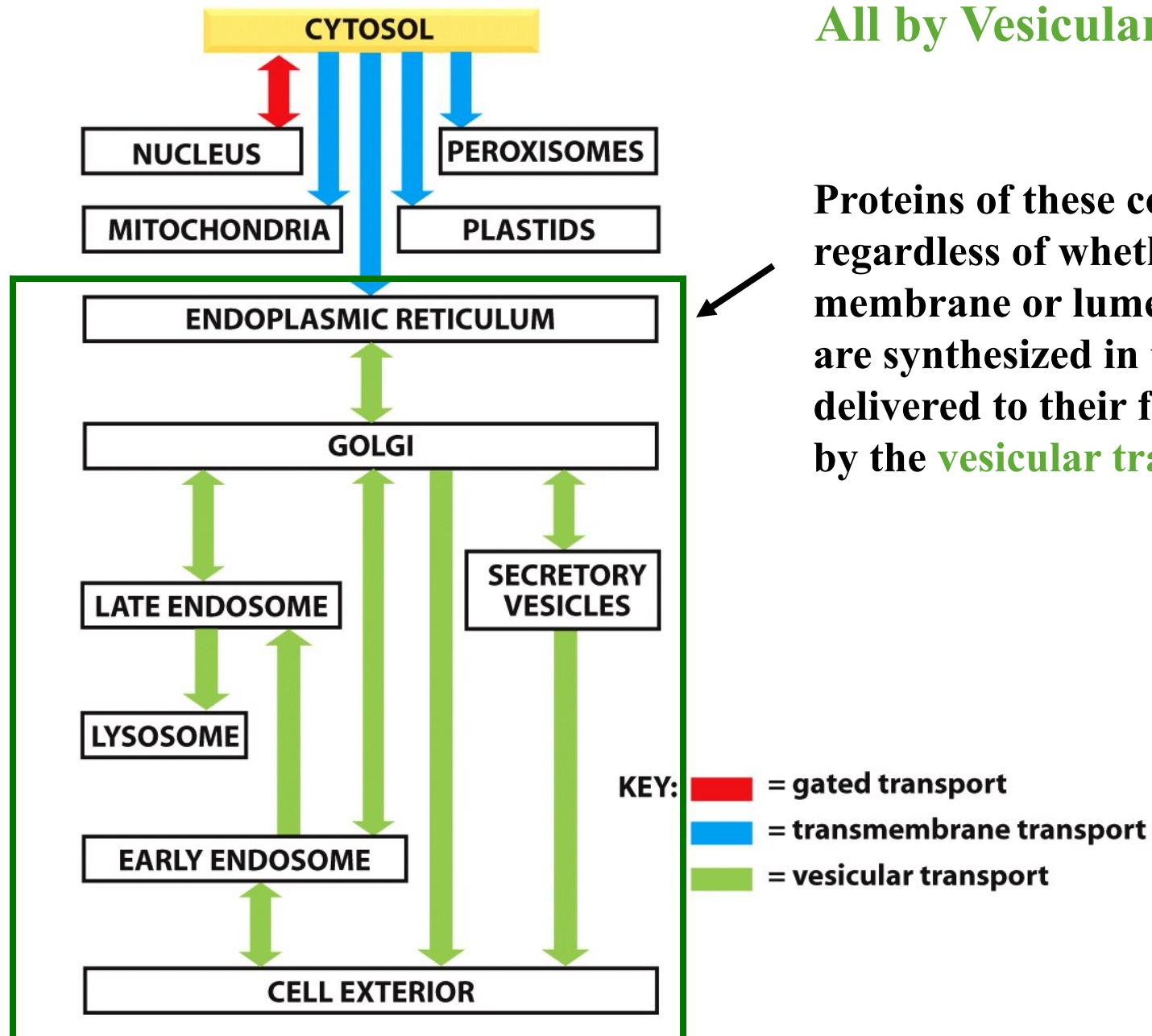


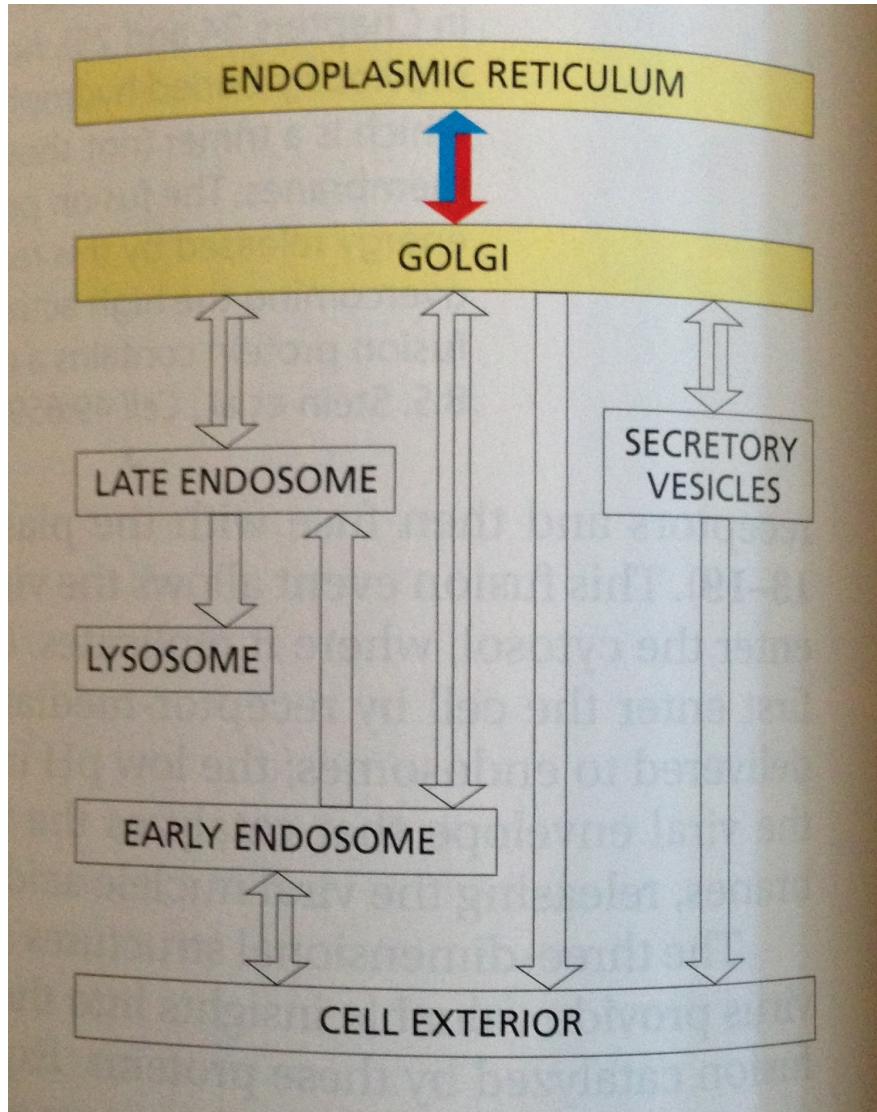
- Transport between the ER and the Golgi
- Transport within the Golgi
- Transport from the Golgi to Late Endosomes and the Lysosome
- Transport from the Golgi to Secretory Vesicles and the Plasma Membrane



All by Vesicular Transport

Proteins of these compartments, regardless of whether they are membrane or luminal proteins, are synthesized in the ER and delivered to their final destinations by the **vesicular transport pathway**.

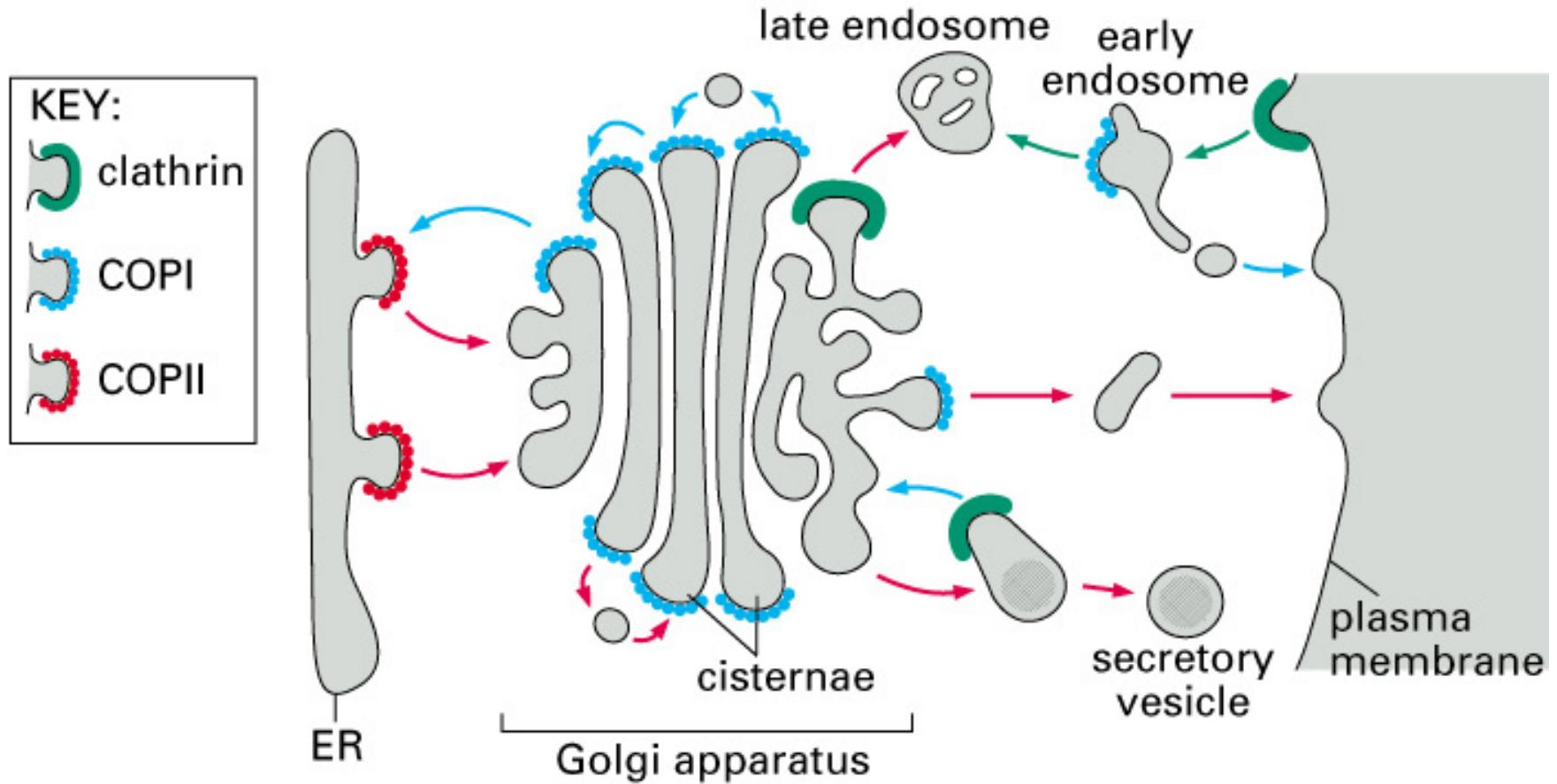
ER-Golgi Transport



**Forward transport,
from ER to Golgi.**

**Retrograde transport
from Golgi to ER.**

Different Coats Are Involved in Different Transport Steps



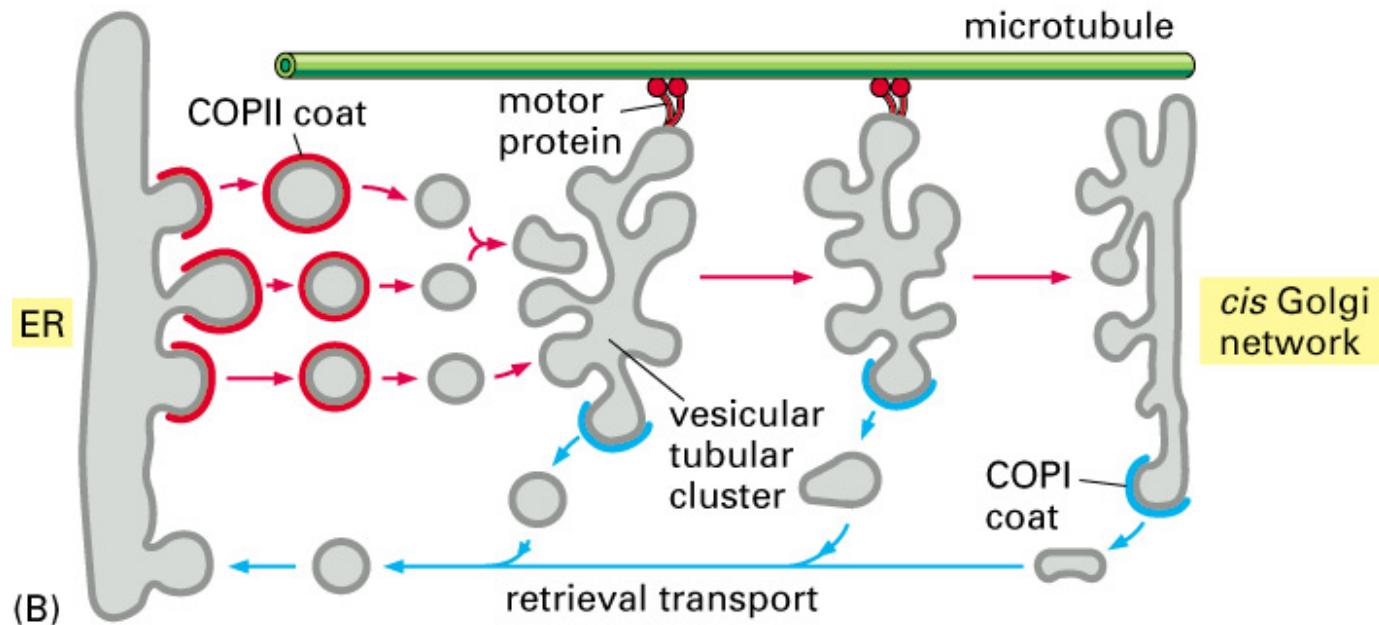
COPII: ER to Golgi

COP1: Golgi to ER, within Golgi, some transport initiated from endosomes

**Clathrin: Transport initiated from Golgi (e.g. Golgi-to-endosome),
plasma membrane (e.g. internalization), and endosomes.**

Transport between the ER and the Golgi Involves the VTC

COPII-derived carrier → VTC → Golgi (forward)

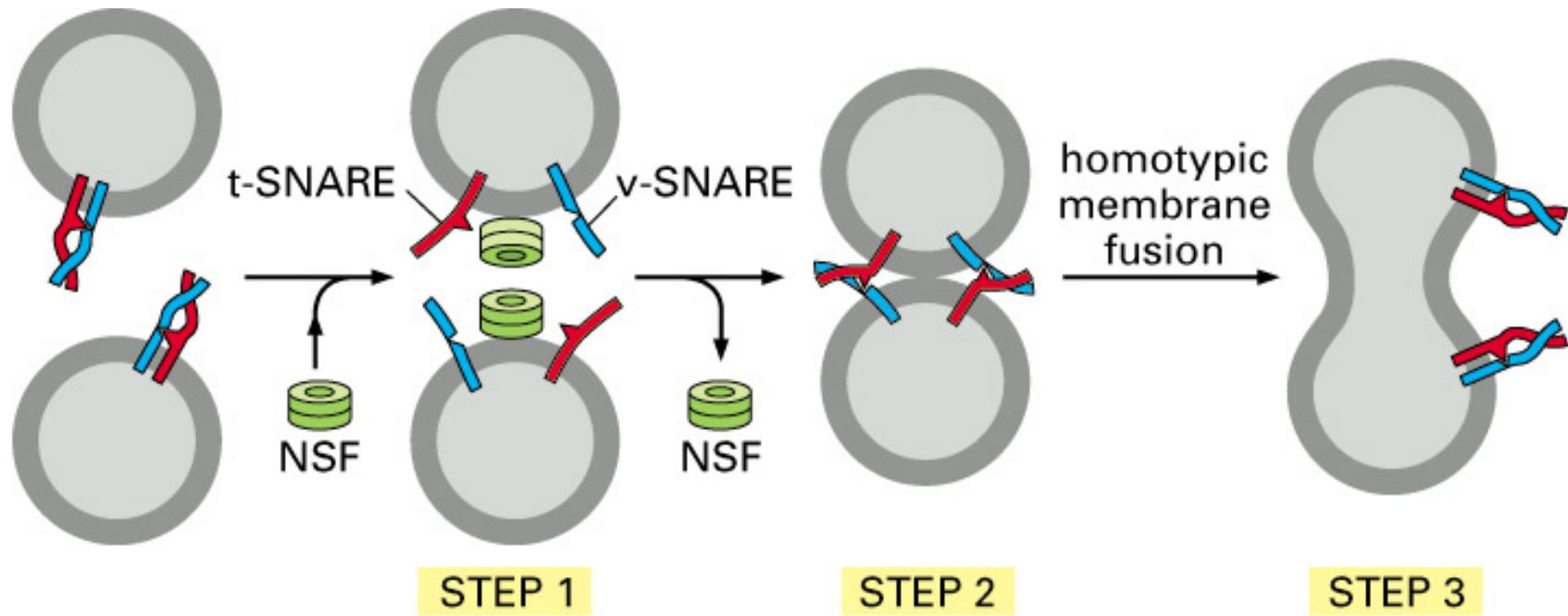


ER ← COPI derived carriers ← Golgi/VTC (retrograde)

VTC (vesicular tubular cluster): an intermediate compartment between ER & Golgi

Formation of VTC via homotypic membrane fusion between ER-derived transport carriers

ER-derived carriers with their COPII coats shed at the ER exit site and undergo homotypic fusion



Homotypic fusion: between two identical compartments

Heterotypic fusion: between two different compartments

Transport between the ER and the Golgi

**Forward transport from the ER to the Golgi
(COPII-mediated)**

**Retrograde transport from the Golgi to the ER
(COPI-mediated)**

Types of Cargo Proteins Packed into the COPII Vesicles

**(1) Proteins destined to later vesicular compartments
(Golgi proteins, plasma membrane or secreted proteins,
lysosomal proteins...)**

**(2) Proteins required for the mobility, tethering/docking, and
fusion of ER-derived carriers on the Golgi membrane
(e.g. v-SNARE, ...)**

