Endomembránový systém.
exocytóza a endocytóza

Transport membrán -Membrane Traffic

How do proteins and lipids move from one compartment to another?

What are the signals that target molecules to their appropriate subcellular location?

How do organelles maintain their identity with the constant flux of lipid and protein? How are resident proteins of an organelle retained there?

What is the molecular basis of vesicular transport?

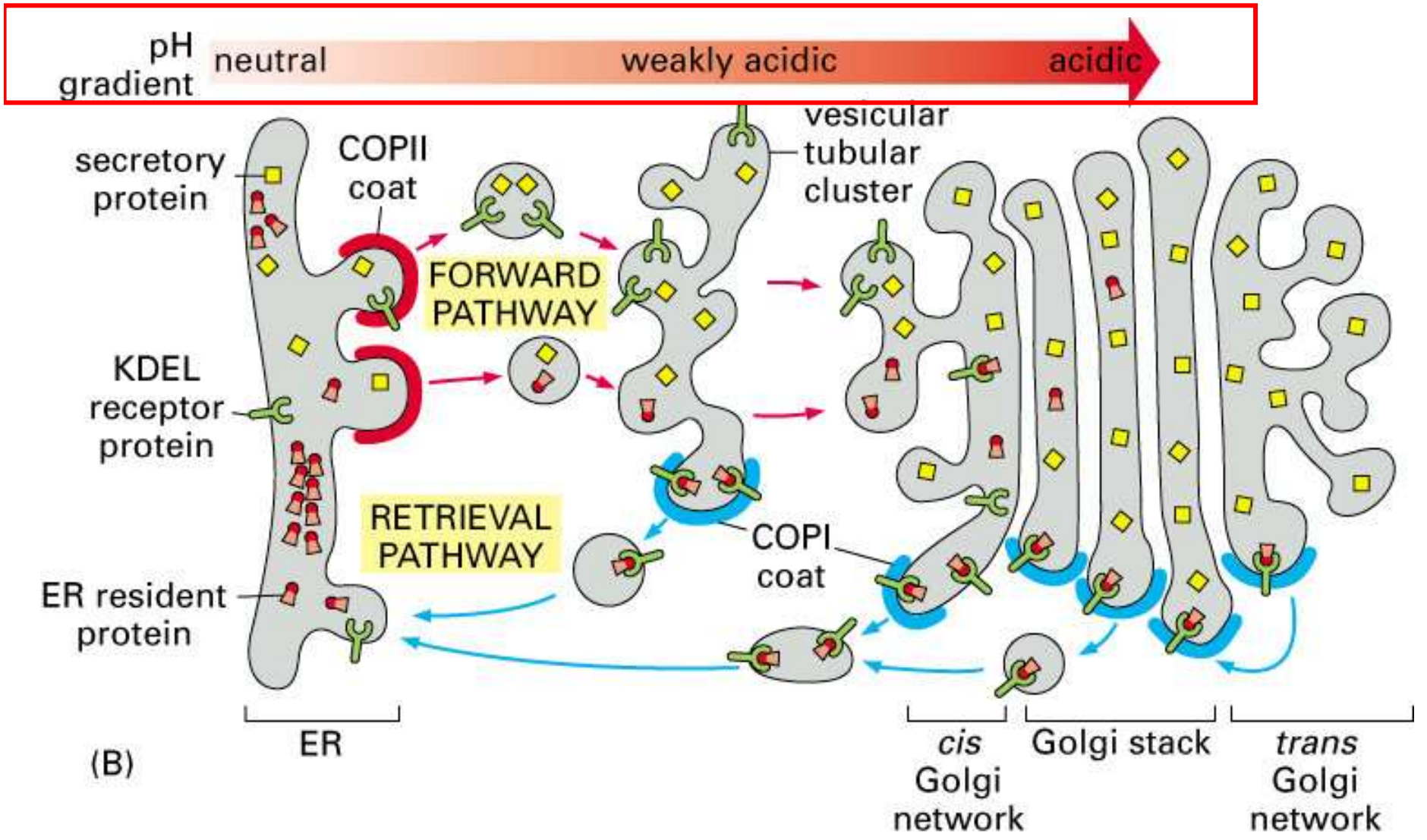


Figure 13–21 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

Early Endos. u rostlin!!

Gradient okyselování sekreční dráhy je klíčový pro její funkci!
Citlivý k monensinu.

The Golgi Apparatus

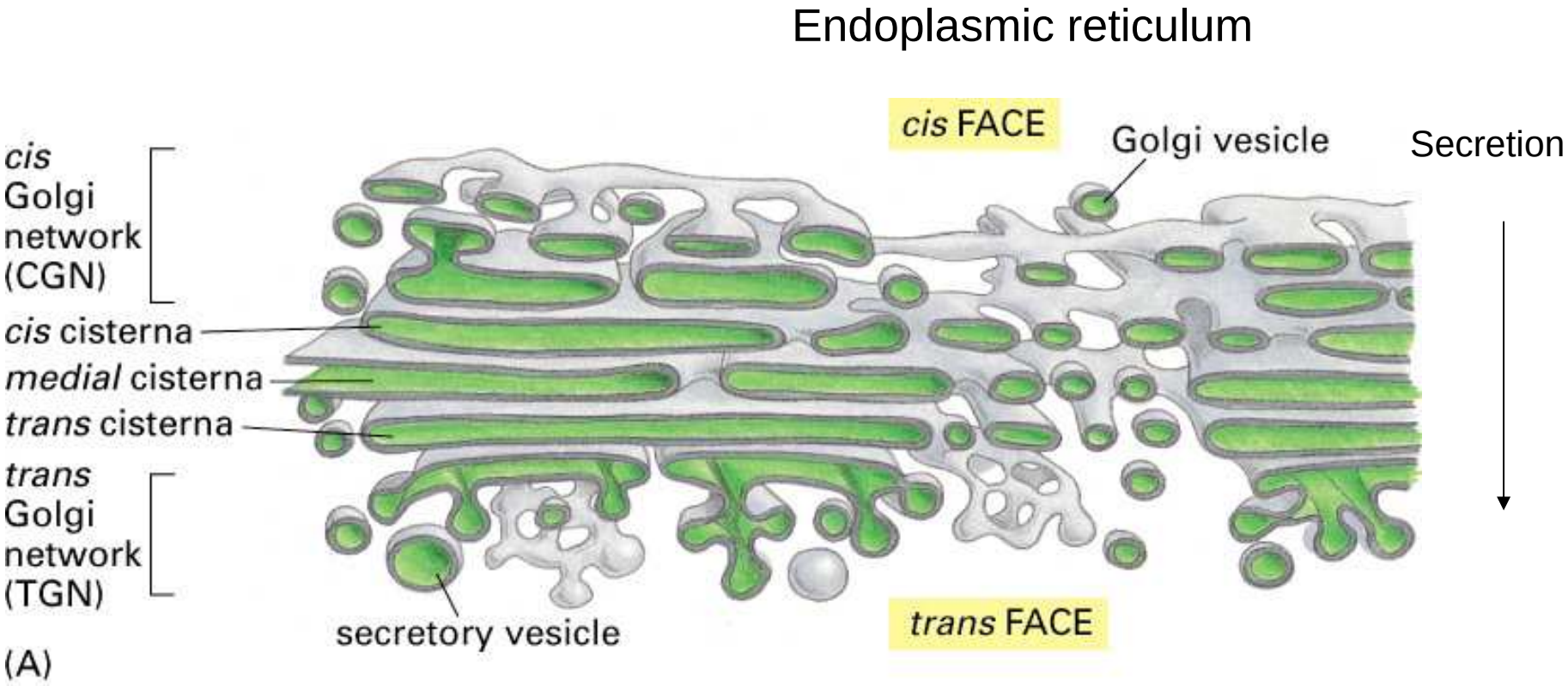
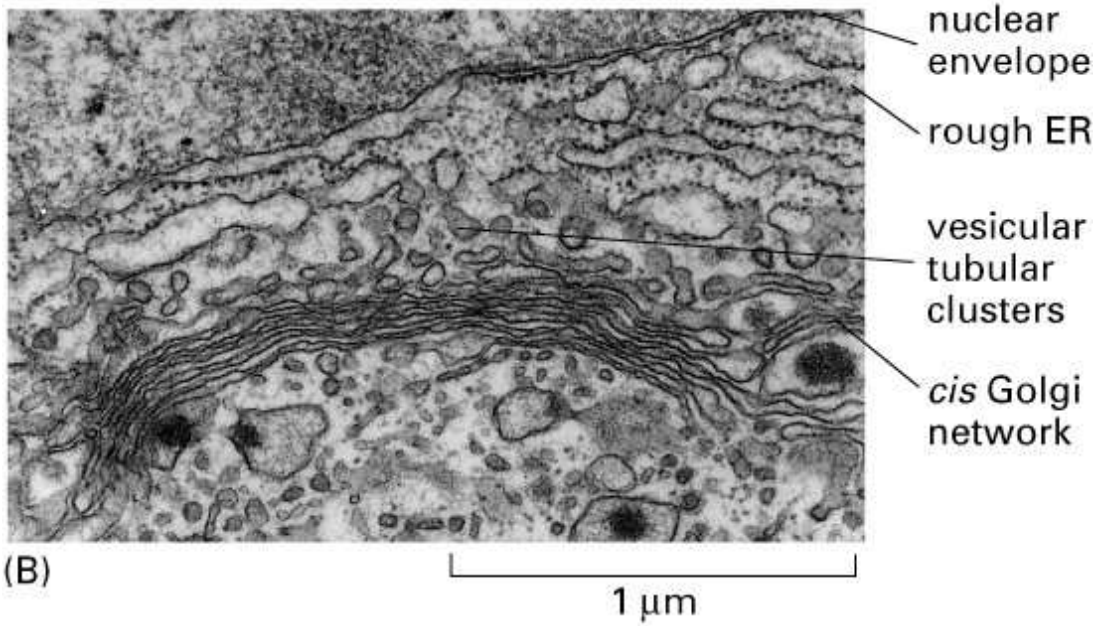
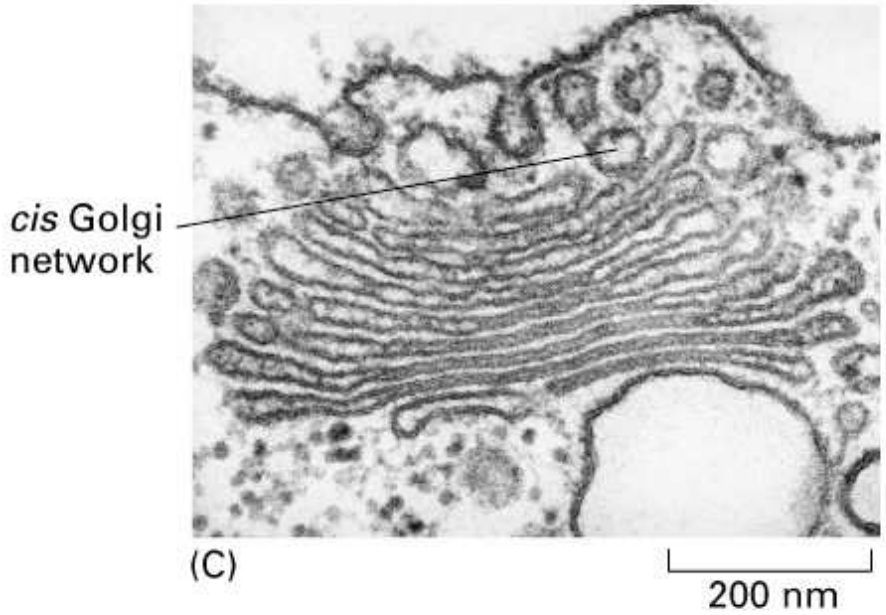


Figure 13–22 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

3-dimensional reconstruction from electron micrographs of the Golgi apparatus in a secretory cell.



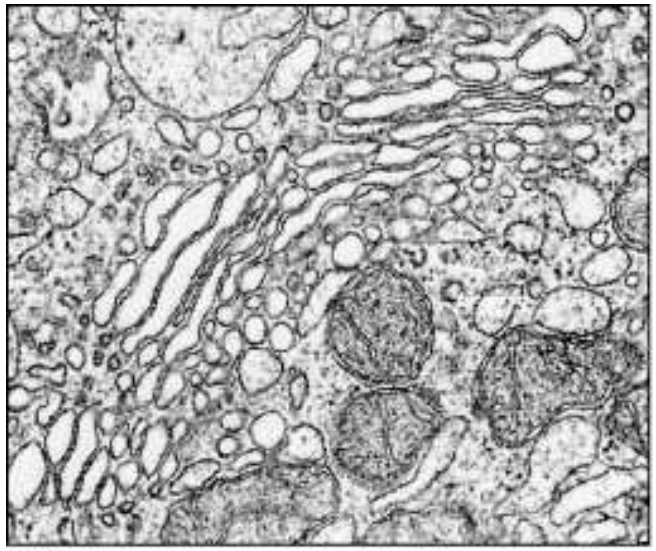
(B) Transitional zone between ER and Golgi in an animal cell



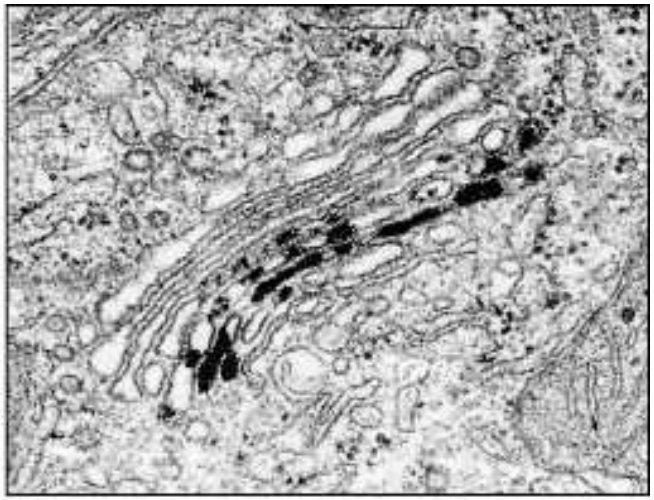
(C) Golgi apparatus in a plant cell

Figure 13–22 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

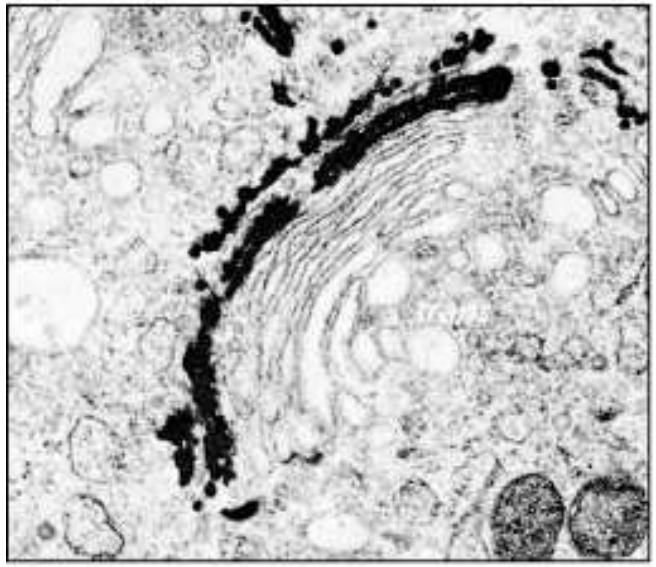
Biochemical Compartmentation Of the Golgi



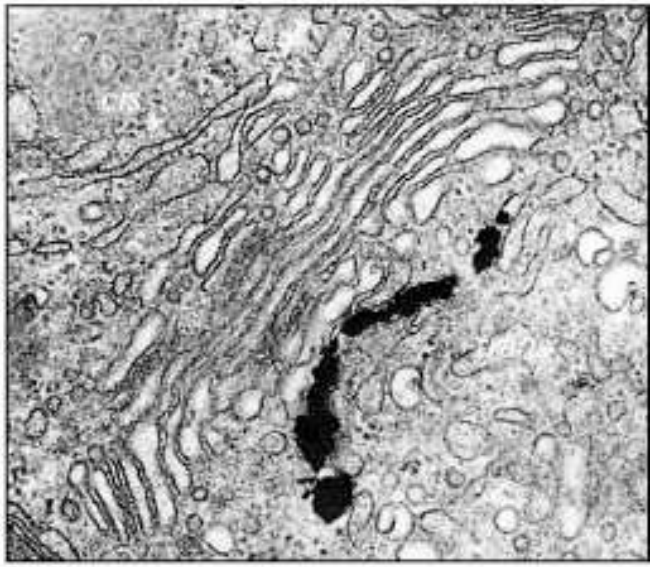
(A) unstained



(C) Nucleoside diphosphatase activity = trans Golgi cisternae



(B)
Stained w/ osmium -cisGolgi cisternae



(D)

1 μm

Figure 13–28. Molecular Biology of the Cell, 4th Edition.

Acid phosphatase activity
= trans Golgi network

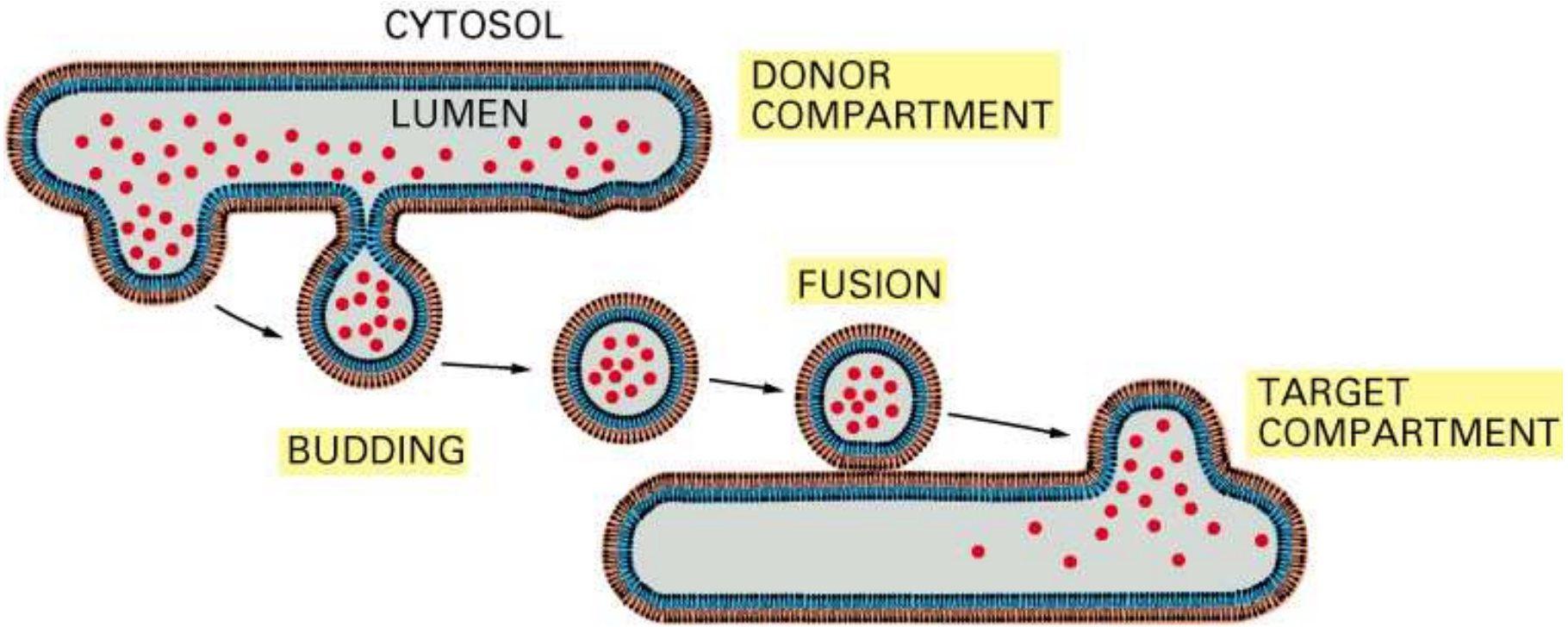


Figure 13–2. Molecular Biology of the Cell, 4th Edition.

A -Vesicular Transport: Mechanism for communication between intramembranous compartments.

Transport carriers have different morphologies - can be small vesicles, pinched off tubules, or other structures.

