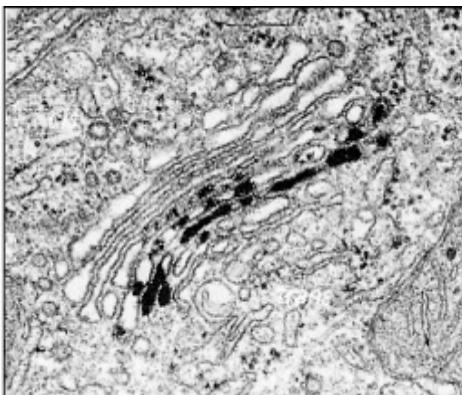
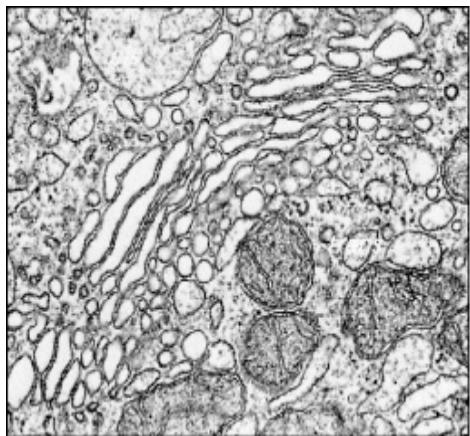
- Hyaluronan (HA)
- Chondroitin sulfate (CS)
- Heparin (HE)
- Heparan sulfate
- Keratan sulfate
- Dermatan sulfate (DS)

# ROLE OF GLYCOSYLATION

- Folding
- Transport of lectins
- Protection from the proteases
- Recognition (selectins) => cell adhesion adhesion
- Signalling (Notch)



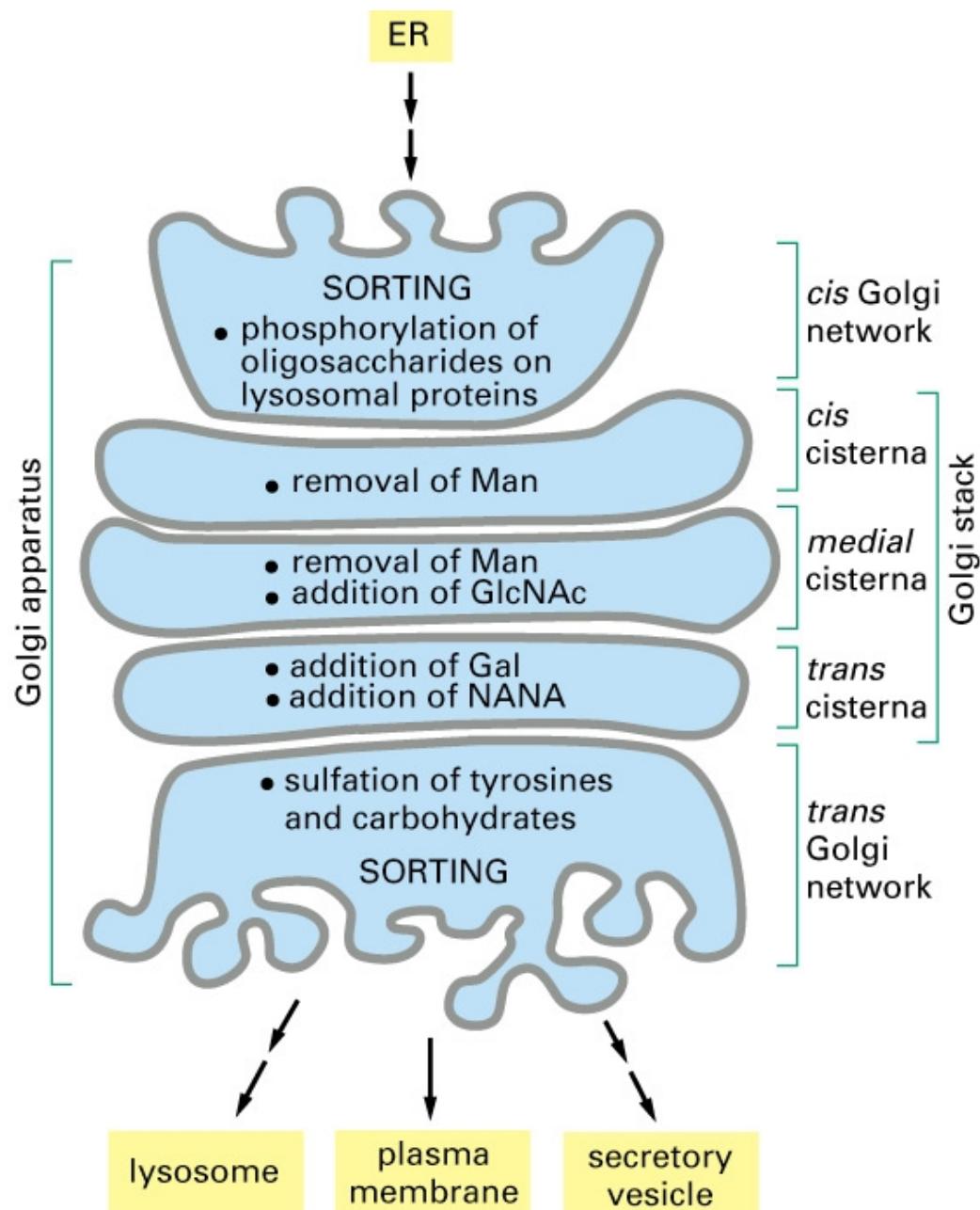
# GA: SERIES OF PROCESSING COMPARTMENTS



1 μm

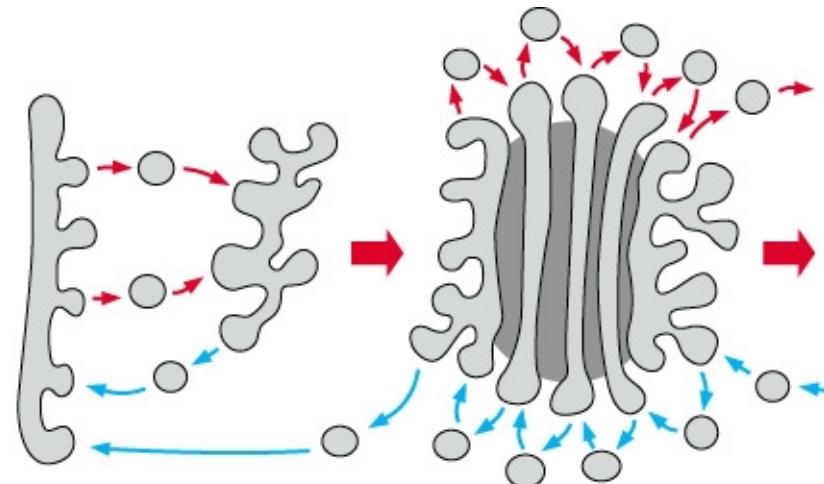
Specific staining of GA compartments

- Compartmentalization:  
specific enzymes concentration



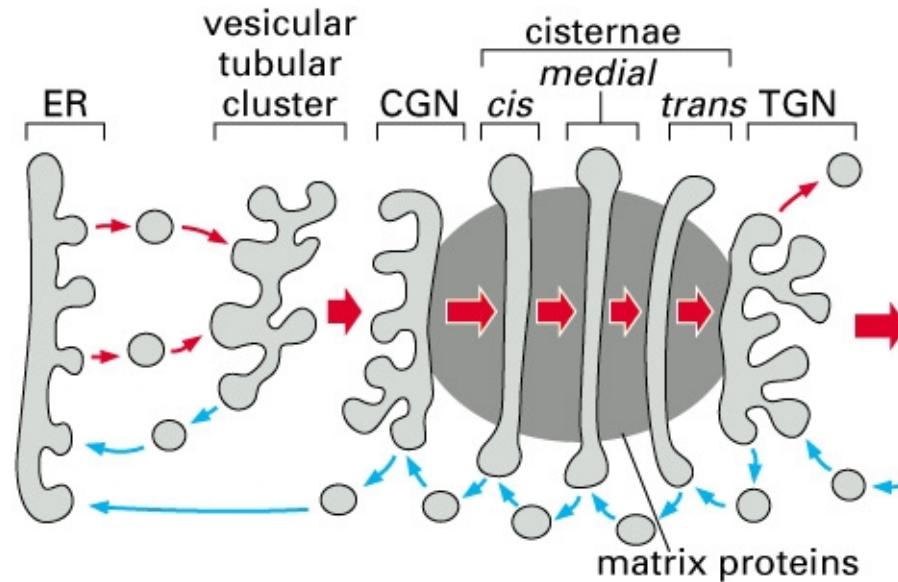
# TRANSPORT THROUGH GA: VESICULAR TRANSPORT VS. CISTERNAL MATURATION

- Specific enzymes are static
- Cargo is moved forward/back



VESICULAR TRANSPORT MODEL

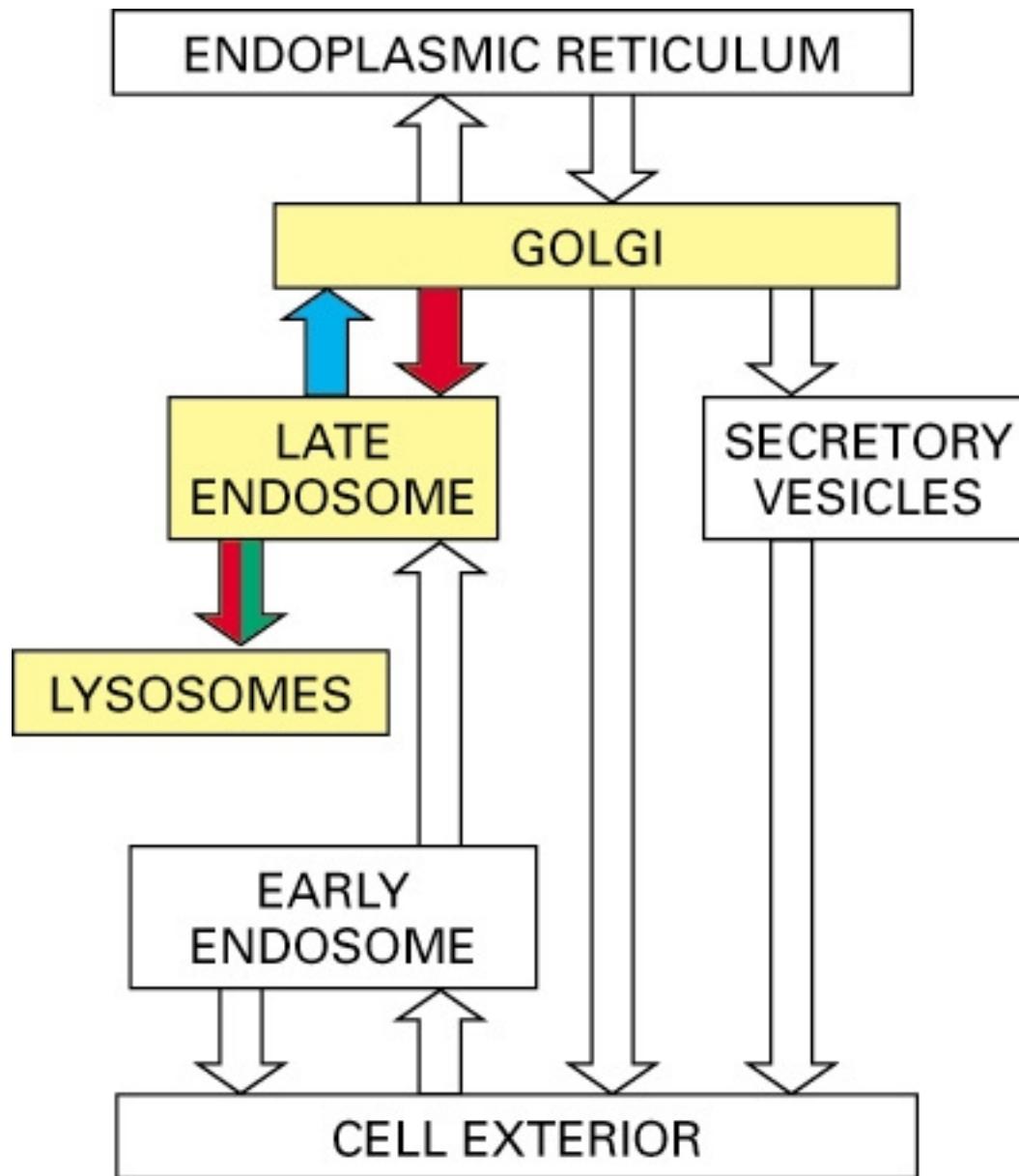
- Enzymes move back
- Cisterna move/change



CISTERNAL MATURATION MODEL

Cytoskeleton/phosphorylation => spatial organization, assembly, disassembly

# TRANSPORT: GA => LYSOSOMES

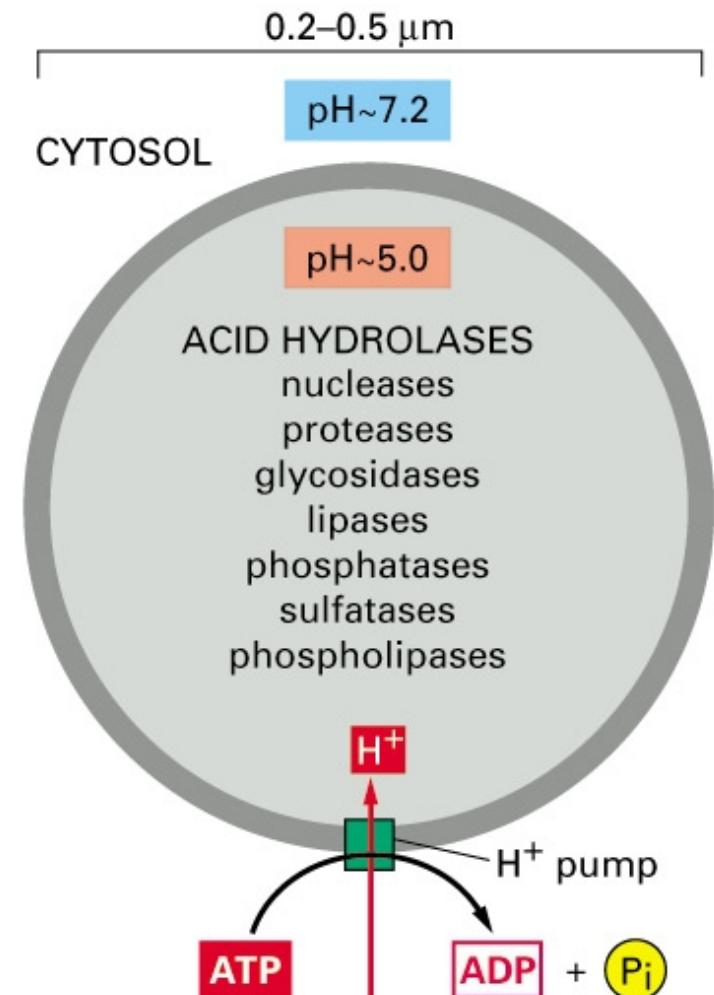


# LYSOSOMES

Function: intracellular digestion of macromolecules

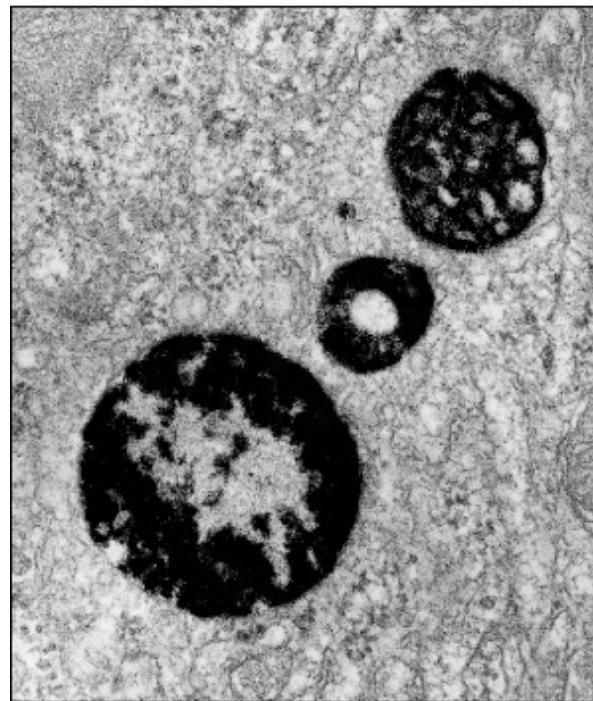
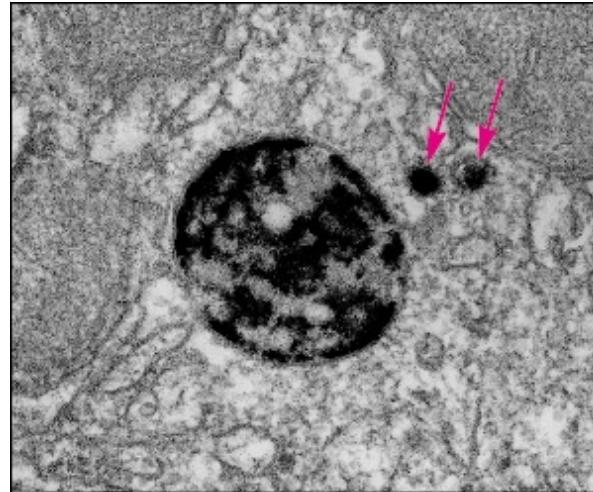
- ~ 40 types of hydrolytic enzymes (acid hydrolases):  
proteases, nucleases, glycosidases, lipases, phospholipases,  
phosphatases, sulfatases