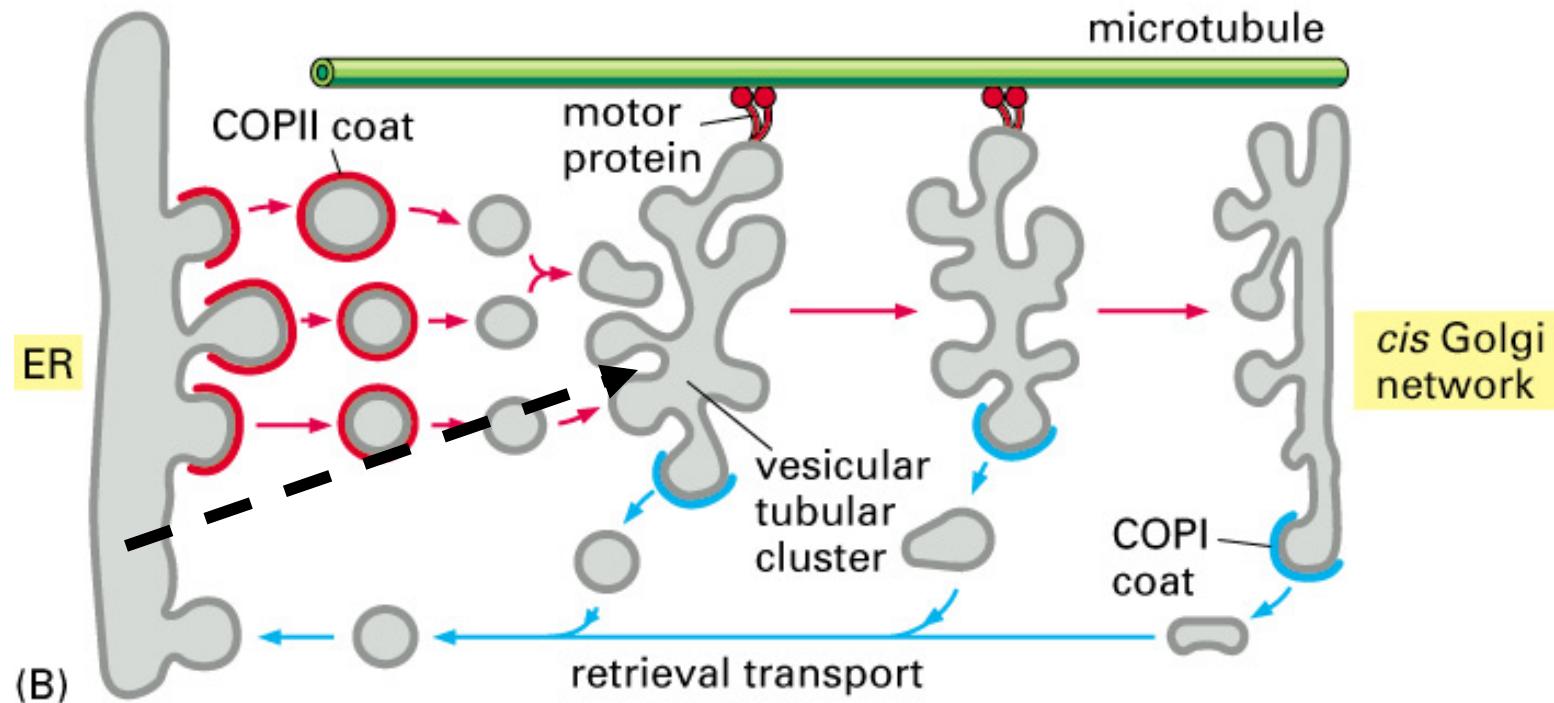
**(3) Proteins required to retrieve escaped ER resident and
sometimes misfolded proteins
(e.g. KDEL receptor)**

Cargo Too Big for COPII Vesicles?

COPII Vesicles → VTC → Golgi

known COPII vesicles: 60-80 nm. Procollagen I bundle: 300 nm

How is the procollagen I bundle transported to the Golgi?



There are two possibilities:

Transport carriers/mechanisms other than COPII vesicles mediate the transport
Different shapes and sizes of COPII vesicles.

Transport between the ER and the Golgi

**Forward transport from the ER to the Golgi
(COPII-mediated)**

**Retrograde transport from the Golgi to the ER
(COPI-mediated)**

Types of Cargo Proteins Packed into the COPI Vesicles

- (1) proteins required for the mobility, tethering/docking, and fusion of Golgi/VTC-derived carriers on the ER membrane**

- (2) escaped ER resident and sometimes misfolded proteins**

Two mechanisms exist to keep ER resident or mis-folded/mis-assembled proteins in the ER

(1) Retention: preventing cargo from leaving ER

- a. the binding of chaperones to mis-folded proteins (quality control mechanism) (e.g. through anchoring or signal masking, and “kin recognition”)
- b. shorter transmembrane domains for resident proteins (e.g. a similar mechanism as in the Golgi)

(2) Retrieval: capturing cargo at a post-ER compartment (i.e. VTC/Golgi) and send it back to the ER

- a. the quality control mechanism at the VTC/Golgi to retrieve mis-folded proteins (not fully understood)
- b. retrieval signals for transmembrane resident proteins: lysine-based (e.g. KKXX-COOH) or arginine-based (e.g. RXRXX)
- c. retrieval signals for ER luminal resident proteins: KDEL-COOH

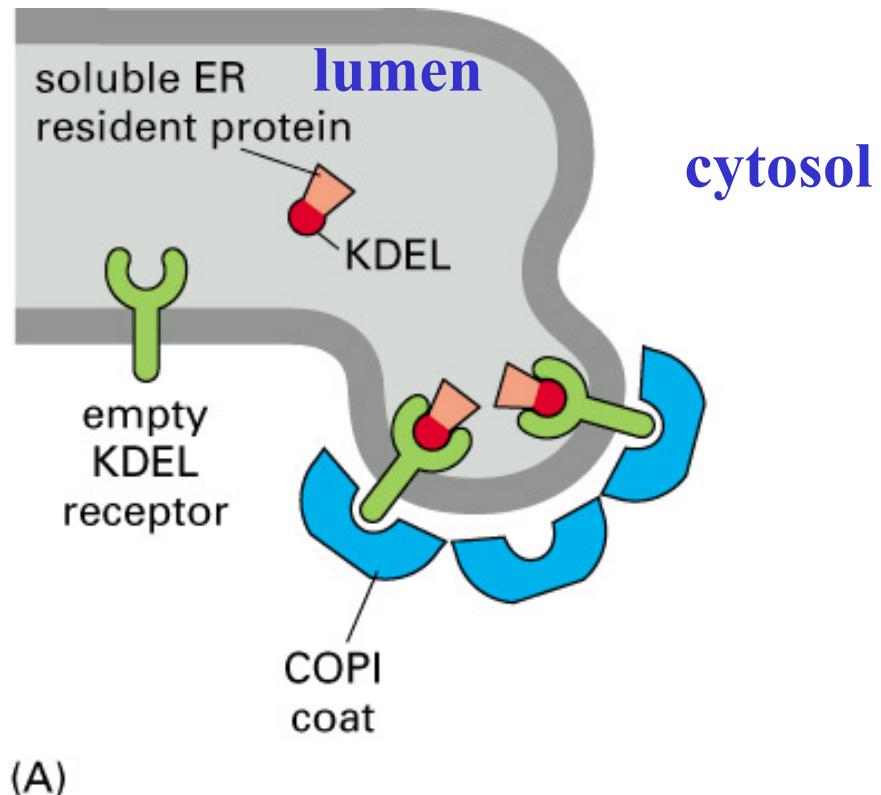
Retrieval of ER resident proteins

For transmembrane resident proteins:
direct binding of KKXX to COPI coat

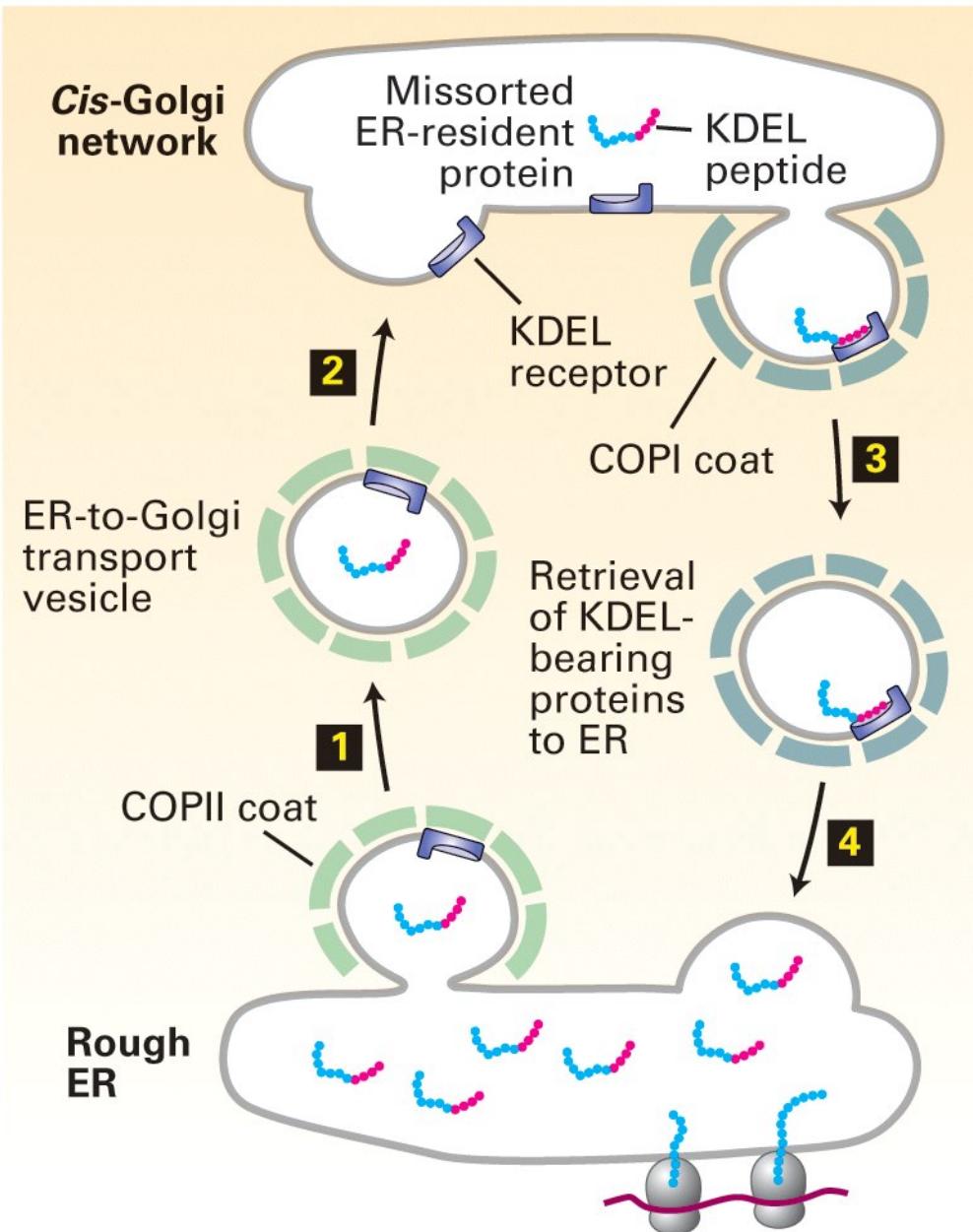
For soluble/lumenal resident proteins:
indirect association between KDEL and COPI coat via the KDEL receptor

KDEL receptor captures the escaped ER lumenal resident proteins at the VTC/Golgi, brings them to the COPI vesicles, and returns them to the ER.

Several intriguing questions
regarding this transport event

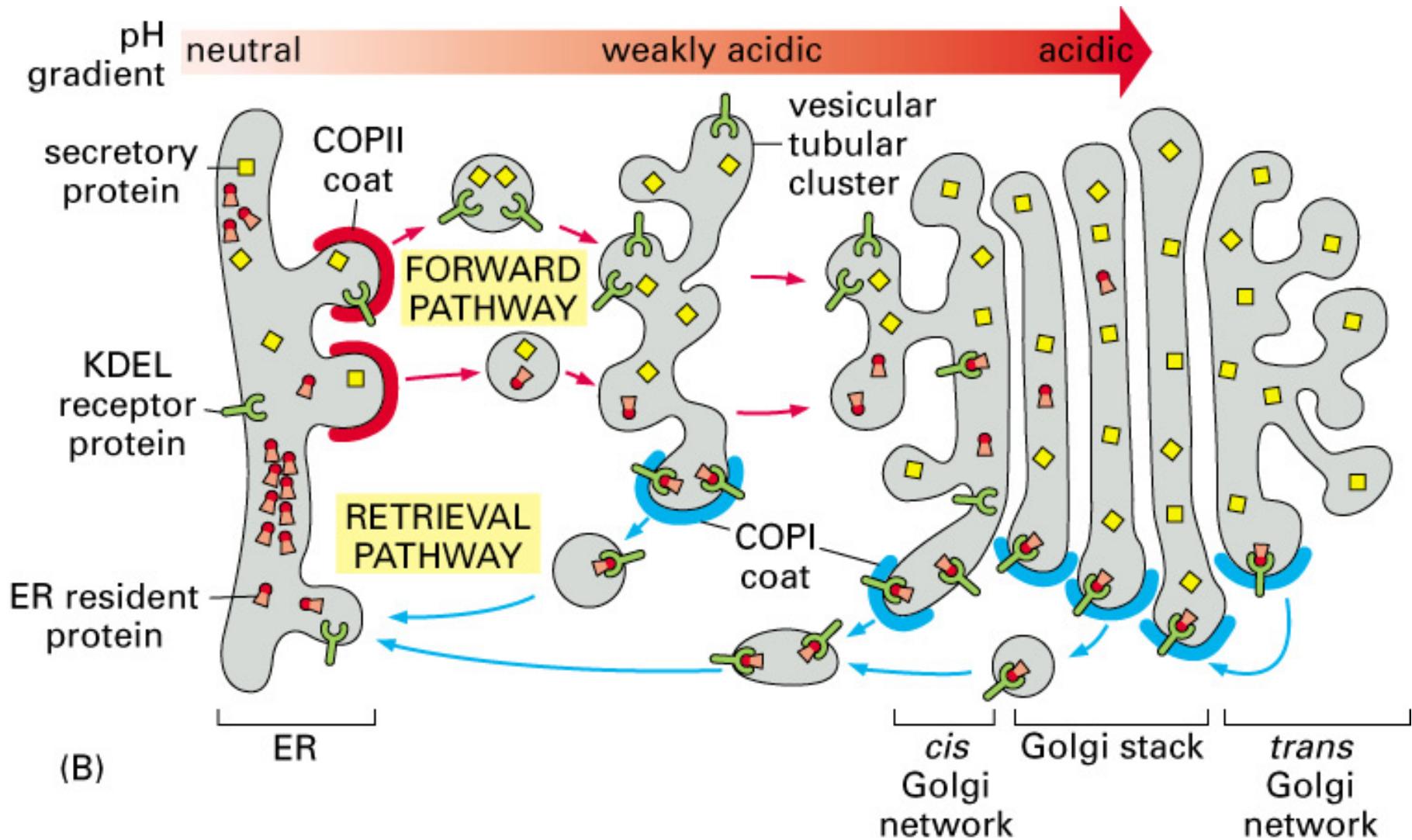


The KDEL receptor is able to bind KDEL-containing cargo at the VTC/Golgi and release the cargo at the ER

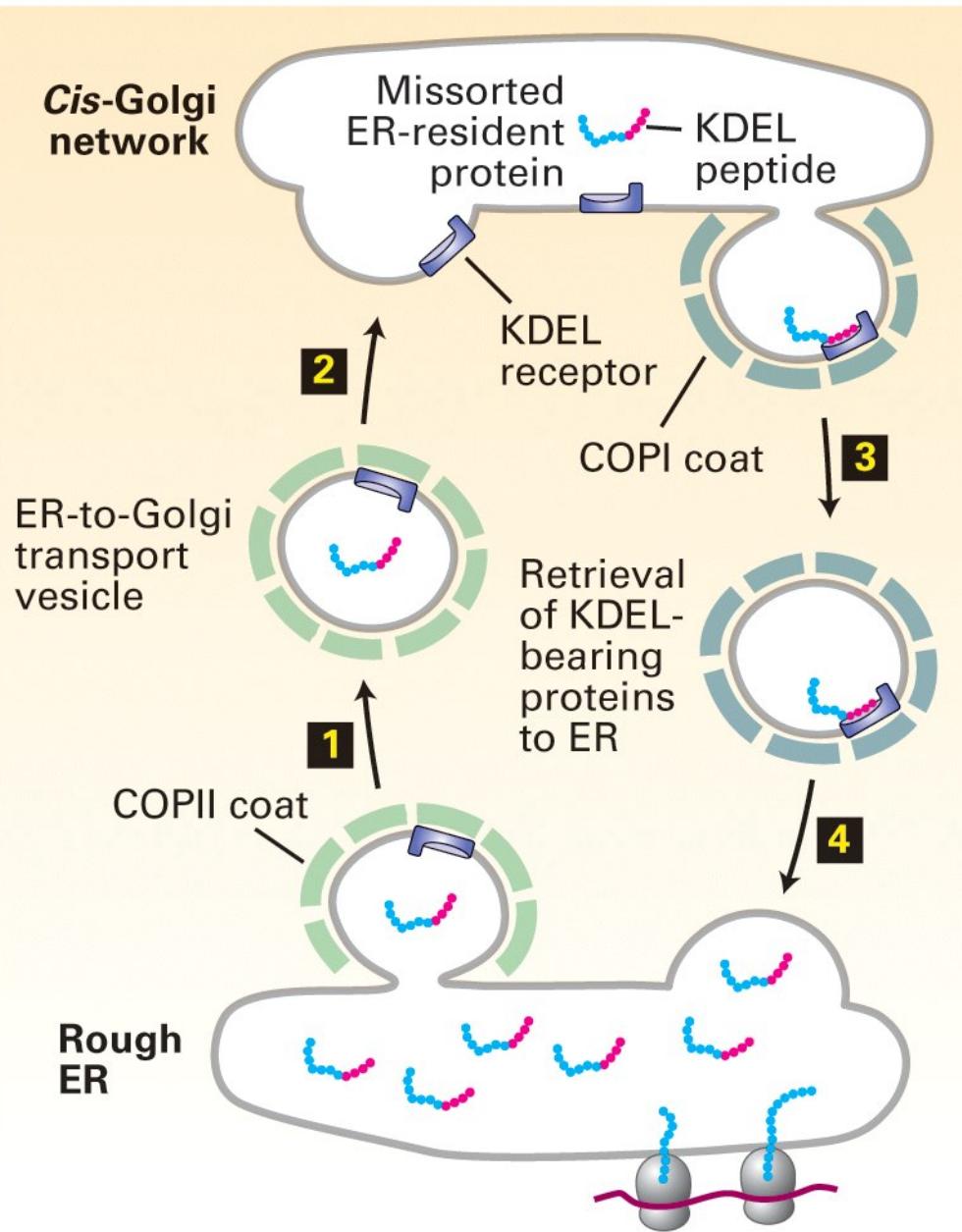


KDEL receptor should bind to its cargo at the Golgi/VTC but not at the ER – how?