B- DOZRÁVÁNÍ CISTEREN

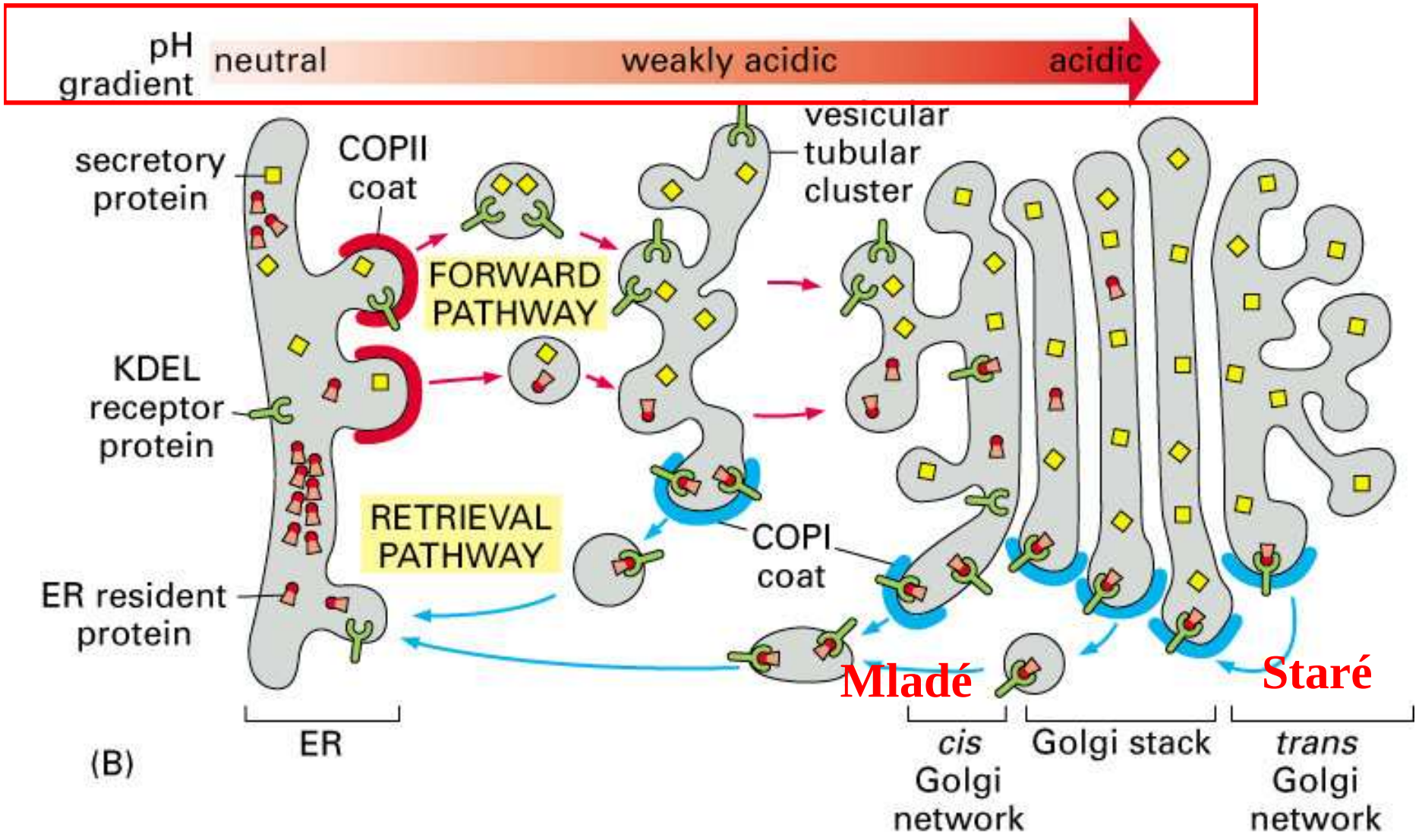
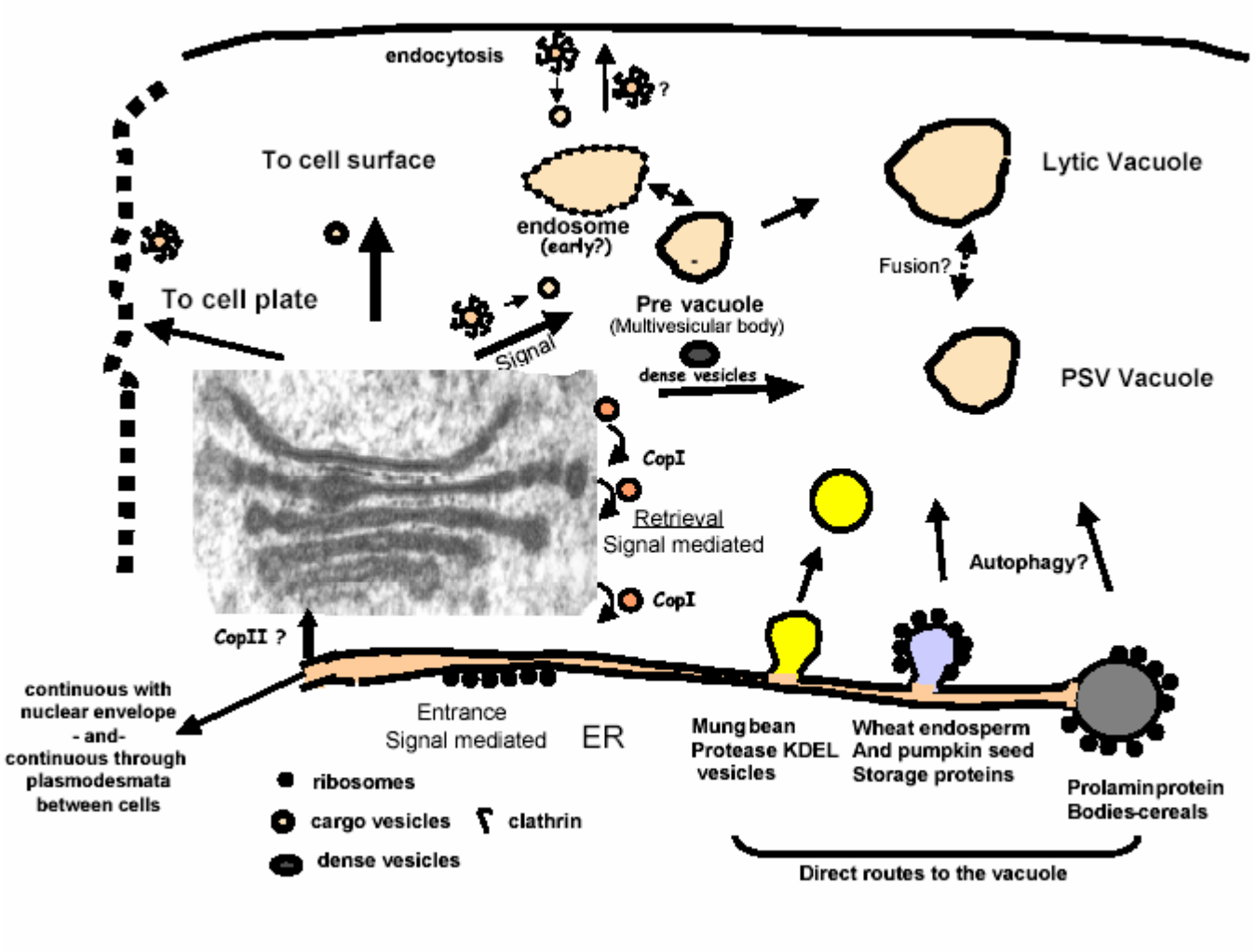


Figure 13–21 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

Early Endos. u rostlin!!

- Součástí endomembránového systému je také nejen jaderná membrána, ale také plasmalema.

Kompartmenty a cesty endomembrán rostlin.



Unique features of the plant secretory system

Minor

1. Plants lack the intermediate compartment between ER and Golgi that is present in animals.
2. Plants may not possess a TGN that is the distinctive sorting compartment is animals.

TGN funguje jako endosom!

Major

3. Plant cells possess many small Golgi rather than the single large perinuclear Golgi of animals.
4. Plant Golgi stream on actin cables in association with the ER.
5. Plant Golgi make non-cellulosic cell wall polymers in addition to glycosylating proteins.
6. Plant cells have two distinct vacuoles with distinct targeting routes. The two vacuoles have been shown to fuse during development.
7. Dividing plant cells assemble a new plasma membrane at the cell plate rather than divide by constriction.
8. Plant cells of certain tissues store proteins in the ER for later direct delivery to the lytic vacuole.

9. GA je funkční při cytokinezi a tedy nedisociuje.

Universal Features

*The plant secretory system consist of one genetic membrane, the endoplasmic reticulum (ER), which synthesizes its polar lipids and acquires all proteins by direct insertion or transport via the Sec translocon.

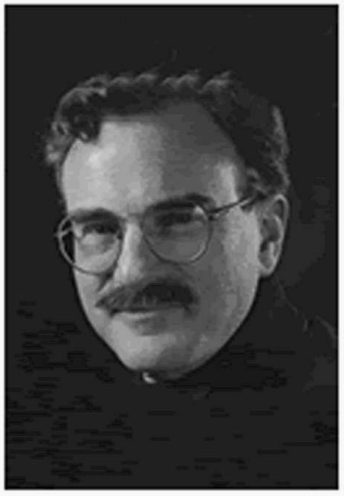
*All other compartments of the secretory system (also called the endomembrane system) are derived membrane-enclosed compartment that ultimately obtain their proteins and membrane bilayer from the ER. Genetic membranes arise by growth and division. Derived membranes, if lost, can be regenerated from the genetic membrane.

Metody studia sekreční dráhy

- Genetic Approach by **Schekman** and coworkers
- Biochemical Approach by **Rothman** and coworkers

2002 Albert Lasker Award for Basic Medical Research

James E. Rothman and **Randy W. Schekman**
For discoveries revealing the universal machinery that orchestrates the budding and fusion of membrane vesicles - a process essential to organelle formation, nutrient uptake, and secretion of hormones and neurotransmitters.



James E. Rothman – Chairman and Paul A. Marks Chair, Cellular Biochemistry & Biophysics Program, Vice Chairman,
Sloan-Kettering Institute

Randy W. Schekman – Investigator,
University of California, Berkeley

Genetické metody studia sekrece

- Genetic: Isolate yeast mutants with temperature-sensitive defects in secretion
- Landmark paper: Novick P., Field C., and Schekman, R. 1980.
  - Identification of 23 complementation groups required for post-translational events in the secretory pathway. *Cell* 21, 205-215

Cell, Vol. 21, 205–215, August, 1980, Copyright ©1980 by Cell Press

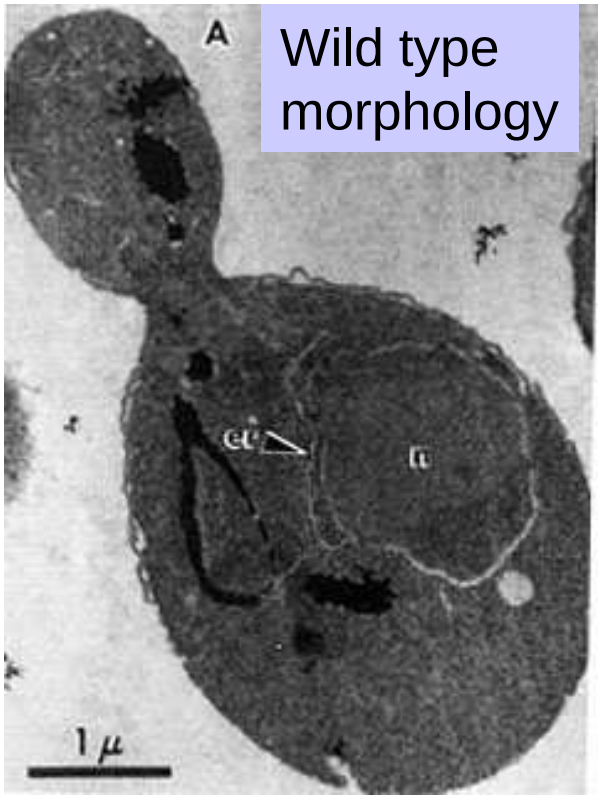
Identification of 23 Complementation Groups Required for Post-translational Events in the Yeast Secretory Pathway

Peter Novick, Charles Field and Randy Schekman*
Department of Biochemistry
University of California, Berkeley
Berkeley, California 94720

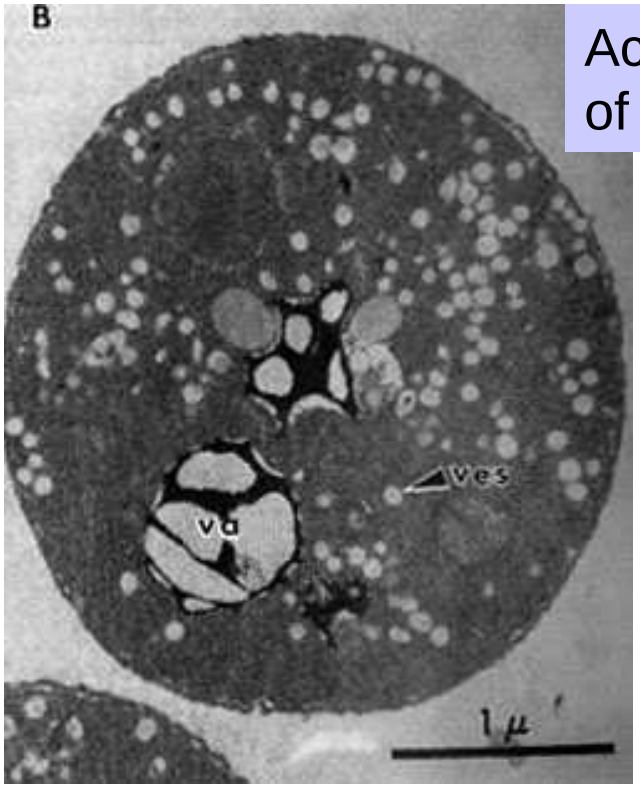
sis pathways, both in identifying intermediate structures and in providing biochemical assays for assembly steps (Wood and King, 1979). We believe that a similar approach may be useful in unraveling a eucaryotic morphogenesis pathway.

Assays and Analysis

- Morphological - thin section electron microscopy



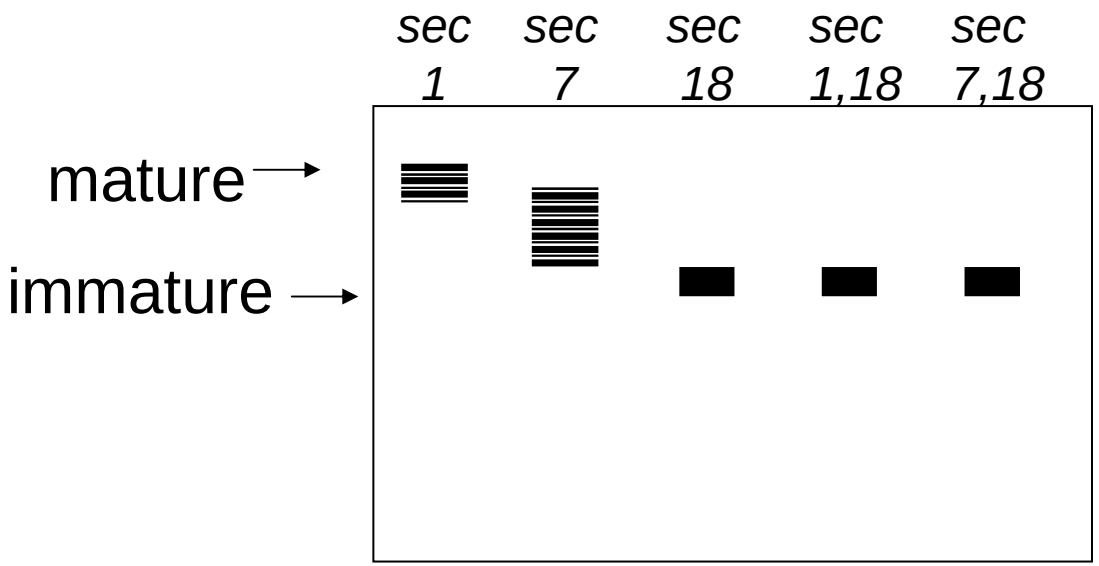
sec4-2 25 ºC



sec15-1 after 2 hr @ 37 ºC

Analýza sekrečního defektu

- Biochemical: secretion of invertase
- Electrophoretic mobility as an indicator of progress through the secretory pathway



Immunoprecipitation of invertase accumulated in single and double mutants

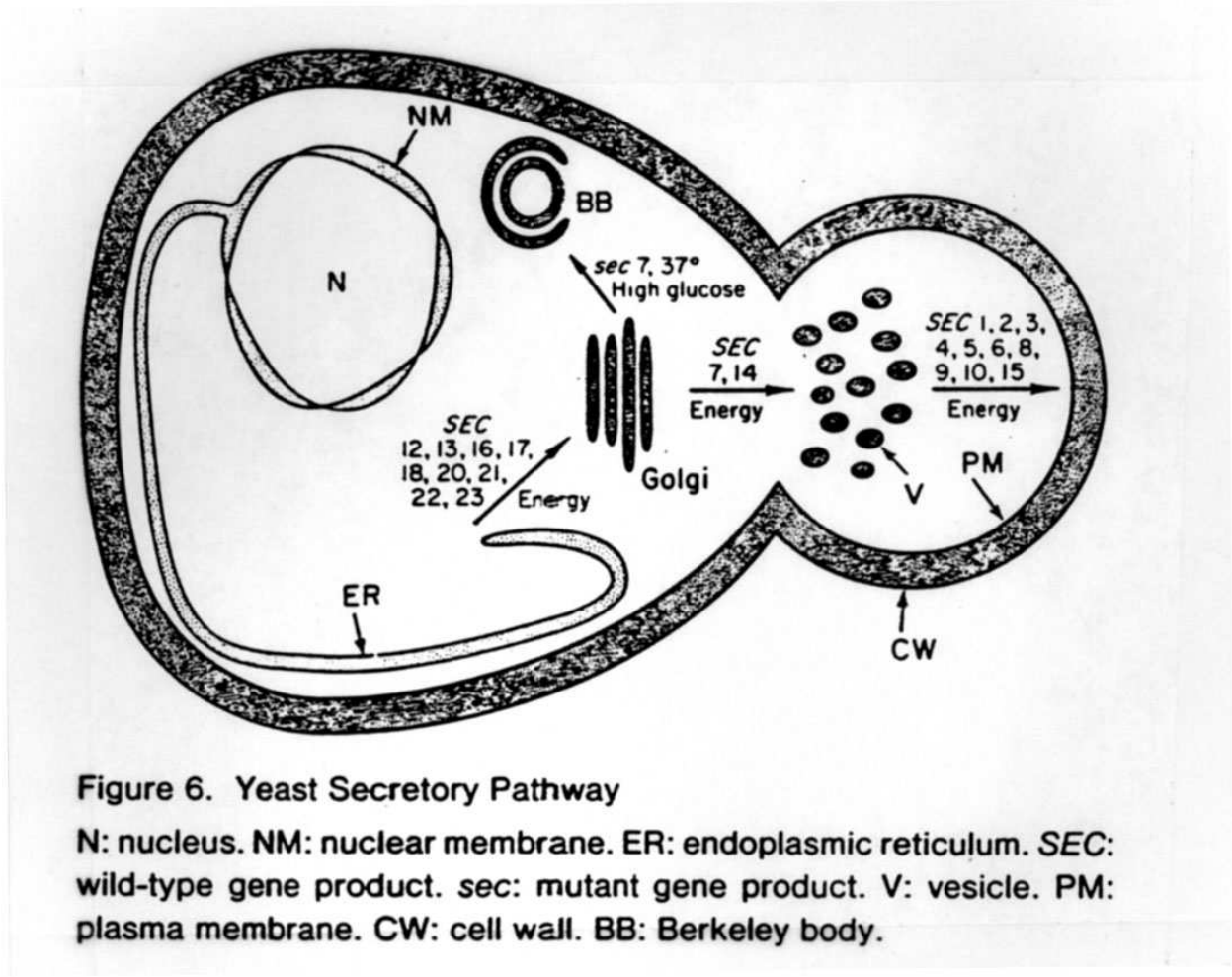


Figure 6. Yeast Secretory Pathway

N: nucleus. NM: nuclear membrane. ER: endoplasmic reticulum. *SEC*: wild-type gene product. *sec*: mutant gene product. V: vesicle. PM: plasma membrane. CW: cell wall. BB: Berkeley body.