- pH ~ 5
- Double protection of cytosolic molecules:
  - pH
  - membrane
- H<sup>+</sup>-pumping ATPase
- Lysosomal secretion in melanocytes



# LYSOSOMES HETEROGENEITY

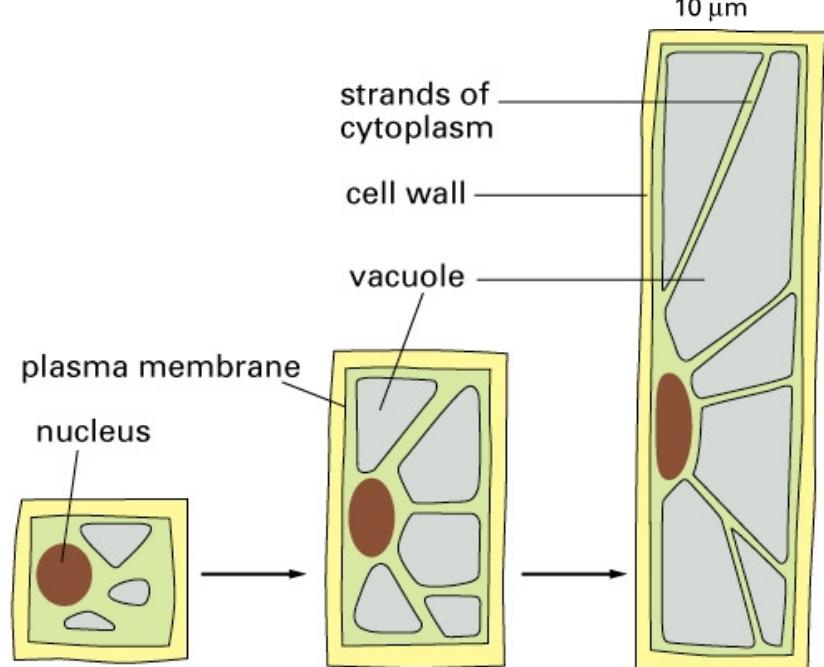
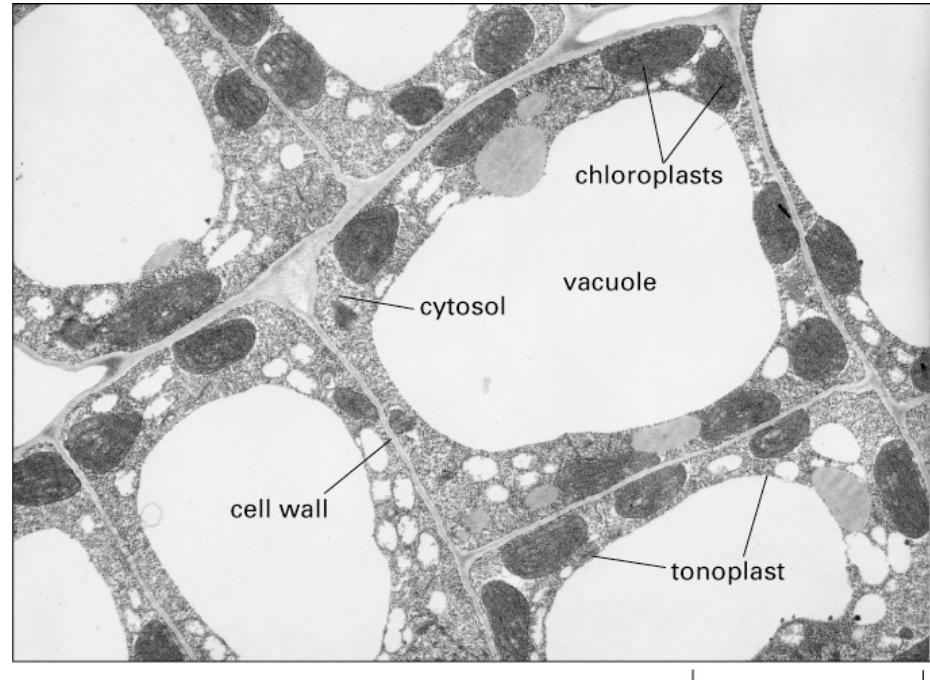
Defined by degestive function



Acid phosphatase marker 200 nm

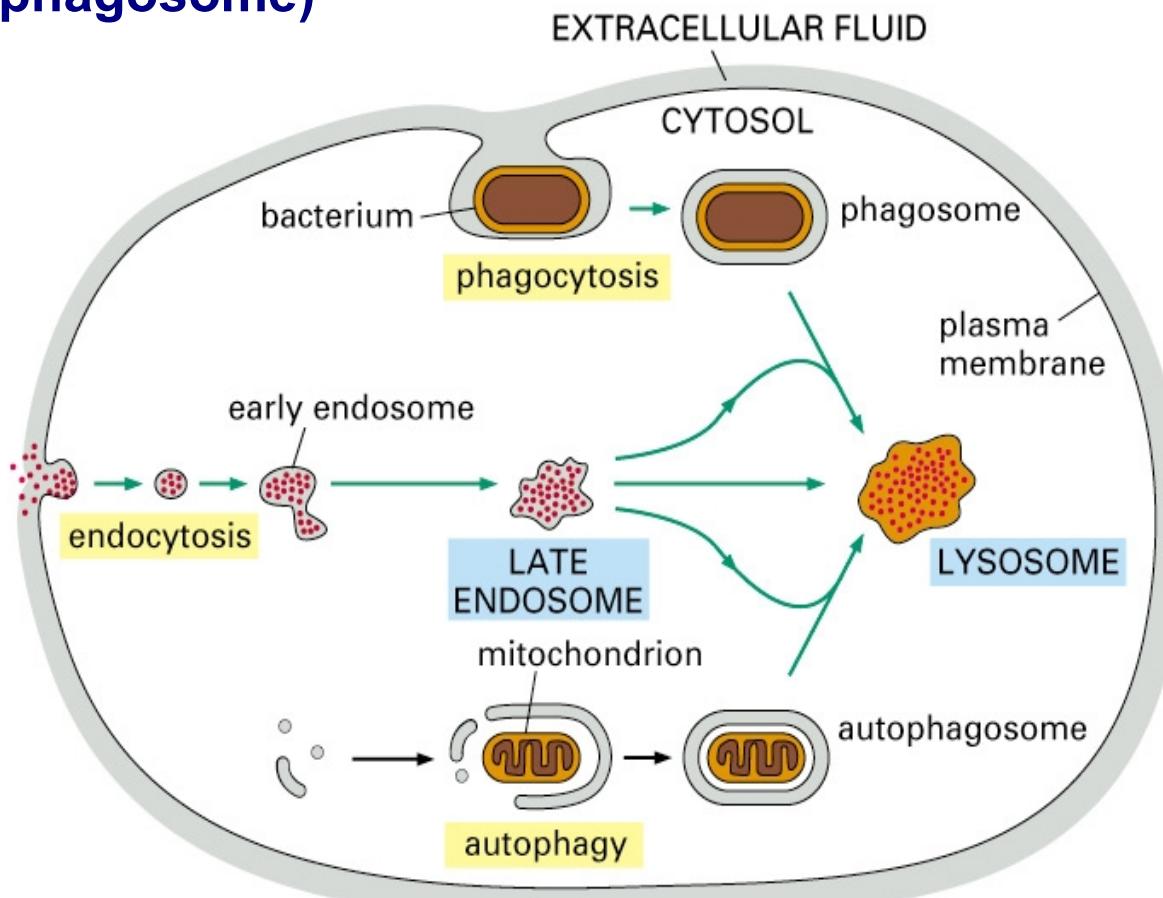
# VACUOLES IN PLANTS: TYPE OF LYSOSOMES

- 30-90% of the cell volume
- Function:
  - hydrolysis
  - storage
  - control of turgor pressure
  - buffering: H<sup>+</sup> balance, osmosis
  - degradation and synthesis



# MULTIPLE PATHWAYS LEAD TO LYSOSOMES

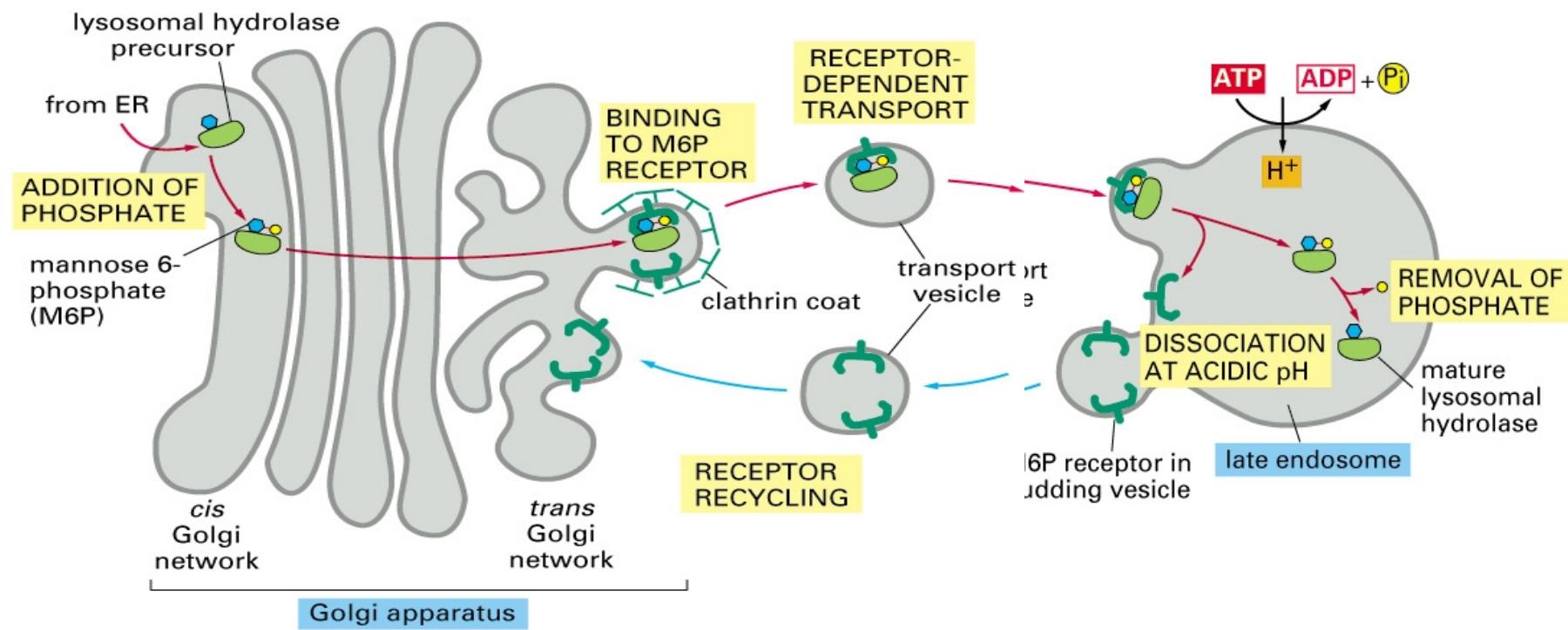
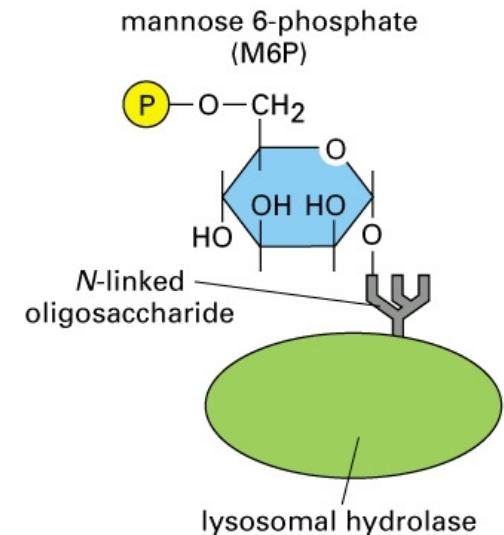
- Enzymes: from ER/GA
- Substances to digest:
  - endocytosis (early => late endosomes), pH gradually decreases
  - autophagy (lifetime of mitochondria ~ 10 days, autophagosome)
  - phagocytosis (phagosome)



# MANNOSE 6-PHOSPHATE SIGNAL

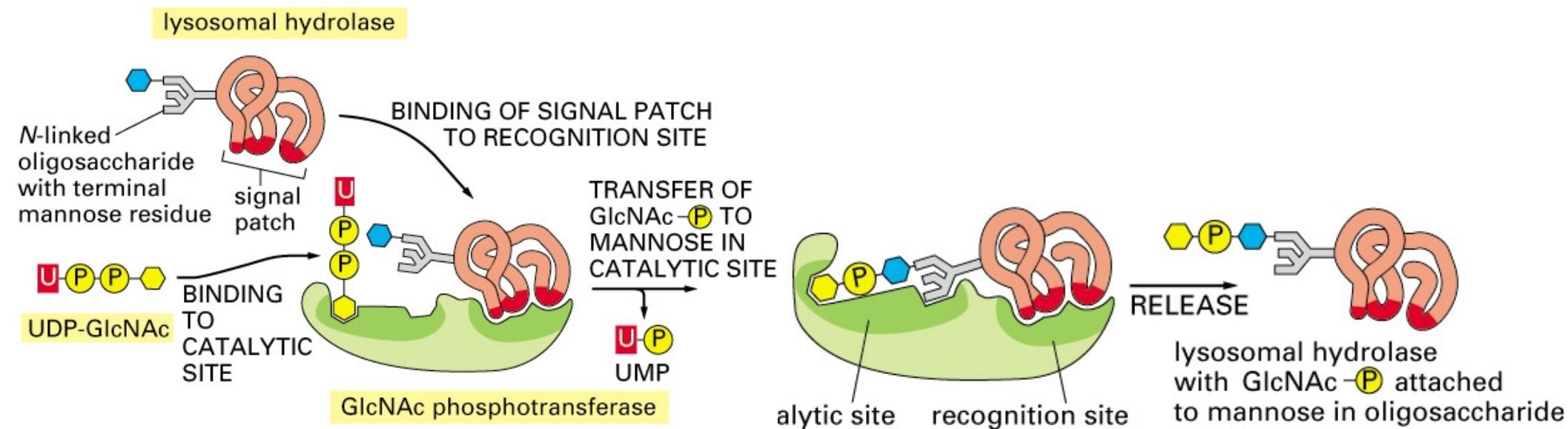
➤ Lysosomal proteins contain a M6P-signal:

- addition in *cis*-GA
- recognition by M6P-receptor in *trans*-GA
- clathrin association on cytosolic side
- pH decreases 6.5 => 6: M6P-receptor releases
- M6P-receptor returns to *trans*-GA