Lumens of Vesicular Compartments Have Different Acidities (pH)

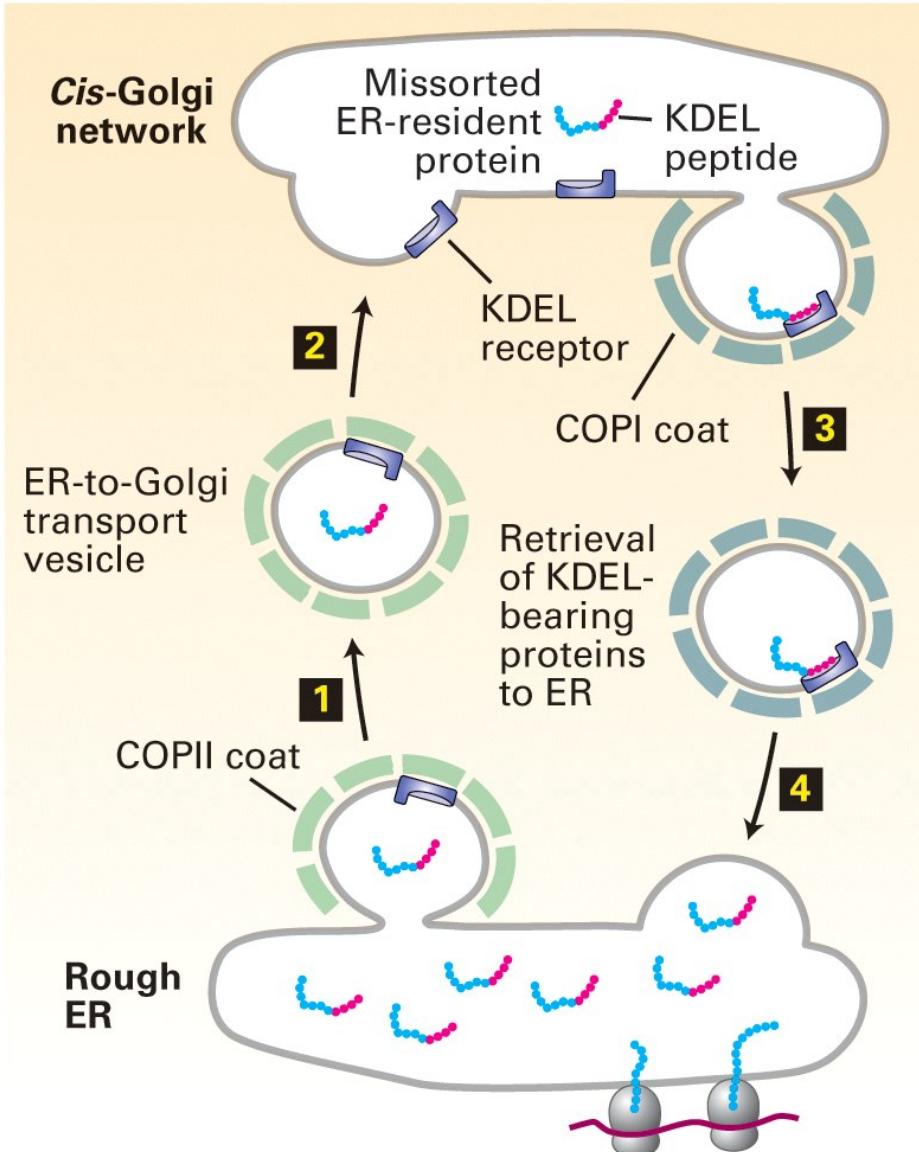


How is the KDEL receptor able to bind KDEL-containing cargo at the VTC/Golgi and release it at the ER?



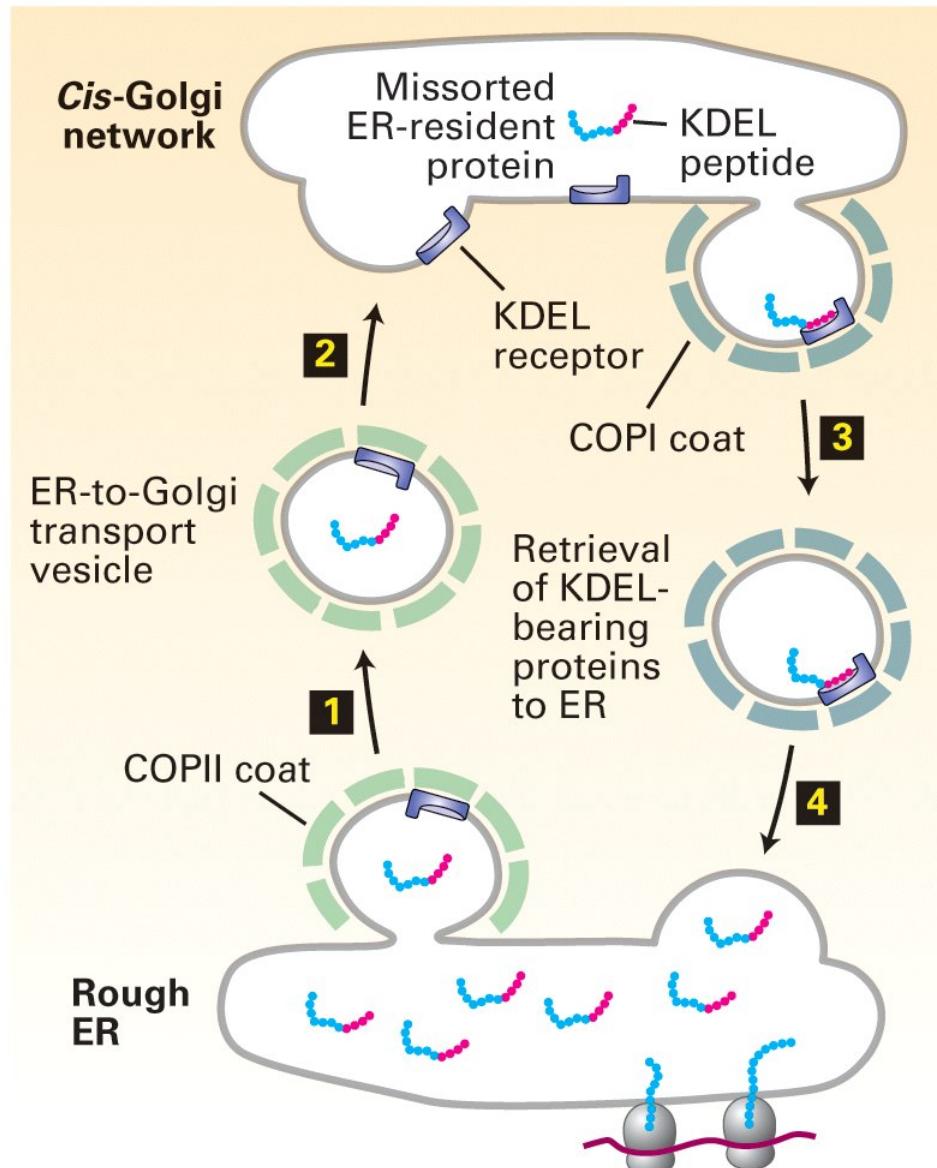
The binding affinity between the KDEL receptor and its cargo is very pH-dependent. The small difference in the pH of the ER and the Golgi favors the binding of KDEL receptor to its cargo at the Golgi but not the ER. As a consequence, the cargo is released from the receptor in the ER.

Whereas the KDEL receptor cycles between ER & Golgi, It is found at a higher level at the Golgi



In order to capture escaped proteins, the KDEL receptor is mainly localized to the *cis*-Golgi region.

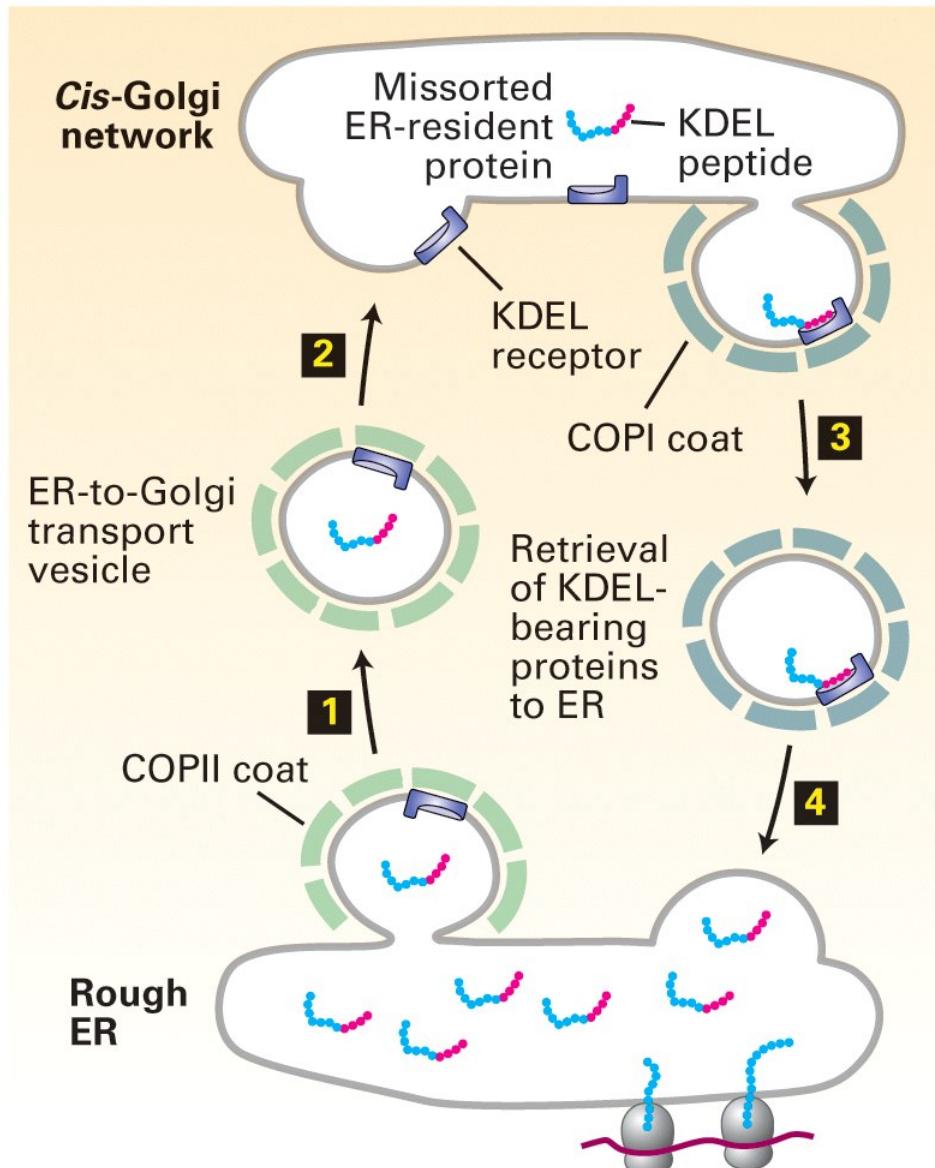
What Causes the KDEL Receptor to Localize to the *cis*-Golgi?



Localization is due to three mechanisms:

- 1. a Golgi-retention mechanism**
- 2. a cargo-induced conformational change which results in the recruitment of the KDEL receptor-cargo complex into the COPI coat vesicle.**
- 3. an ER export mechanism to return KDEL receptor to the Golgi**

What Types of Trafficking Signals Does the KDEL Receptor Have?



Three trafficking signals:

Golgi retention signal

Golgi-to-ER retrieval signal (regulated by the cargo binding)

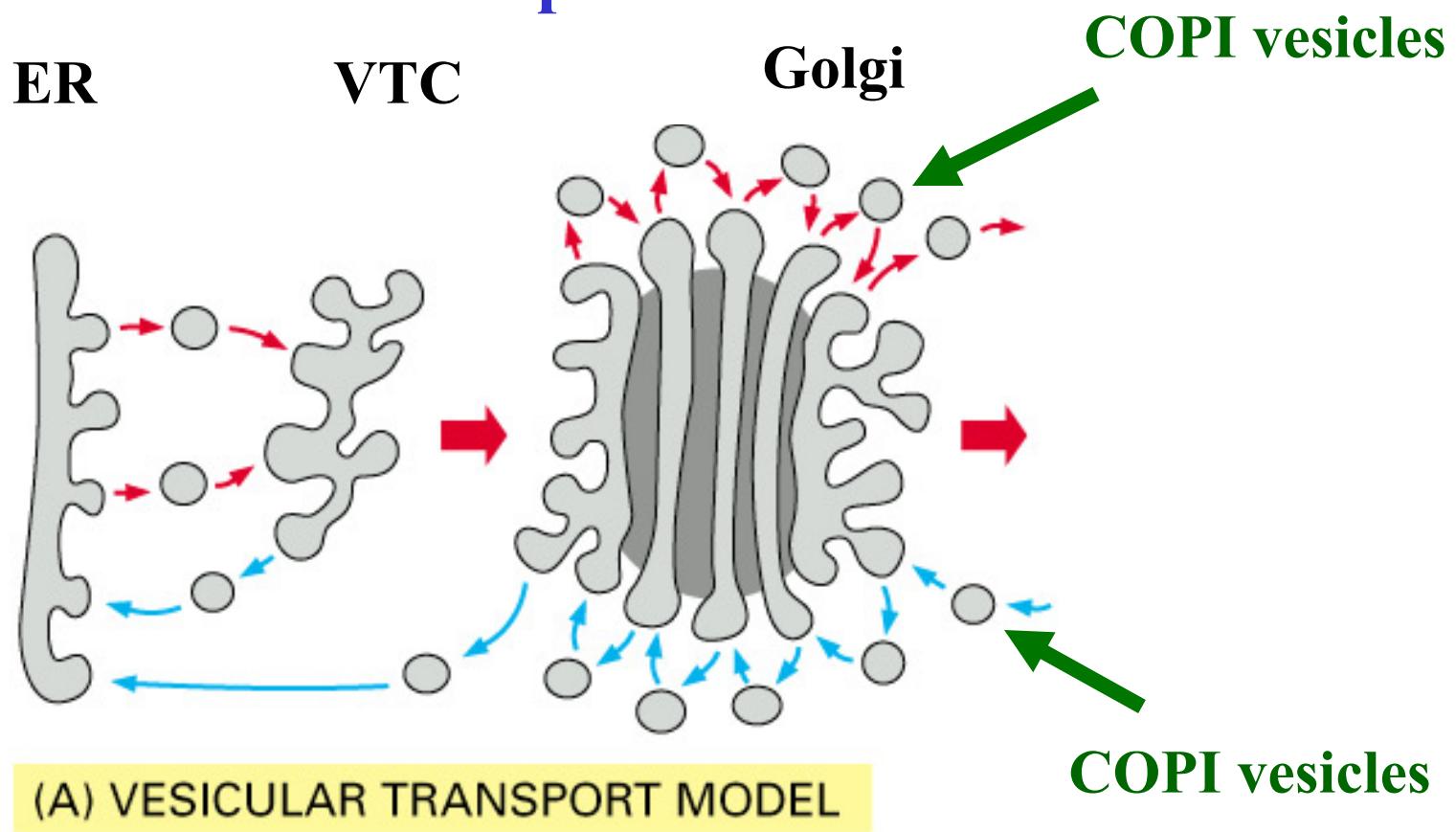
ER export signals

Transport within the Golgi

Vesicular Transport Model

Cisternal Maturation Model

Vesicular Transport Model

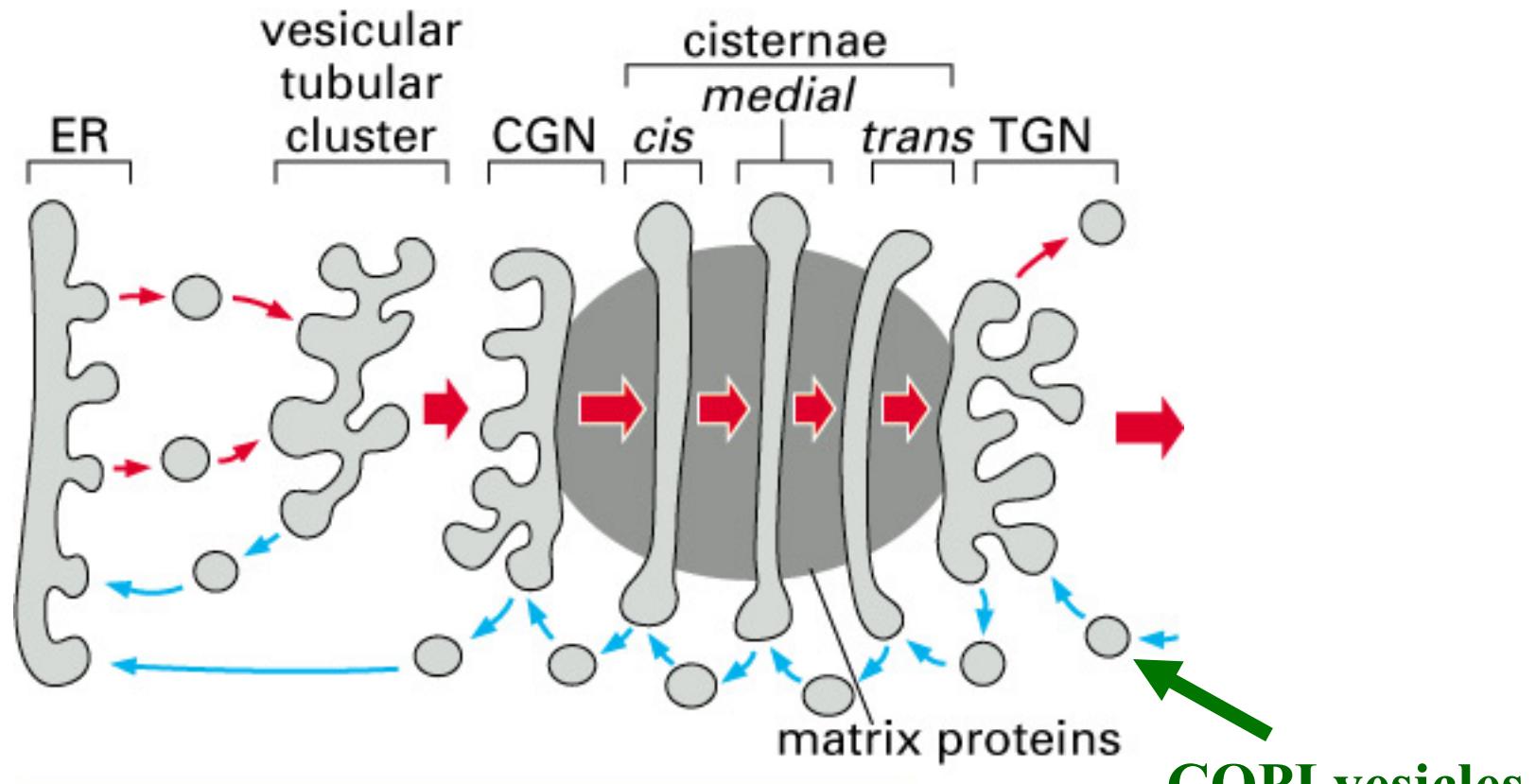


1. Each Golgi cisterna is a static structure
2. Cisterna-specific proteins are held in their places by retention mechanisms
3. COPI vesicles mediate both forward and retrograde transports of cargo in transit

In this model, an intriguing question is how the same COPI coat can mediate the bi-directional transport within the Golgi cisternae?