Novick P, Ferro S, and Schekman R; 1981

Hledání mechanismů -

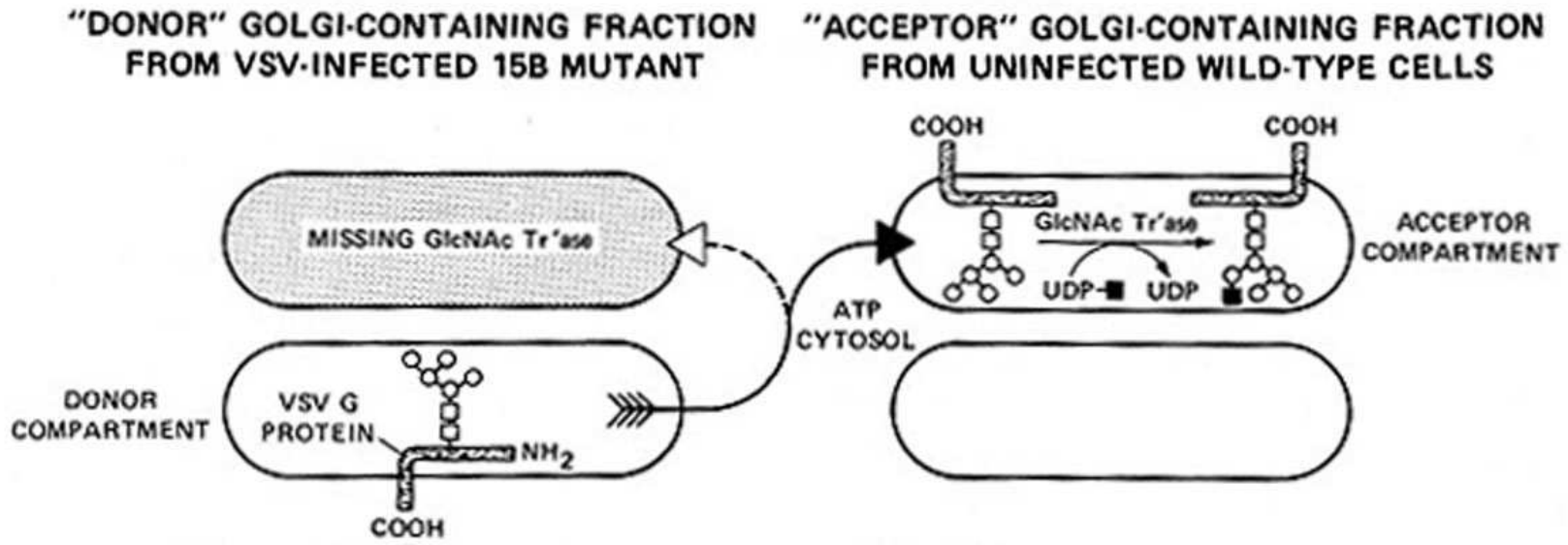
- Clone *SEC* genes
- Guess function by sequence homology
  - *SEC4* = monomeric GTPase
- Characterize gene products
  - Localization
  - Assay activity
- More genetic screens to isolate additional players in the pathway
  - Multicopy suppression
- Reconstitute transport step *in vitro*

Biochemické metody studia sekrece

- Biochemical: Cell-free assays of vesicular transport
- Landmark paper: Balch W.E., Dunphy W.G., Braell W.A., and Rothman J.E. 1984
  - Reconstitution of the transport of protein between successive compartments of the Golgi measured by the coupled incorporation of N-acetylglucosamine. *Cell* 39, 405-416

Bezbuněčný systém studia sekrece *in vitro.*

Goal: Reconstitute transport between two organelles *in vitro*



VSV = vesicular stomatitis virus

Golgi Transport Assay

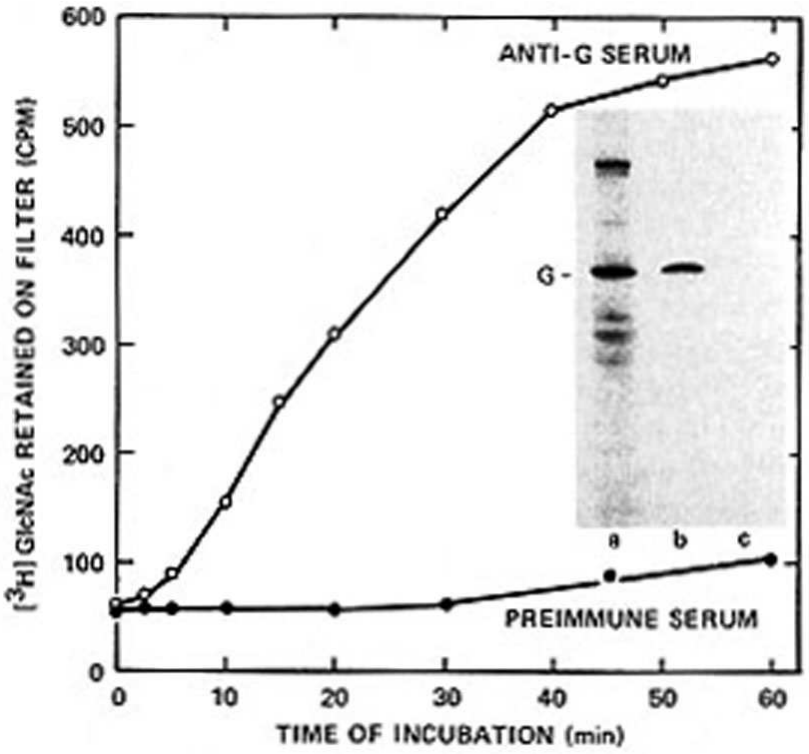


Figure 2. Incorporation of ^{3}H-GlcNAc into G Protein during Incubations of the PNS of VSV-Infected 15B Cells with the PNS of Uninfected Wild-Type Cells

What is measured?

Incorporation of
[^{3}H]GlcNAc
into VSV G protein

INSET:
Lane a = total reaction
Lane b = IP w/ anti G serum
Lane c = IP w/ preimmune serum

PNS = post nuclear supernatant

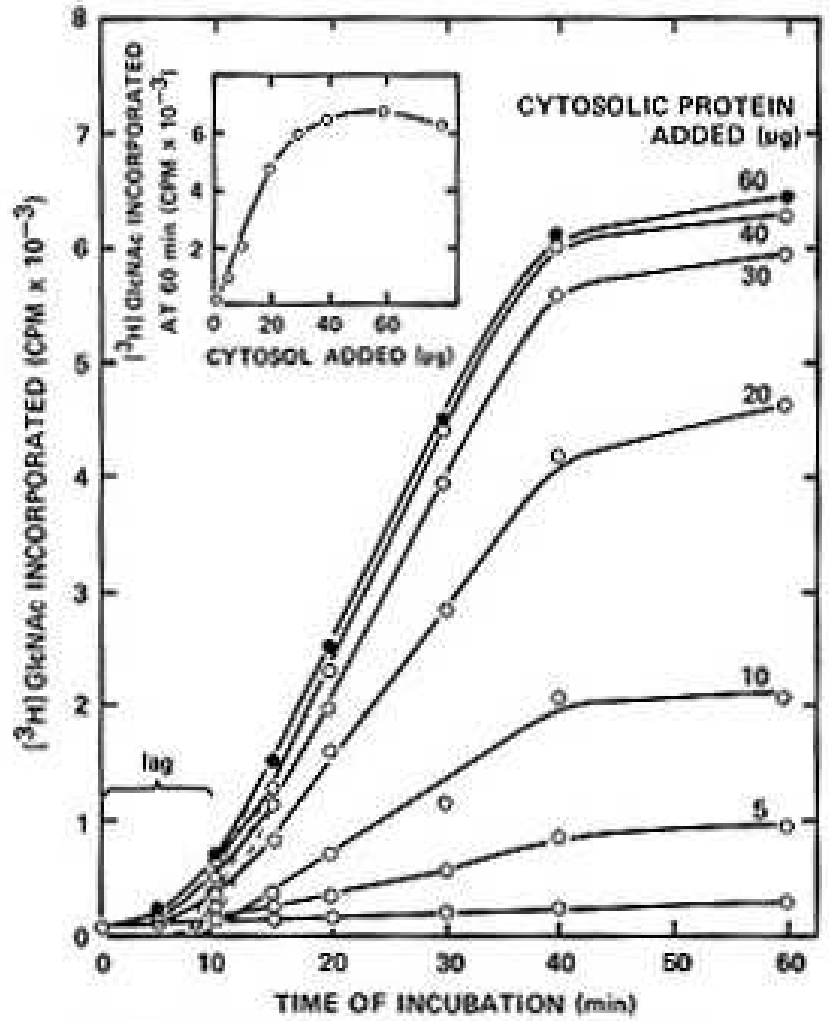


Figure 7. Effect of Cytosol Concentration on the Rate and Extent of Transport

Each incubation (50 µl) contained 2.5 µg donor membrane fraction, 5 µg of acceptor fraction, and gel-filtered CHO cytosol in the indicated amount in the standard assay cocktail. Incubations were stopped after various times at 37°C, and the ^{3}H-GlcNAc incorporated into G protein was determined. Inset: ^{3}H-GlcNAc incorporated into G protein at the plateau of incorporation as a function of the amount of cytosol protein added.

Golgi Transport Assay

- Components in the assay required for activity
  - Golgi membranes
  - Energy
  - Acyl-Coenzyme A
  - Cytosolic proteins

Konvergence genetické a biochemické analýzy

In vitro Golgi transport assay

↓

Purify mammalian NSF

↓

cDNA cloned → Homology to *SEC18*

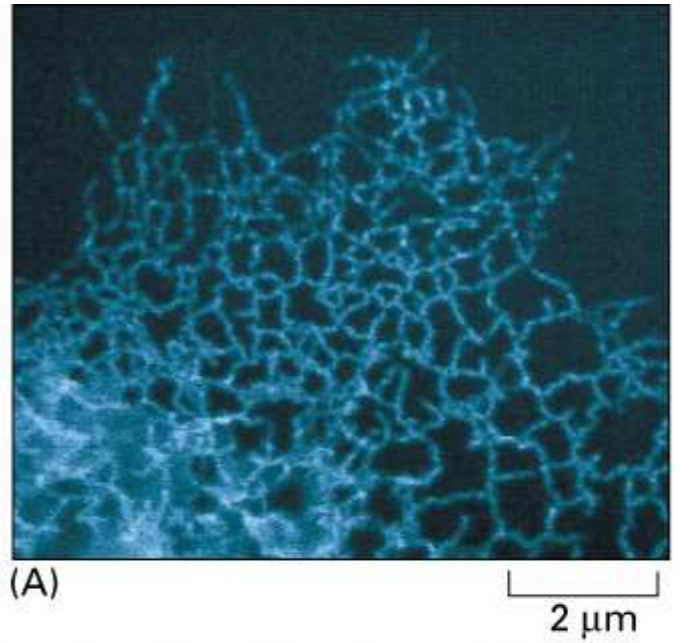
↓

Yeast Sec18p reconstitutes activity in the Golgi transport assay.

SEC Mutants

dál uvidíme u SNARE.

Endoplasmatické retikulum

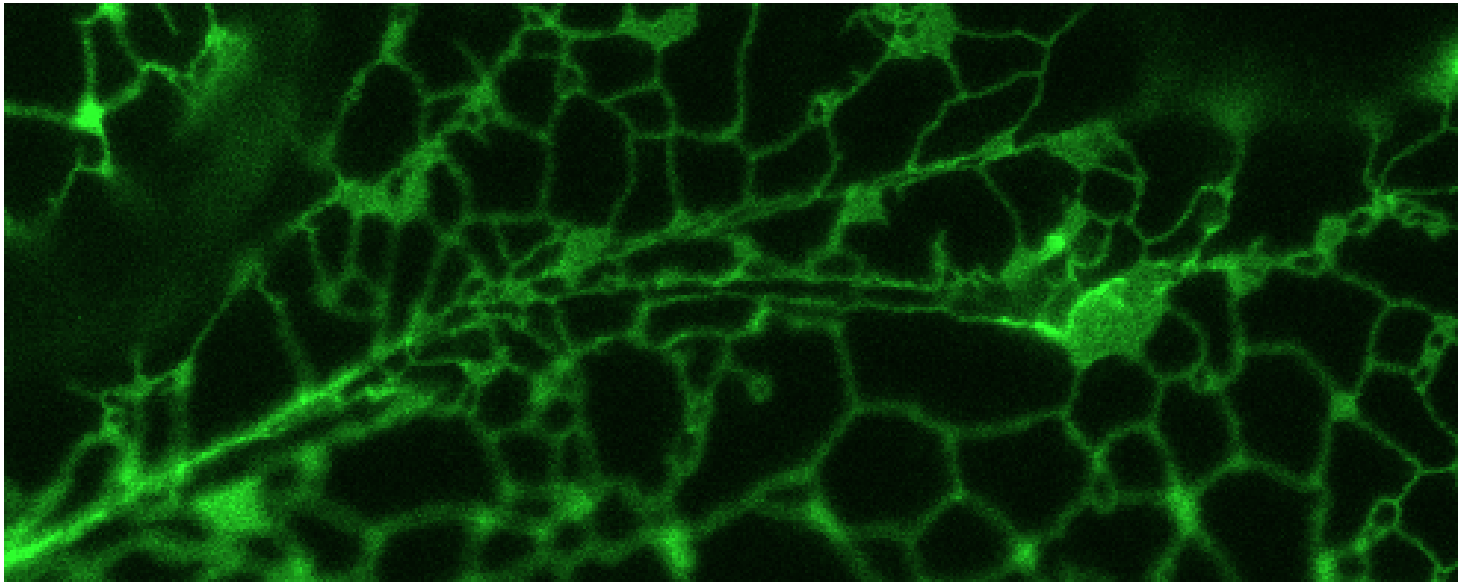


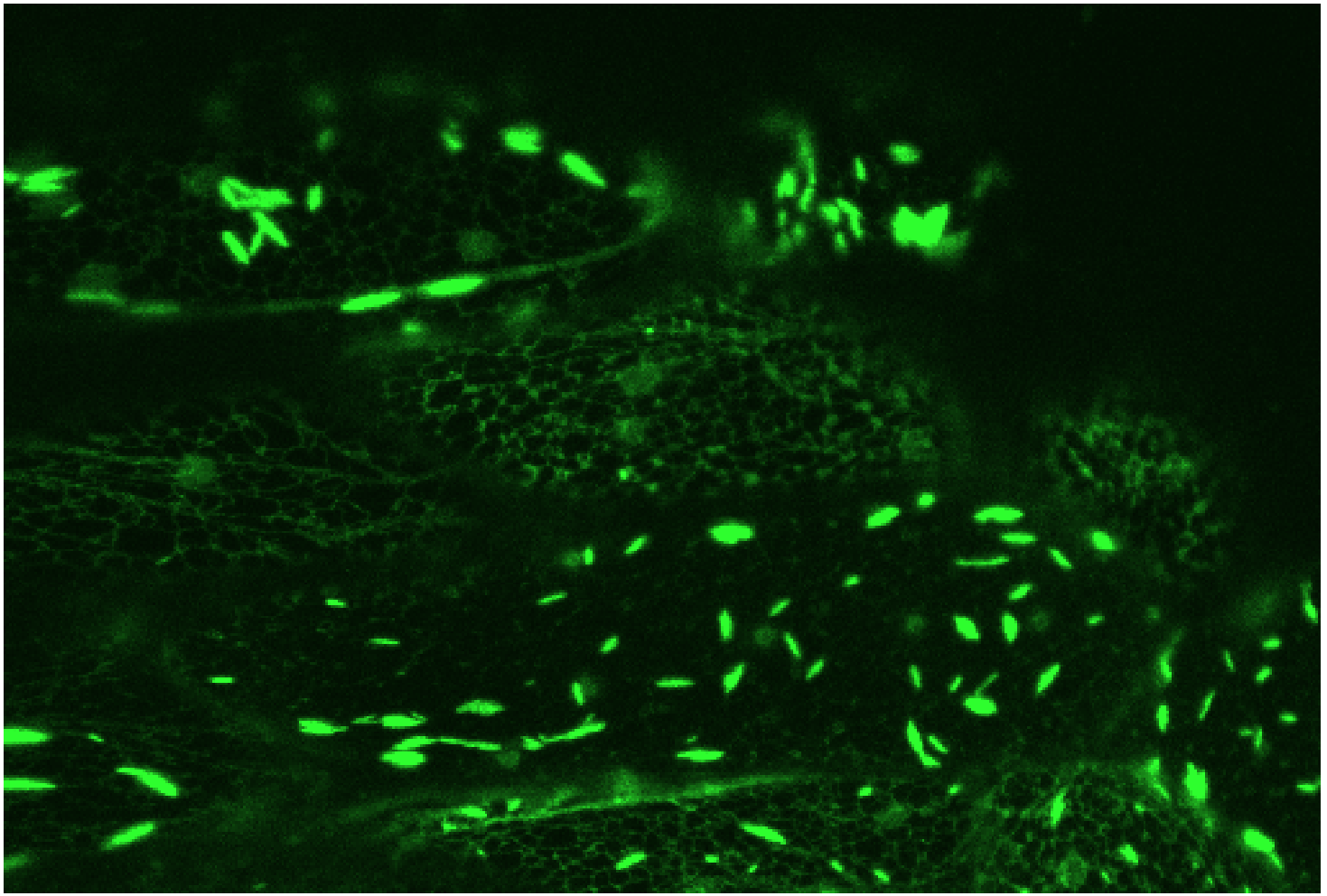
12-35 ENDOPLASMIC
ΓICULUM. Cultured mammalian
l (Part A) stained with blue
orescent antibody against protein
ained in the endoplasmic
iculum (ER). Part B also shows ER
fluorescence, but this time the
ne for the protein was genetically
gineered to extend the sequence
h green fluorescent protein and
constructed gene introduced into
nt cells.

een fluorescent protein from
yfish *Aequorea victoria* has
come an essential tool in cell
logy (search the web for GFP).

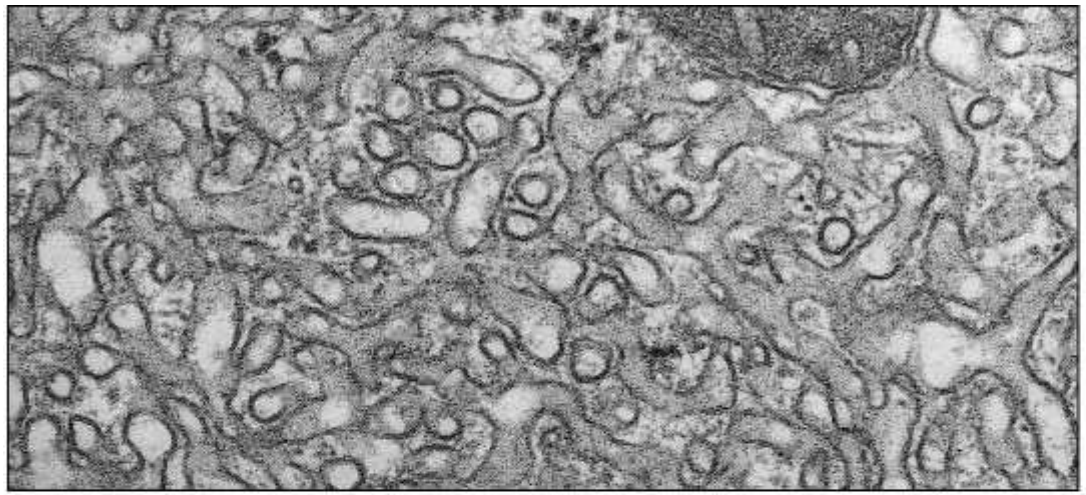
://pantheon.cis.yale.edu/~wfm5/gfp_gateway.
l

Kontrola kvality bílkovin
Má důležitou signální funkci - regulovaný Ca2+ výtok.





ER-tělíska (ER bodies) - zvl. některých rostlinných buněk.
V listech - obsahují proteázy, které se účastní reakcí na stress (i biotický!!) a senescence.