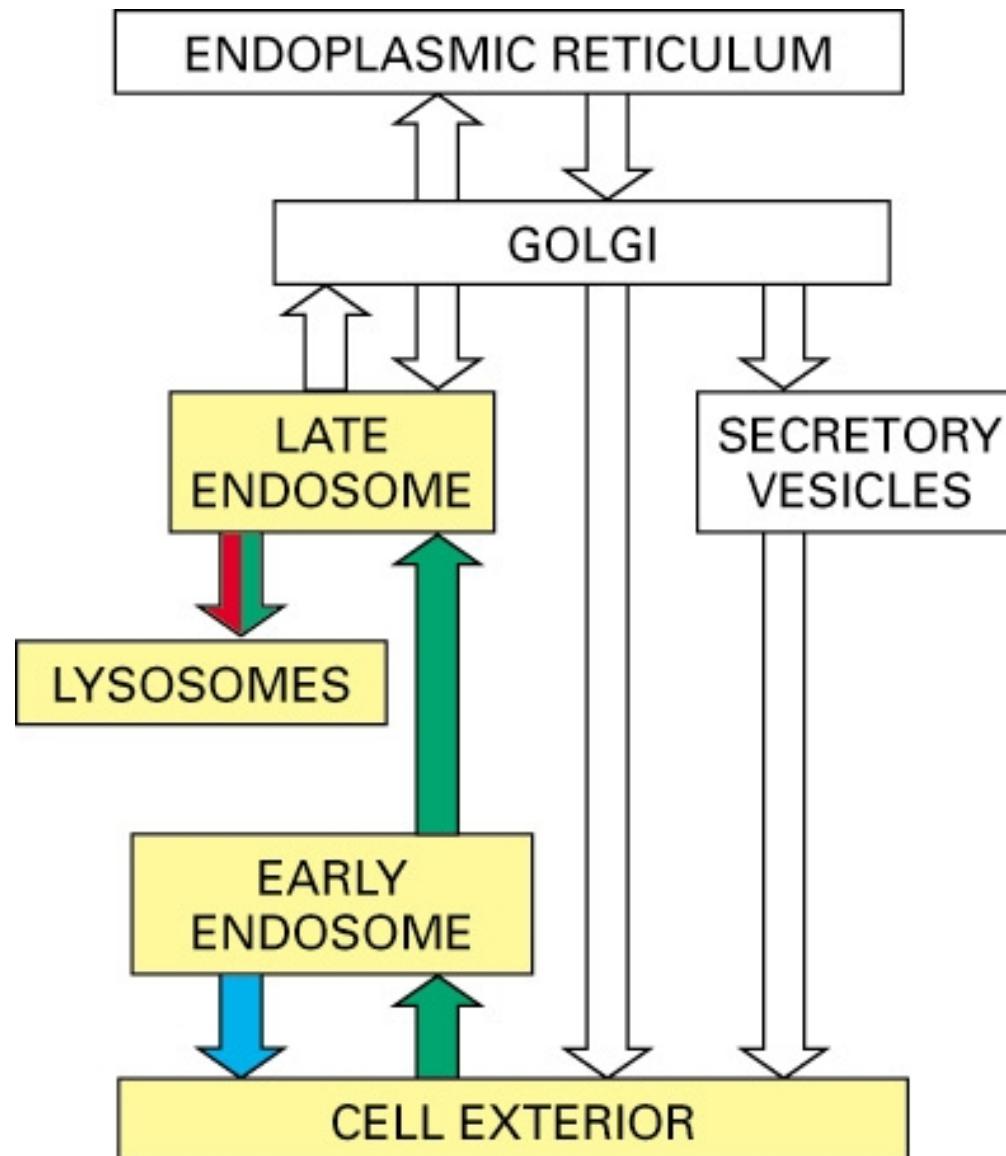
# MANNOSE 6-PHOSPHATE SIGNAL

- Some lysosomal proteins with no M6P => PM => lysosome
- Not lysosomal => PM by default
- M6P is made from the core N-linked oligosaccharide: aa signal patch
- Defects in GlcNAc phosphotransferase => lysosomal storage diseases:
  - Hurler's disease (GAGs breakdown )
  - inclusion cell disease (hydrolyses are in blood, one recessive mutation, complement mechanisms)



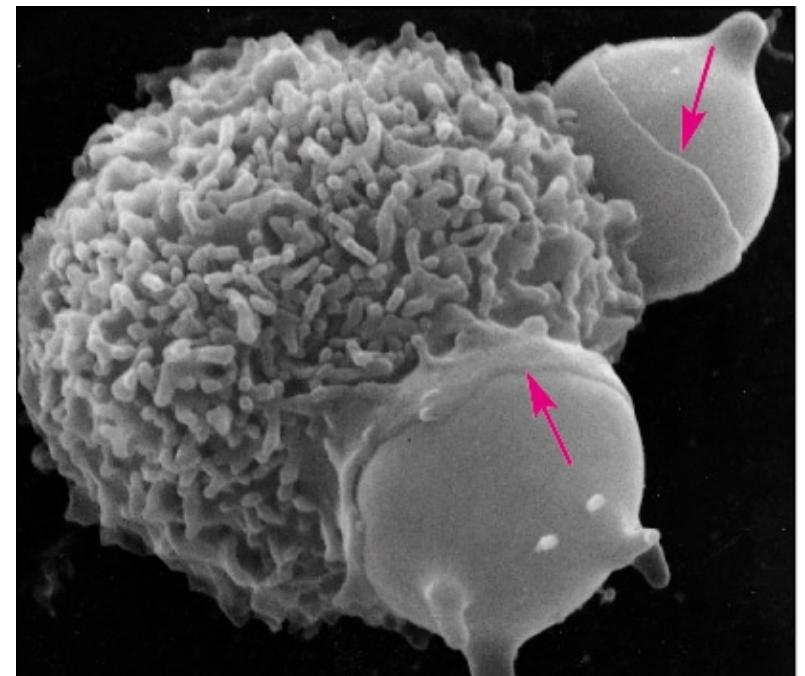
# ENDOCYTOSIS

- Phagocytosis: digestion of large particles (phagosome > 250nm)
- Pinocytosis: digestion of liquids and small particles (pinosome ~100nm)



# PHAGOCYTOSIS

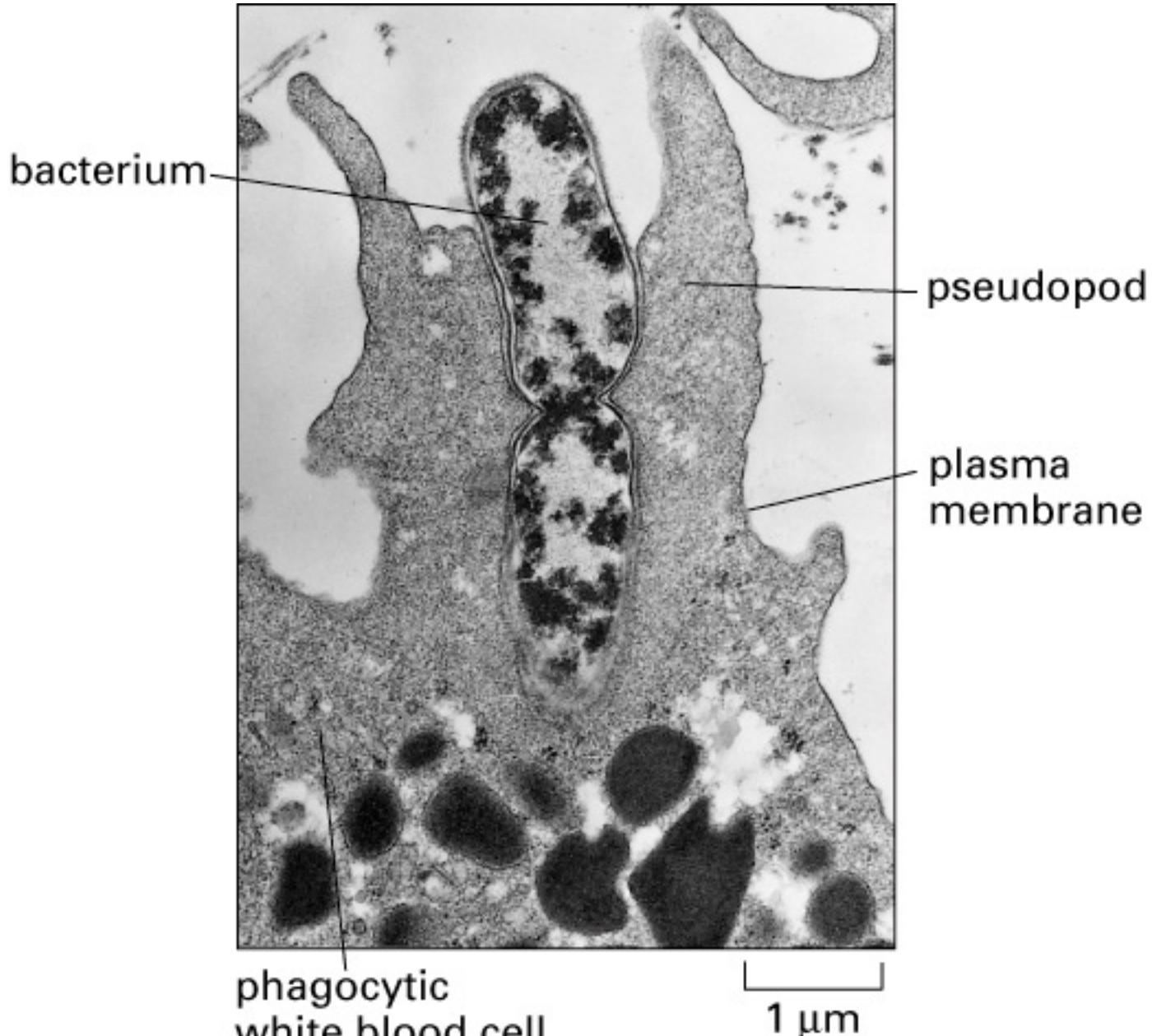
- Protozoa: particles => cytoplasm => lysosomes
- Eukaryotes: food first broken down extracellularly in most cells
- Phagocytes:
  - macrophages (~ $10^{11}$  red blood cells/day)
  - neutrophils
  - dendritic cells
- Phagosomes => lysosomes
- Specialized surface receptors for:
  - antibodies (Fc)
  - saccharides
  - complement compounds
  - asymmetry of lipids in apoptotic cells
- “Eating by default” principle (Tyr-phosphatases)



5 μm

Phagocytosis by macrophage

# PHAGOCYTOSIS



Phagocytosis by neutrophil

# PINOCYTOSIS

➤ Most of cells (macrophages: 100% of own membrane per 30 minutes)

➤ Endocytic-exocytic cycle

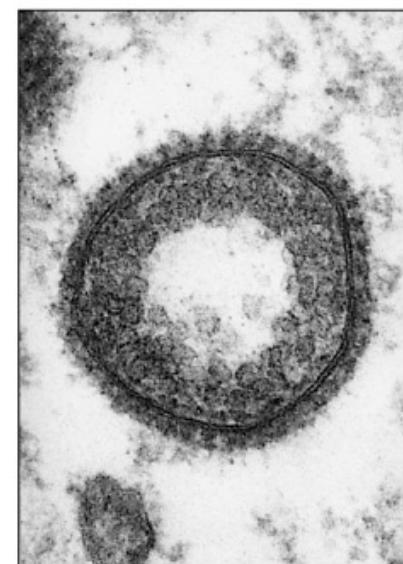
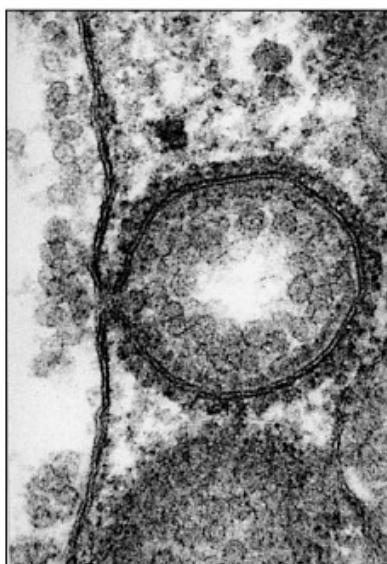
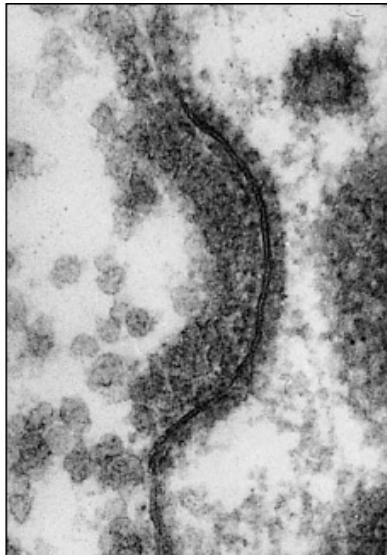
➤ Clathrin-coated pits:

- 2% of total membrane

- lifetime ~ minute

- ~2500 vesicles a minute

➤ Fluid-phase endocytosis

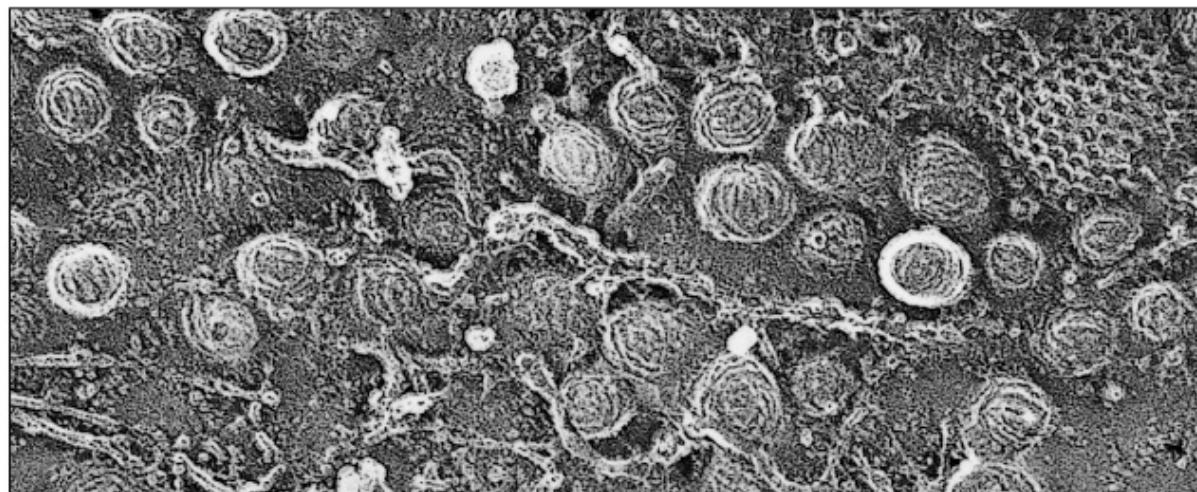
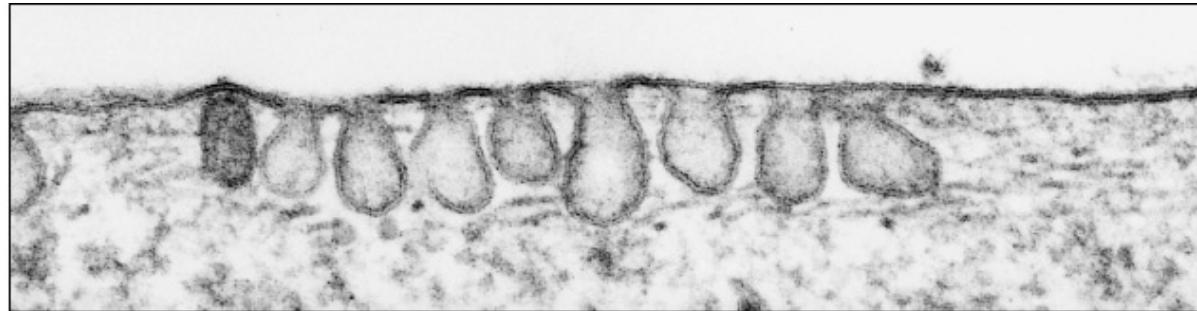


0.1 μm

Clathrin-coated vesicles formation

# PINOCYTOSIS

- Caveolae pathway: endothelial cells lining blood vessels
- Lipid rafts rich in cholesterol, glycosphingolipids, GPI-anchored membrane proteins
- Caveolin: multipass integral TM protein
- No protein complexes assembling mechanisms