The other problem is that it cannot explain how some large cargo proteins are moved through the Golgi cisternae.

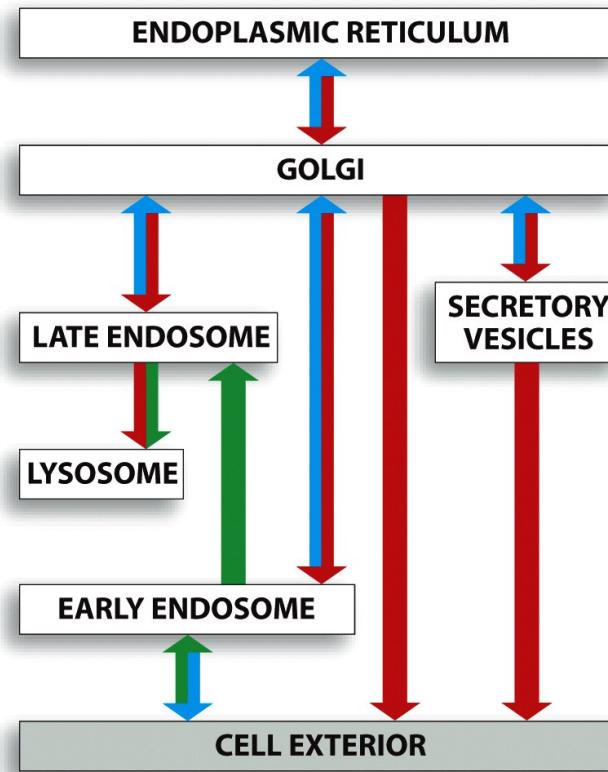
Cisternal Maturation Model



1. Each Golgi cisterna is a dynamic structure w/ a forward movement
2. No vesicle-mediated forward transport between cisternae
3. COPI vesicles mediate retrograde transport by removing specific Golgi resident proteins from the “older” cisterna to the “younger” ones.
4. Each cisterna “matures” as it moves outward by selective retrieval.

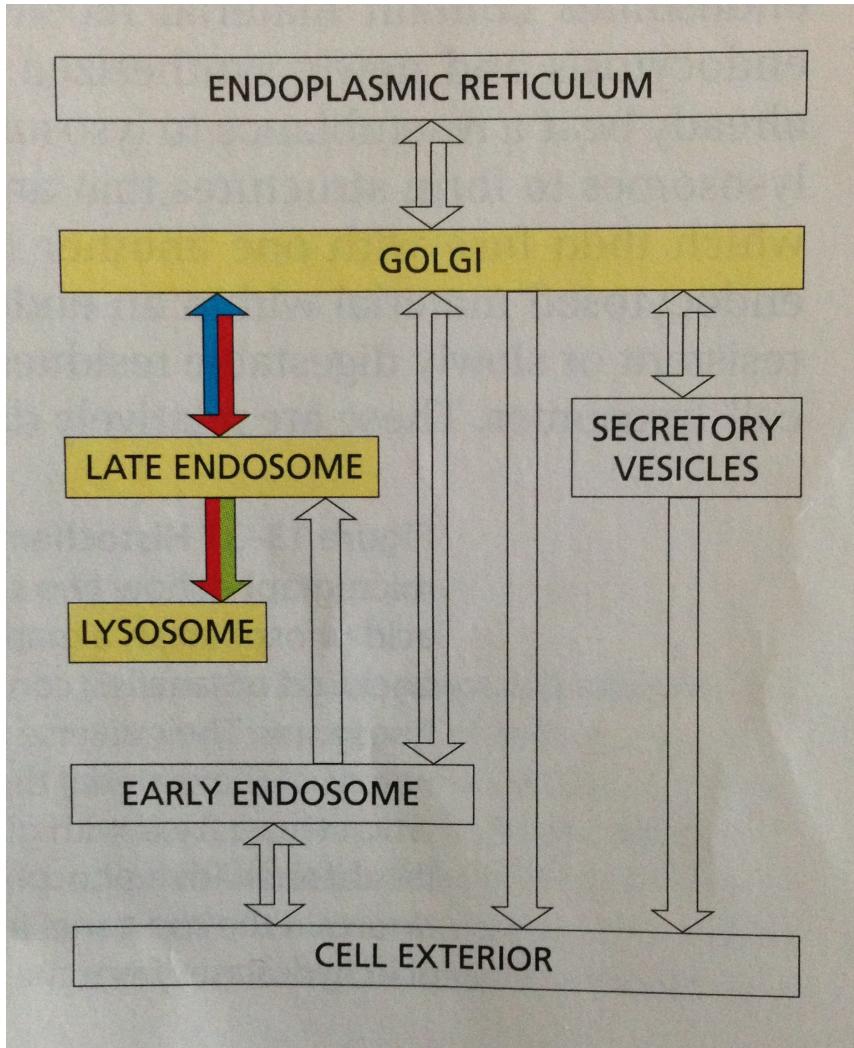
This model is supported by the observation that some large cargo proteins can be transported through the Golgi

The Golgi Lies in the Crossroad of Both Biosynthetic and Recycling Pathways



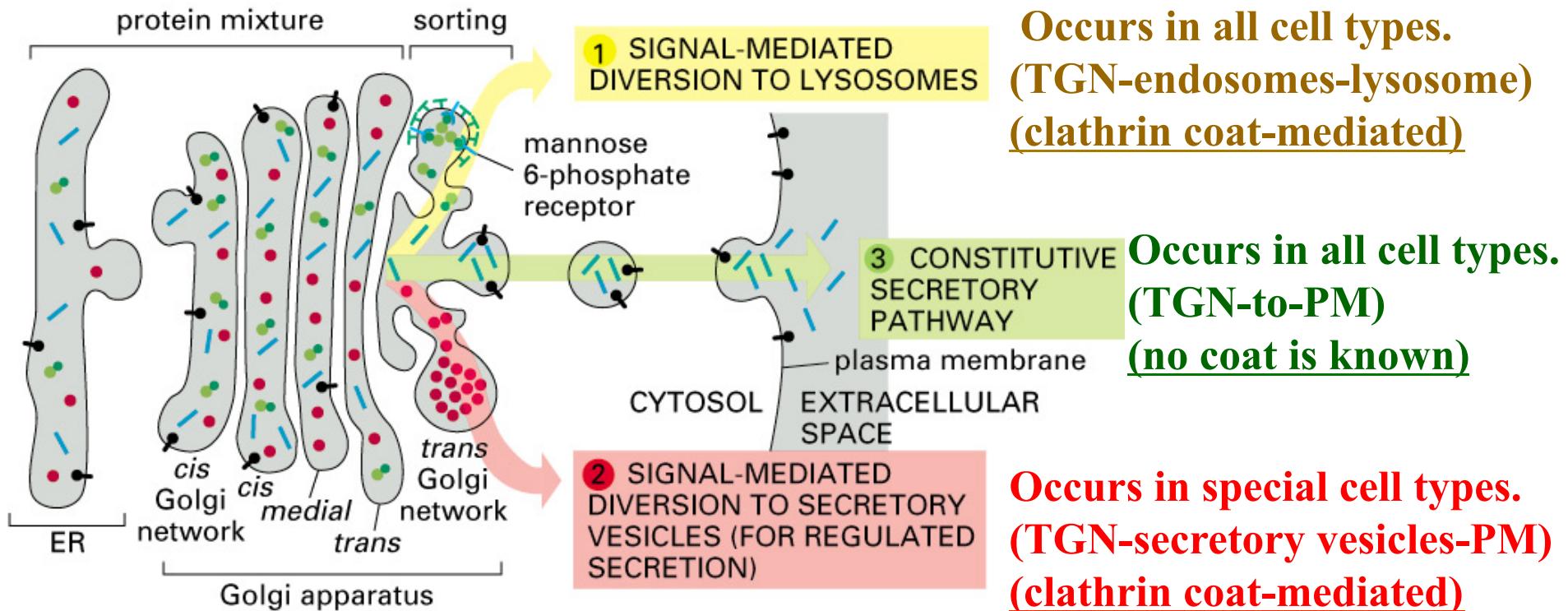
The Trans Golgi Network (TGN) is the main sorting station of the Golgi apparatus.

Transport from the Golgi to Late Endosomes and the Lysosome



The TGN is the Major Sorting Station in Biosynthetic Pathways

There are three major sorting pathways at the TGN



Occurs in all cell types.
(TGN-endosomes-lysosome)
(clathrin coat-mediated)

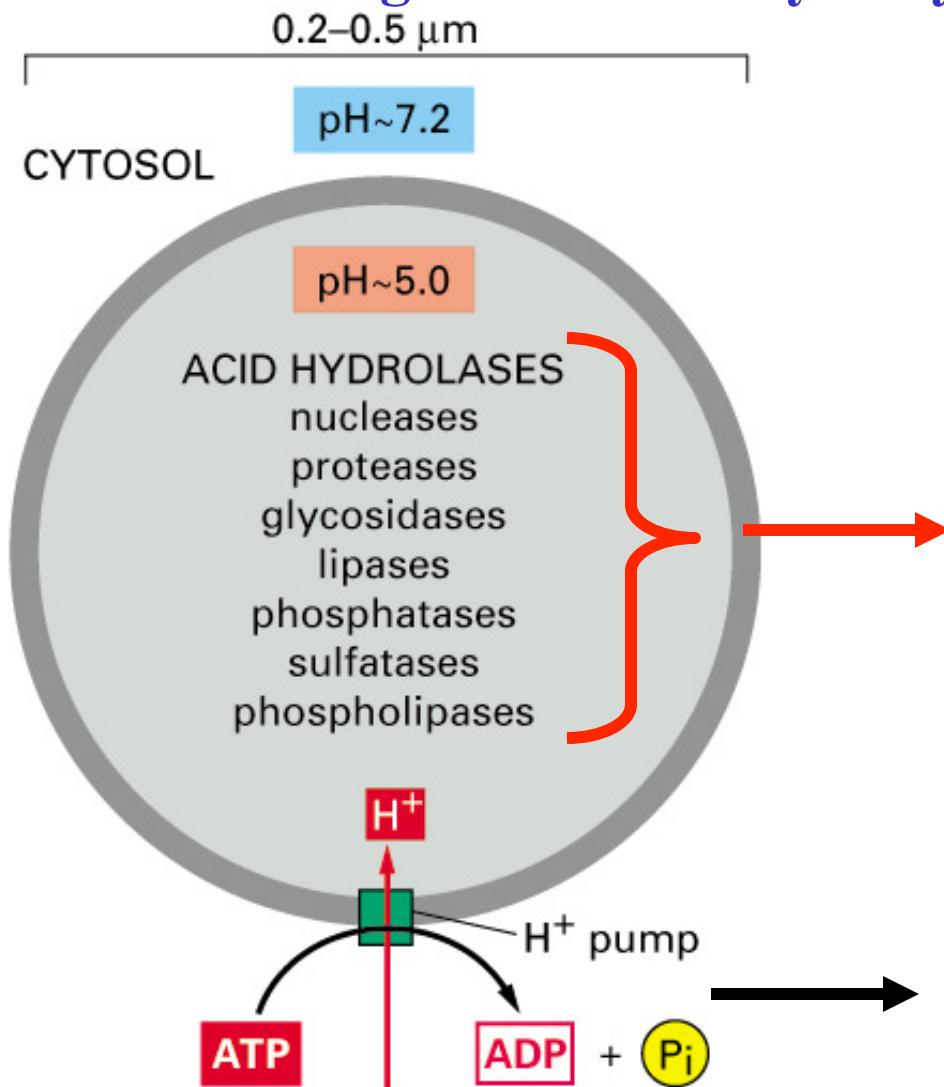
Occurs in all cell types.
(TGN-to-PM)
(no coat is known)

Occurs in special cell types.
(TGN-secretory vesicles-PM)
(clathrin coat-mediated)

New coats may be involved in the above transport pathways.

Recent studies also show a possible role of the *trans*-Golgi cisterna in sorting.

Lysosomes are a collection of heterogeneous organelles with high content of hydrolytic enzymes (hydrolases)



Hydrolases are luminal enzymes

Different types of hydrolases have different substrate specificities

Since these hydrolases display optimal enzymatic activity at the acidic pH, they are also called acid hydrolases

Acid hydrolases are often heavily glycosylated

to generate acidic pH

How do acid hydrolases get to lysosomes?

Let's Talk About "Mannose 6-Phosphate"



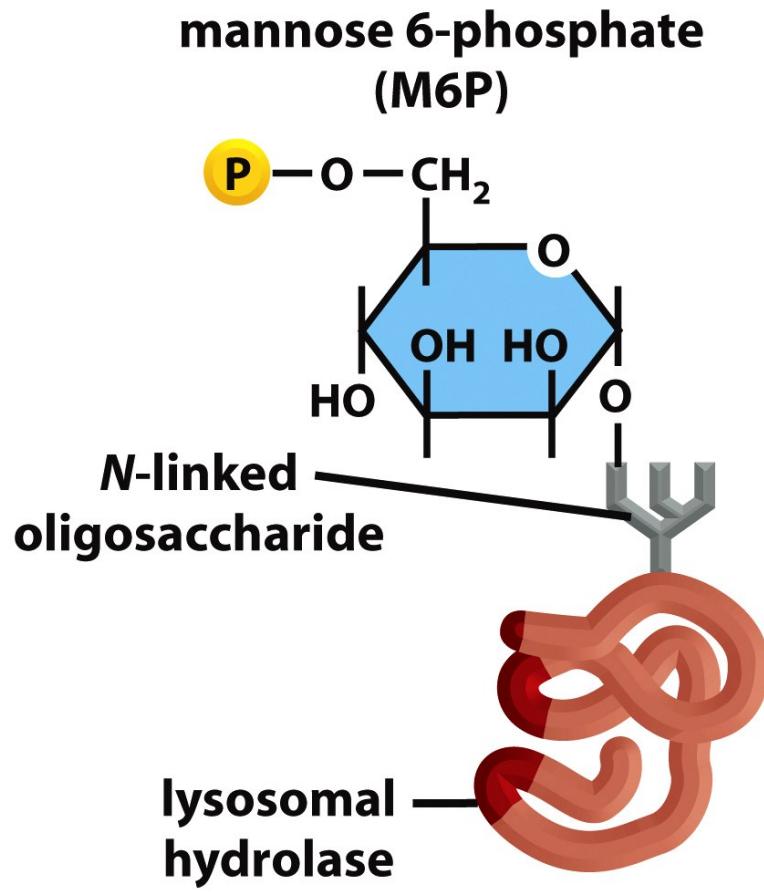
From "Extraordinary Measures"

“What’s the Matter ..., Not Up on Your Glycobiology?”



From “Extraordinary Measures”

Acid Hydrolases are Sorted to Lysosomes from TGN by Endosomes by the Mannose-6-Phosphate (M6P) Signal and M6P Receptor



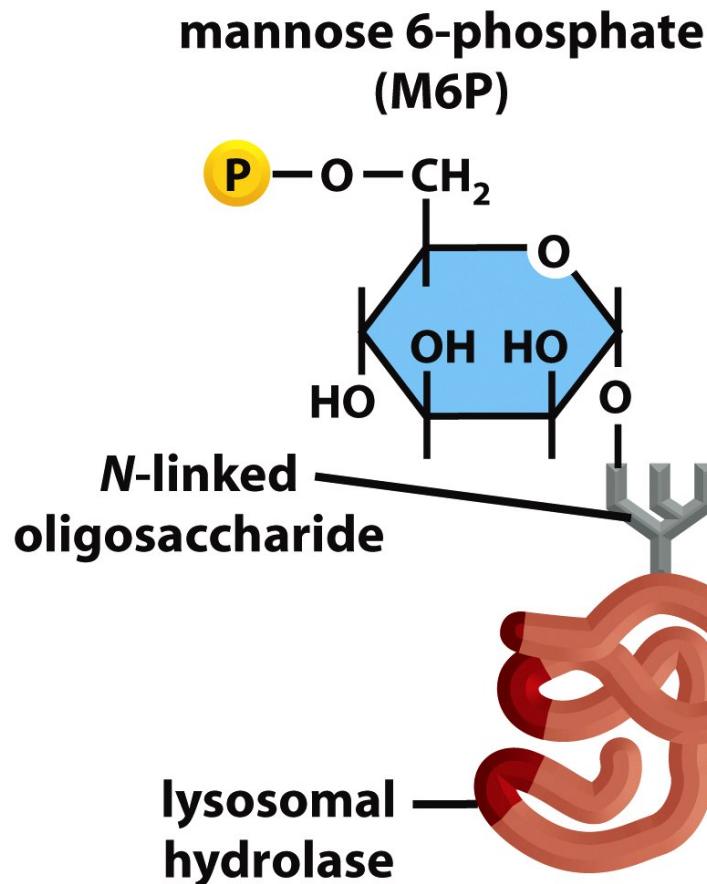
The C6 position of the mannose residue of the N-linked glycan of an acid hydrolase includes a phosphate group attached (M6P). This structure is produced in the cis-Golgi (CGN).

M6P then functions as a sorting signal to allow acid hydrolases to be packed into clathrin coated vesicles at the TGN.

These vesicles then fuse with late endosomes leading to the release of acid hydrolases. These endosomes are subsequently targeted to lysosomes.

Several intriguing questions regarding this transport event...

Why is the M6P tag only added to the lysosomal acid hydrolases?

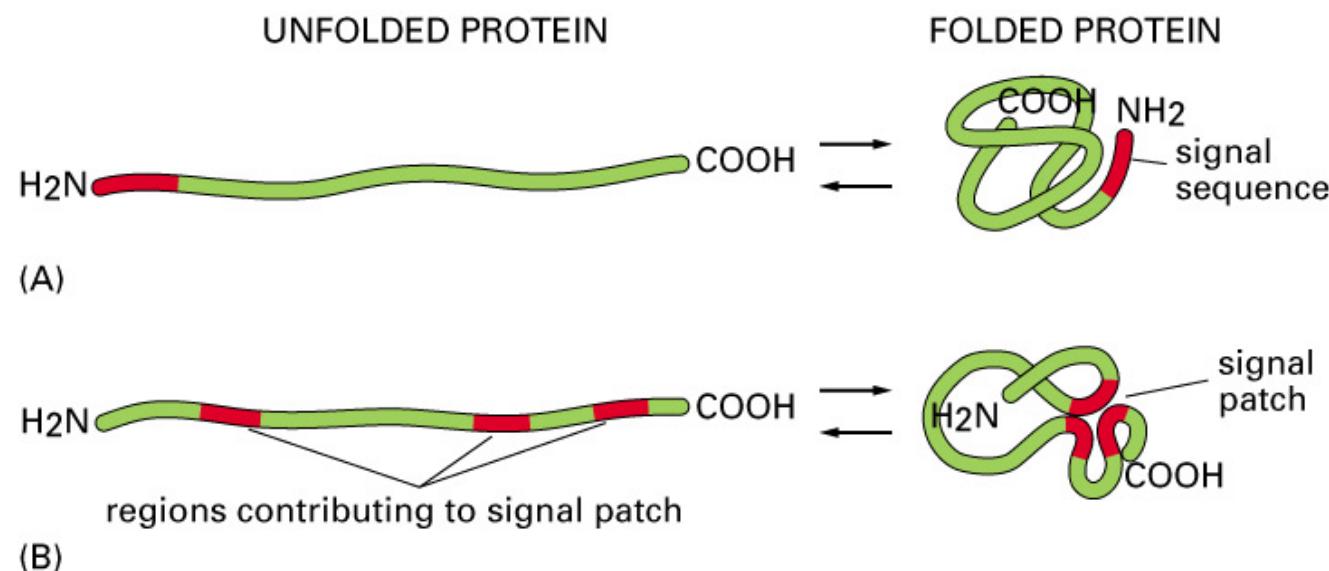


The presence of phosphate on mannose is due to the GlcNAc phosphotransferase at the CGN.