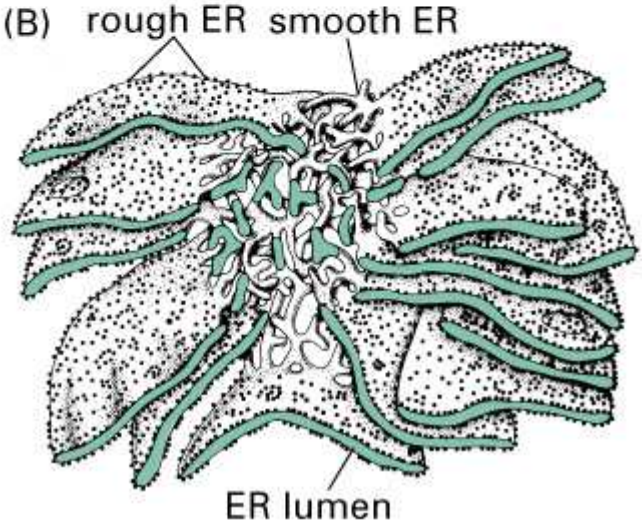


Figure 12–38. Molecular Biology of the Cell, 4th Edition.

Hladké ER (smooth) u rostlin mj. doména produkce lipidů a membrán.

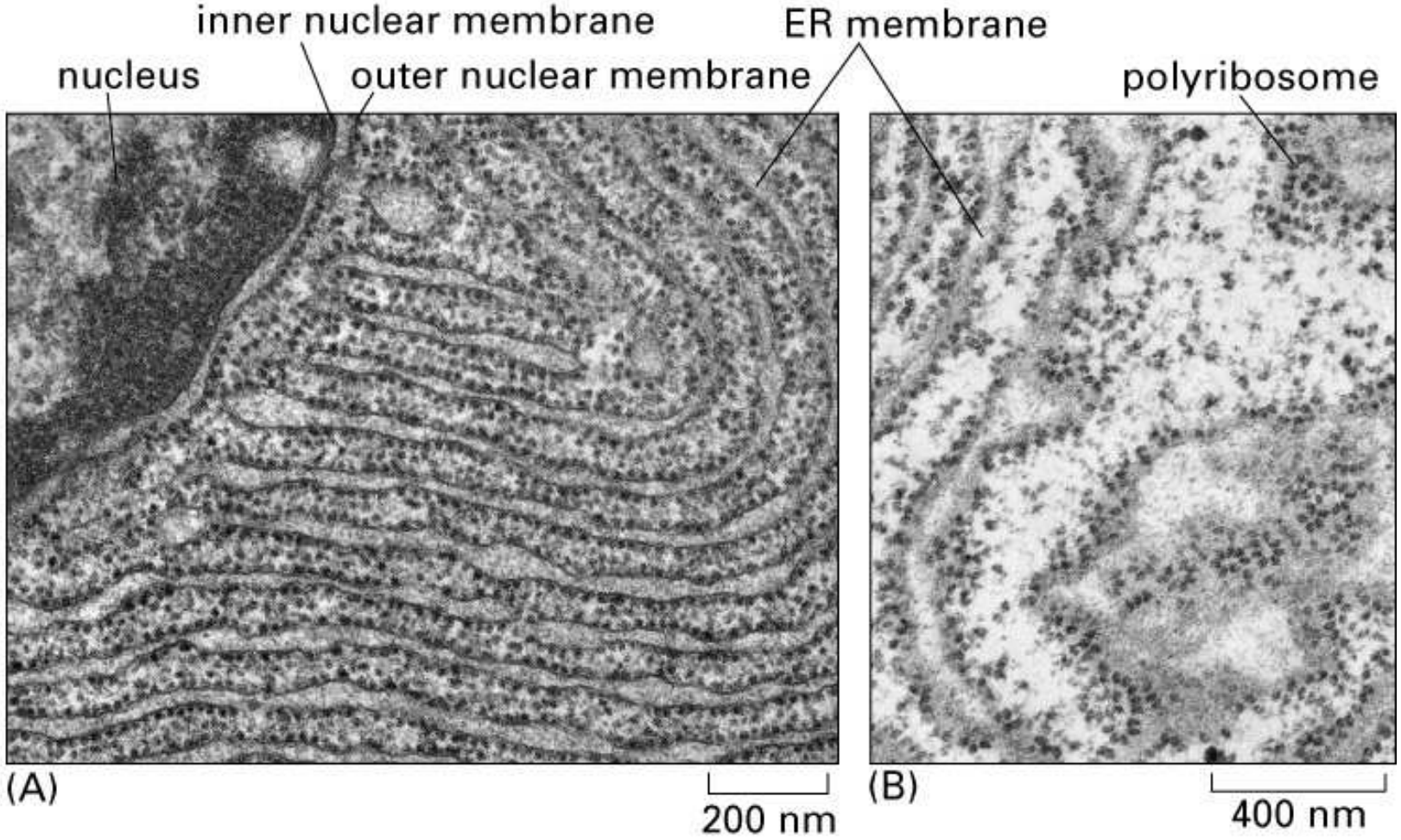


Figure 12–36. Molecular Biology of the Cell, 4th Edition.

TEM of rough endoplasmic reticulum (RER). Extensive cellular network of membranes. In this case the membranes are involved in protein synthesis and ribosomes are associated with the membranes, giving them a “rough” appearance. The inside of the compartment is called “cisterna”, or “cisternal space”.

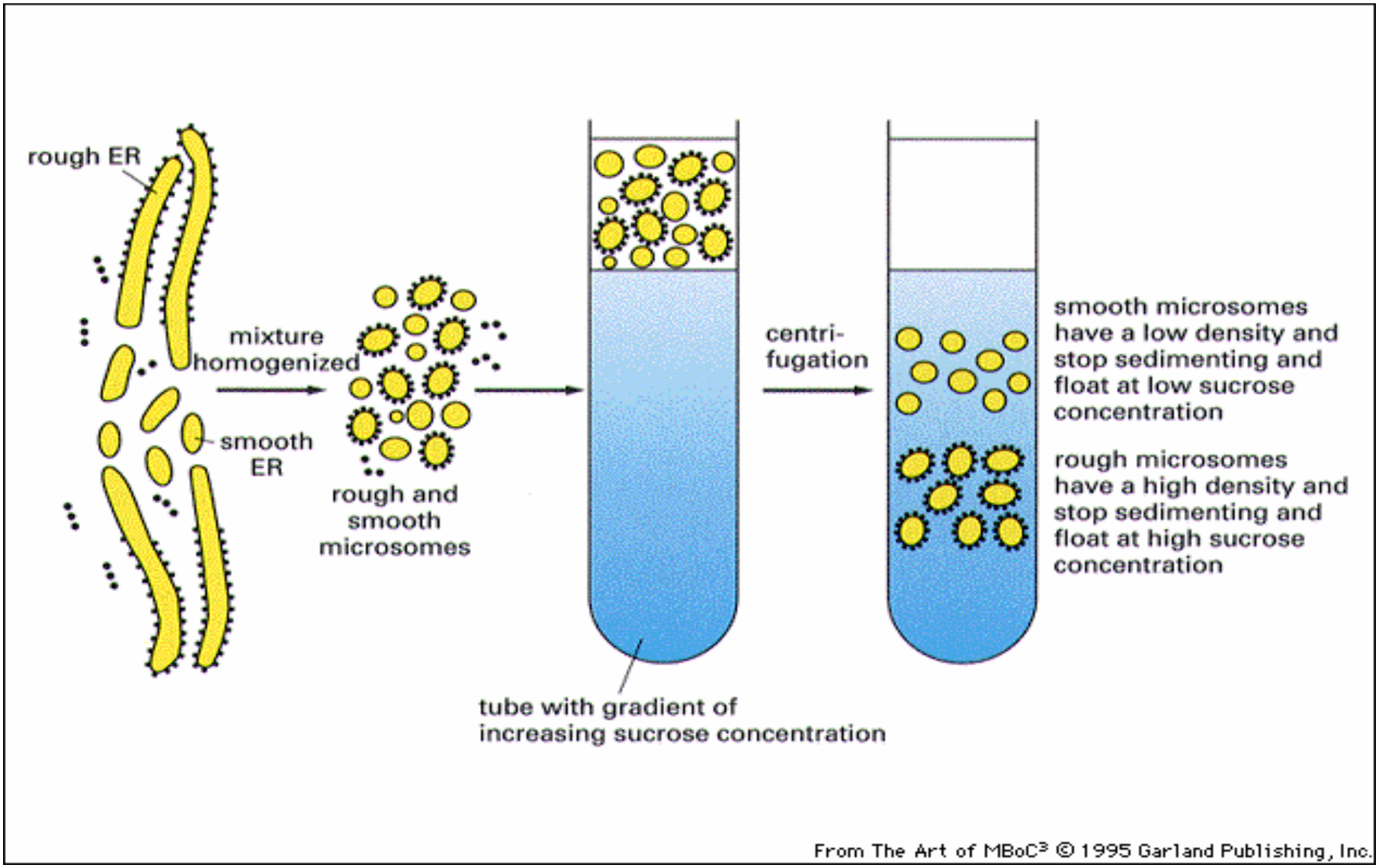


Fig. 12-39 Separation of smooth and rough ER as “microsomes” on sucrose gradient. Different conditions may have to be used to separate peroxisomal microbodies and other vesicles from the smooth microsomes.

The pool of free ribosomal subunits in the cytoplasm and their association into (1) free and (2) endoplasmic reticulum bound polyribosomes. Note that the association with the ER membrane is via the signal sequence on the newly forming protein.

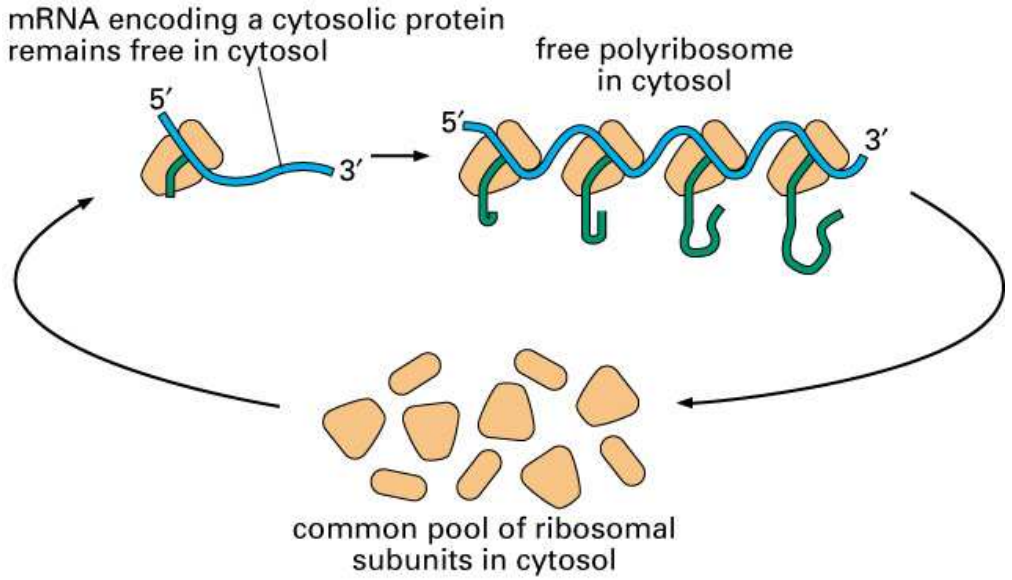


Figure 12–37 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

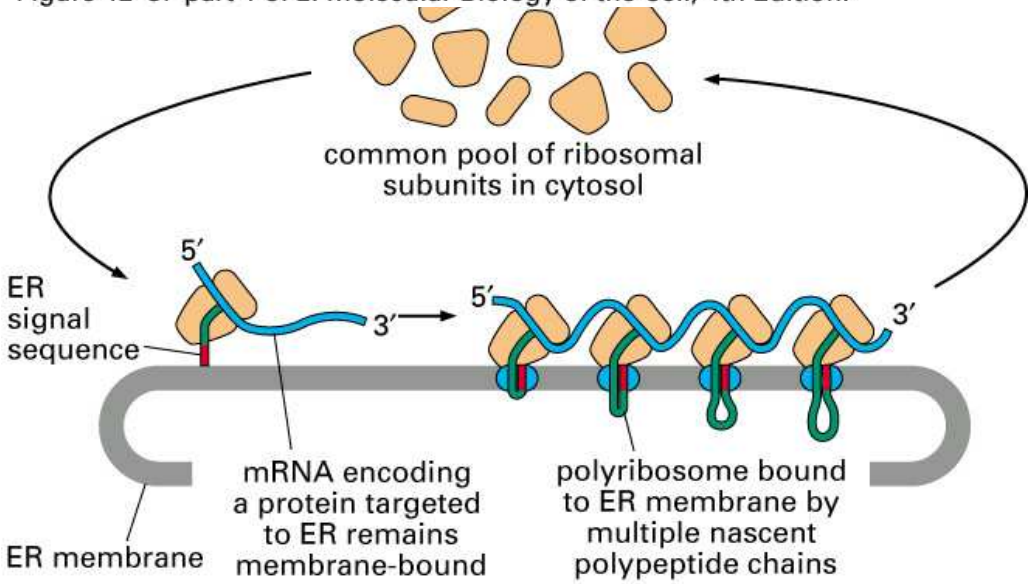


Figure 12–37 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

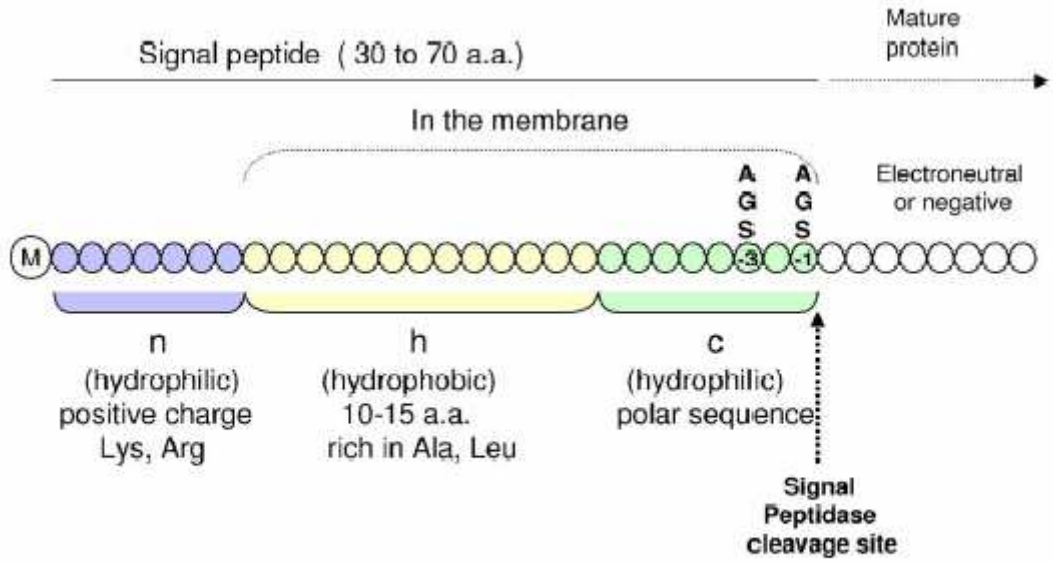
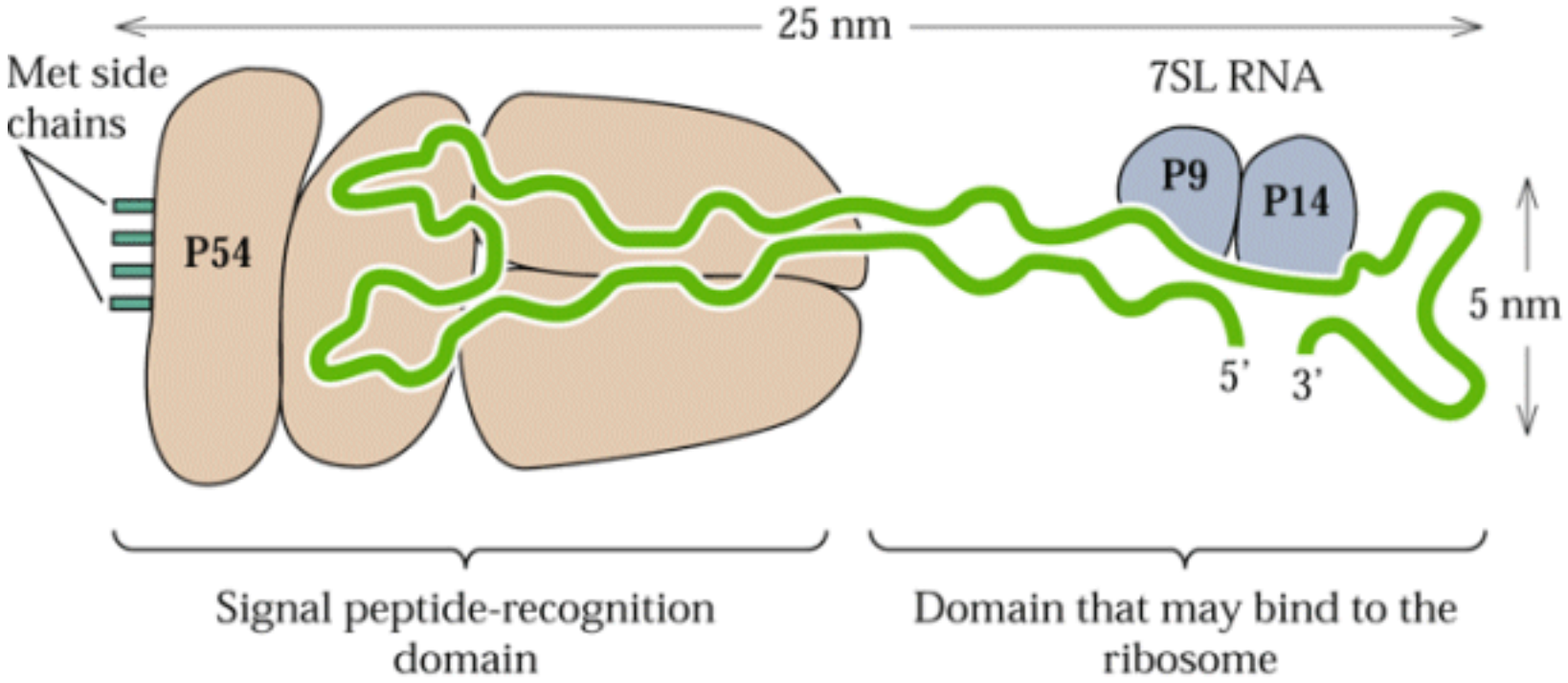


Fig. 3. Typical tripartite structure of the N-terminal signal peptide of preproteins targeted to the ER.

SRP


25 nm
Met side chains
P54
7SL RNA
P9
P14
5 nm
5'
3'
Signal peptide-recognition domain
Domain that may bind to the ribosome

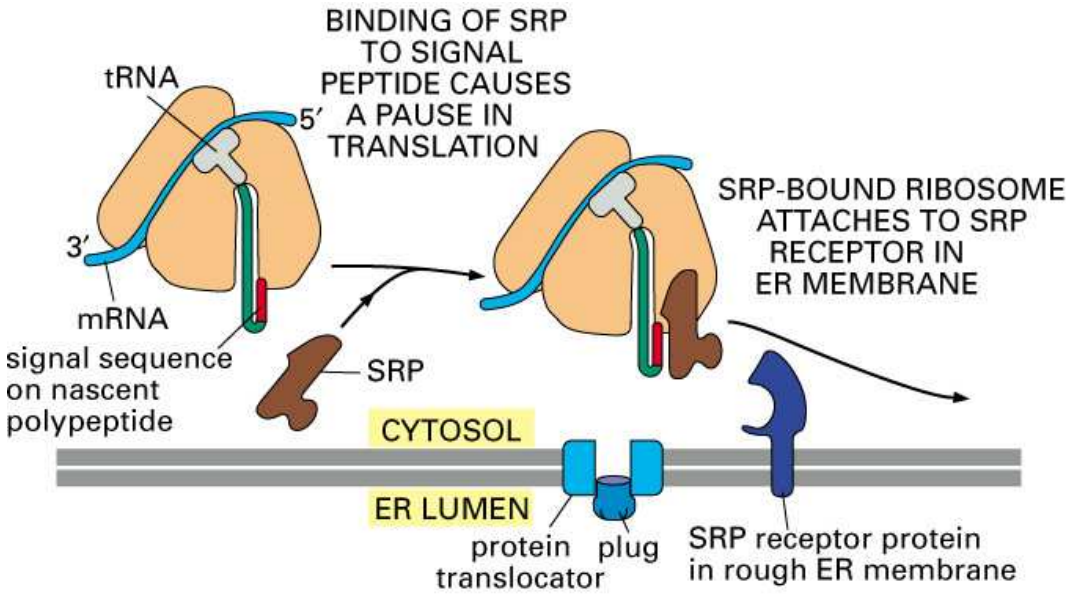


Figure 12–42 part 1 of 2. Molecular Biology of the Cell, 4

ER signal peptides and SRP direct ribosomes to the ER membrane.