0.2 μm

# RECEPTOR-MEDIATED ENDOCYTOSIS

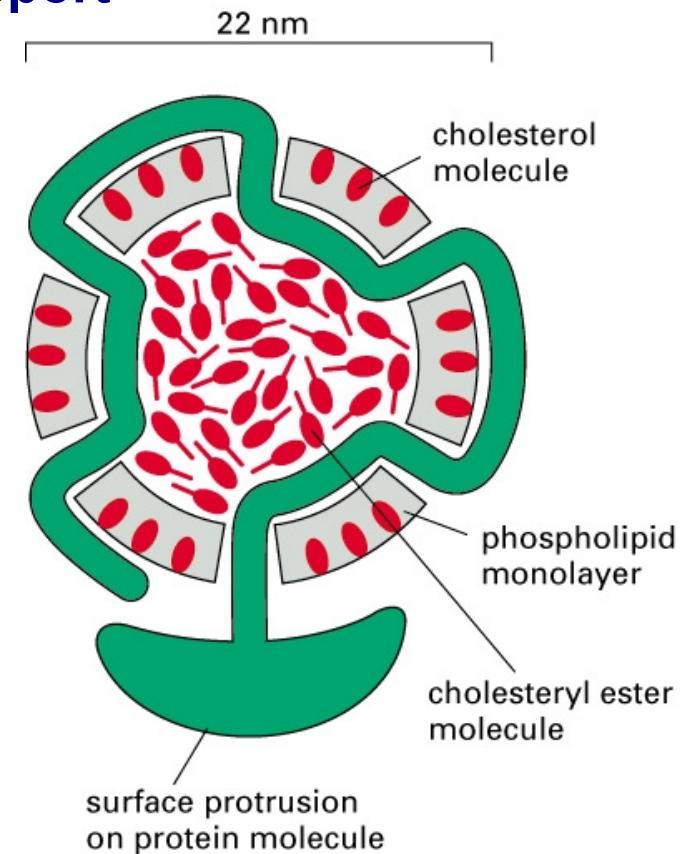
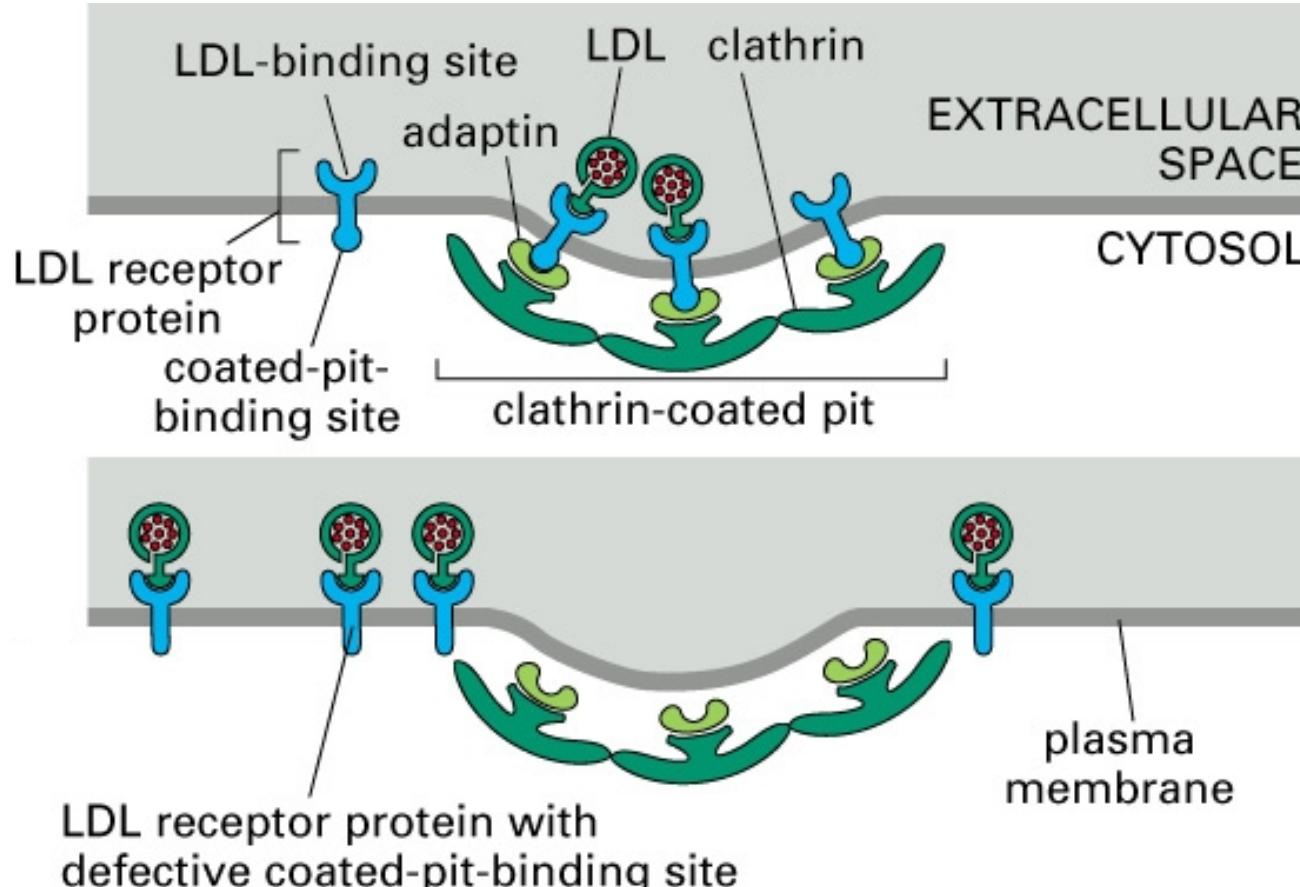
➤ Concentration of cargo => more effective transport

➤ Low-density lipoproteins (LDL):

- single protein chain

- ~  $10^3$  cholesterol molecules

➤ Clathrin dissociates after pinching off



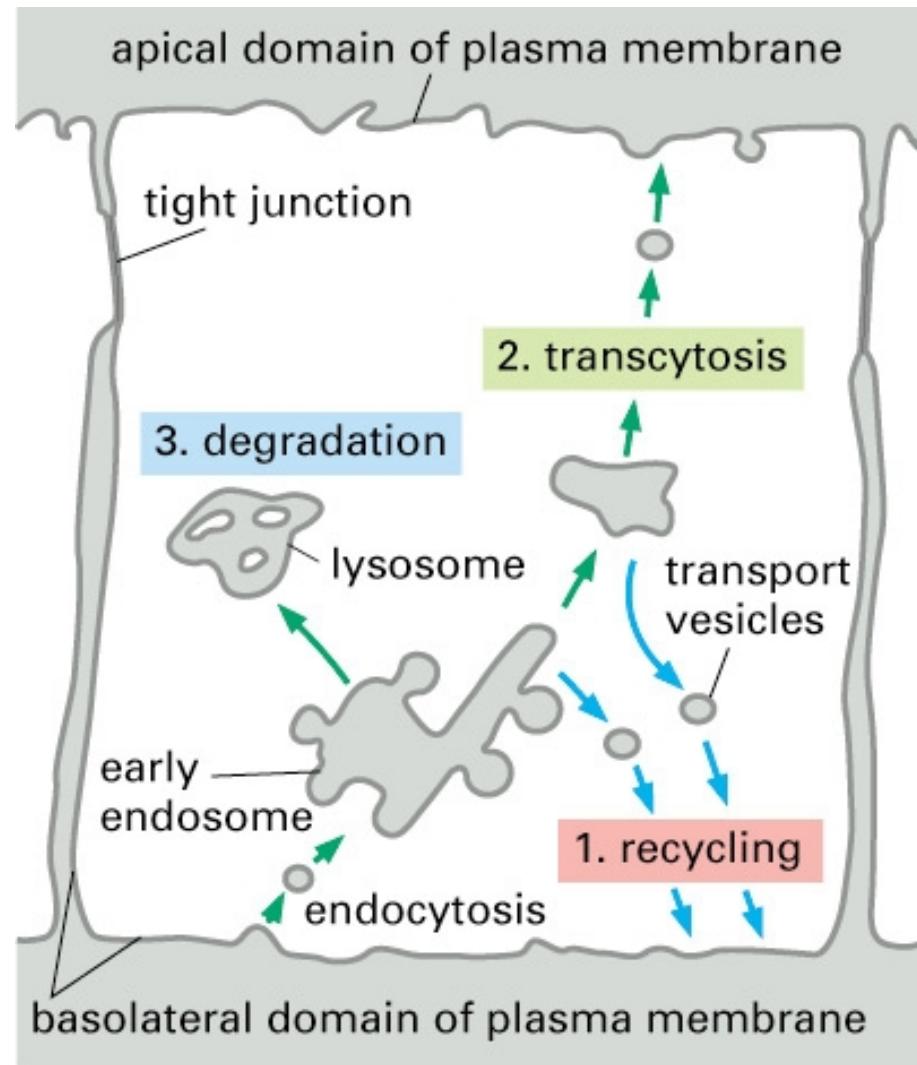
# RECEPTOR-MEDIATED ENDOCYTOSIS

- Cholesterol esters are hydrolyzed to cholesterol
- Too much cholesterol => synthesis decreases
- Atherosclerosis: blocked uptake of cholesterol
- 25 known receptors for receptor-mediated endocytoses:
  - prebound to pits
  - are activated by ligand binding
  - have Y-X-X-Ψ signal to bind to adaptins in clathrin-coated pits
- Clathrin-coated pits are the same for all receptors
- One pit ~ 1000 receptors

# DESTINY OF RECEPTORS AND LIGAND IN ENDOSOMES

## Receptors

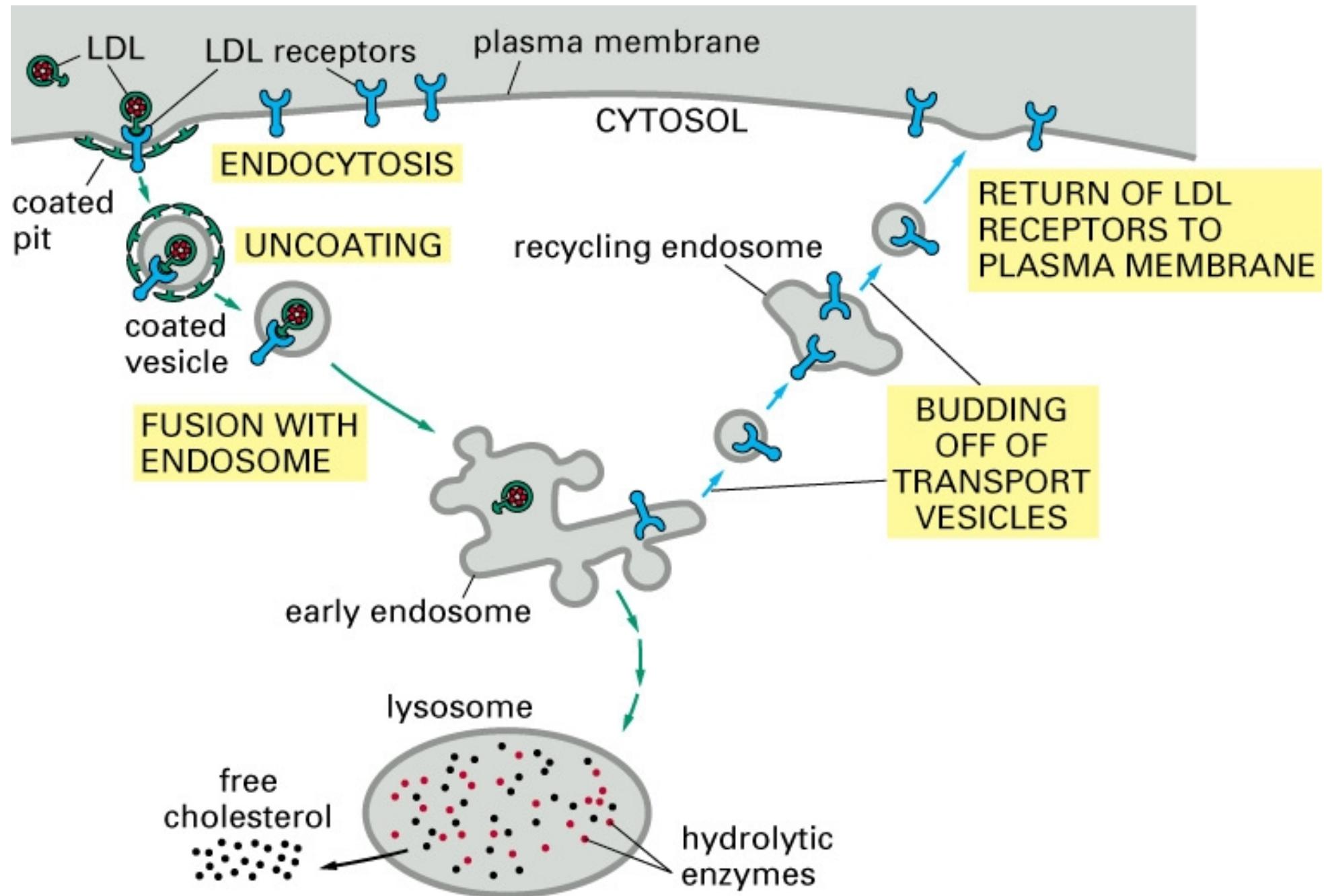
- Retrieved from endosome:
  - recycling (LDL, transferrin)
  - transcytosis (Fc)
- Degraded in lysosome
  - (f.i.: epidermal growth factors)



## Ligands

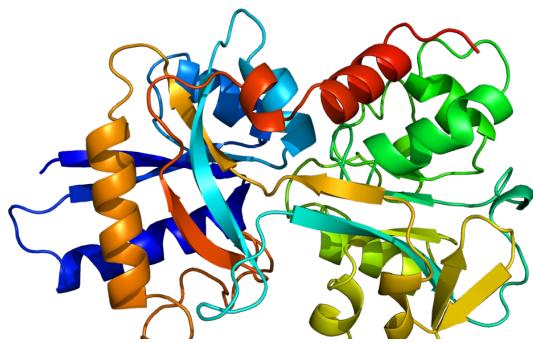
- Dissociated from the receptor => degraded
- Bound to the receptor => follow fate of receptor

# LDL ENDOCYTOSIS

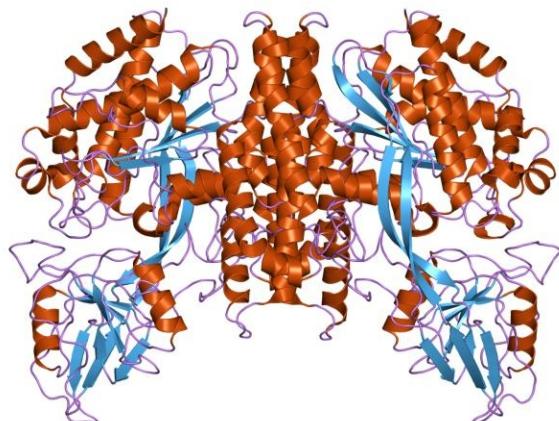


# TRANSFERRIN ENDOCYTOSIS

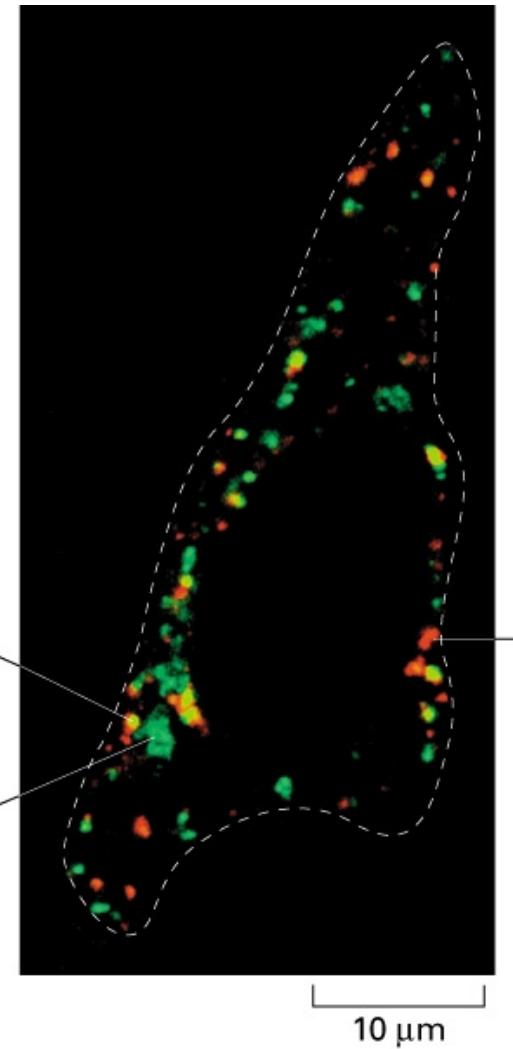
- Transferrin: 0.1% of all iron, 2 Fe(III) BS
- Transferrin + receptor => endosome
- pH decreases: iron is unbound
- Recyclization => PM => receptor unbinding => iron uptake