However, many plasma membrane or secreted proteins are also *N*-glycosylated. In other words, they also contain mannose residues.

How does the GlcNAc phosphotransferase distinguish acid hydrolases from other proteins with the same or similar *N*-linked glycosylation?

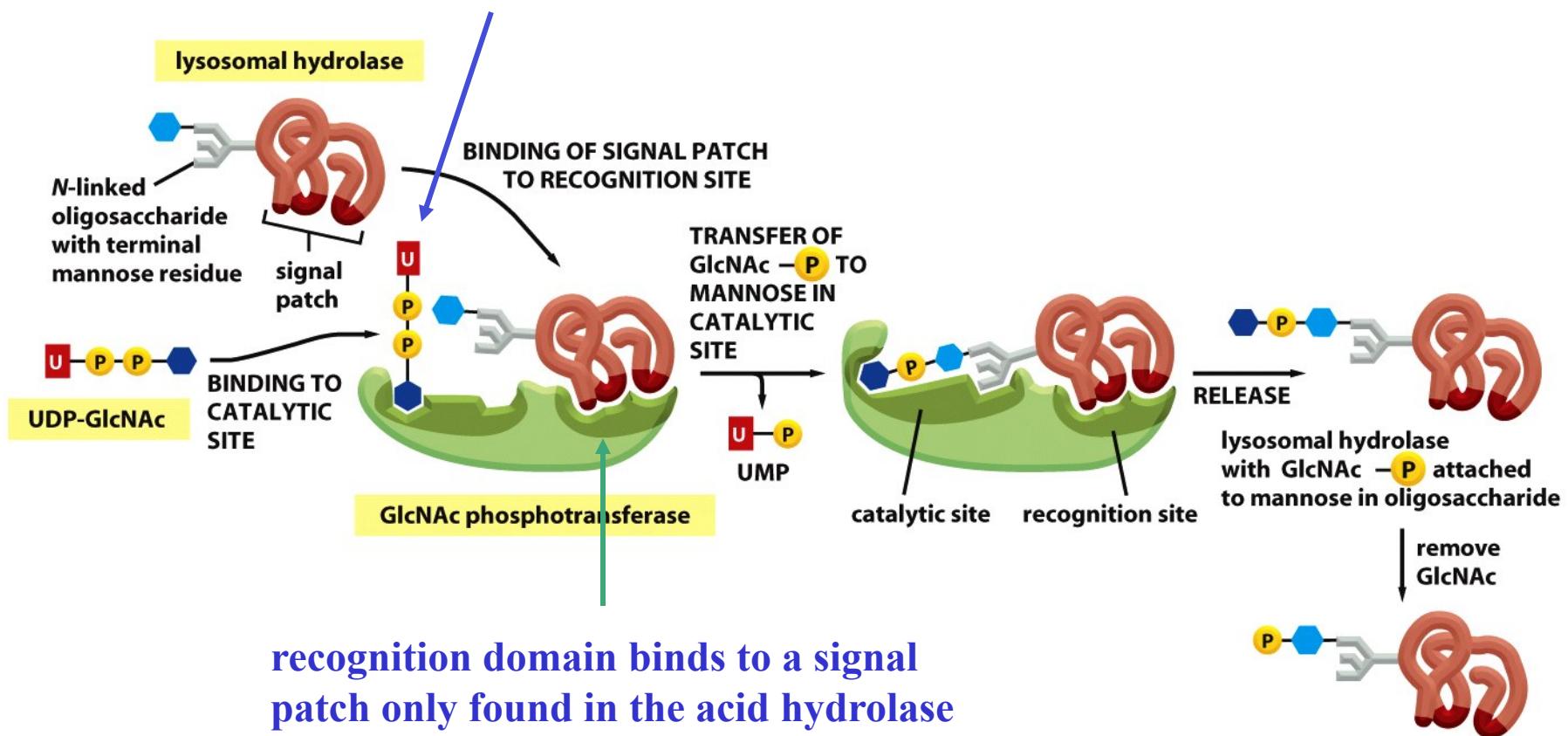
Recall Two Types of Signals: Signal Sequences and Signal Patches



The GlcNAc phosphotransferase first recognizes a signal patch which is present only in acid hydrolases via its recognition domain in the cis-Golgi

The GlcNAc phosphotransferase has two domains: recognition and catalytic

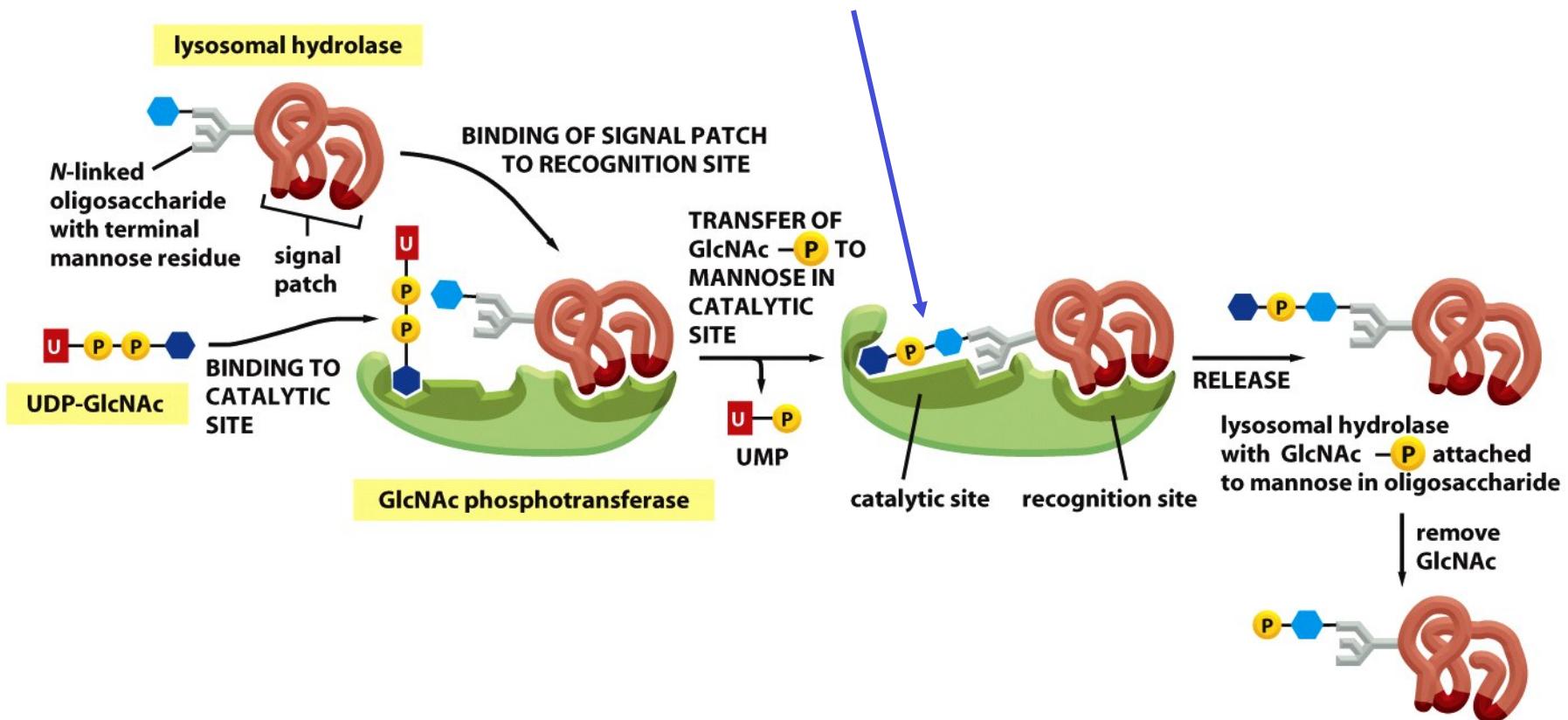
The catalytic domain binds to the donor of the phosphate group



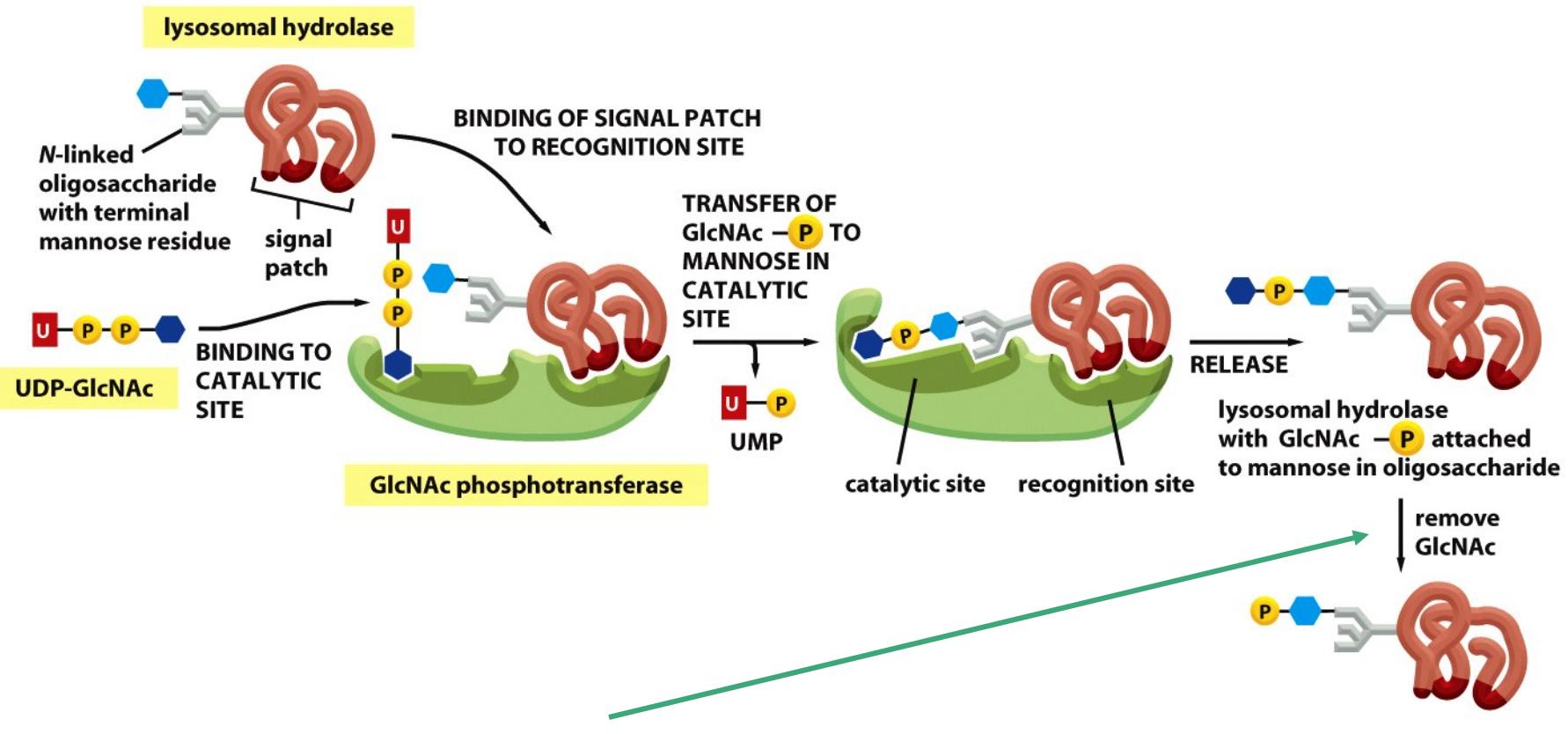
The GlcNAc phosphotransferase then adds GlcNAc-P to the mannose residue of an acid hydrolase

A GlcNAc phosphotransferase has two domains: recognition and catalytic

catalytic domain transfers GlcNAc-phosphate from the UDP-GlcNAc donor to the acid hydrolase



A Second Enzyme Removes the GlcNAc From the Acid Hydrolases at the Trans-Golgi to Leave the M6P

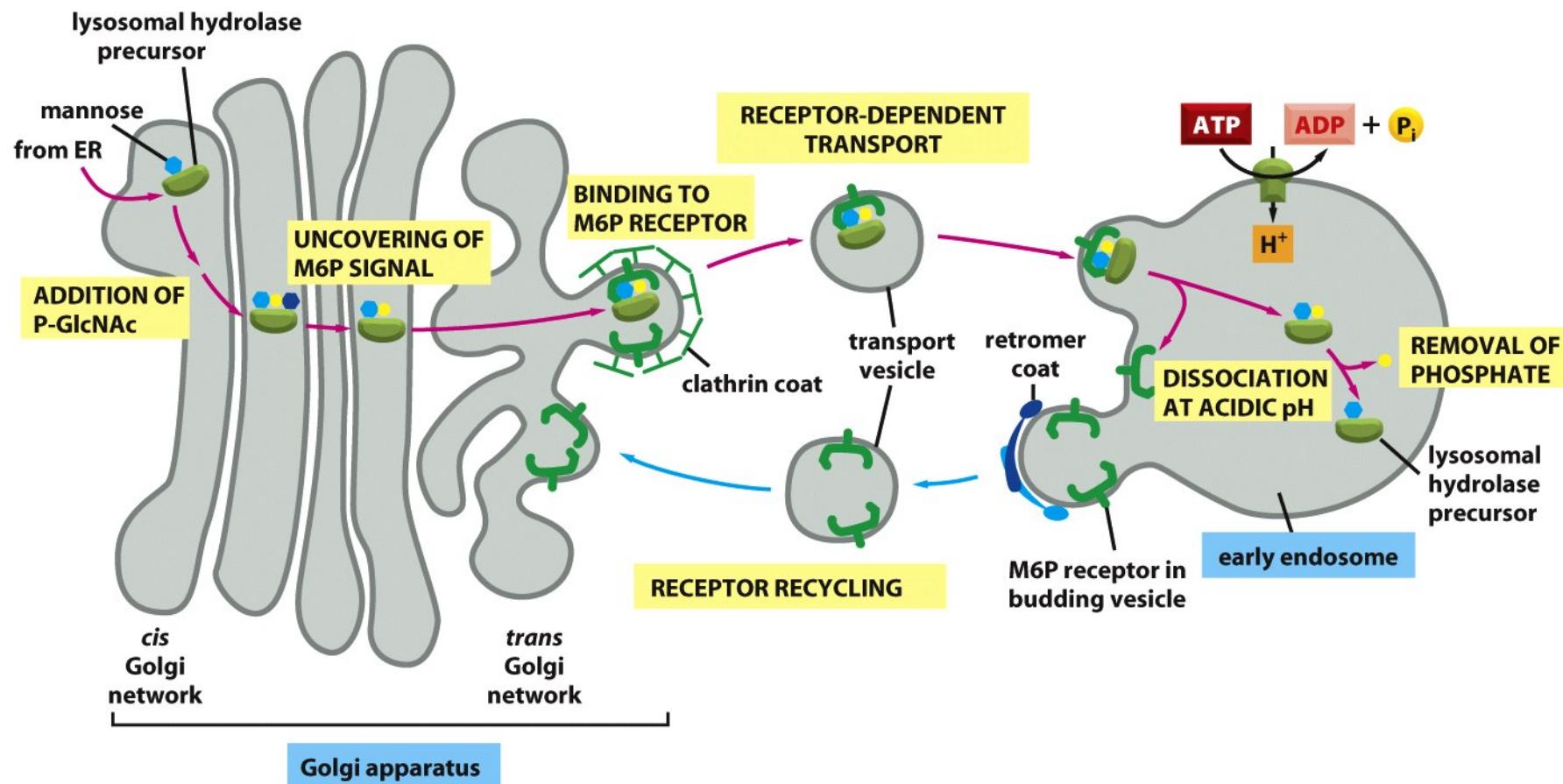


cleavage of GlcNAc and exposure of M6P by a glycosidase

How is M6P recognized by the sorting machinery?