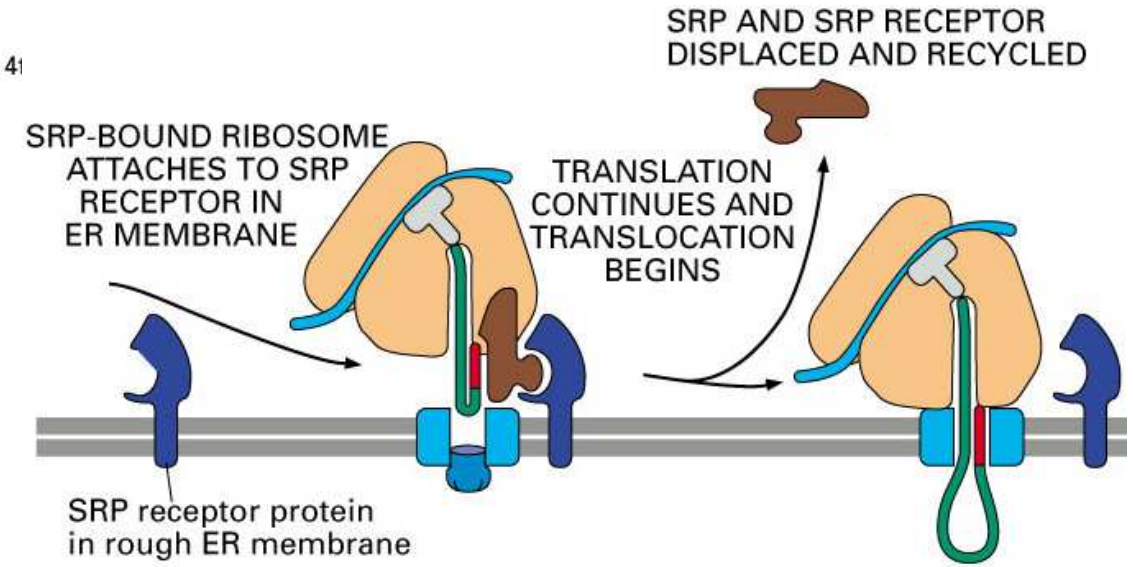


Figure 12–42 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

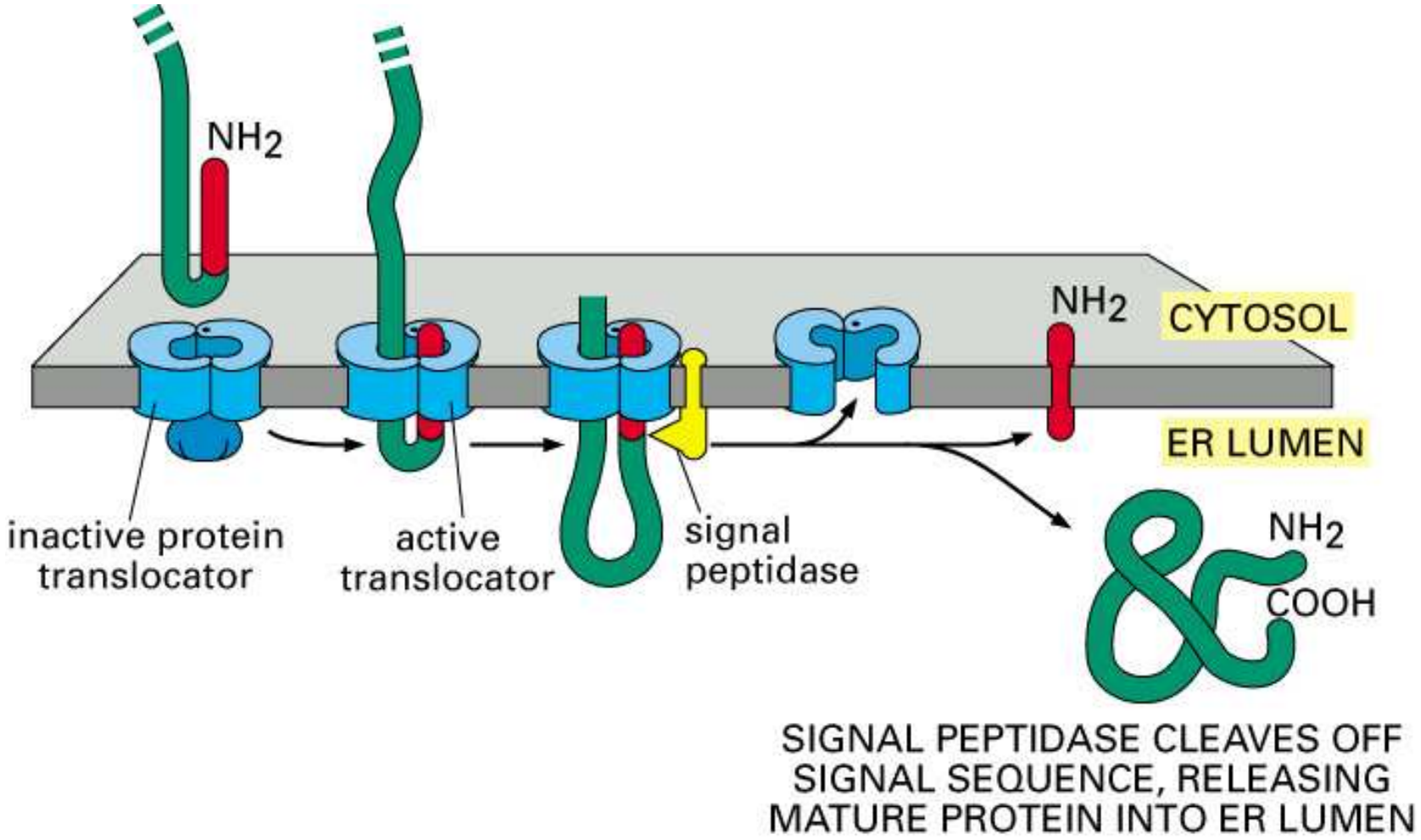


Figure 12–46. Molecular Biology of the Cell, 4th Edition.

Import of a protein destined for the LUMEN of the ER and possibly further translocation. Signal peptidase on inner surface of ER membrane cleaves off the amino end (and the initiator methionine) of the imported protein.

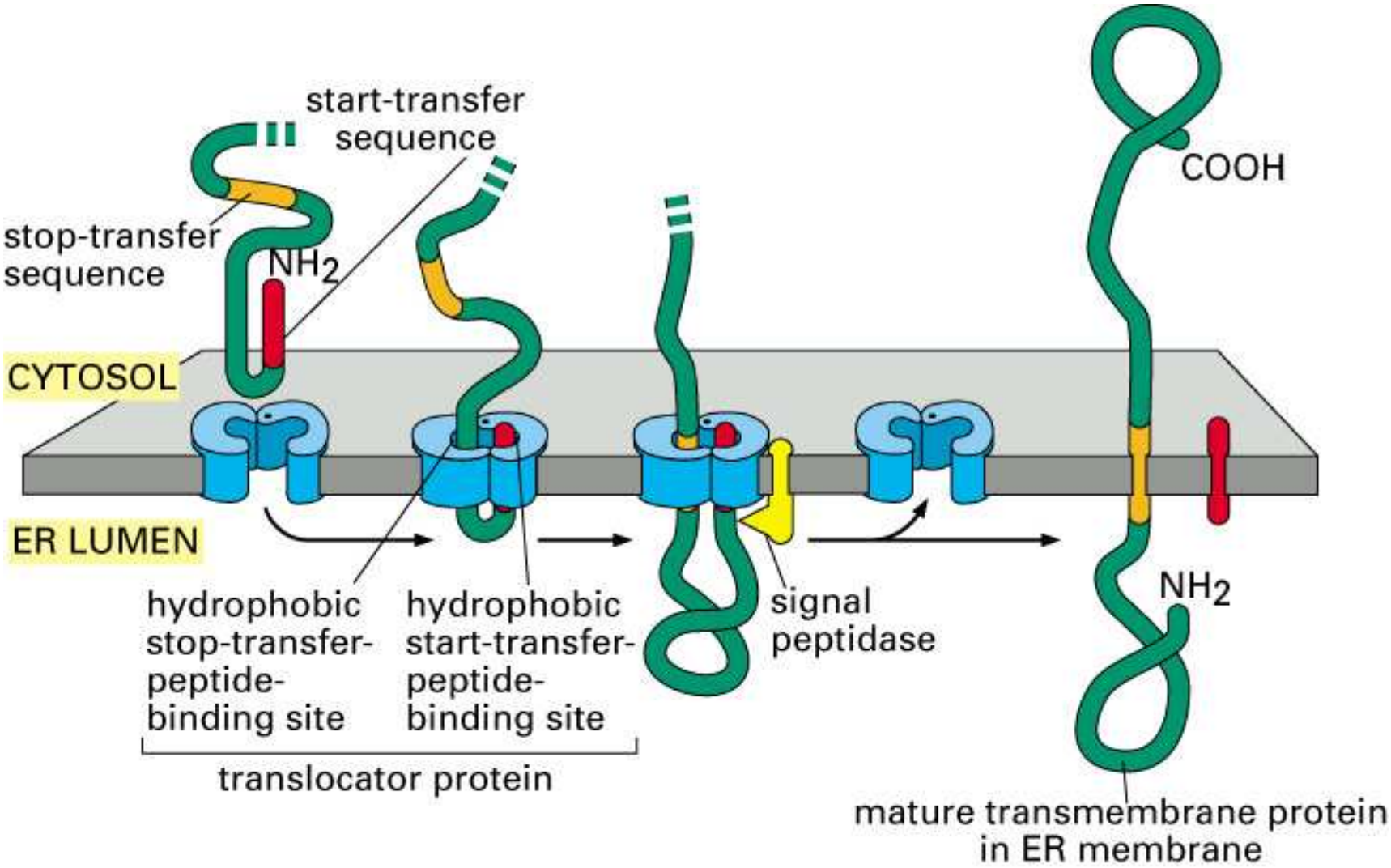


Figure 12–47. Molecular Biology of the Cell, 4th Edition.

The need for "stop transfer" signal sequence in proteins which have to remain integral inside the membrane. Proteins which span the membrane SEVERAL TIMES, need several of these start and stop transfer sequences (Fig. **12-49, 50**).

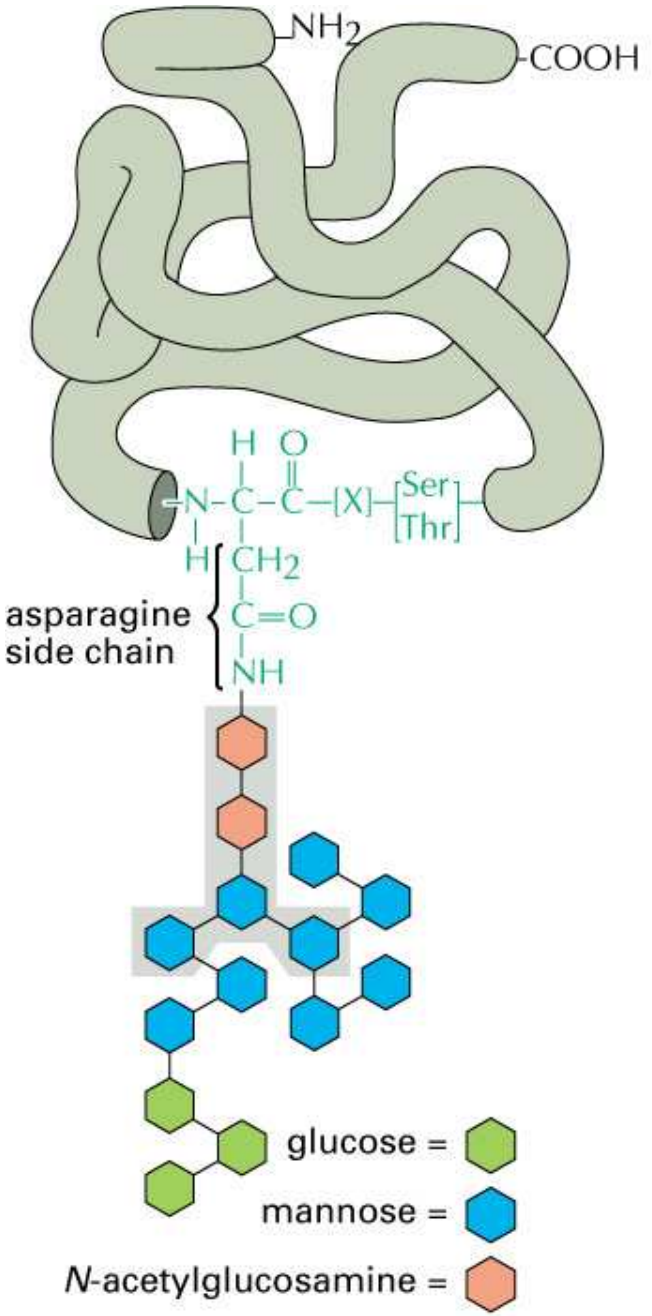


Figure 12–51. Molecular Biology of the Cell, 4th Edition.

Fig. **12-51** The asparagine-linked (N-linked) oligosaccharide that is added to most proteins in RER. This is called "core" glycosylation. The reactions which synthesize the "christmas tree" of sugars take place elsewhere on the membrane - dolichol molecule - and the entire structure is transferred to the asparagine by enzyme called oligosaccharyl transferase.

Further processing of the sugars takes place in the Golgi body and many glycoproteins end up on the surface of the cell, in the glycocalyx, which characterizes the organism as "self". Antibodies recognize "non-self" glycosylation patterns and attack "foreign" cells.

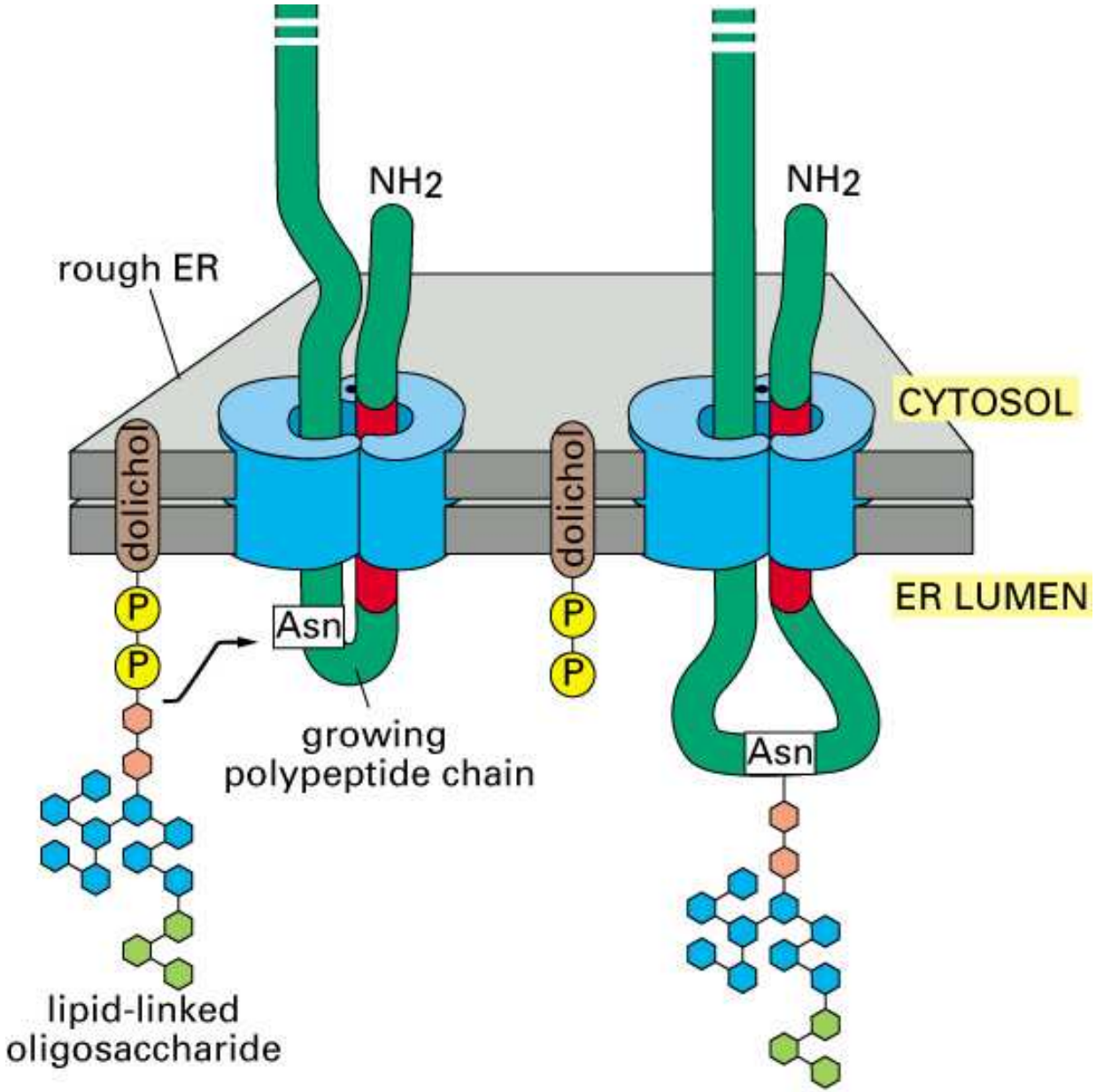


Figure 12–52. Molecular Biology of the Cell, 4th Edition.