Transferrin



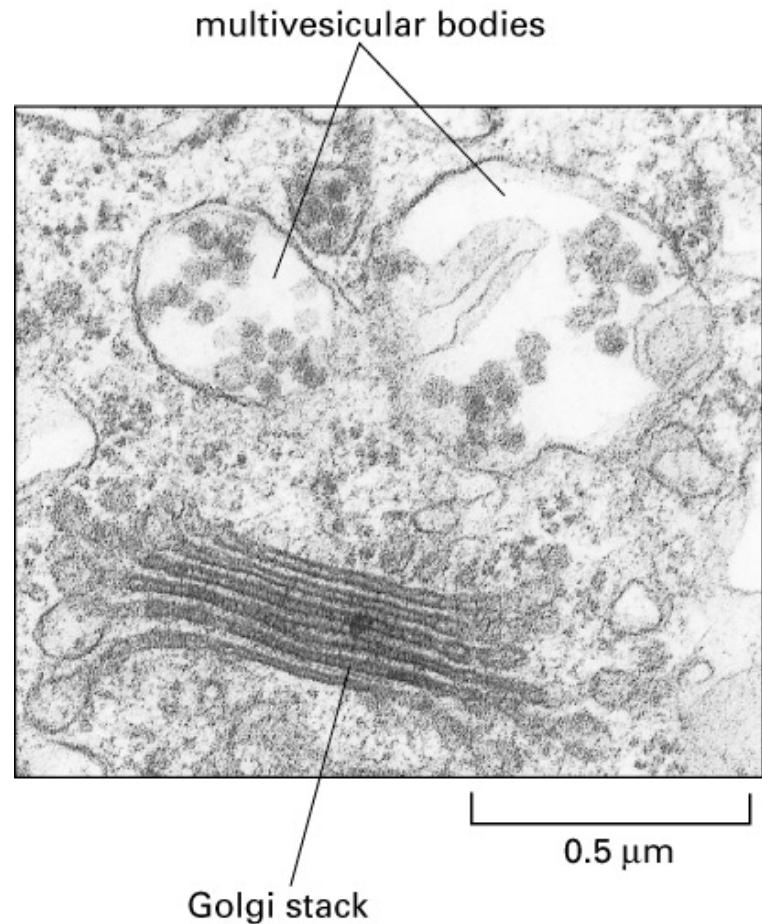
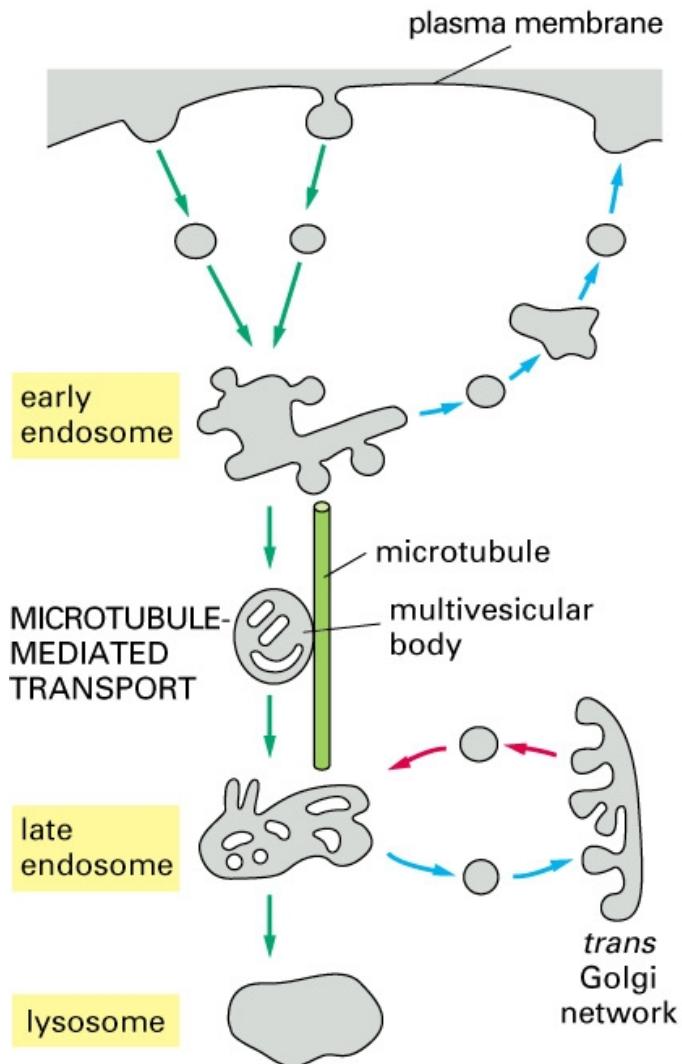
Transferrin +  
transferrin receptor



Transferrin receptor  
Opioid receptor  
Both receptors

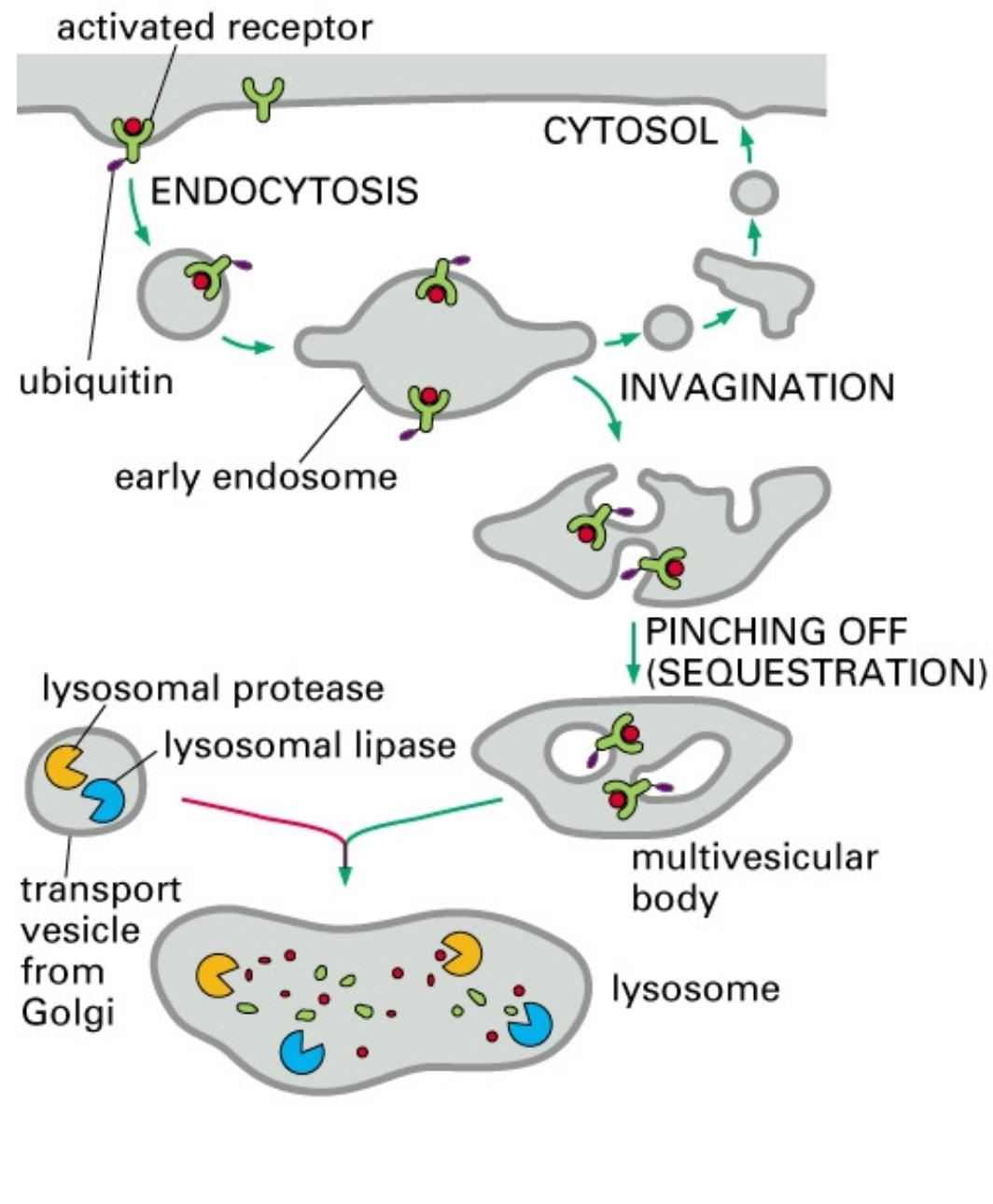
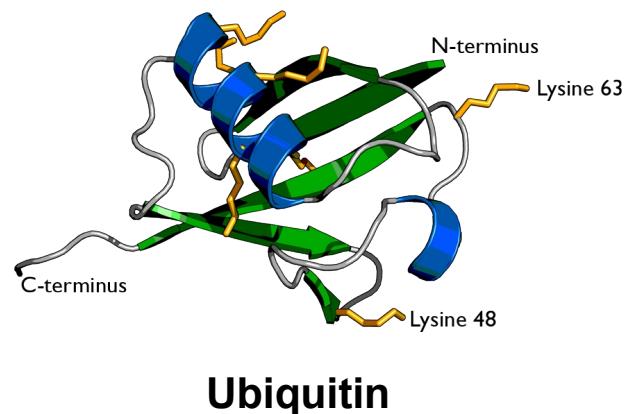
# MULTIVESICULAR BODIES

- Fused migrated endosomes
- Moment of fusion is unknown
- Movements along microtubules

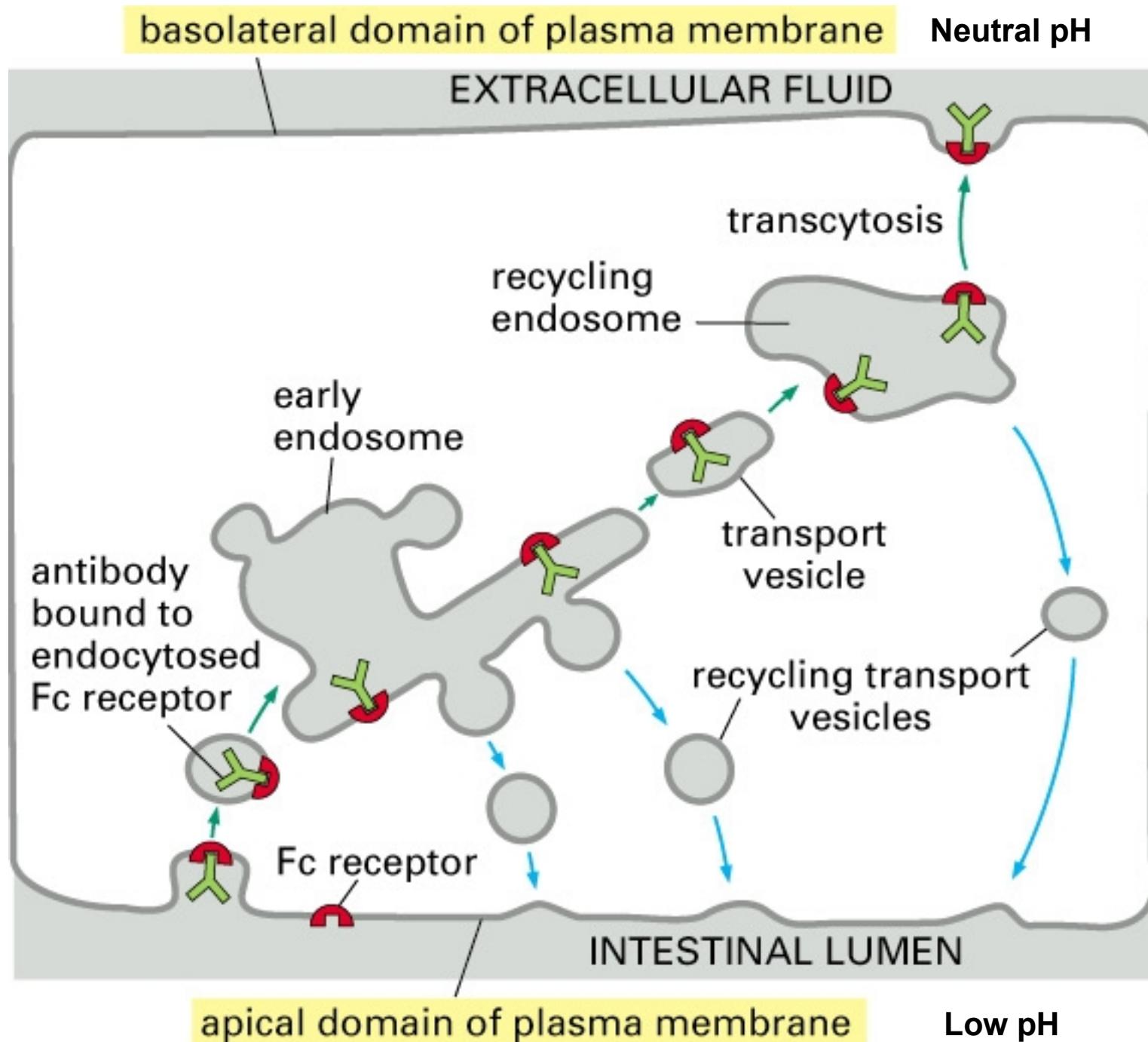


# FATE OF NON-RECYCLED RECEPTORS IN MULTIVESICULAR BODIES

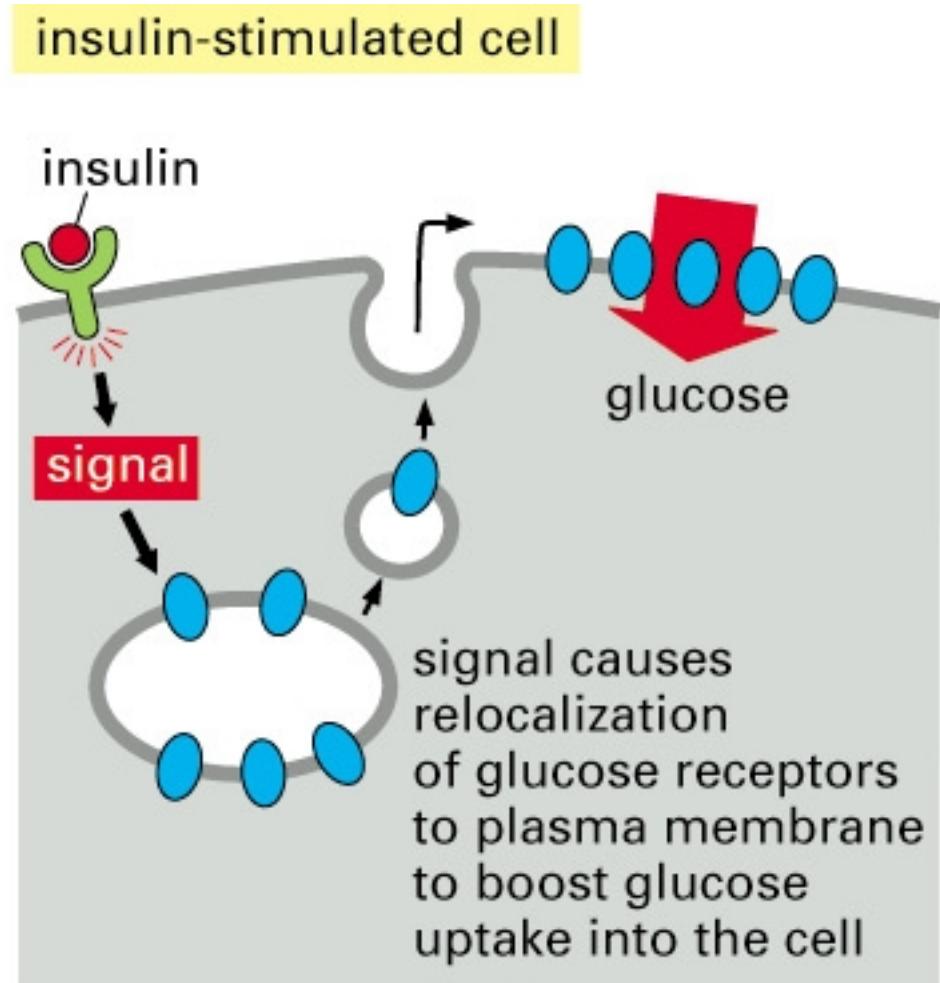
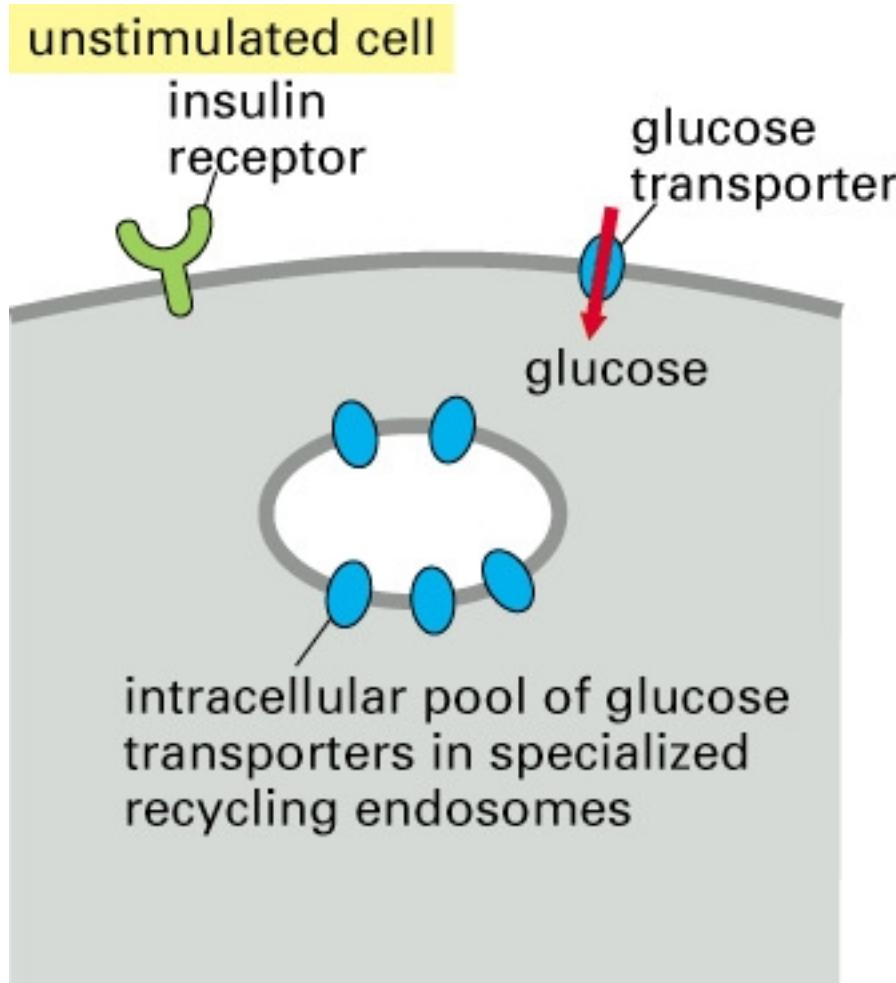
- Invagination => digestion
- Ubiquitination of membrane proteins:
  - single ubiquitin addition
  - uptake to vesicles
  - sorting to internal membrane of multivesicular bodies
- Lipid kinase => phosphatidylinositol
  - docking sites for proteins activating invagination