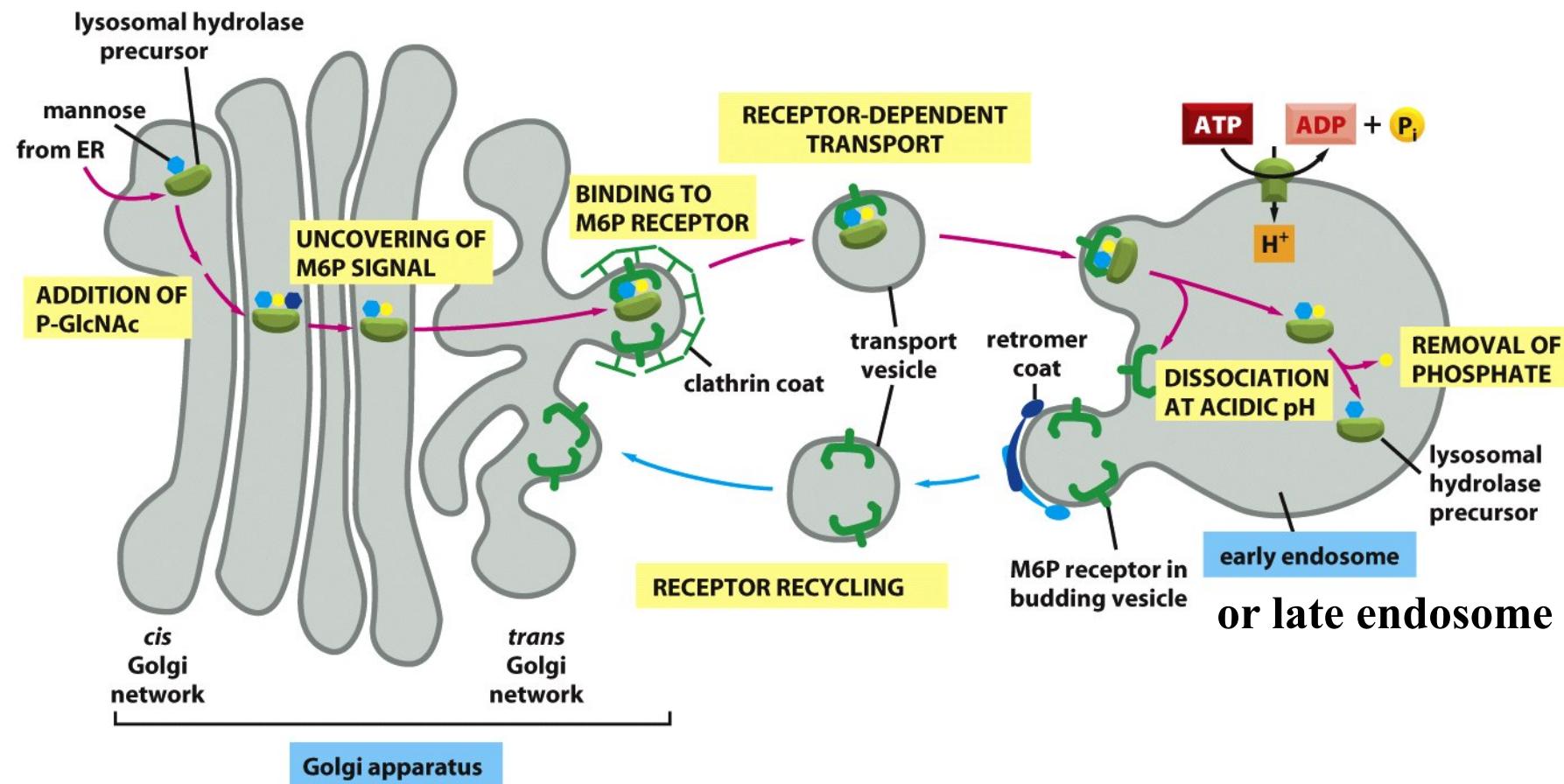
As a luminal cargo, the M6P signal is recognized by the M6P receptor at the TGN

acid hydrolase → M6P receptor → clathrin (textbook)
acid hydrolase → M6P receptor → GGA coat (a new coat)



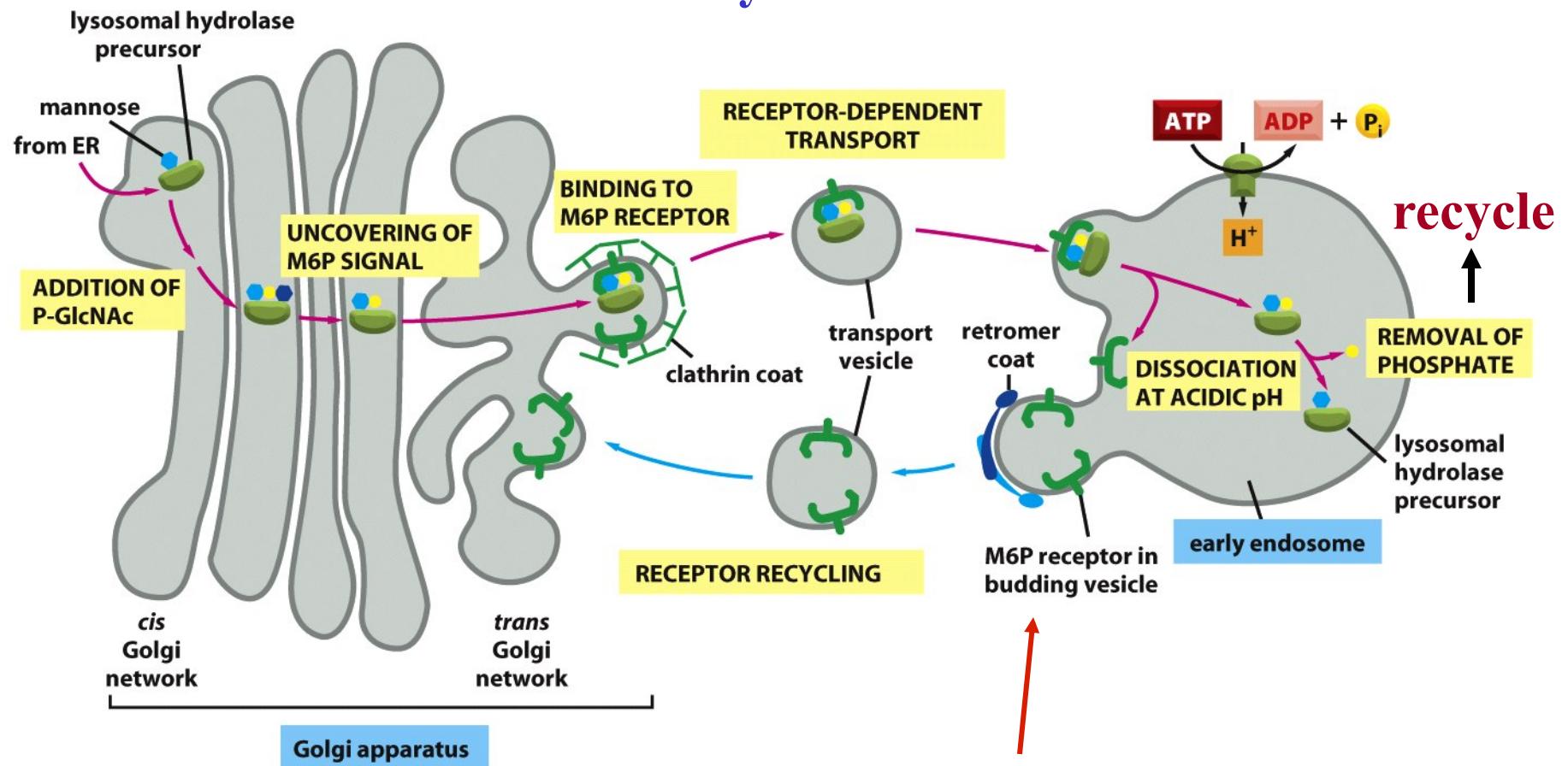
How does M6P receptor release its cargo in the endosome & recycle to the TGN?

Some endosomes have a lower pH compared to the TGN, which causes dissociation between M6P and M6P receptor.



=> pH-Dependent Sorting

How to Recycle the M6PR ?



A retromer coat protein interacts with free M6P receptors at the endosomes and brings them back to TGN for re-use.

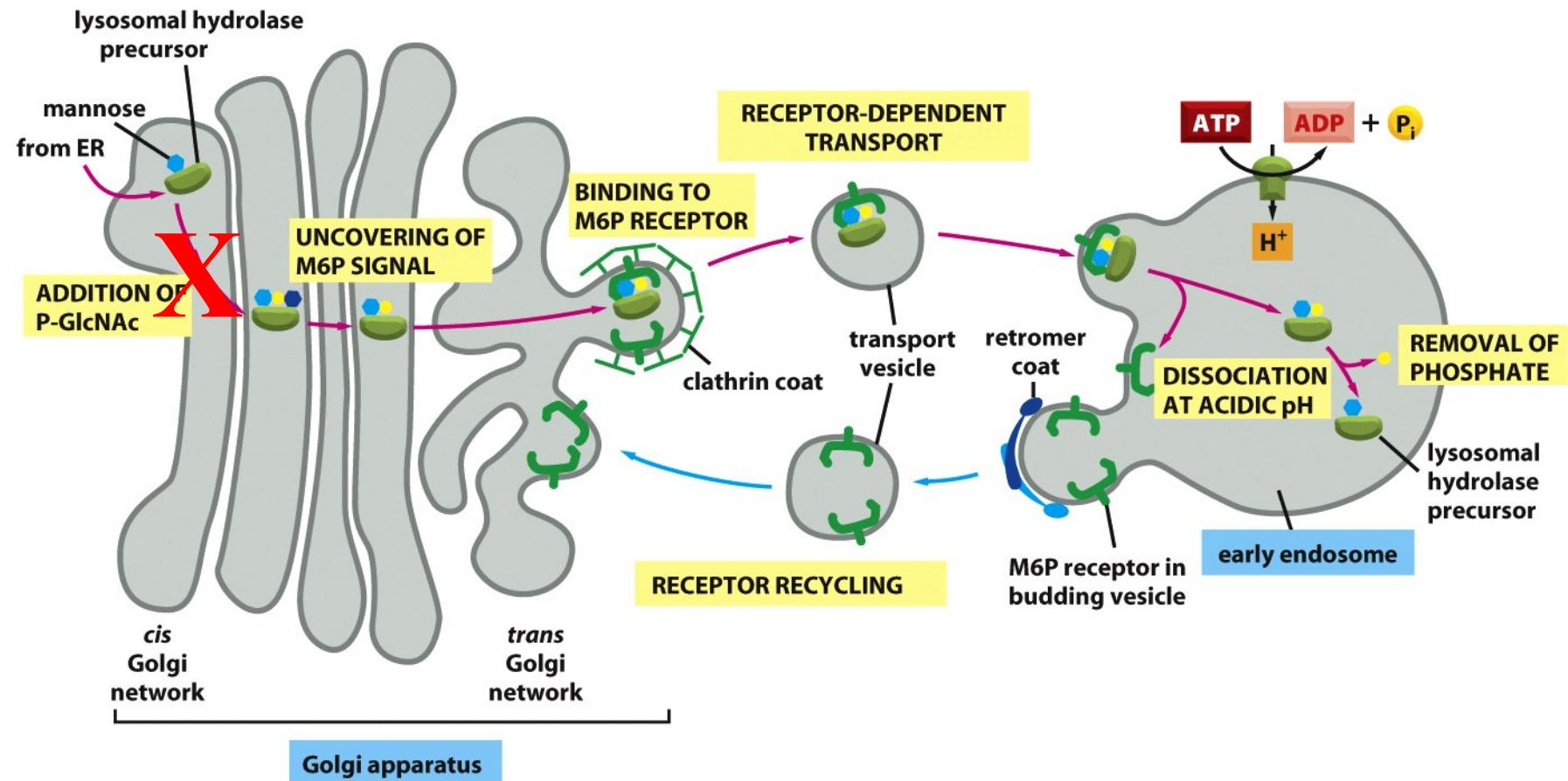
=> Recycling of transport machinery

How to Capture Escaped Acid Hydrolases?

A fraction of M6PR escapes to the plasma membrane and is retrieved via another type of M6PR that can bind to M6P at neutral pH.

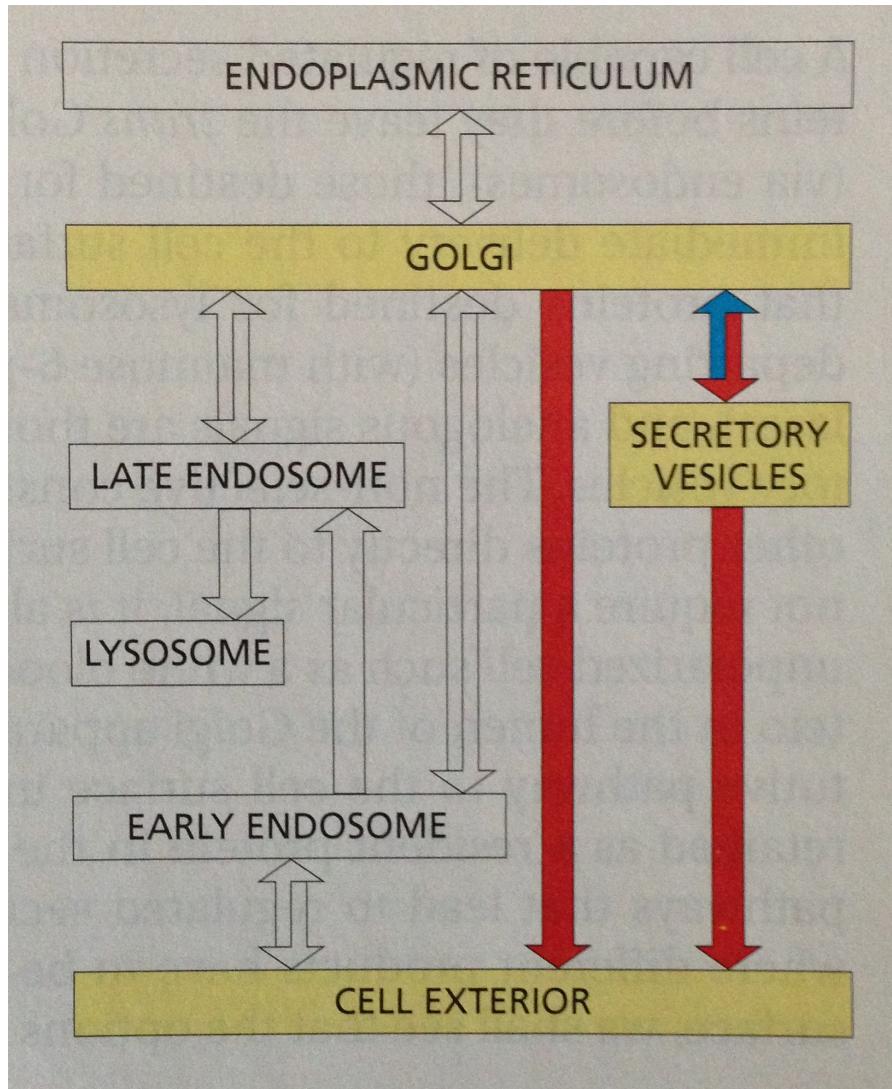
Multiple mechanisms assure the specificity and/or efficiency of transport

Inclusion Cell Disease: A recessive genetic disease resulting in the loss of GlcNAc phosphotransferase activity



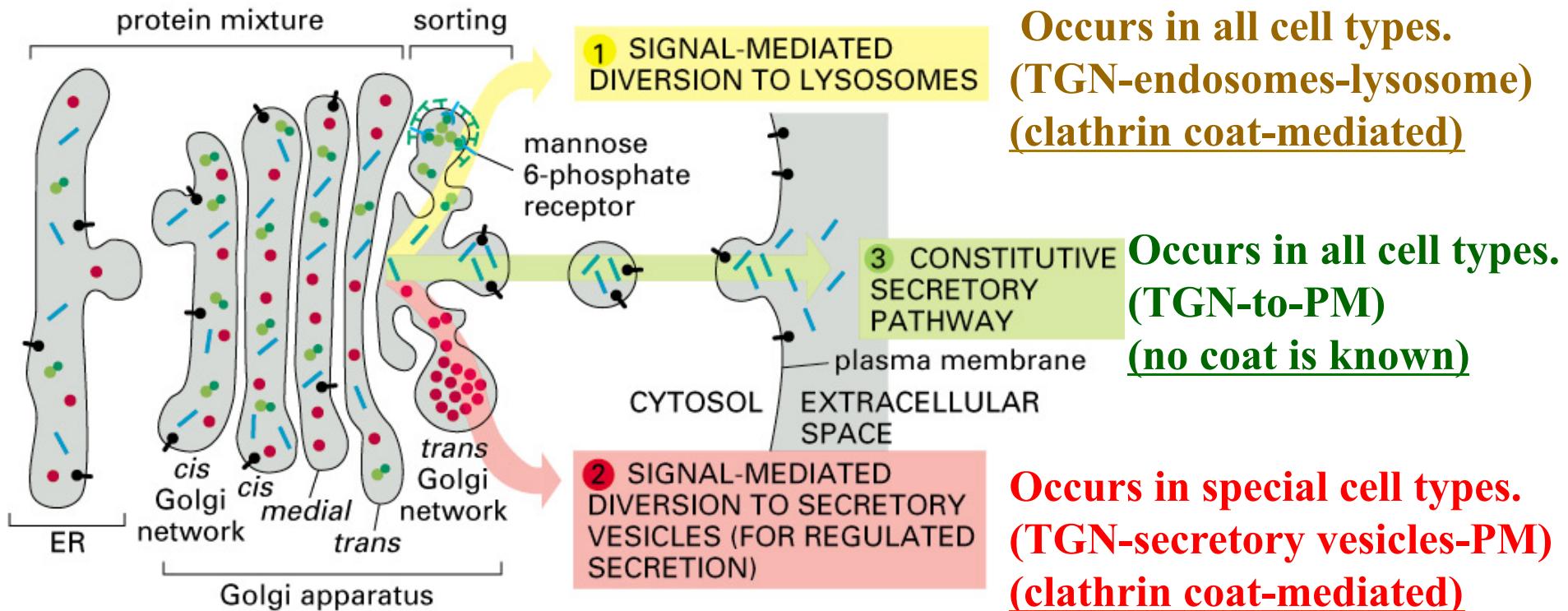
What would happen to the acid hydrolases in I-cell patients?

Transport from the Golgi to Secretory Vesicles and the Plasma Membrane/Cell Exterior



The TGN is the Major Sorting Station in Biosynthetic Pathways

There are three major sorting pathways at the TGN



Occurs in all cell types.
(TGN-endosomes-lysosome)
(clathrin coat-mediated)

Occurs in all cell types.
(TGN-to-PM)
(no coat is known)

Occurs in special cell types.
(TGN-secretory vesicles-PM)
(clathrin coat-mediated)

New coats may be involved in the above transport pathways.

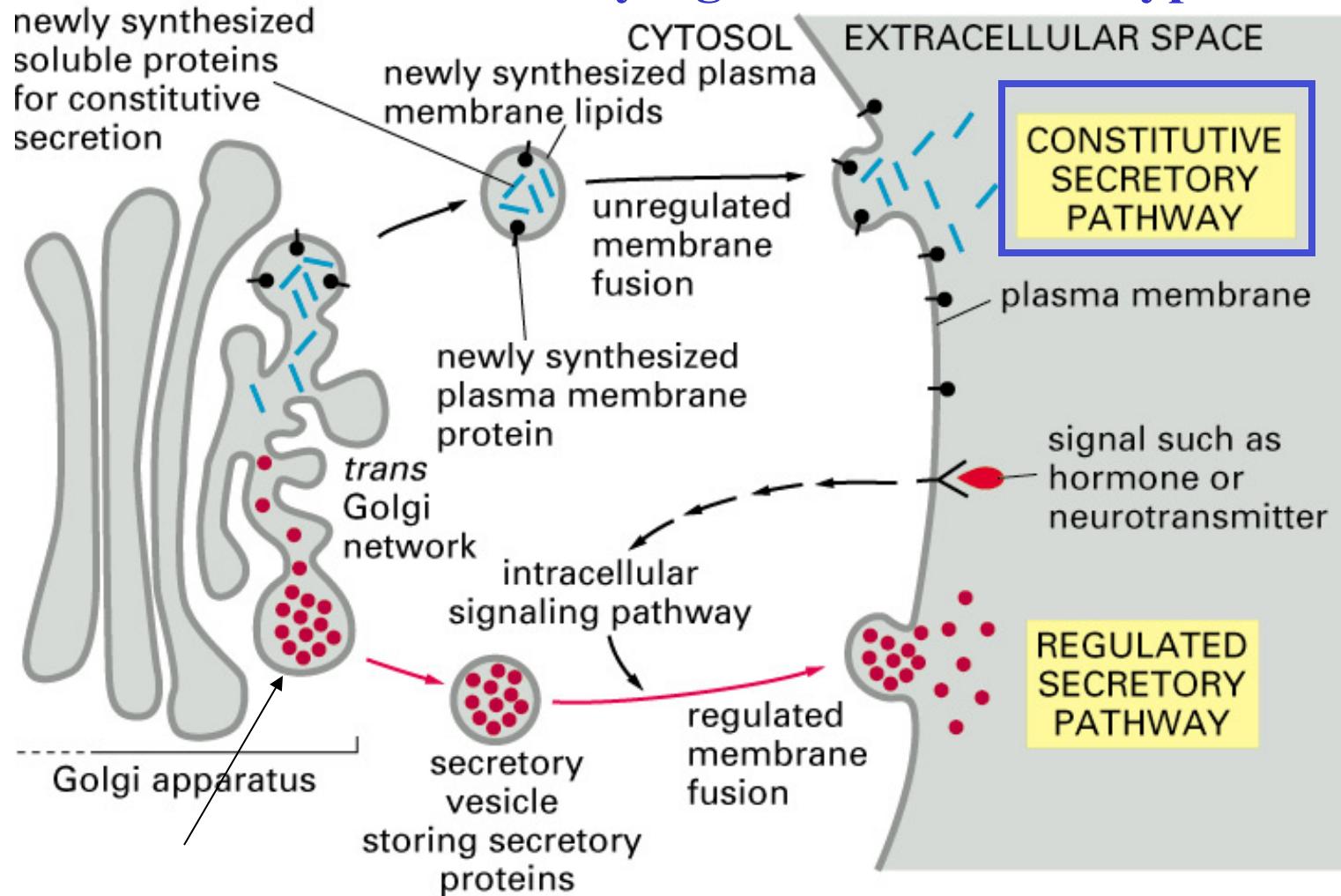
Recent studies also show a possible role of the *trans*-Golgi cisterna in sorting.