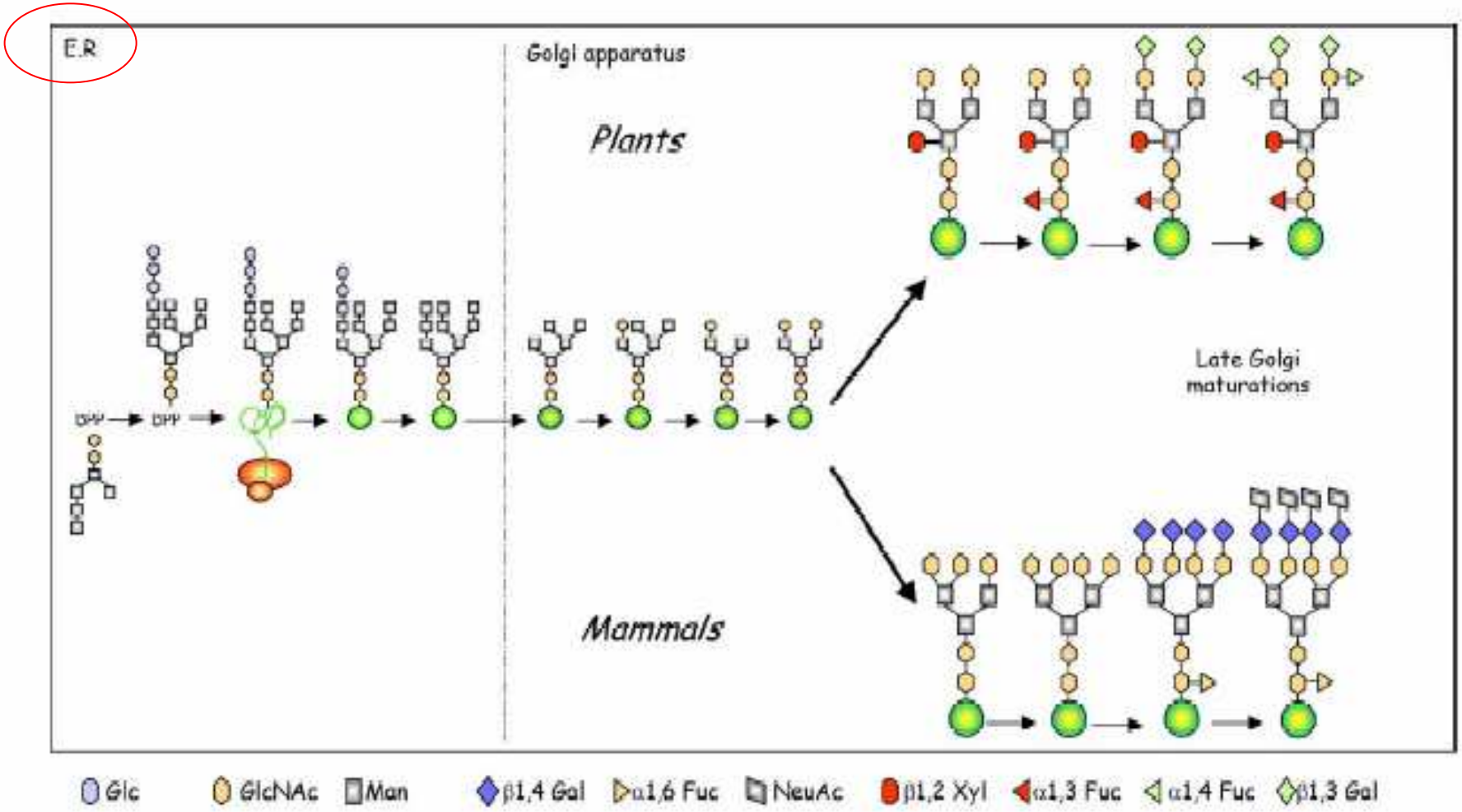


Fig. 1. Addition and processing of N-linked glycans in the endoplasmic reticulum (ER) and Golgi apparatus of plant and mammalian cells. A precursor oligosaccharide assembled onto a lipid carrier is transferred on specific Asn residues of the nascent growing polypeptide. The N-glycan is then trimmed off with removal of glucosyl and most mannosyl residues. Differences in the processing of plant and mammalian complex N-glycans are late Golgi maturation events.

Retikuloplasminy

podílejí se na regulaci konformace bílkovin v lumen ER.

BiP,calnexin, calreticulin, PDI (prot. disulf. isomeráza)…

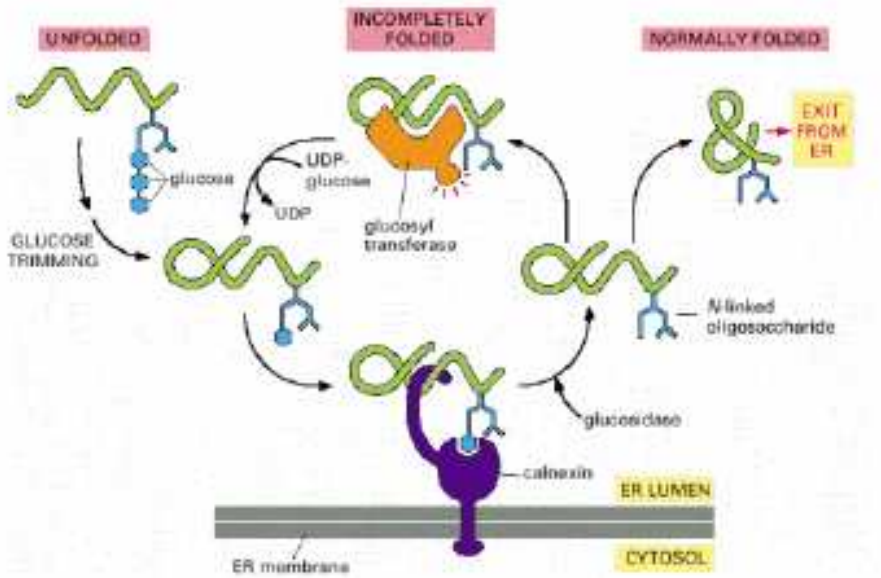


Figure 12-54. The role of *N*-linked glycosylation in ER protein folding. The ER-membrane-bound chaperone protein calnexin binds to incompletely folded proteins containing one terminal glucose on *N*-linked oligosaccharides, trapping the protein in the ER. Removal of the terminal glucose by a glucosidase releases the protein from calnexin. A glucosyl transferase is the crucial enzyme that determines whether the protein is folded properly or not: if the protein is still incompletely folded, the enzyme transfers a new glucose from UDP-glucose to the *N*-linked oligosaccharide, renewing the protein's affinity for calnexin and retaining it in the ER. The cycle repeats until the protein has folded completely. Calreticulin functions similarly, except that it is a soluble ER resident protein. Another ER chaperone, ERp57 (not shown), collaborates with calnexin and calreticulin in retaining an incompletely folded protein in the ER.

Calnexin se podílí na kontrole kvality

ve srov. s cytoplasmou je v ER oxidační prostředí

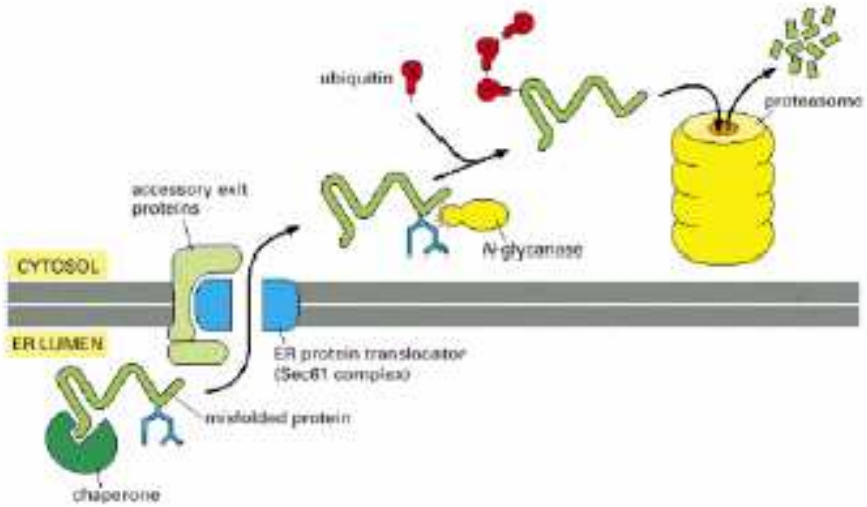


Figure 12-55. The export and degradation of misfolded ER proteins. Misfolded soluble proteins in the ER lumen are translocated back into the cytosol, where they are deglycosylated, ubiquitylated, and degraded in proteasomes. Misfolded membrane proteins follow a similar pathway. Misfolded proteins are exported through the same type of translocator that mediated their import; accessory proteins that are associated with the translocator allow it to operate in the export direction.

a nepodařené bílkoviny jsou exportovány ven z ER a degradovány po ubikvitinaci PROTEASOMEM.

Řízená degradace bílkovin proteasomem (příp. COP/signalosomem) je stejně důležitý regulační pochod jako jejich syntéza.

Unfolded **P**rotein **R**esponse se uplatňuje i u rostlin

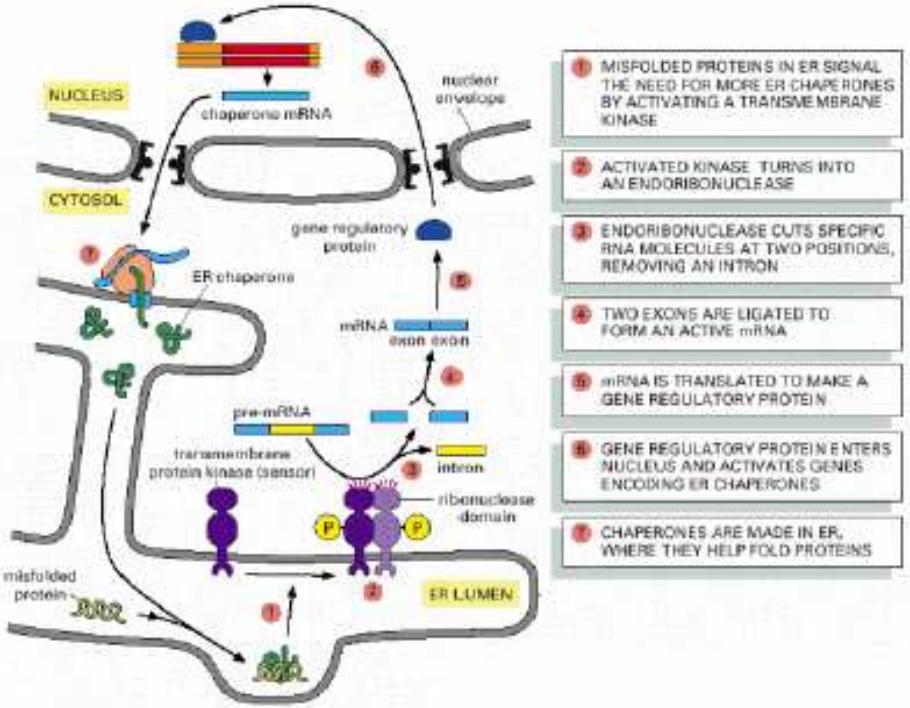


Figure 12-56. The unfolded protein response in yeast. By this novel intracellular signaling pathway, the accumulation of misfolded proteins in the ER lumen signals to the nucleus to activate the transcription of genes that encode proteins that help the cell to cope with the abundance of misfolded proteins in the ER.

Denat. bílkoviny váží BiP, který normálně drží IRE1 neagregovanou; po agregaci aktivovaná IRE1 vystřihne intron TF XBP1. Vedle toho PERK kináza (ER TM bílk.) inhibuje translaci (fosf. eIF2α). ATF6 je TM TF v membr. ER, který je aktivován také uvolněním z BiPu, transportem do GA a po odštěpení proteázou putuje do jádra.

Retrograde Traffic - Required for retention of ER-resident proteins

- Signal-mediated mechanism
  - Lumenal proteins = rozpustné
    - KDEL sequence at carboxyl-terminus; HDEL in *S. cerevisiae*
    - (http://www.ergito.com/lookup.jsp?expt=pelham)
  - Membrane proteins (reviewed in Cosson and Letourneur 1997)
    - **Dilysine motif - KKXX**

Udržování spec. rozp. obsahu ER.

- Residentní bílkoviny ER jsou udržovány v tomto kompartmentu C´term značkou - **retenčním signálem**
- His(Lys)-Asp-Glu-Leu = **H(čiK)DEL**
- rozponávaným rec. **ERD2**
- **COPI váčky dopravují retikuloplasminy uniklé do GA zpátky do ER.**

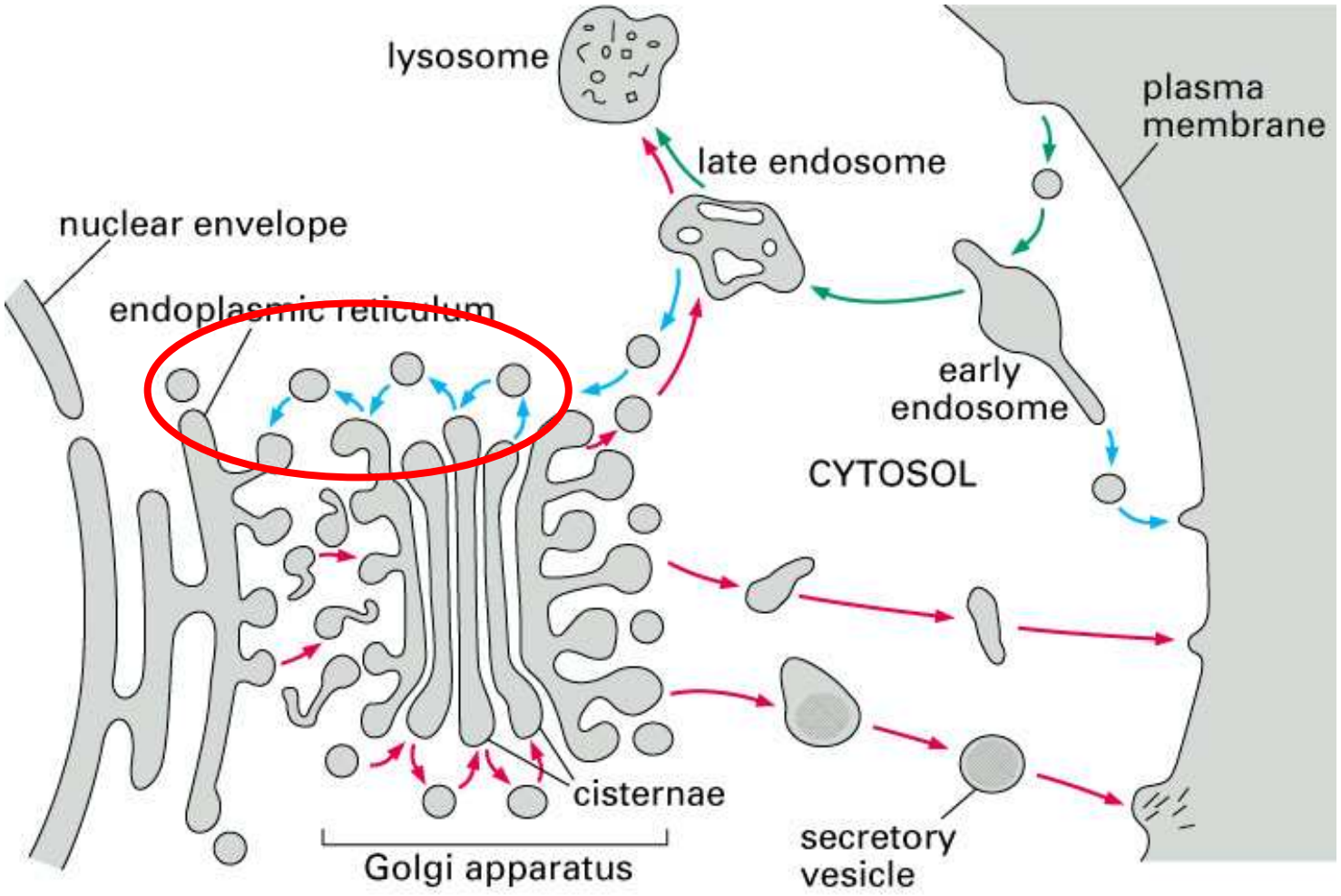
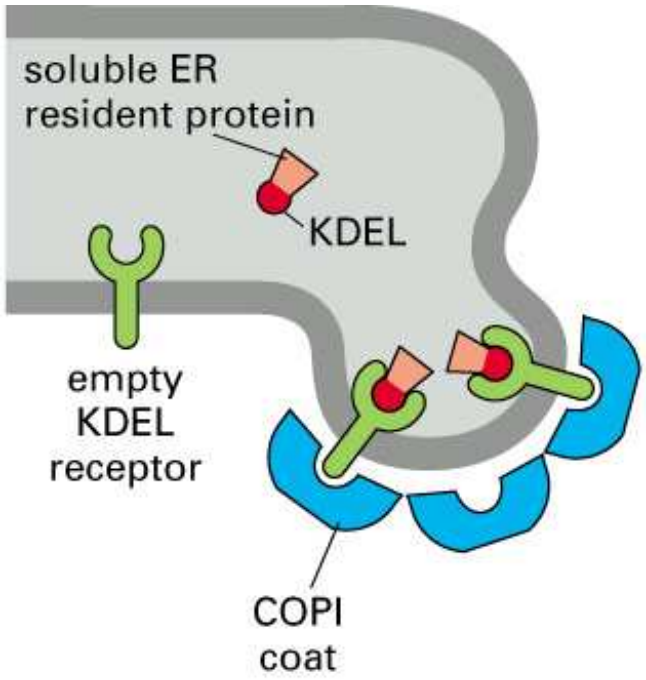


Figure 13–3. Molecular Biology of the Cell, 4th Edition.

Vesicular traffic and the role of Golgi apparatus (body) as a “traffic controller”. Protein modifications take place in the Golgi, which result in acquisition of appropriate signals, and hence packaging into correct vesicles. Hydrolytic enzymes are sent to lysosome, export vesicles to plasmalemma and ER membranes and proteins are recycled back to ER (**Fig 13-21**).

Retrograde Traffic - Required for retention of ER-resident proteins



- Receptor-mediated mechanism for retrieval
- HDEL receptor identified in yeast - *ERD2*; multispanning transmembrane protein
- Dilysine motif in C-terminal tail binds to COPI coat

Figure 13–21 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

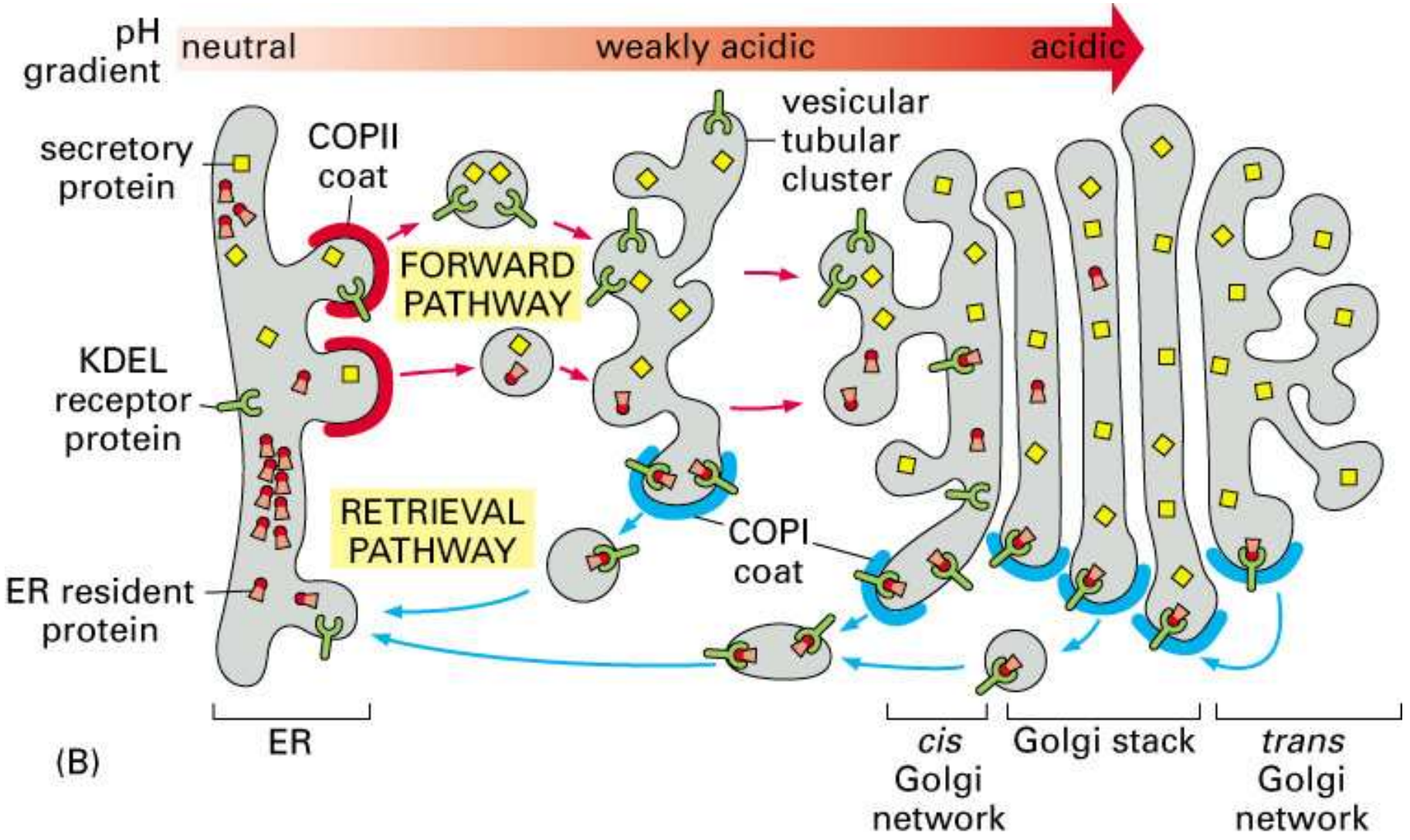


Figure 13–21 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

Membránový transport začína na ER, **ale také se do něj vrací.**

- Vesicular Transport - how are transport vesicles formed?
  - Golgi transport assay - COPI vesicles
  - ER to Golgi transport in yeast - COPII
- Transport through the Golgi
  - Cisternal maturation vs. vesicles