# TRANSCYTOSIS

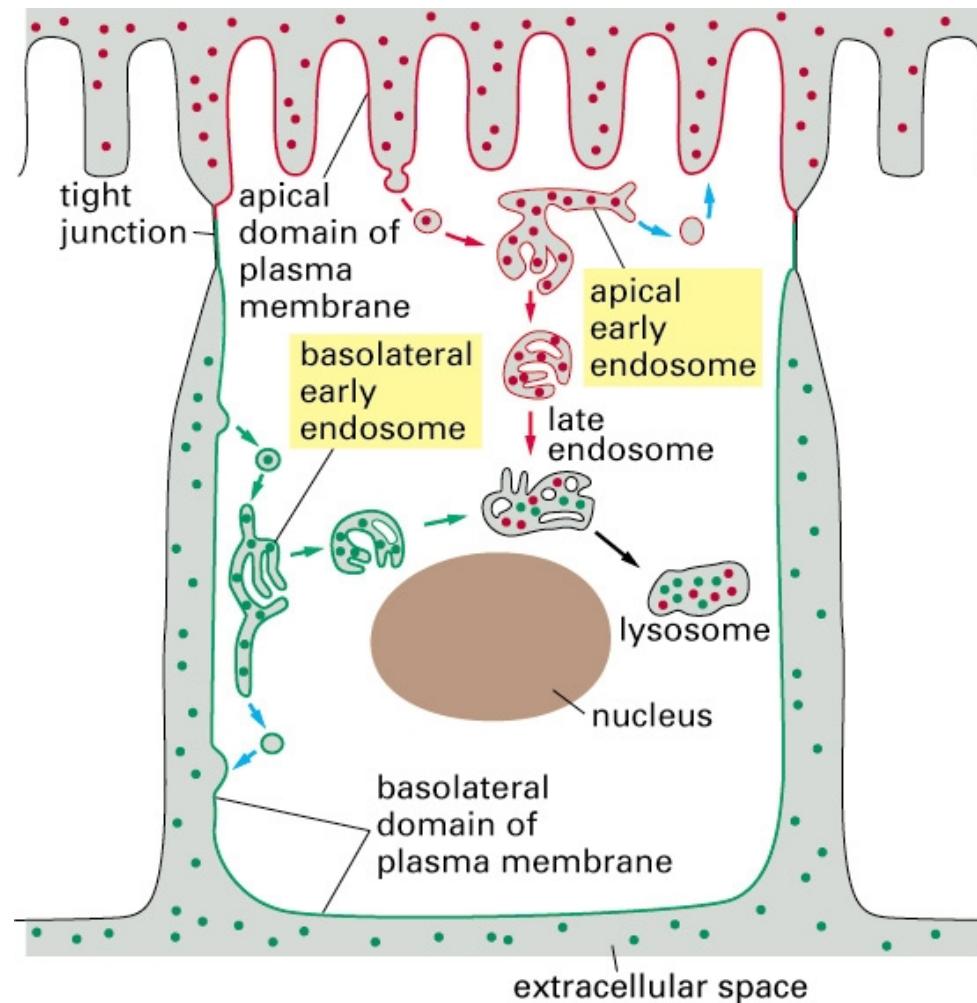


# RECYCLING ENDOSOMES: STORAGE AND REGULATION



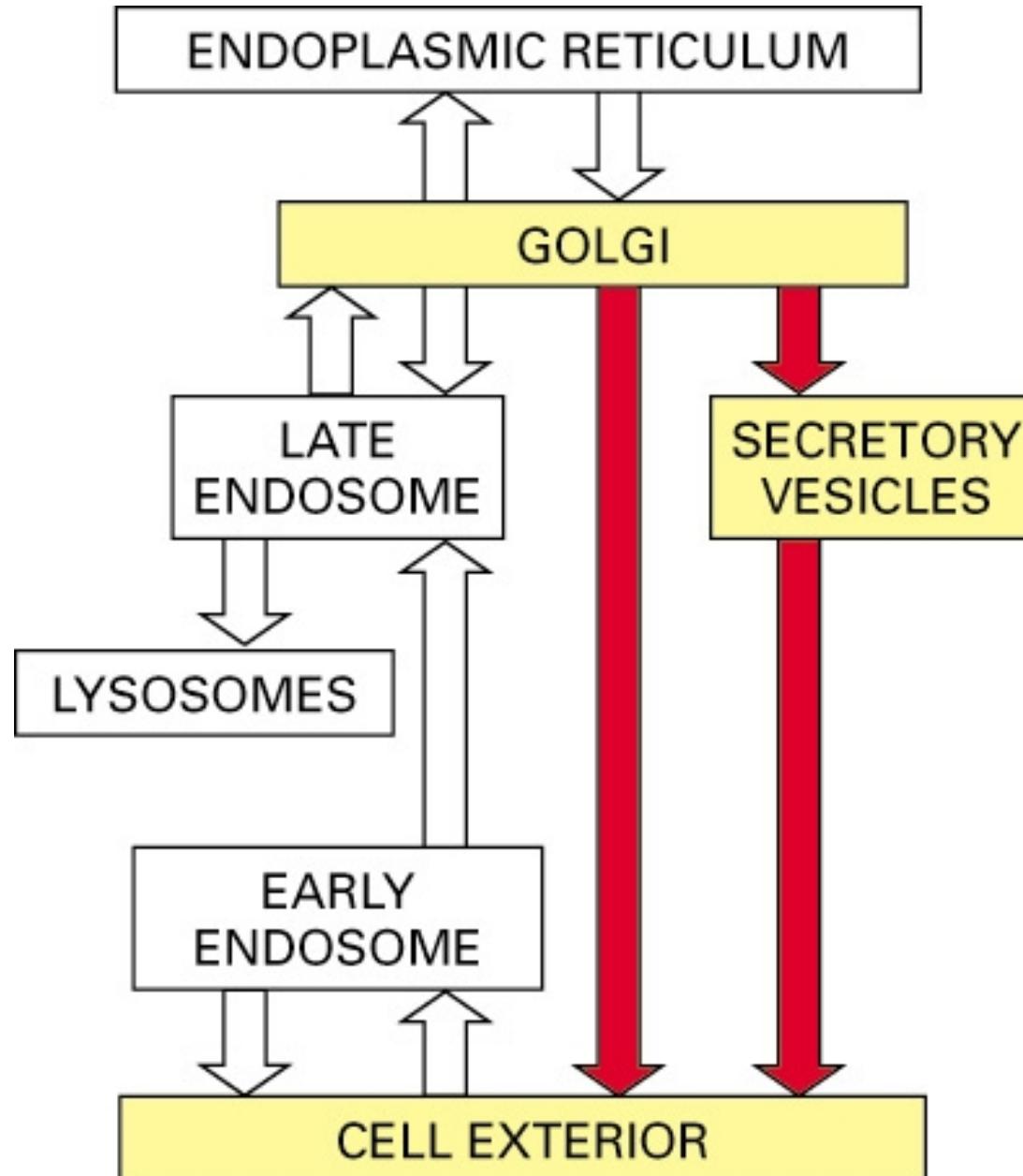
# EPITHELIAL CELLS: DISTINCT EARLY BUT COMMON LATE ENDOSOMES

- Different receptors in basalateral and apical domains
- Correct receptors recycling



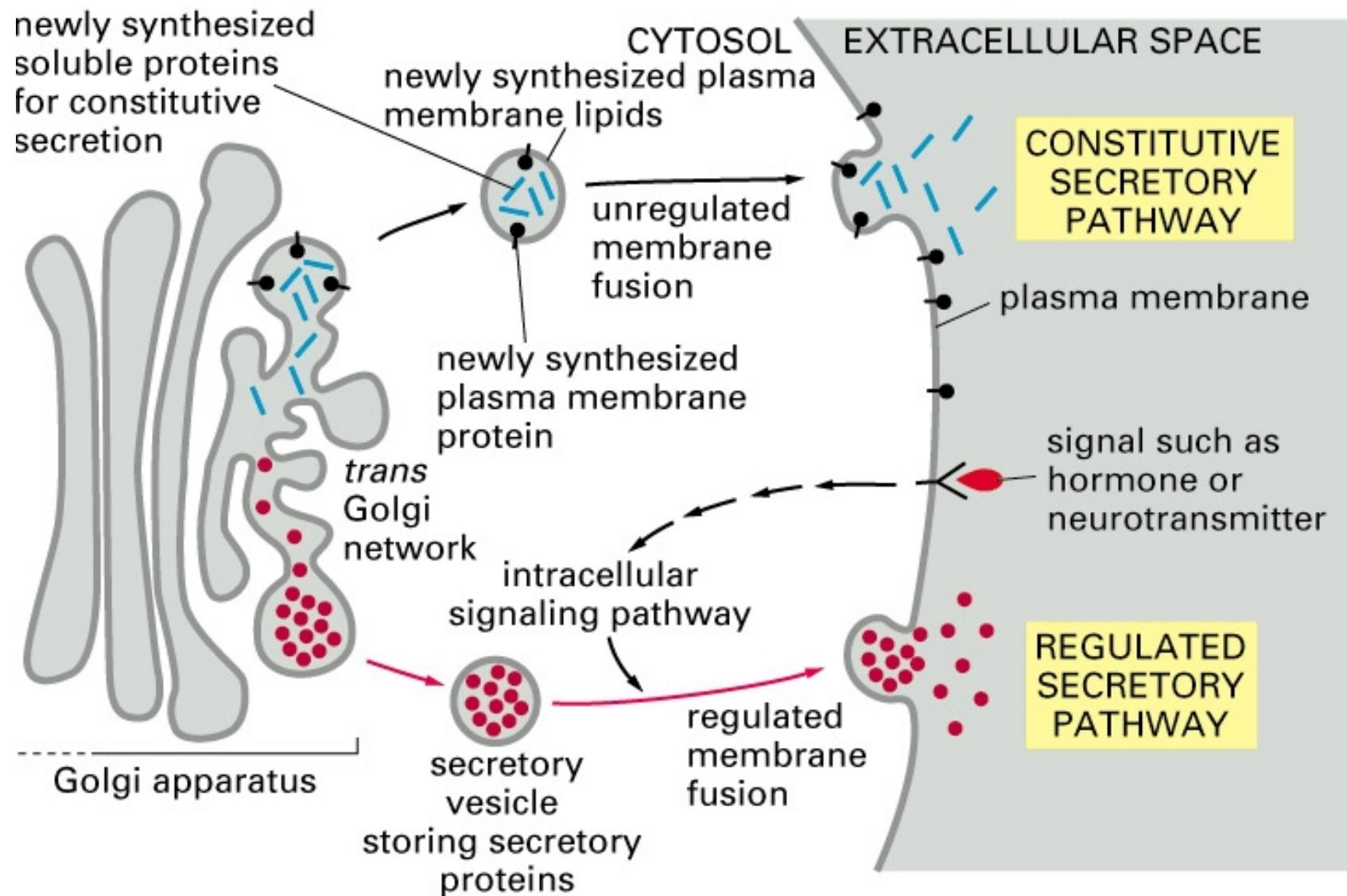
# EXOCYTOSIS

Fusion of the vesicles with plasma membrane



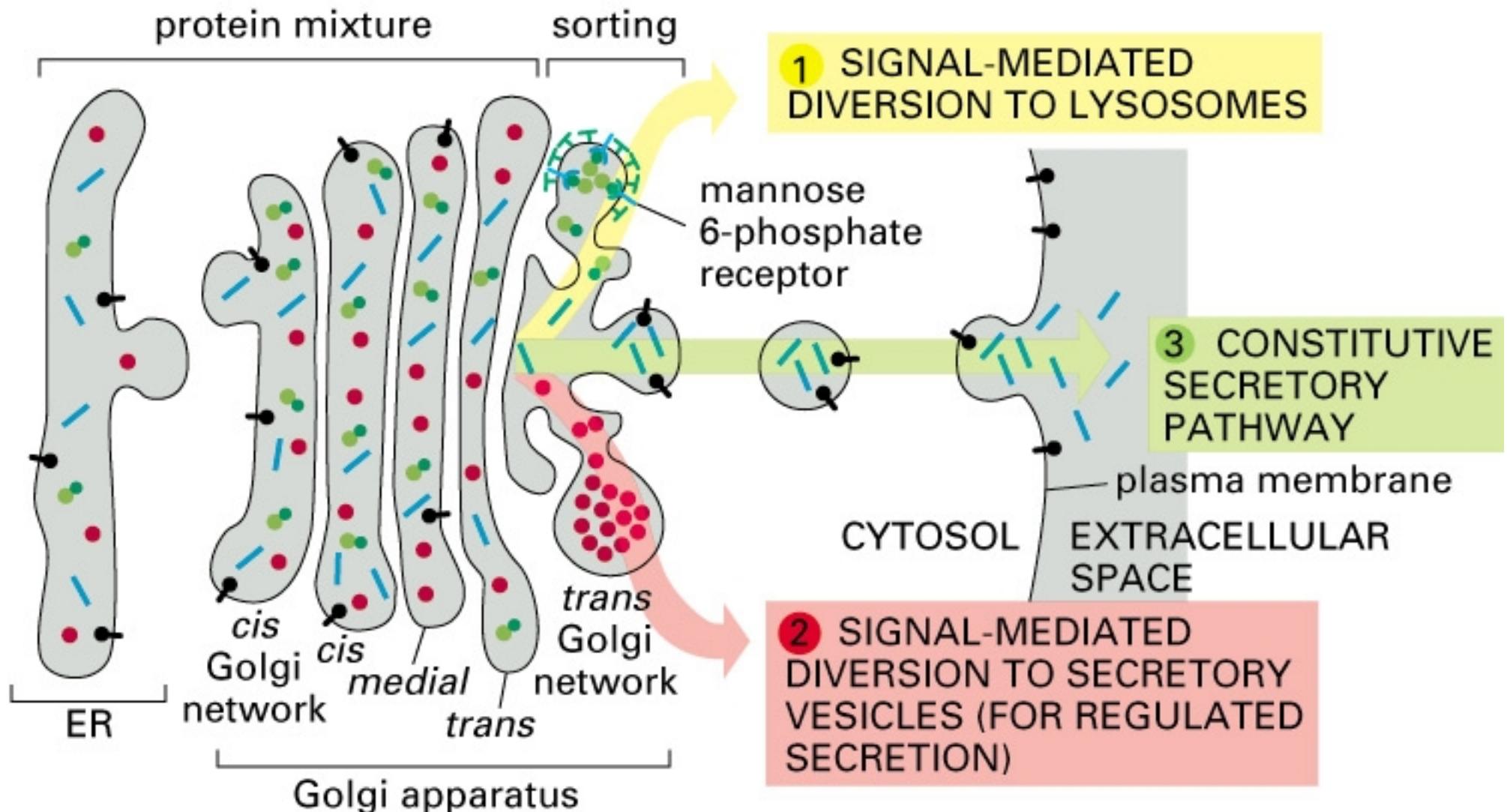
# EXOCYTOSIS

- Constitutive secretory pathway (all cells)
- Regulated secretory pathway (specialized cells)