TGN-to-Plasma Membrane Transport

“Constitutive” pathway

Regulatory pathway

Constitutive secretion is generally thought to be a default pathway and not mediated by signals in most cell types



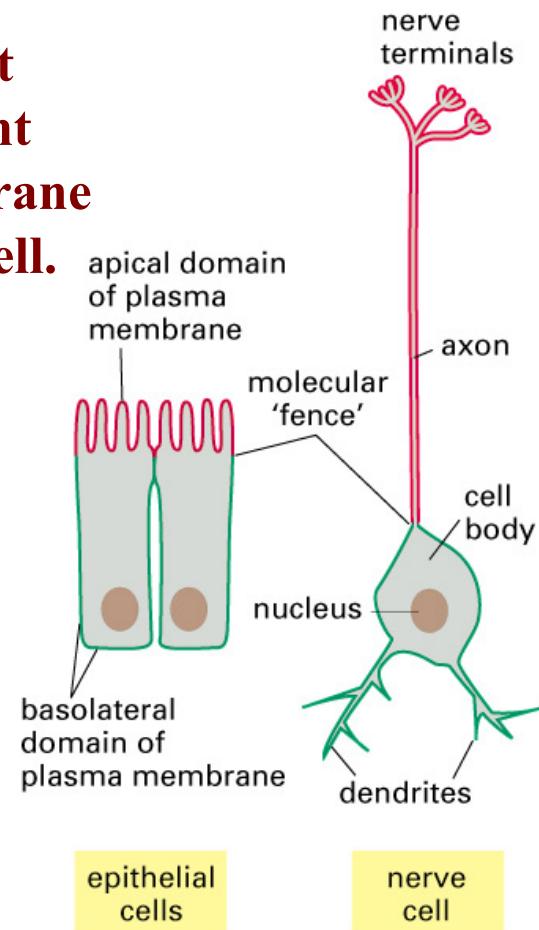
However, this pathway is regulated in polarized cells such as epithelia cells or neurons.

Intracellular Trafficking & Cell Polarity

A polarized cell (e.g. a neuron or epithelial cell) has two membrane domains, which are structurally and functionally asymmetric.

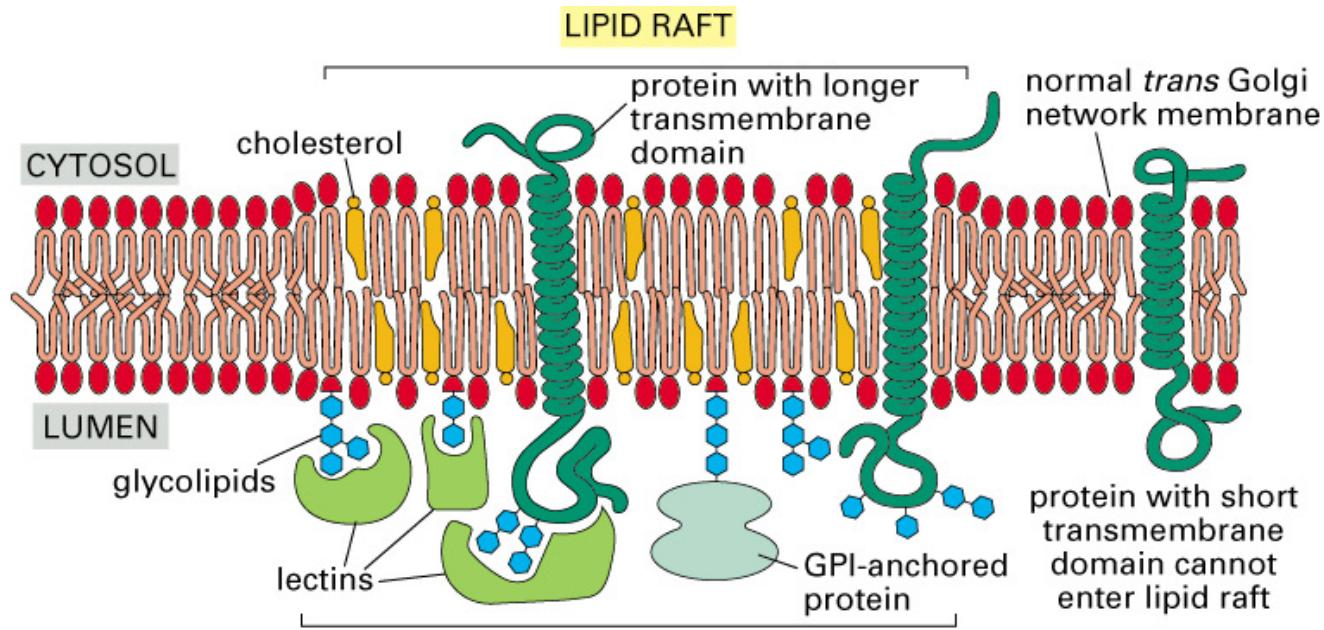
Since these two membrane domains have different protein compositions, there must be signals present to allow the selective targeting of a plasma membrane protein into different membranes in a polarized cell.

apical ←→ axon
basolateral ←→ dendrite



Polarized trafficking is an important mechanism for creating cell polarity.

Polarized TGN-to-Plasma Membrane Transport

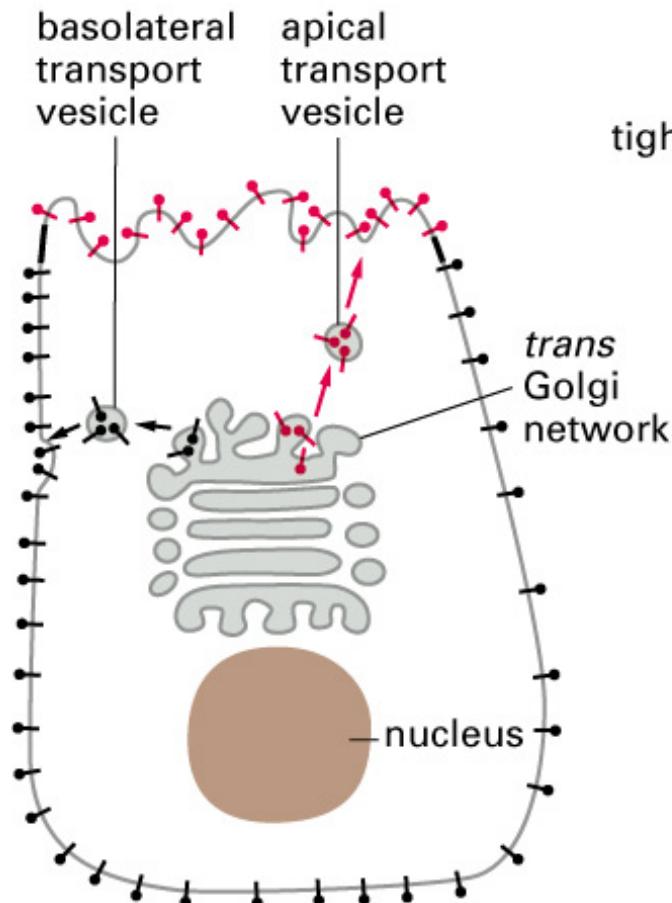


Lipid rafts are assembled at the Golgi and are selectively targeted from TGN to the apical domain of PM (GPI can serve as an apical-targeting signal).
Recent studies suggest that the assembly of lipid rafts may begin at the ER.

Lipid rafts remain the only characterized apical-targeting signal.

In comparison, several basolateral-targeting signals composed of short a.a. stretches have been identified.

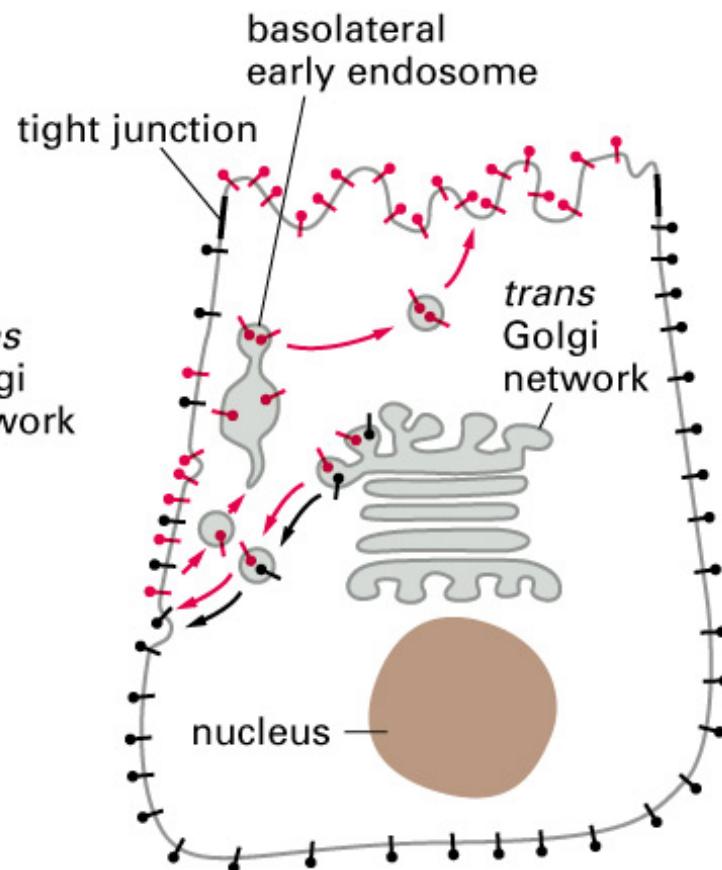
Multiple ways to generate polarity by polarized transport



(A) DIRECT SORTING OF
MEMBRANE PROTEINS IN
THE TRANS GOLGI NETWORK

signals in TGN-to-PM transport

signals in internalization or recycling



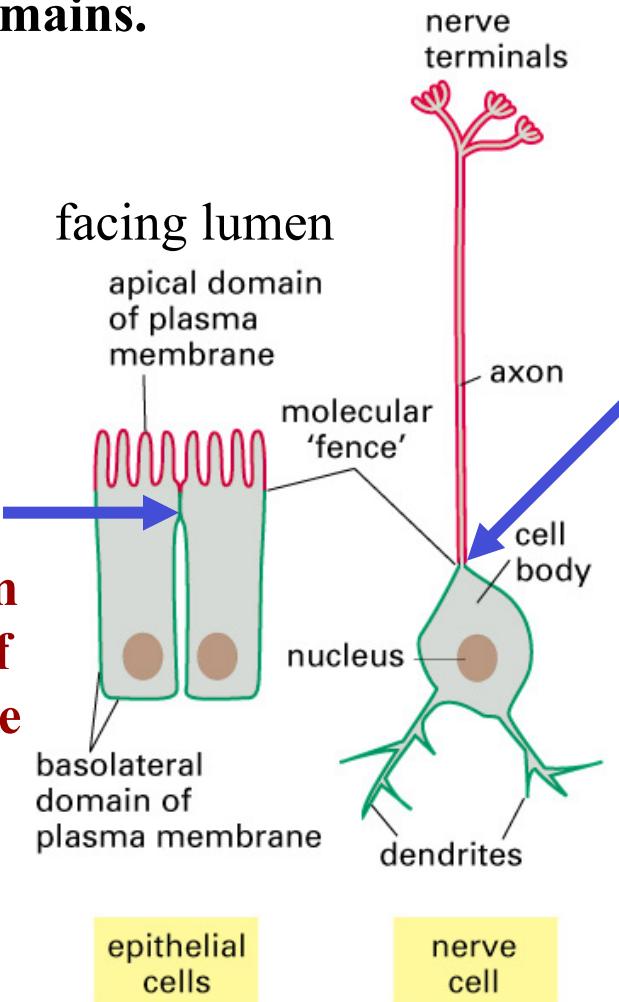
(B) INDIRECT SORTING VIA ENDOSOMES

another possibility: signals in fusion at the PM

In addition to the polarized trafficking, molecular fences are important for maintaining the polarity of the plasma membrane

Molecular fences are structures consisting of tightly associated membrane proteins and the actin cytoskeleton and they prevent the mixing of plasma membrane proteins between two domains.

tight junction
A structure seals cells together in an epithelium to prevent the leakage of small molecules from one side to the other



axonal hillock

This structure separates the cell body of a neuron from its axon. It is where an action potential originates. It also functions as a permeability barrier between the cell body and the axon.

TGN-to-Plasma Membrane Transport

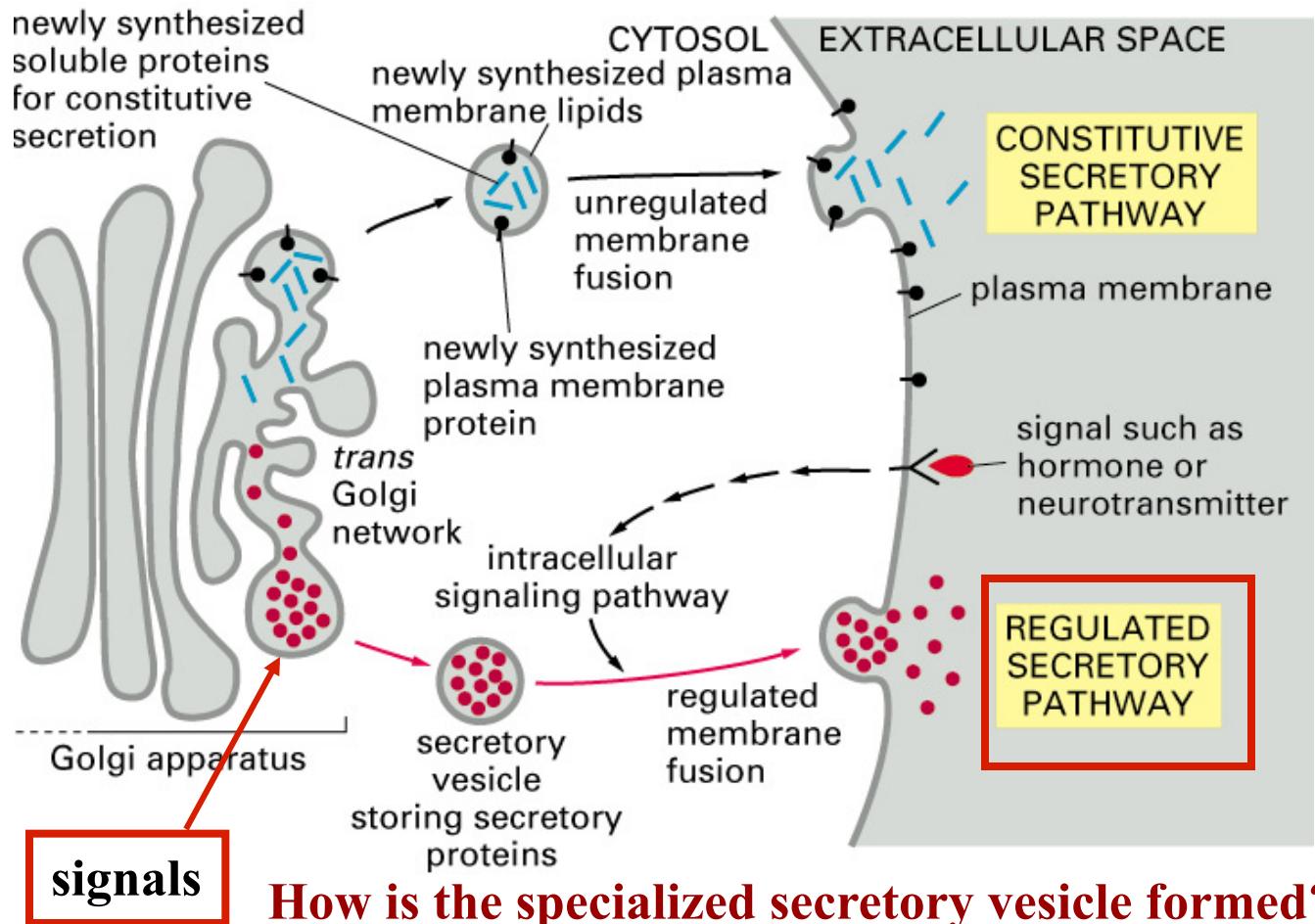
“Constitutive” pathway

Regulatory pathway

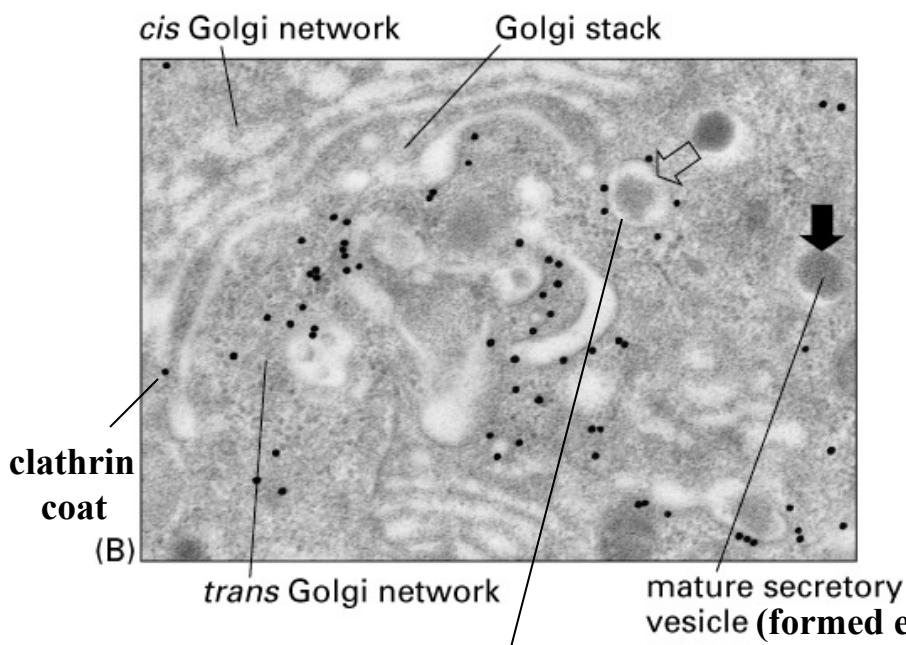
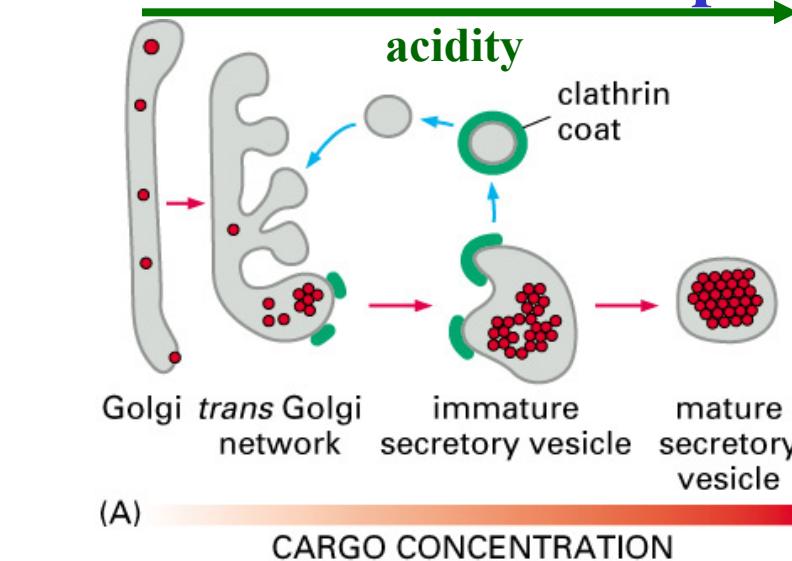
Regulated TGN-to-PM Transport

Specific secreted proteins are packed, concentrated, and stored in specialized secretory vesicles.