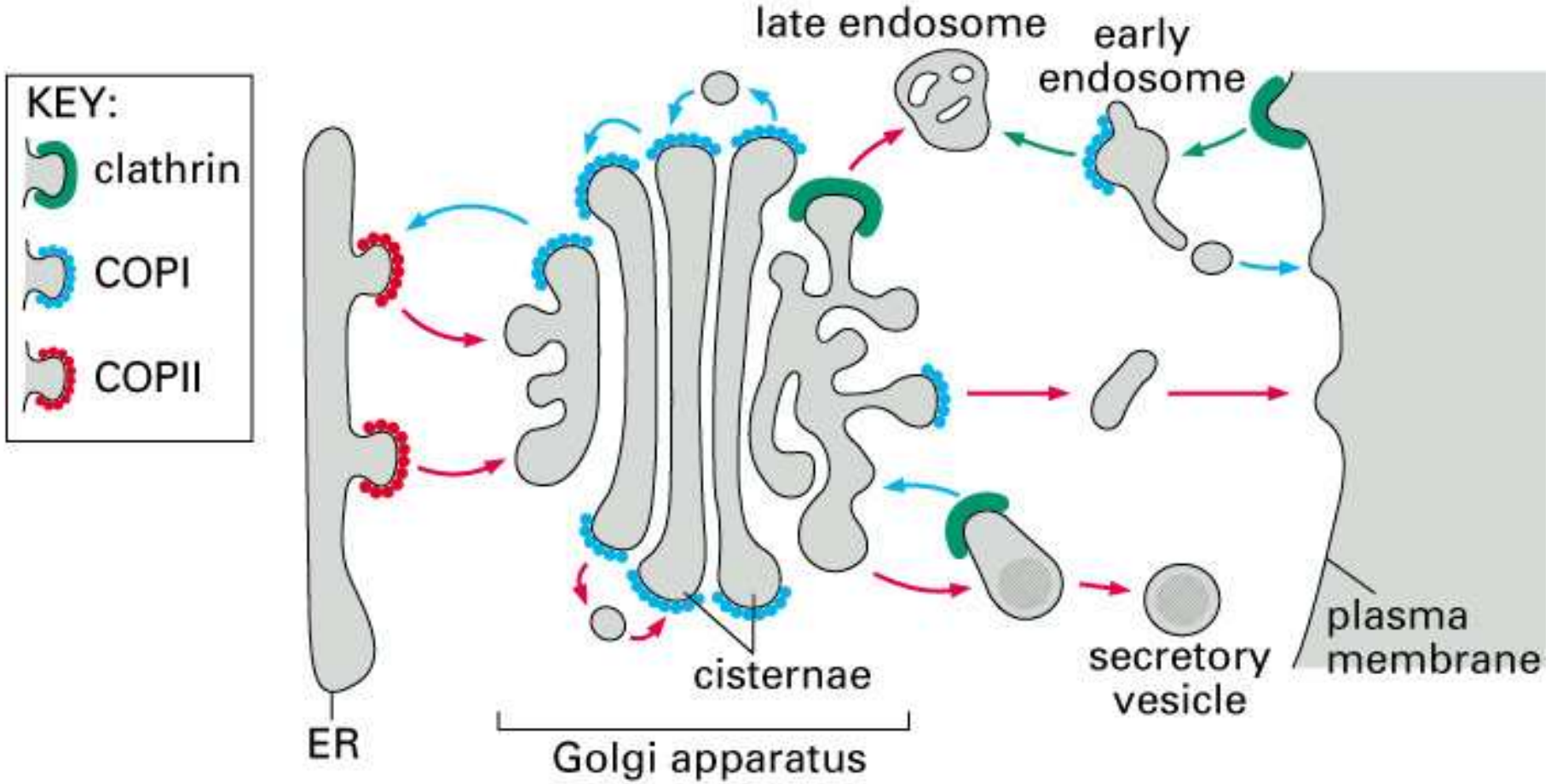


Figure 13–5. Molecular Biology of the Cell, 4th Edition.

COPII mediates ER to Golgi transport.

COPI mediates retro-transport through the Golgi to ER, but also forward GA transport.

Vesicle formation in the transport assay

- Addition of GTPγS to the Golgi transport assay inhibited transport
- Immunoelectron microscopy of Golgi membranes incubated with GTPγS showed an accumulation of coated vesicles
- Vesicles were isolated and protein coat purified

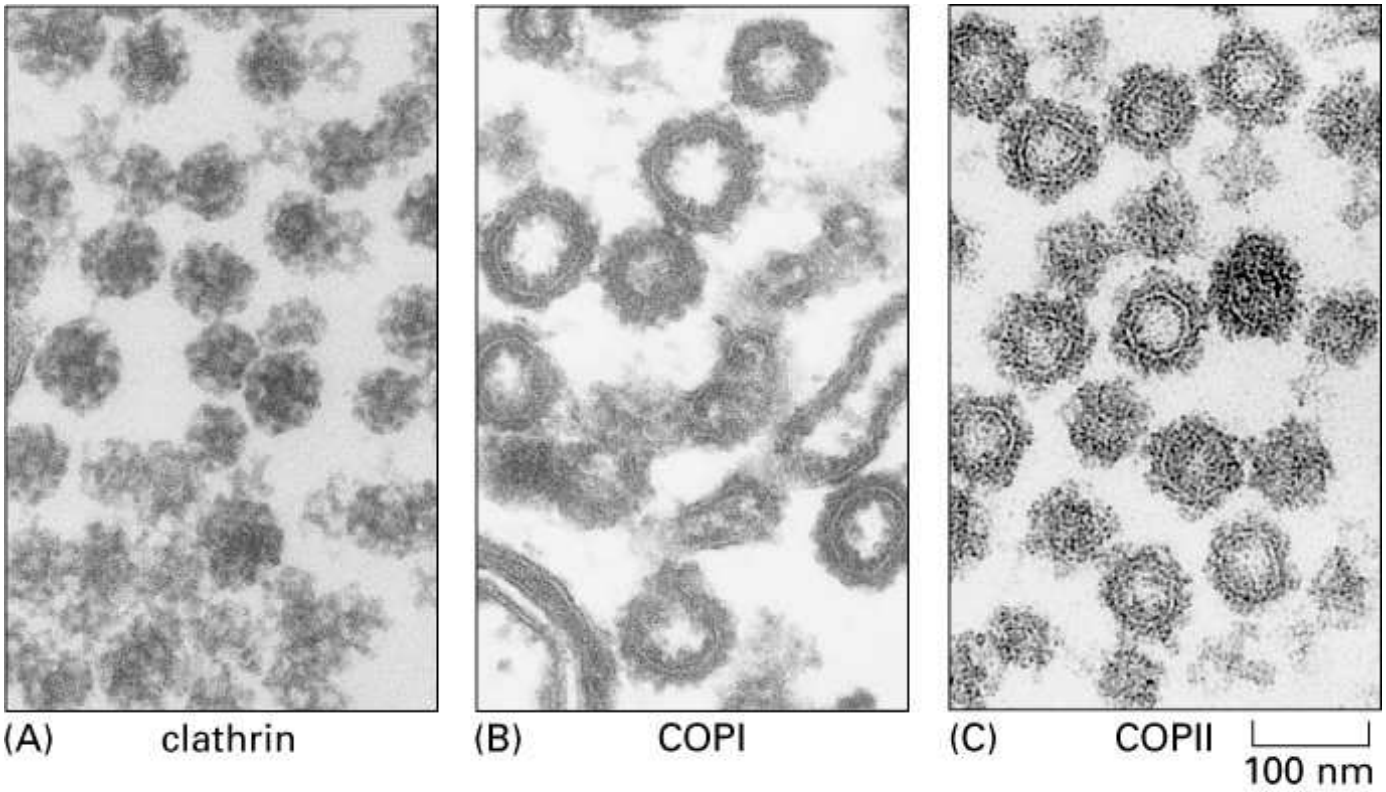


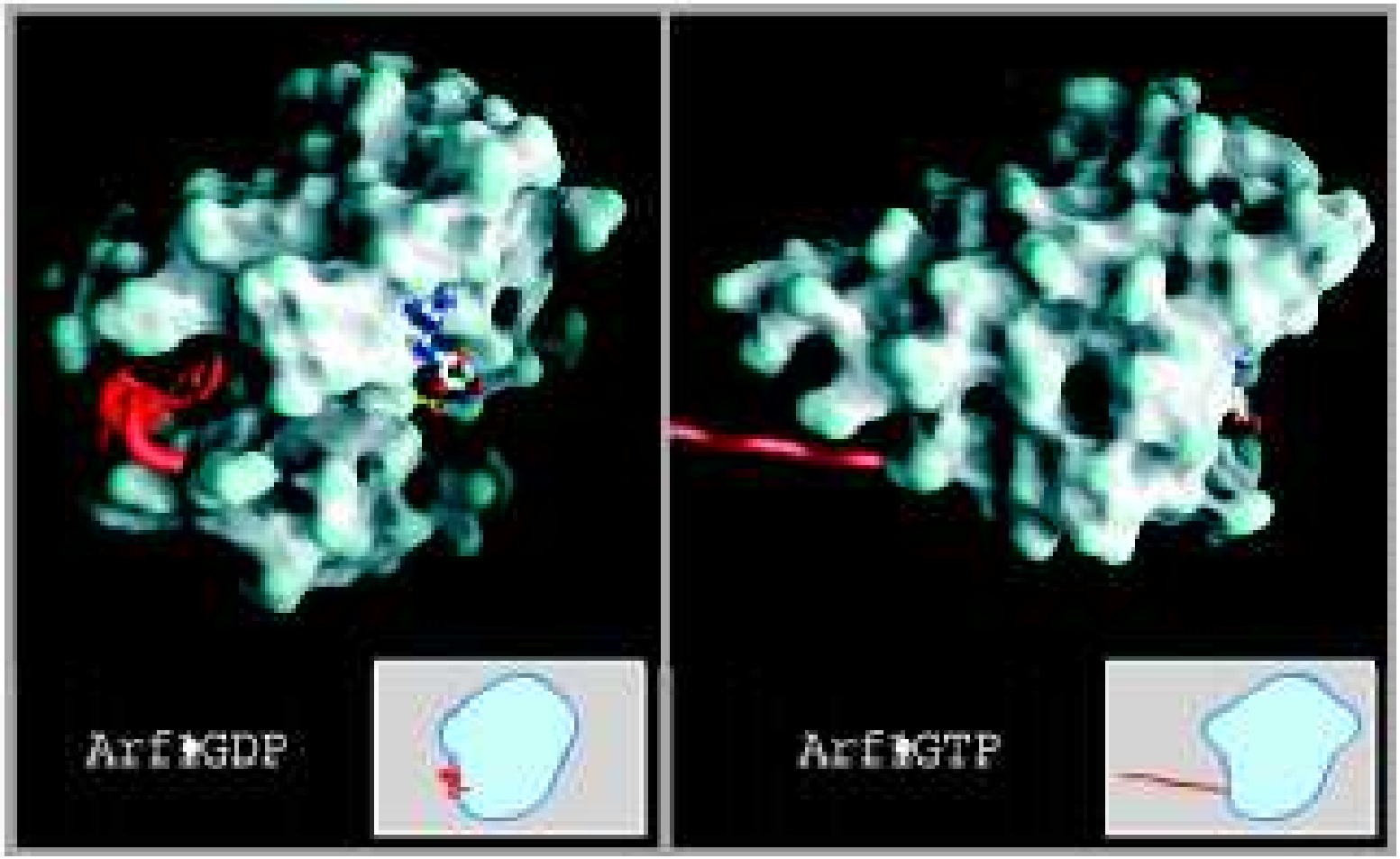
Figure 13–4. Molecular Biology of the Cell, 4th Edition.

COPI = coat protein or coatomer
Coatomer is a 7 subunit complex preassembled in
the cytoplasm that is recruited to membranes through a GTPase.

ARF -ADP ribosylation factor

- GTPase switch
  - Active = GTP form
  - Inactive = GDP form
- Cycles between cytoplasm and membrane
  - Modified at its N-terminus with fatty acid myristate (see handout on post-translational modifications)
  - Myristoyl-GTP switch
    - In GDP form, hydrophobic myristate is buried
    - In GTP form, myristate is extruded and interacts with membranes

Nucleotide-dependent confomational changes in Arf1

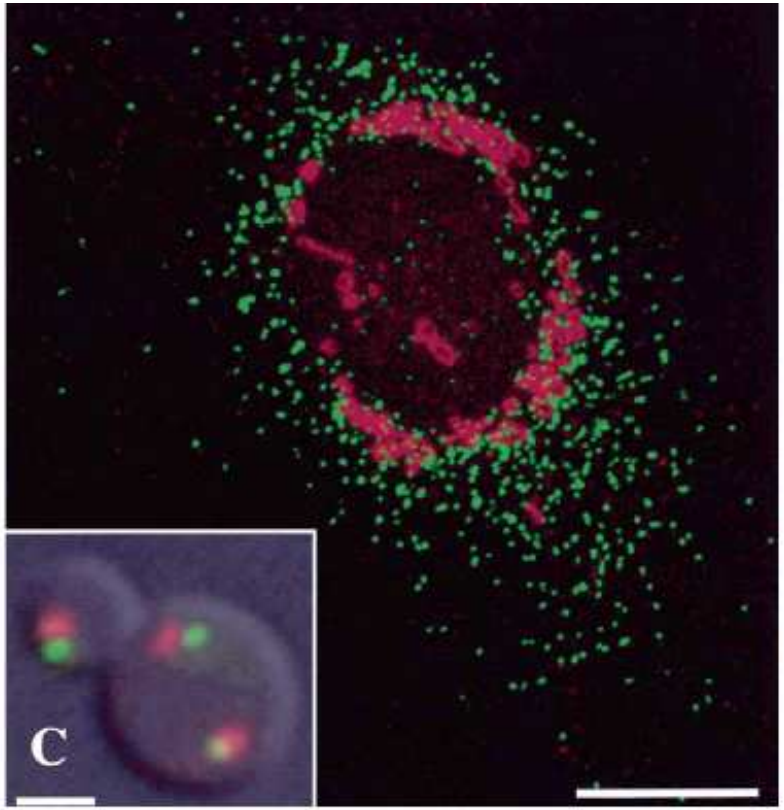


Lee et al. 2004

Malé GTPázy regulují tvorbu obalů

- Sar1 – COPII.
- ARF1 a další ARF homolgy – COPI a také CCV = clathrin coat.ves.
- Další neprostudované typy obalů mohou využívat další GTPázy.

ER to Golgi Transport - Yeast vs. Mammalian Cells

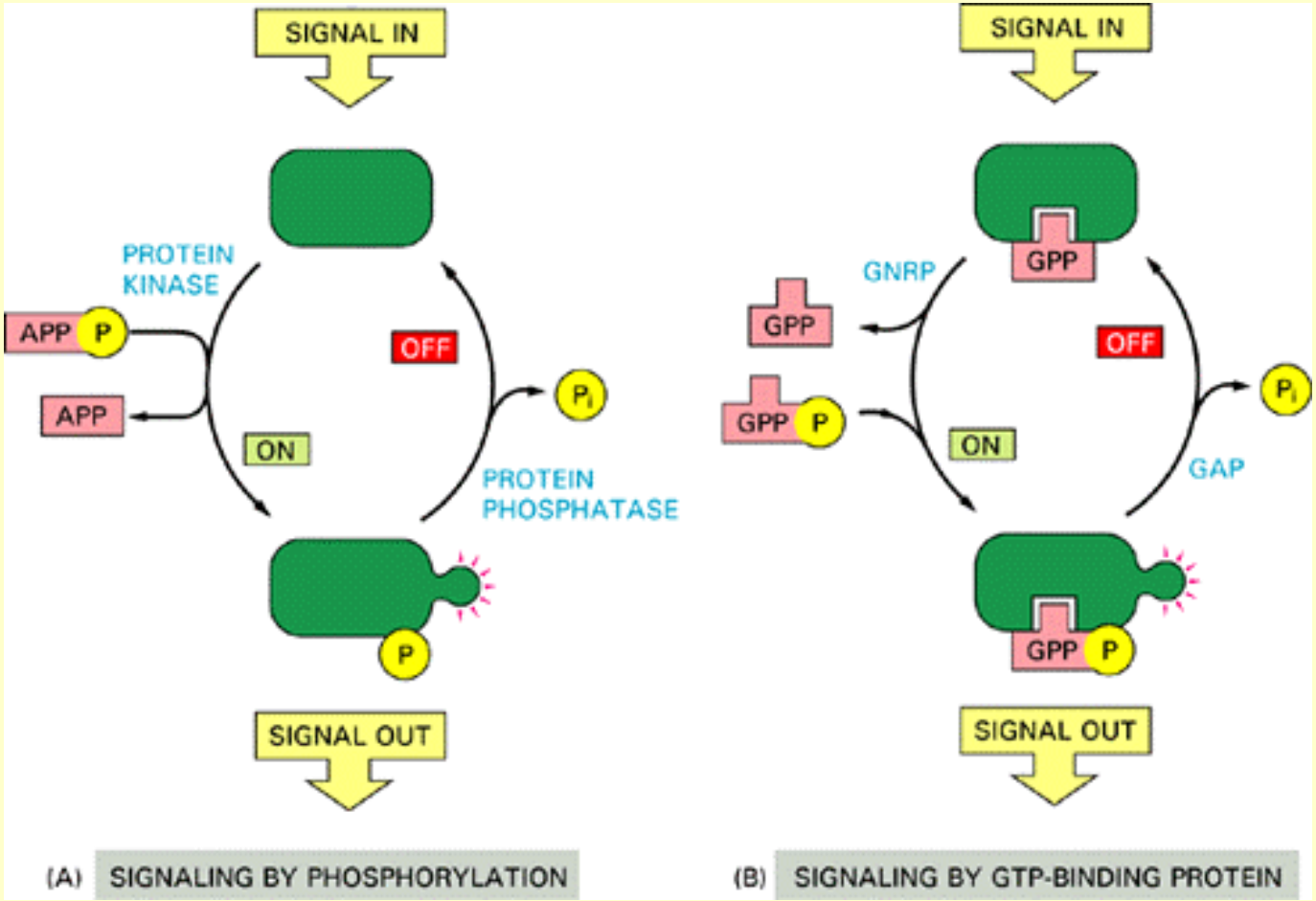


- Mammalian NRK cell stained for **ER exit sites** (COPII component mSec13, green) and cis-Golgi marker Giantin, red).
- Yeast cell stained for transitional ER (COPII - Sec13p) and Cis-Golgi marker Sec7p (red).
- Note differences in physical distances

JAK JE TO U ROSTLIN?