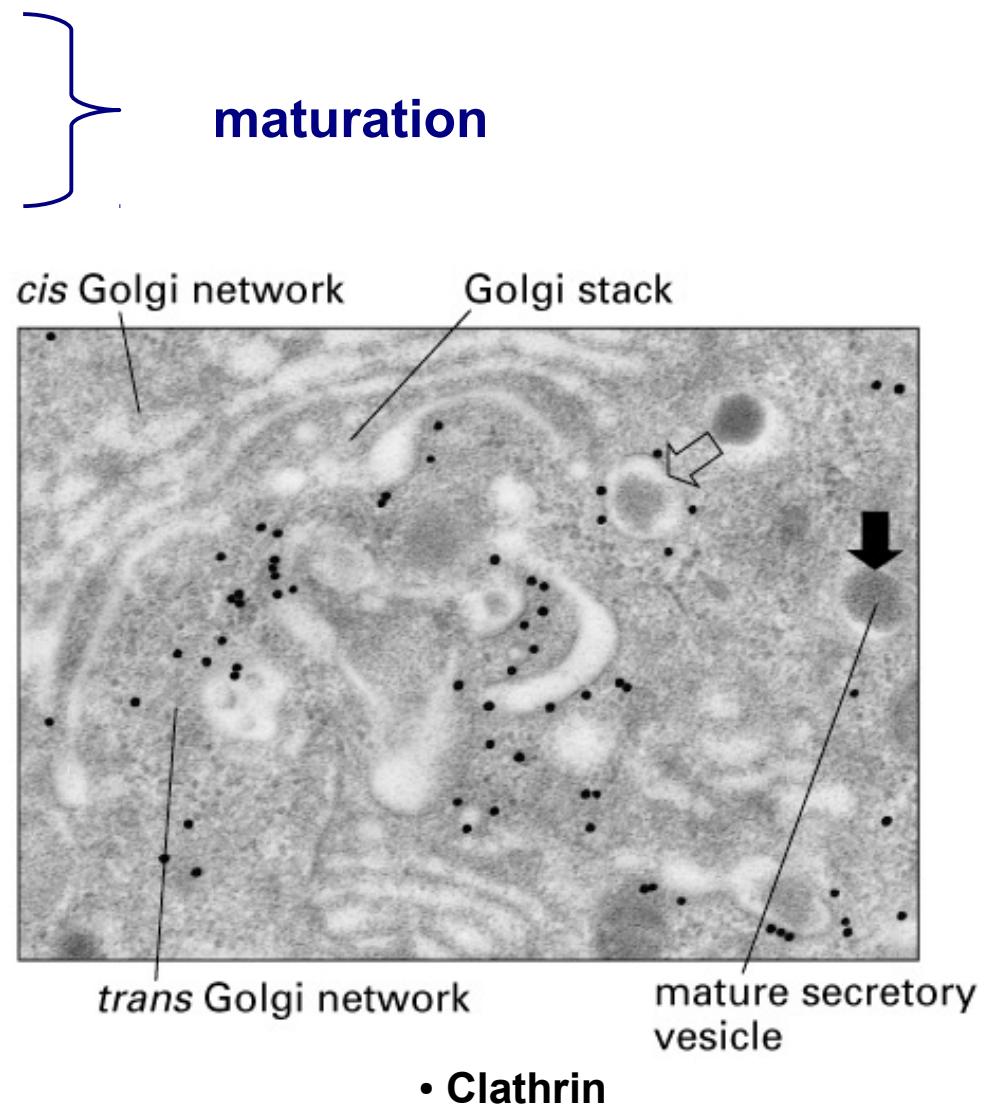
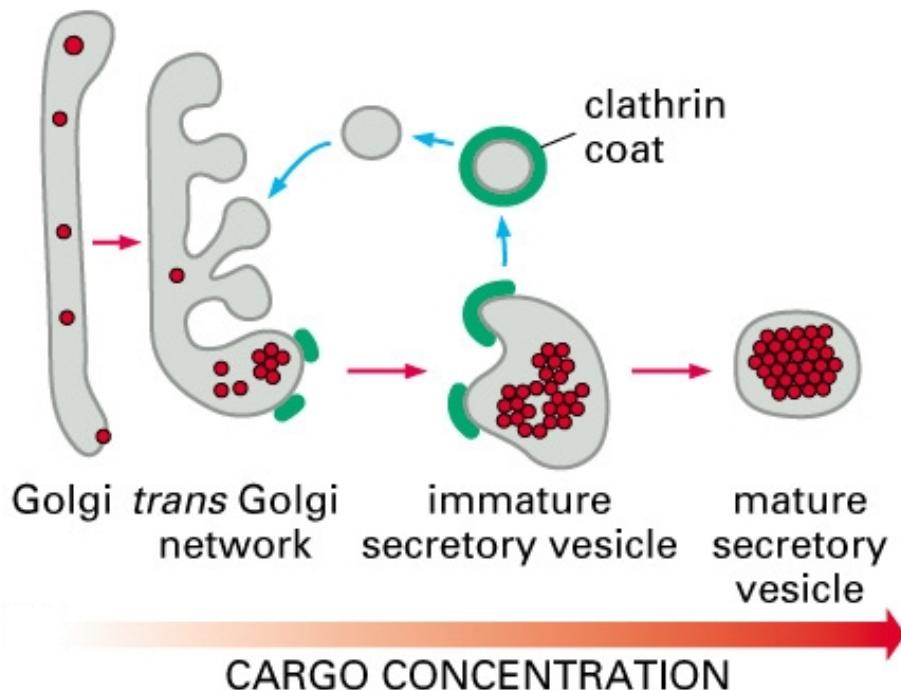
# SECRETORY PROTEINS

- Automatically secreted/default pathway in unpolarized cells (f.i.: blood, fibroblasts)

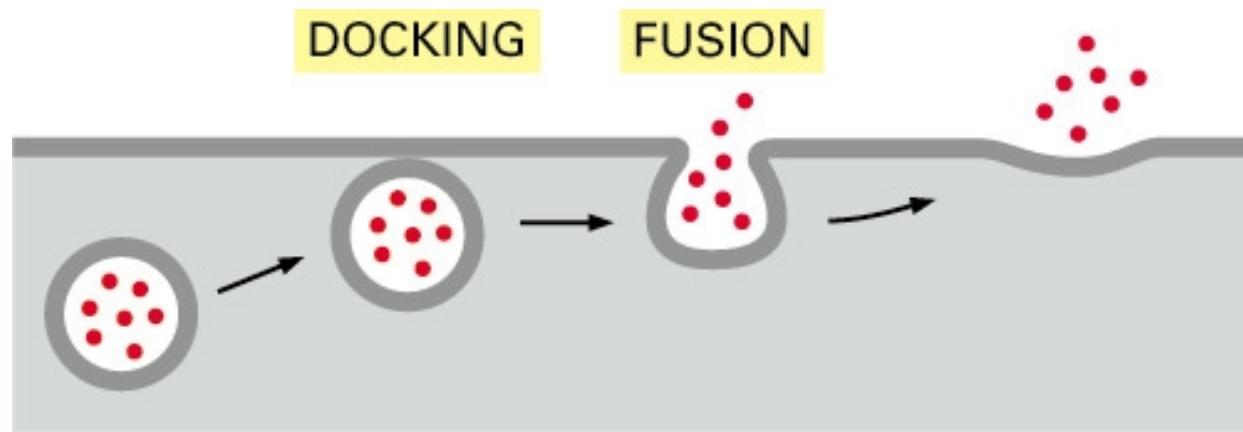


# SECRETORY VESICLES

- Sorting signal is the same for all
- Variable size of cargo (from histamine to proteins)
- Highly concentrated cargo ( $\sim 10^3$  fold):
  - acidity effect ( $H^+$  ATPase)
  - retrieval of membrane excess



# SECRETORY VESICLES



0.2  $\mu\text{m}$

# PROTEOLYSIS IN SECRETORY VESICLES

➤ Initial form of synthesised protein is inactive:

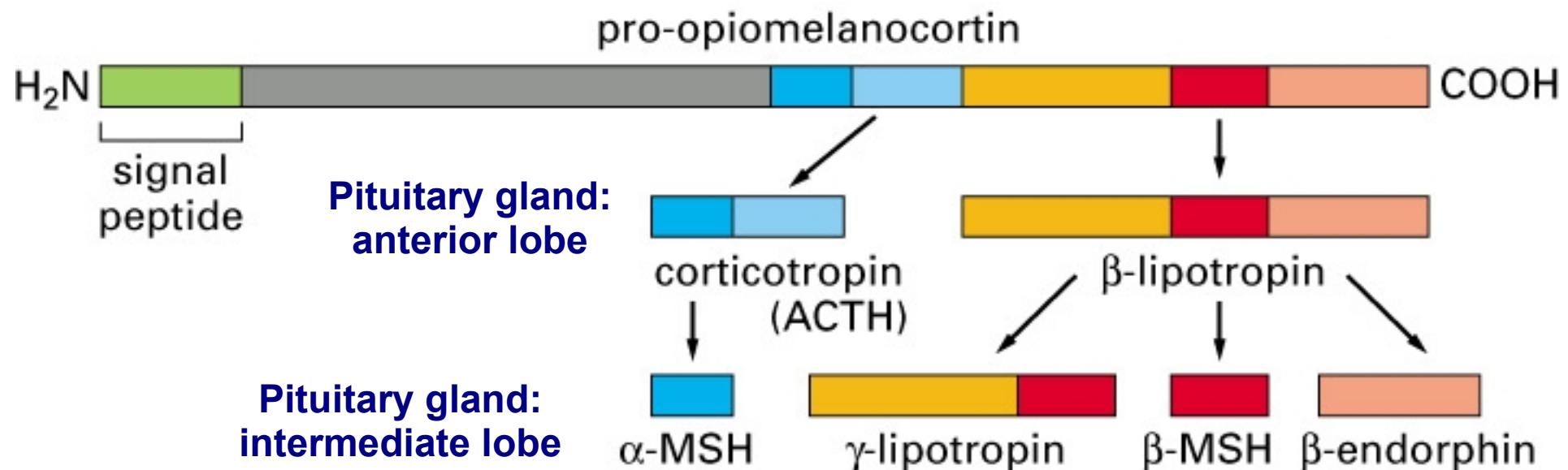
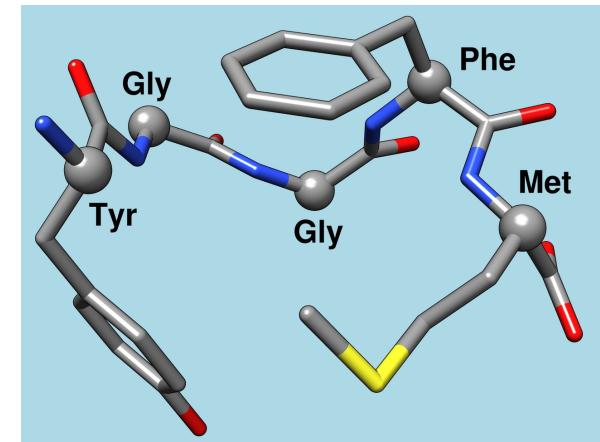
- Propeptide at N-terminus => pre-pro-peptides

- Polyproteins: multiple copies

➤ Cellular specificity

➤ Targeted activity

➤ Enkephalins (YGGF[M,L]): too short to be transported to ER



Pathways of prohormone proopiomelanocortin

# SECRETORY VESICLES RELEASE

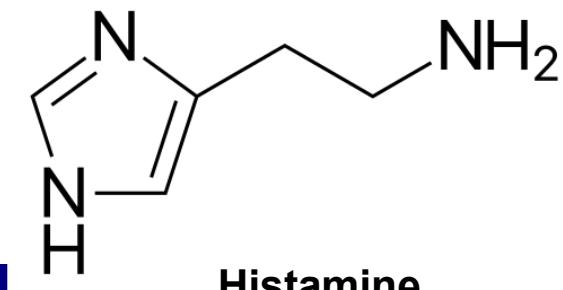
➤ External signal (f.i.: to release neurotransmitters in nerve cells):

- chemical messenger (f.i.: hormone) =>  $[Ca^{2+}]_{\text{inside}}$

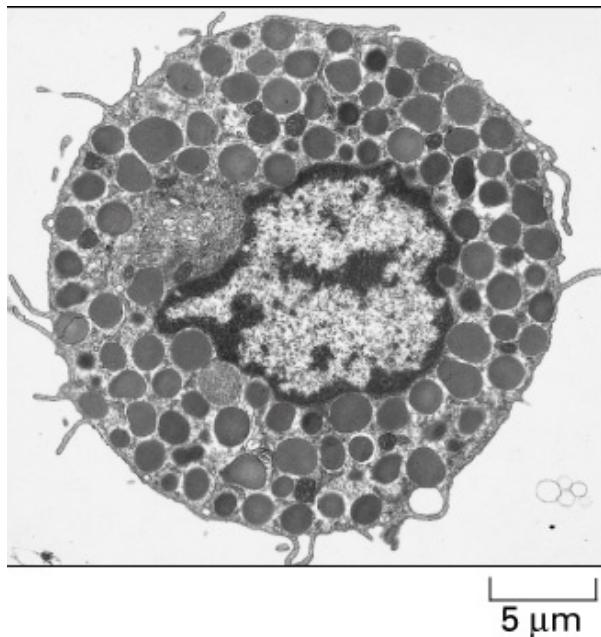
- electric excitation =>  $Ca^{2+}$  channels

➤ Fusion of SNAREs is not complete until  $Ca^{2+}$  is released

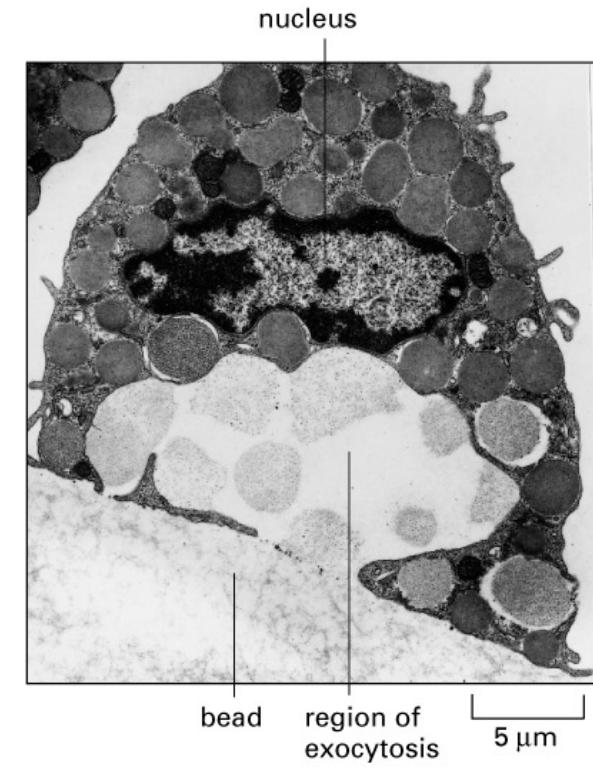
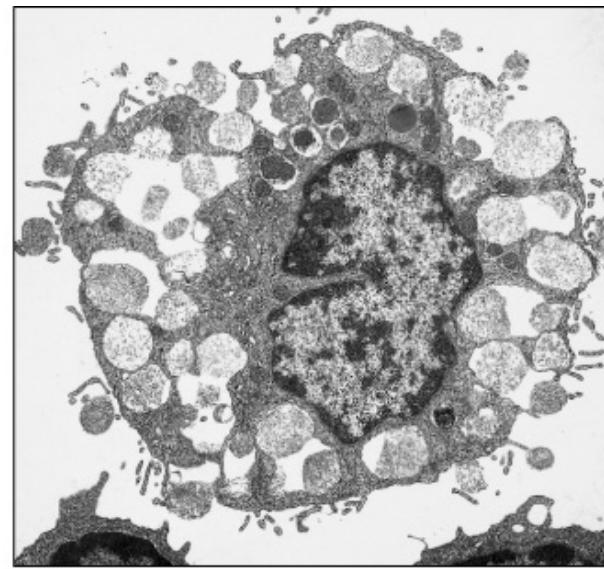
➤ Exocytosis can be local (mast cells, histamine)