Secretion is controlled by a physiological stimulus such as a neural (e.g. an action potential) or hormonal (e.g. insulin) signal.



Formation of Specialized Secretory Vesicles



immature secretory vesicle (formed more recently, w/ coat, less condensed)

Insulin-secreting β cell in the pancreas

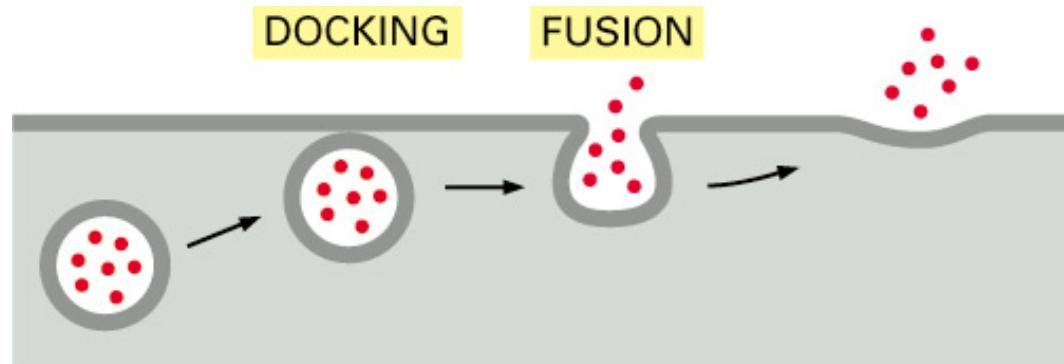
Specialized secretory vesicles are formed from the TGN in many cases, and these immature vesicles gradually mature while the vesicles become more acidic. At the same time, the cargo also becomes more concentrated.

Two concentrating mechanisms:

- (A) signal-mediated formation of aggregates in TGN, which is enhanced by acidification as vesicles mature (unknown signal)
- (B) retrieval of membrane and luminal contents as vesicles mature

Docking/fusion as a critical control switch during the regulated TGN-to-PM secretion

physiological signals



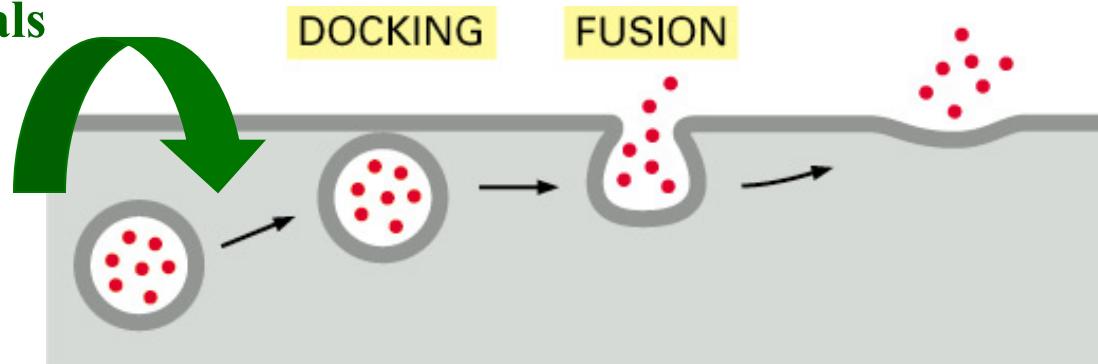
What might be the advantage for a cell
to control the regulated secretion at the docking/fusion step?

0.2 μm

What might be the advantage for a cell to control the regulated secretion at the docking/fusion step?

physiological signals

rapid



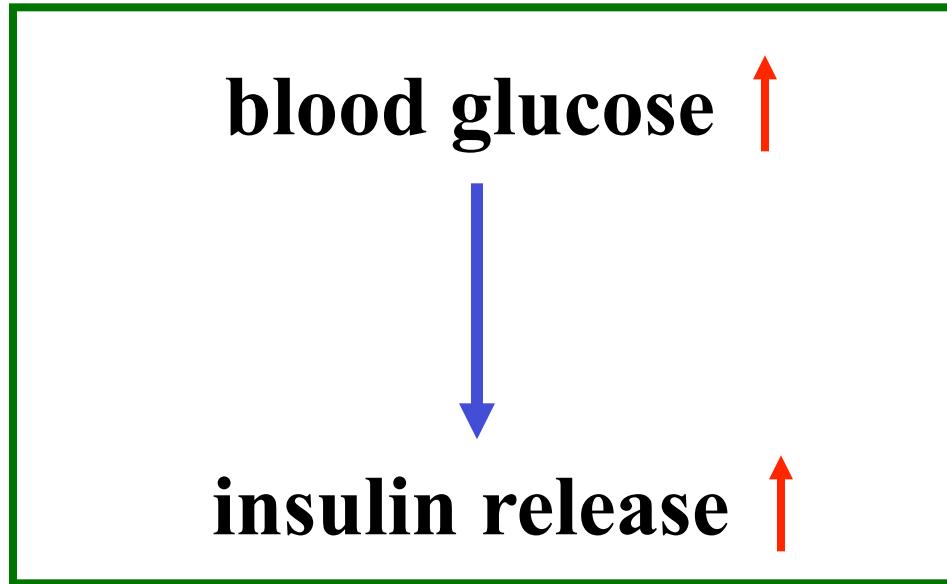
large amount



0.2 μm

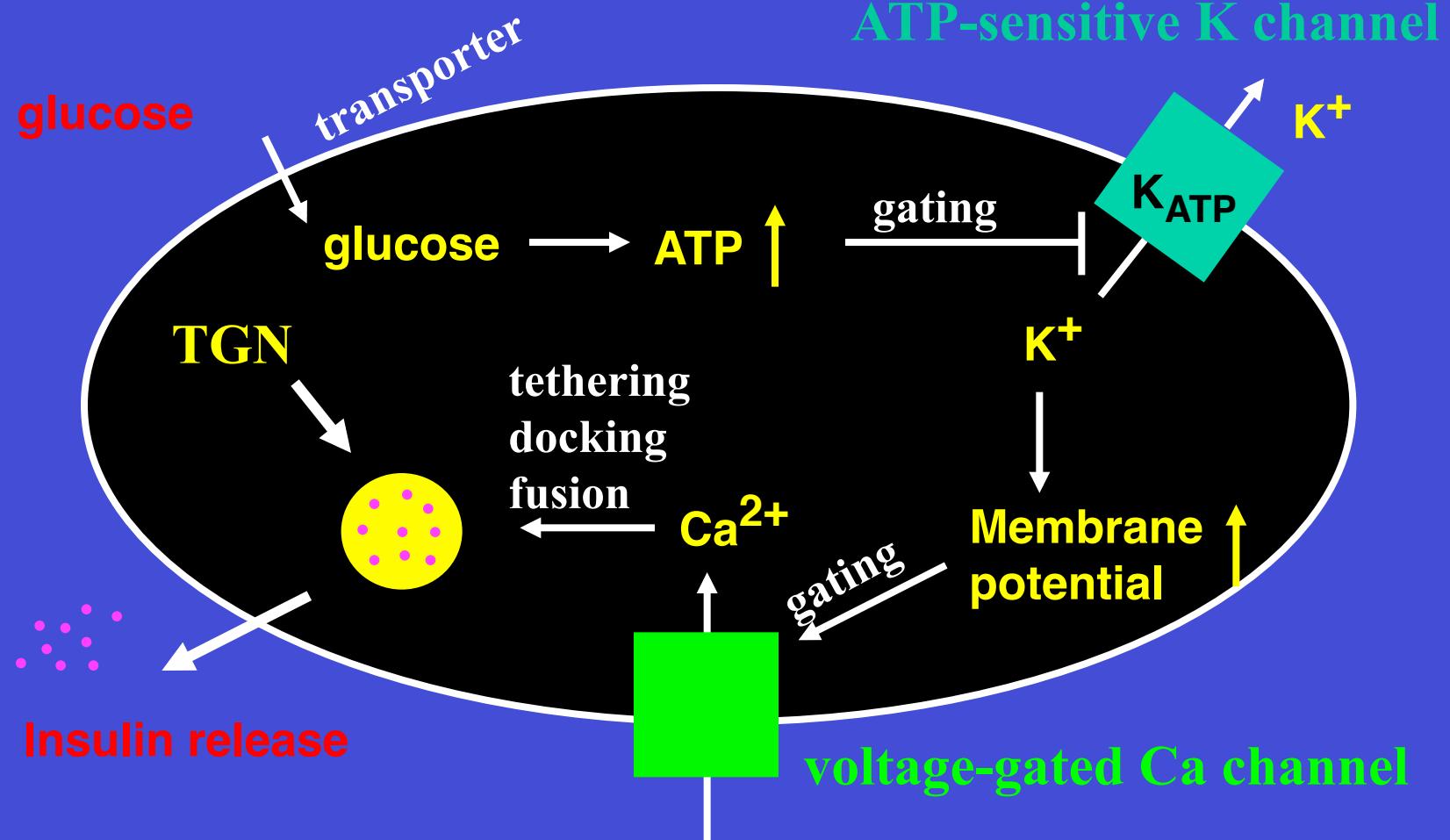
How does a physiological signal regulate the docking/fusion of specialized secretory vesicles?

one example: insulin release in response to increased blood sugar



(pancreatic β -cells)

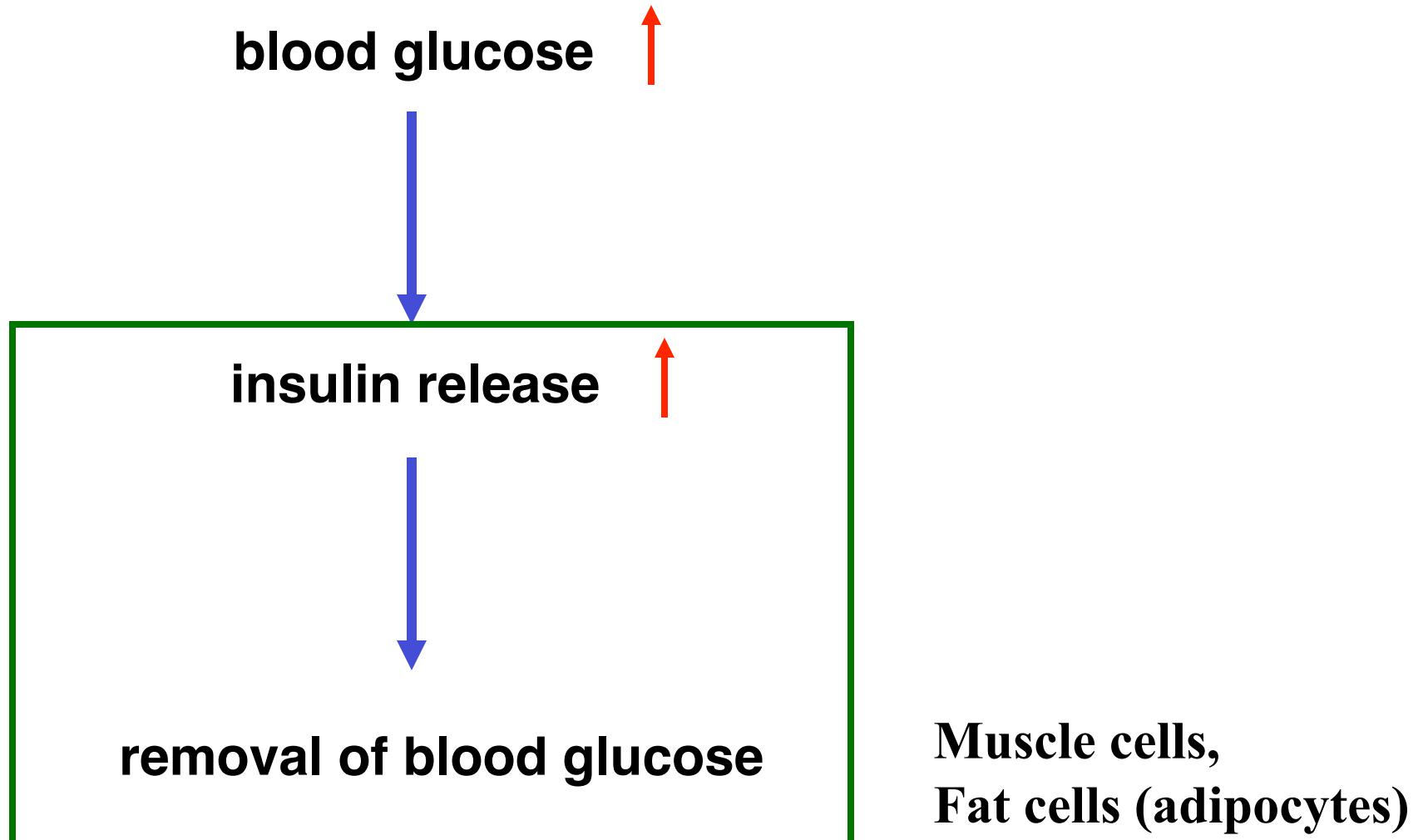
How to link blood glucose level to insulin secretion?



Increased intracellular Ca²⁺ conc. promotes docking/fusion
(mainly SNARE-mediated fusion)

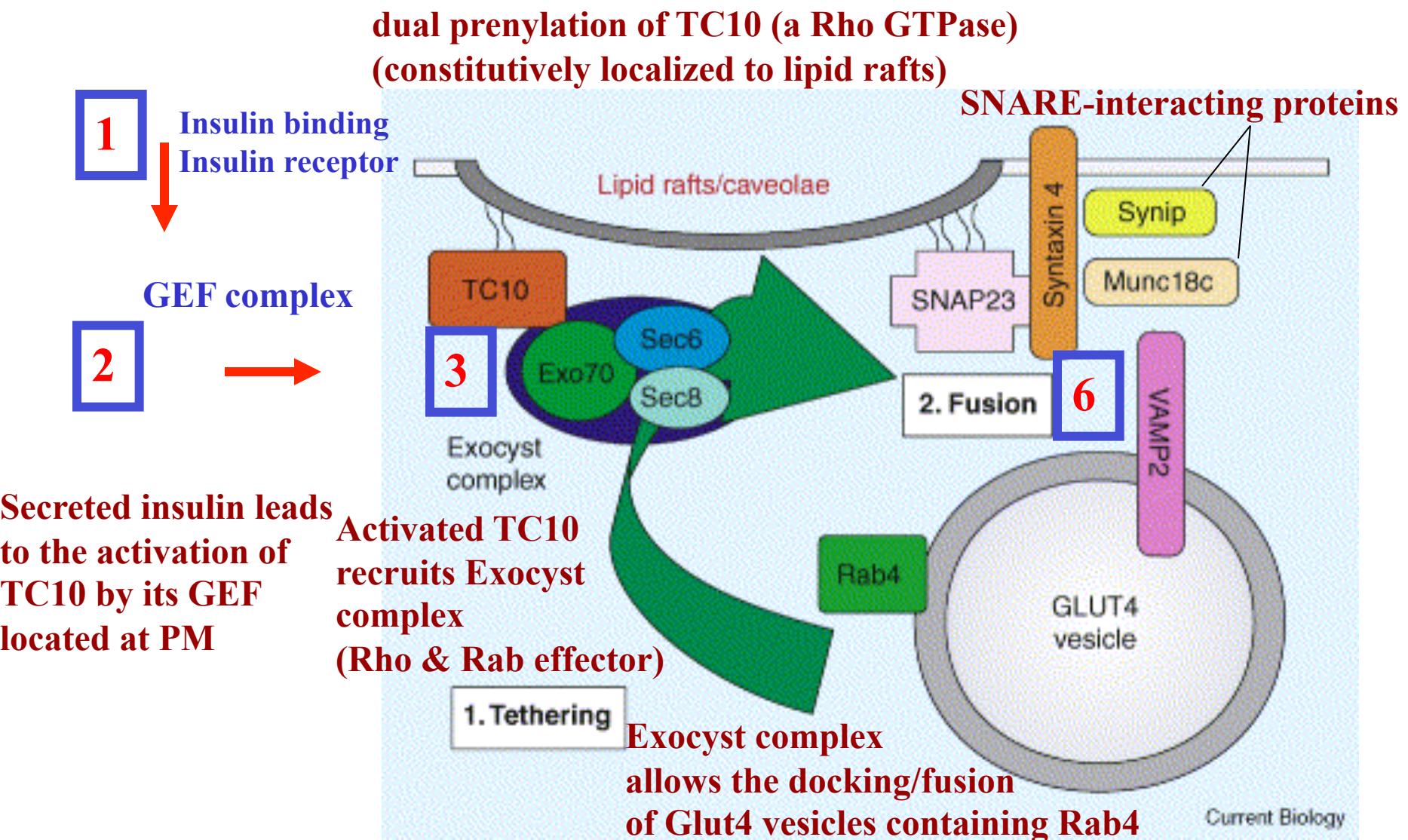
How does a physiological signal regulate the docking/fusion of specialized secretory vesicles?

another example: released insulin to remove increased blood sugar



Adipocytes and Muscle Cells Remove Blood Sugar by Insulin-Induced Trafficking of Glucose Transporters to the PM

Glut4 is a glucose transporter cargo that traffics between secretory vesicles & PM



Allergic Reactions: Another Example of Regulated Secretion

Exocytosis of Histamine in Mast Cells Triggered by a Soluble Extracellular Stimulant