Histamine



Activation of rat mast cell =>



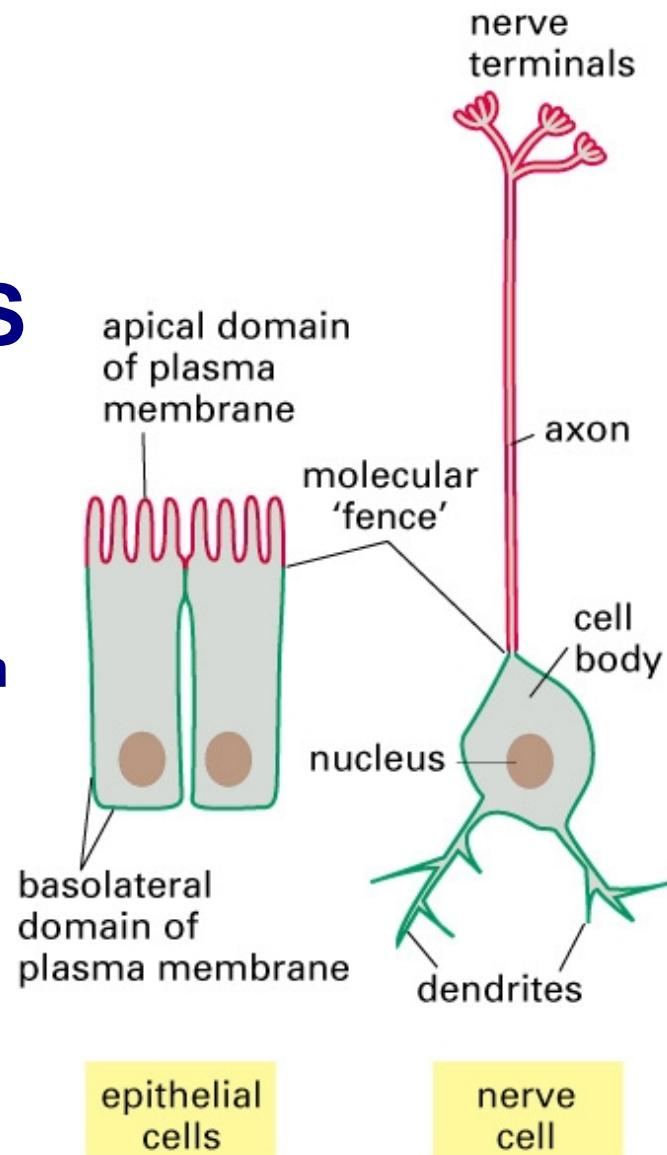
Local exocytosis

# SECRETORY VESICLES FATE AFTER RELEASE

- Fusion with PM
- Increase of PM size ~ 30 times in glands
- Secretory vesicle receptors => lysosomes
- Controlled balance of exocytosis <=> endocytosis

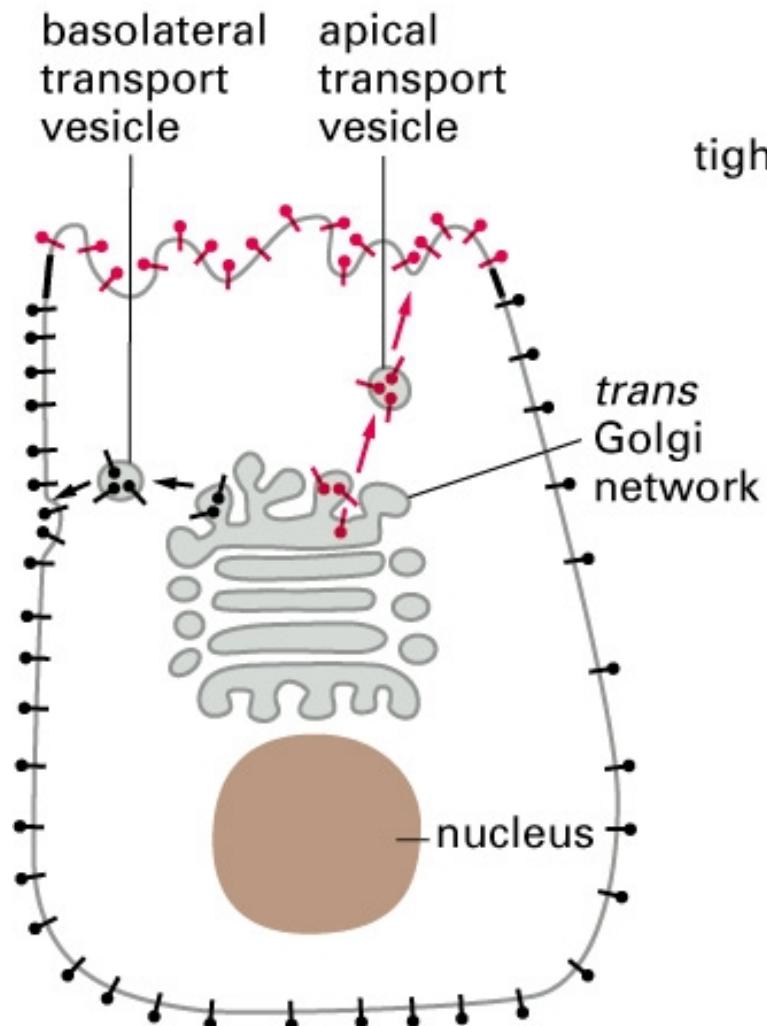
## POLARIZED CELLS

- Several plasma membrane domains
- Different vesicle types in distinct domains
- Tight junctions: prevention of lipid/protein diffusion

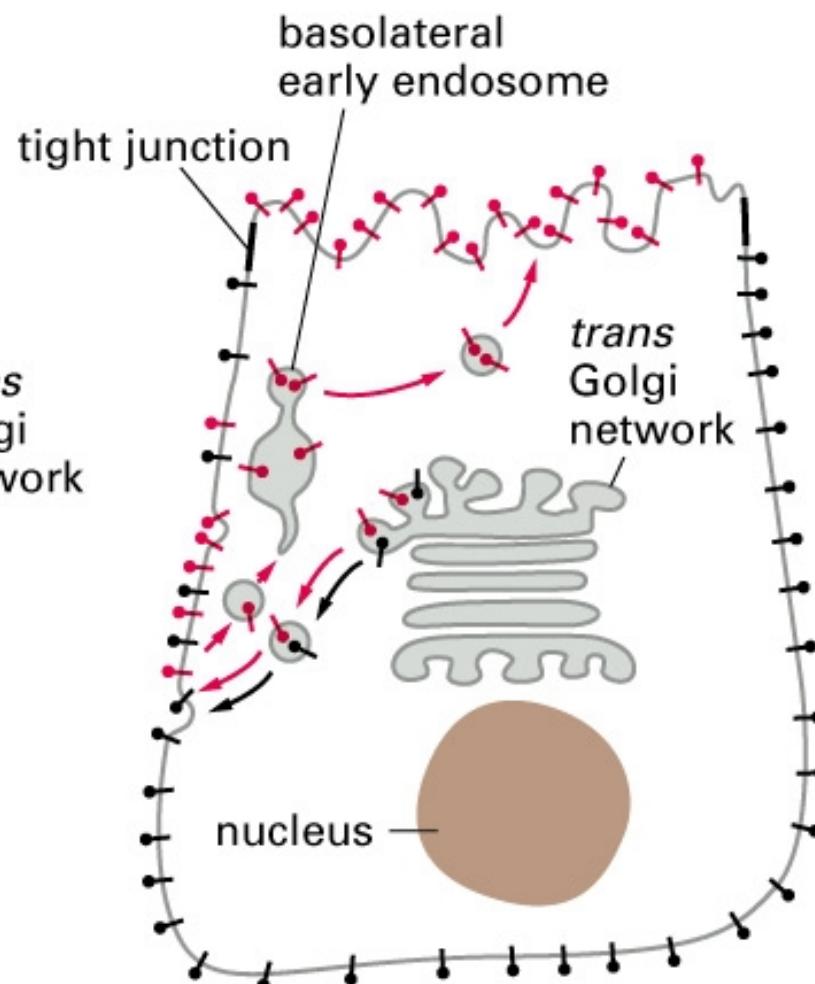


# PROTEINS SORTING IN POLARIZED CELLS

- Proteins targeted to different domains are transported together ER=>GA
- Sorting signals



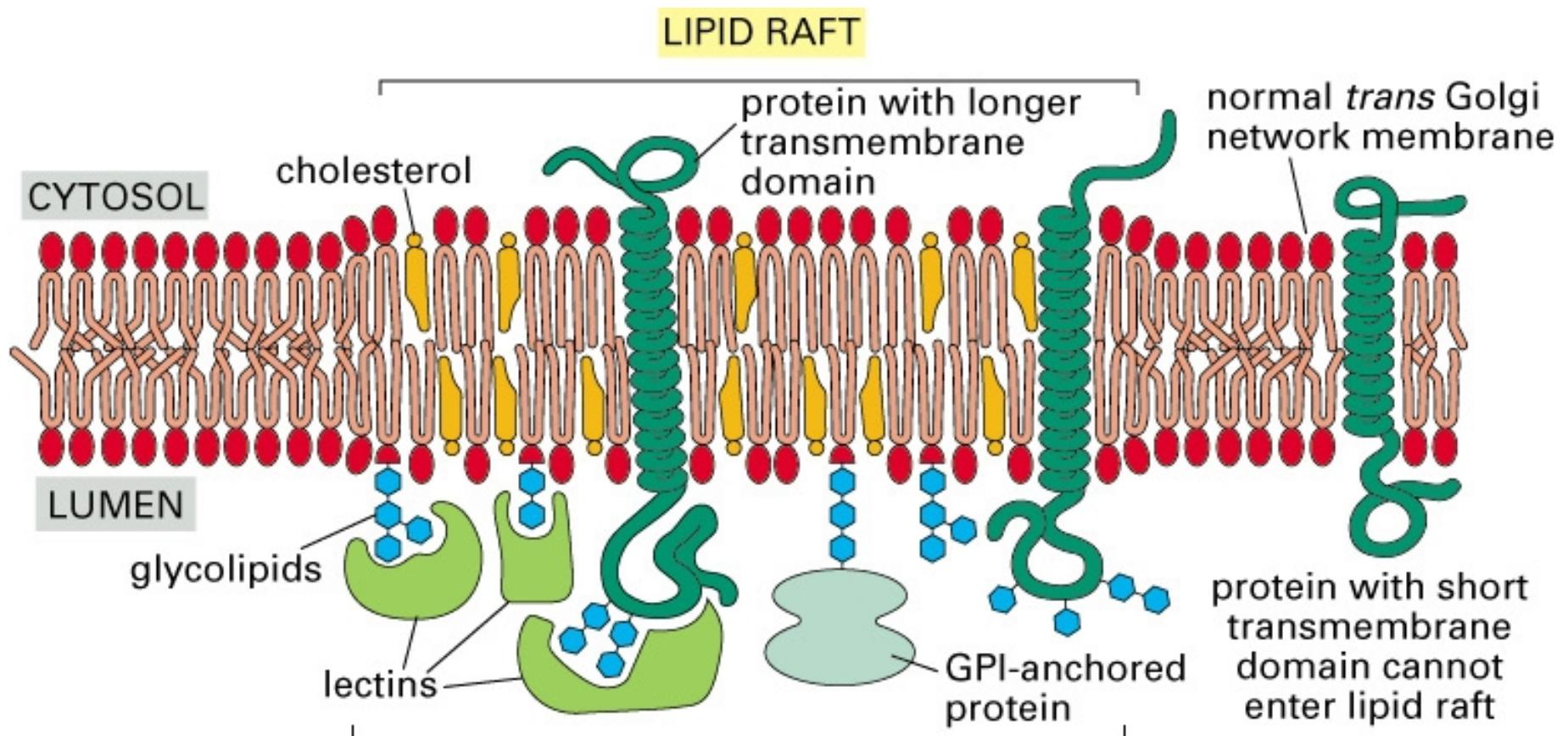
DIRECT SORTING OF  
MEMBRANE PROTEINS IN  
THE TRANS GOLGI NETWORK



INDIRECT SORTING VIA ENDOSOMES

# LIPID RAFTS IN SORTING GLYCOSPHINGOLIPIDS AND GPI-ANCHORED PROTEINS

- Apical plasma membrane of most cells is rich in:
  - glycosphingolipids (protection from digestion and low pH)
  - glycosylphosphatidylinositol (GPI)-anchored protein are targeted there
  - lectins



# ENDOCYTIC VESICLES => SYNAPTIC VESICLES

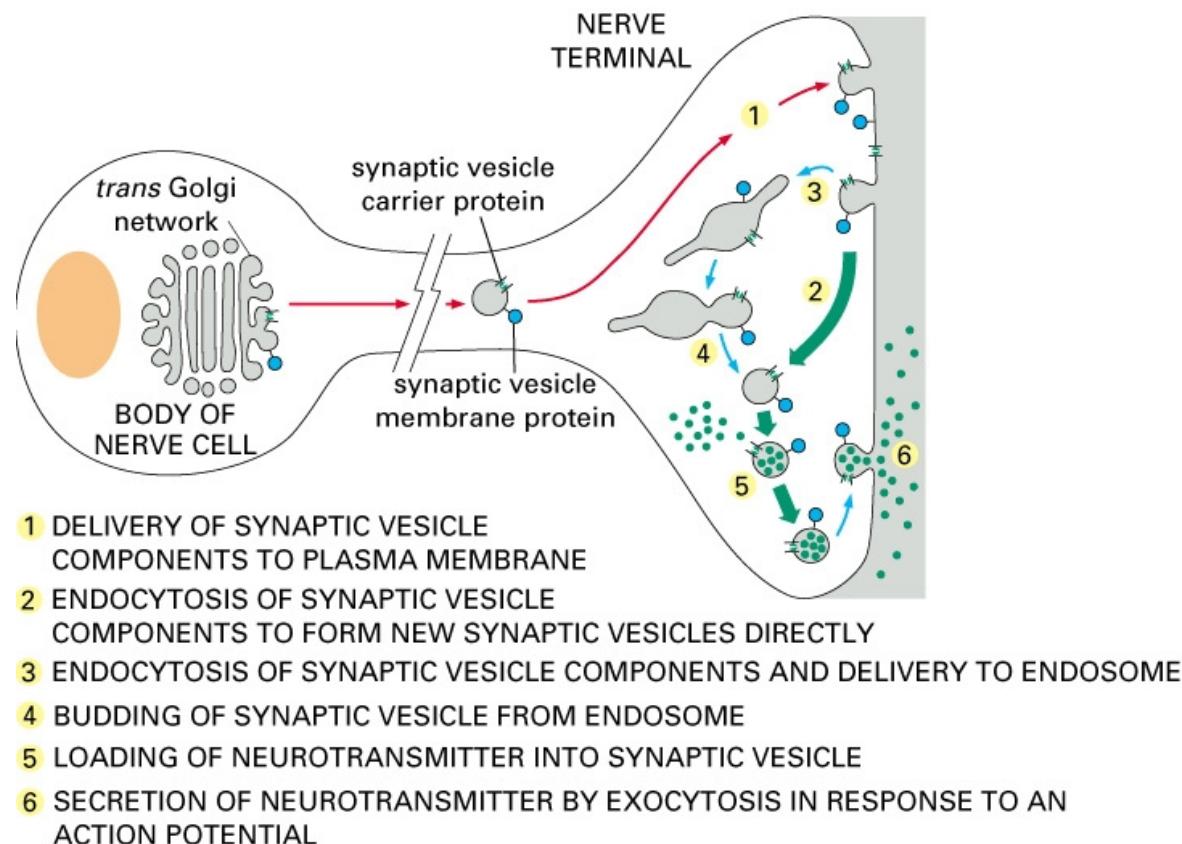
➤ Nerve cells vesicles:

- standard secretory vesicles

- synaptic vesicles (~50 nm) contain neurotransmitters

➤ Fast regeneration for synaptic vesicles is needed ( $10^3$  firing events/second)

=> regeneration from plasma membrane to endosomes ( $H^+$  gradient)



# LECTURES 17-18: VESICULAR TRANSPORT

- Introduction: basic concepts
- Molecular mechanisms and cell compartments diversity
- Transport ER => GA
- Transport GA => lysosomes
- Endocytosis
- Exocytosis