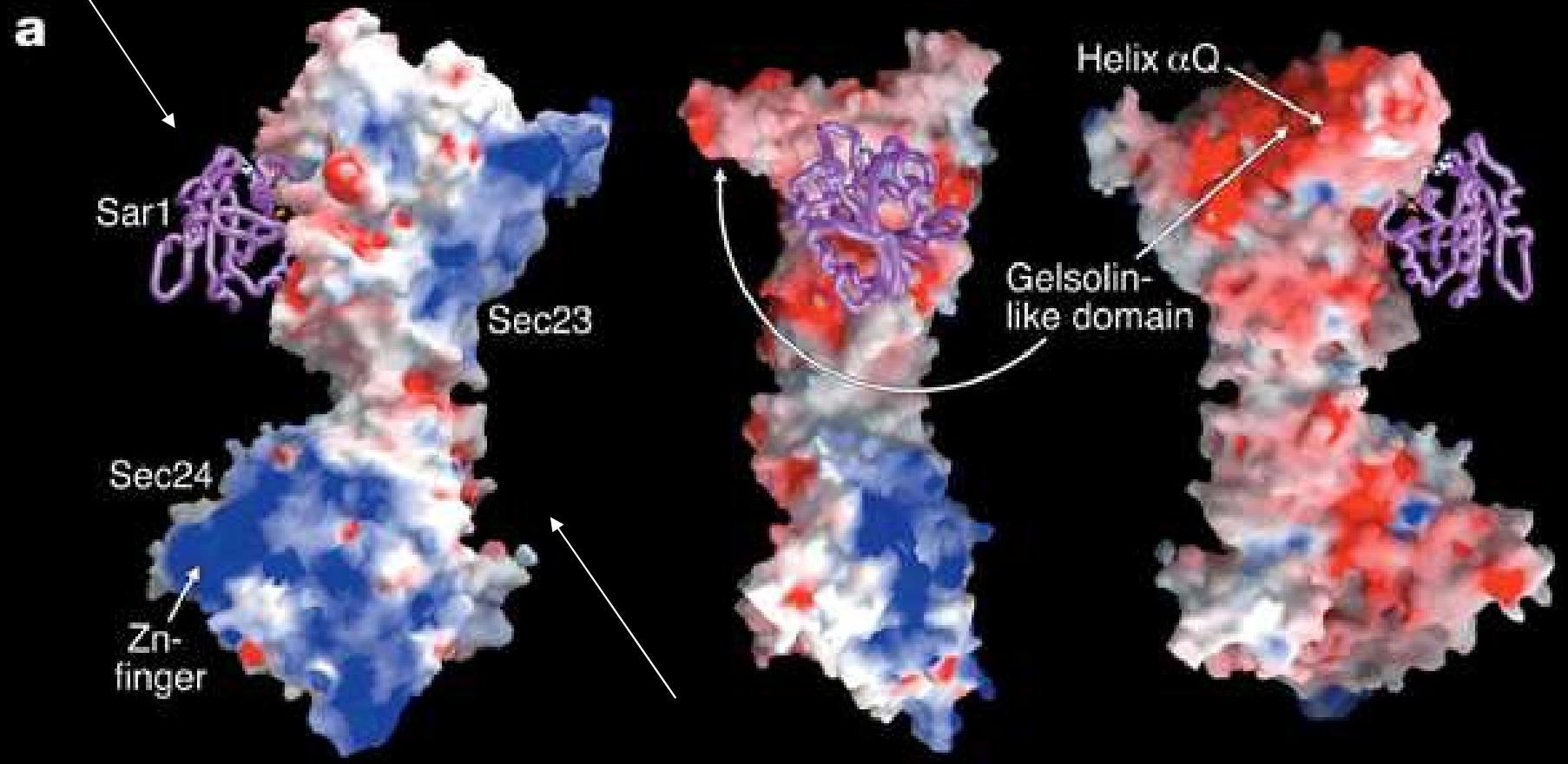
Duden 2004

Two major signaling mechanisms of eucaryotic cells



Molecular Biology of the Cell, 3rd edn, by B. Alberts, D. Bray, J. Lewis, M. Raff, K. Roberts, and J.D. Watson

Conformational change in Sar1
N-terminus with GDP/GTP exchange - promotes insertion into membrane



Membrane binding surface is basic.

SAR1-dependent binding of Sec23/24 to synthetic liposomes requires acidic phospholipids.

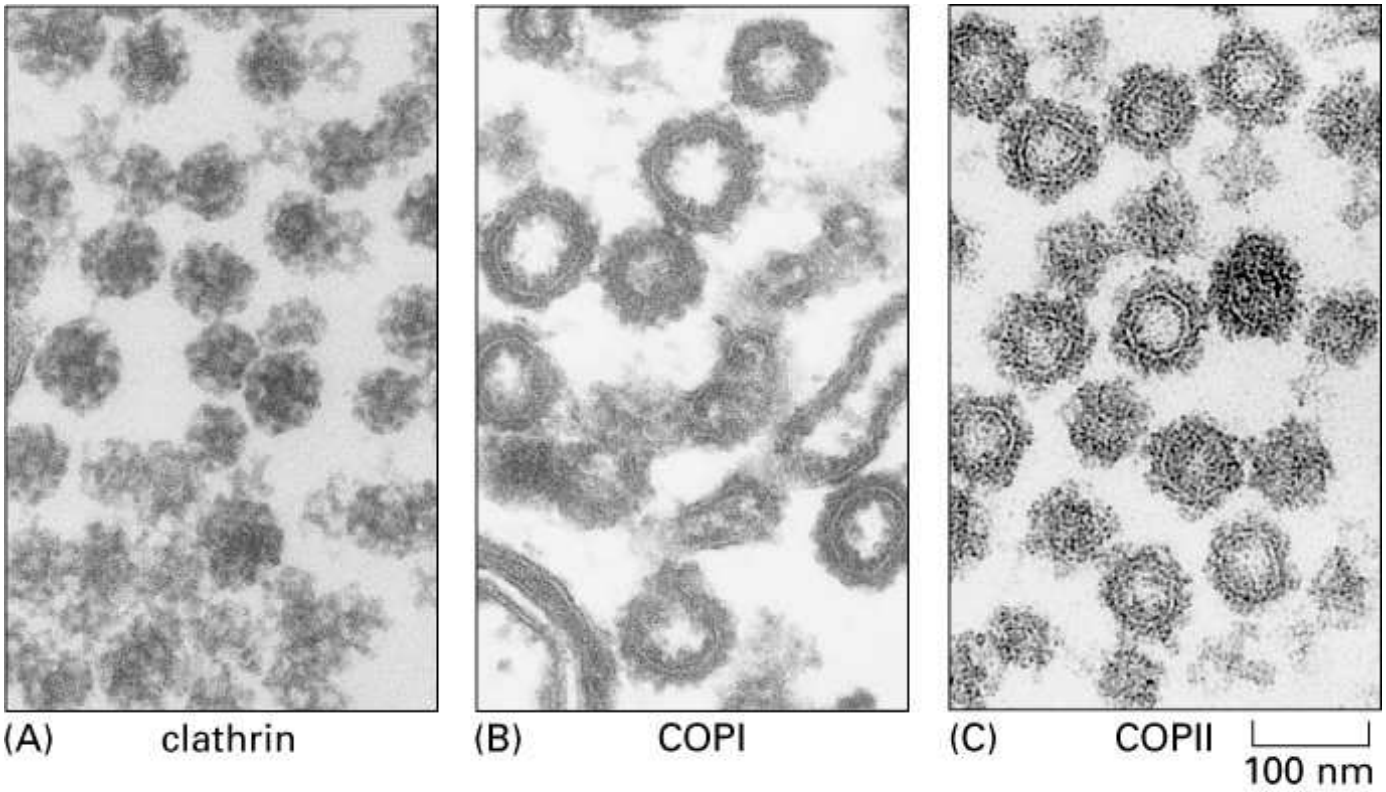
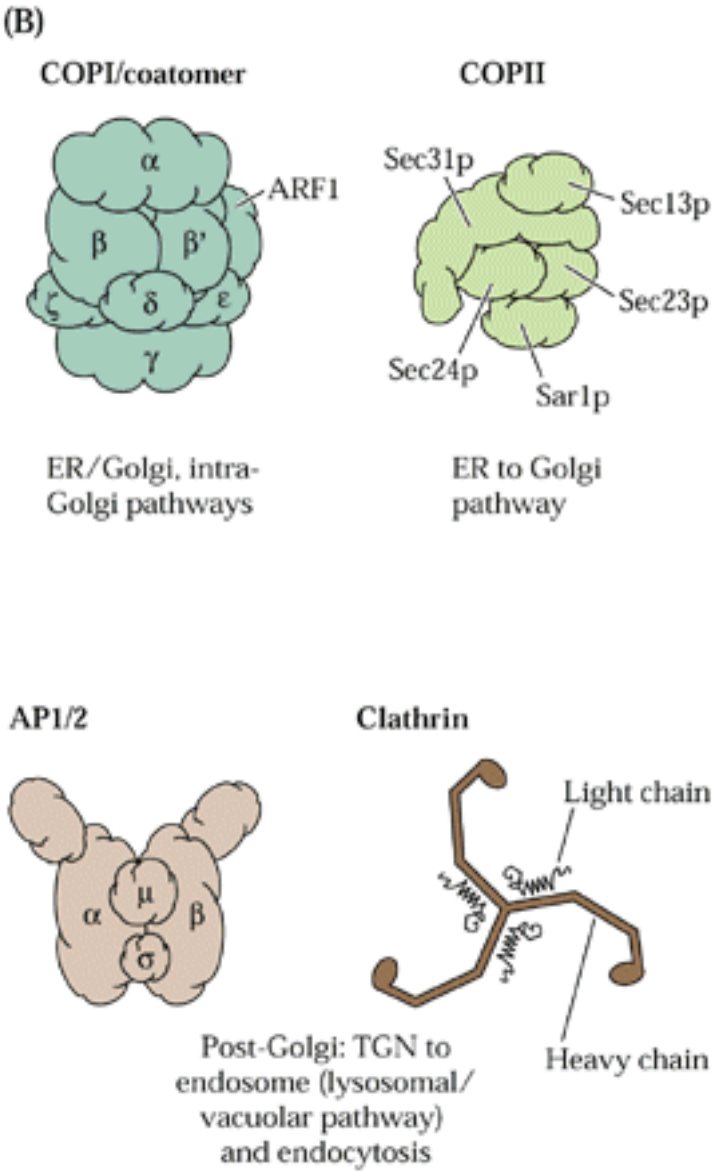
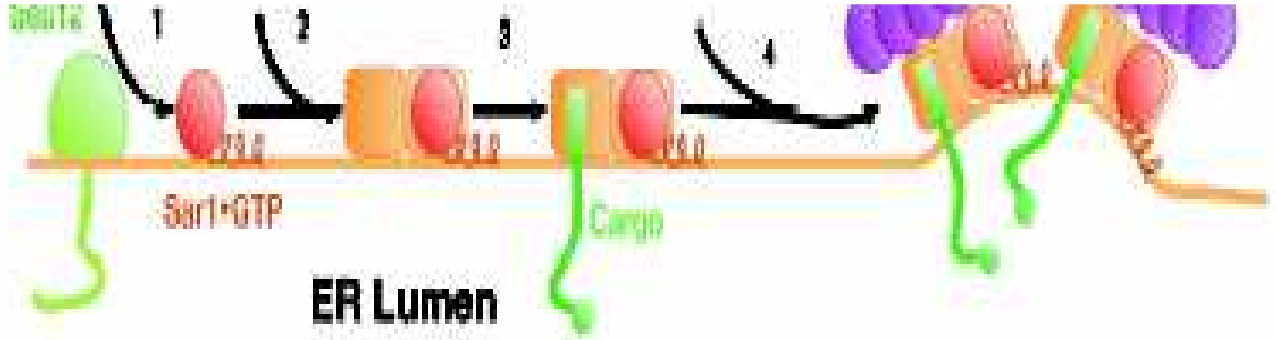


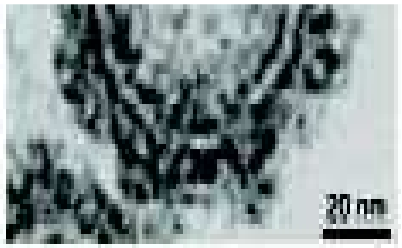
Figure 13–4. Molecular Biology of the Cell, 4th Edition.

COPII = protein coat that forms to vesiculate ER membranes

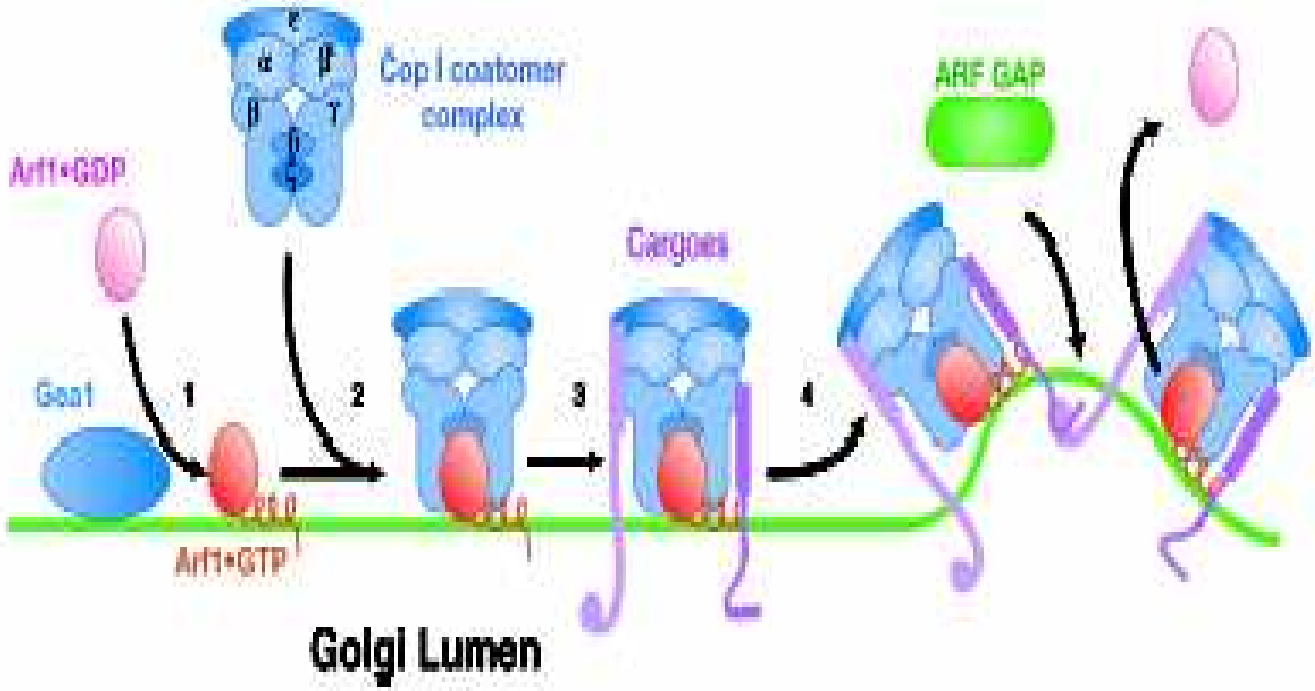
(B)


COPI/coatomer
α
ARF1
β
β'
ζ
δ
ε
γ
ER/Golgi, intra-Golgi pathways
COPII
Sec31p
Sec13p
Sec23p
Sec24p
Sar1p
ER to Golgi pathway
AP1/2
α
μ
β
σ
Clathrin
Light chain
Heavy chain
Post-Golgi: TGN to endosome (lysosomal/vacuolar pathway) and endocytosis





b Retrograde transport: COPI vesicles



CopI
cis Golgi to ER
and intra Golgi

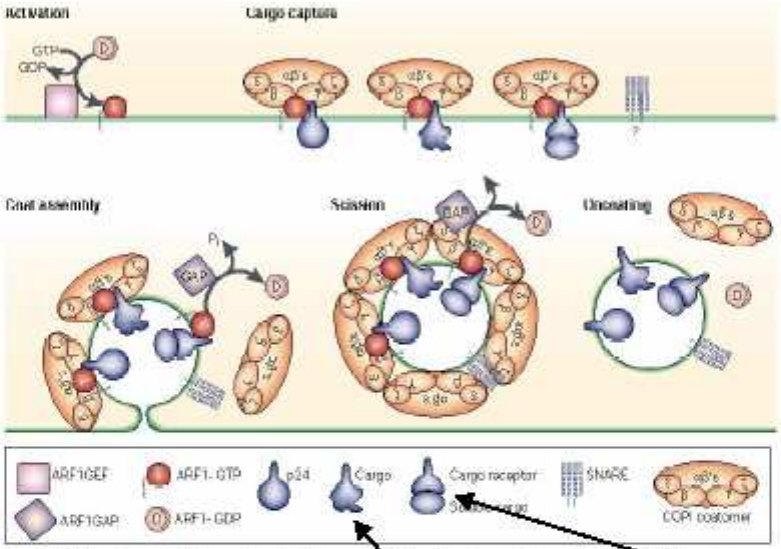


Figure 3 | **The key steps in the formation of COPI-coated vesicles.** Coat assembly is activated by the recruitment of ARF1-GTP to the membrane. This allows the binding of the COPI coatomer and the recruitment of cargo. GTP hydrolysis is slow when ARF1 is bound to its preferred cargo, allowing kinetic regulation of coat recruitment. Membrane deformation occurs at the same time as coat recruitment. When the coat is complete, the vesicle buds. The GTPase activity of ARF1 is enhanced by ARF1GAP, which acts as a timer, leading to inactivation of ARF1 and uncoating. (ARF1, ADP-ribosylation factor 1; ARF1GAP, ADP-ribosylation factor 1 GTPase activating protein; ARF1GEF, ADP-ribosylation factor 1 guanine exchange factor.)

e.g., ERD2
and KDEL protein

1. ARF1=small GTPASE, initiates coating in GTP form (myristoylated)
2. ARF1-GEF=marks the spot This is a brefeldin A target
3. ARF1-GAP=stimulates ARF1 GTPase

4. Cargo membrane protein = KKXX motif in C-terminus
5. Cargo receptor, e.g. KDEL receptor
6. Coatamer = coat recruited by ARF1-GTP and cargo membrane protein. γ subunit recognizes KKXX

Signals combined with the machinery for vesicle trafficking give the overall specificity.

Kirchhausen T. Three ways to make a vesicle. Nat Rev Mol Cell Biol. 2000 Dec;1(3):187-98. Review.

CopII
ER to cis Golgi

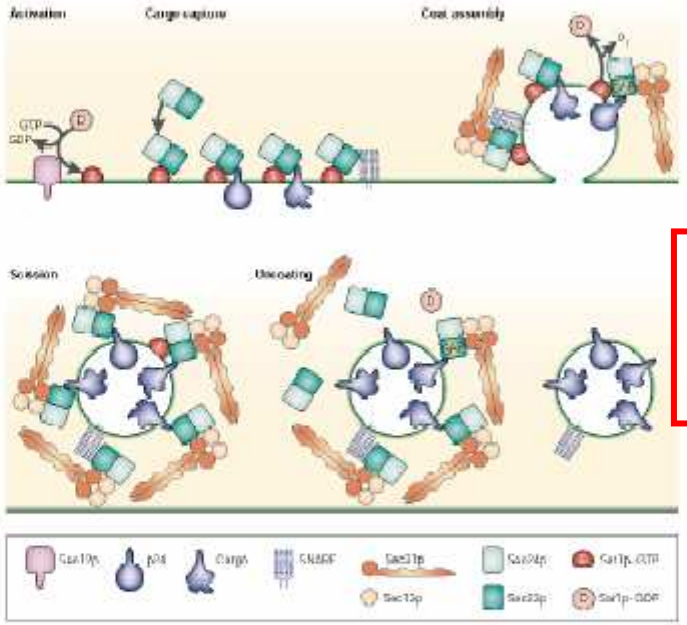
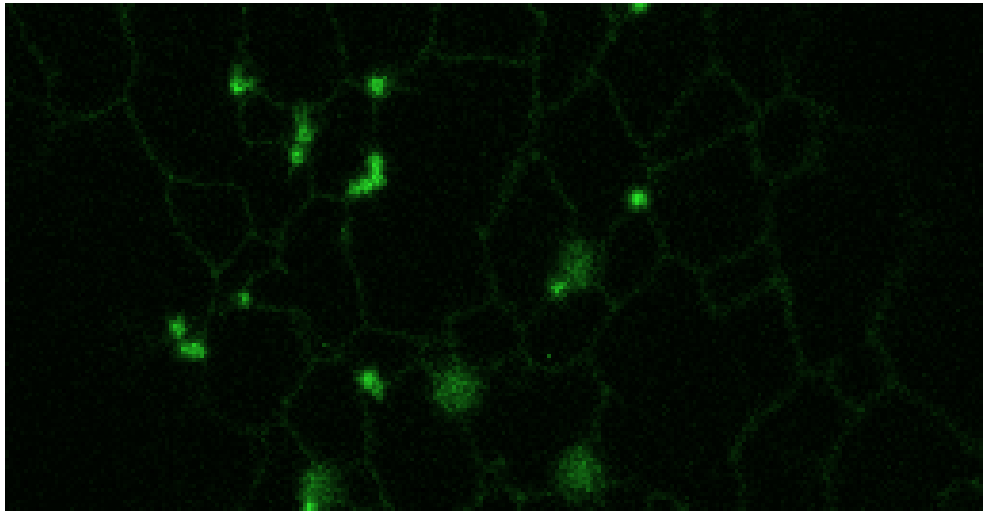


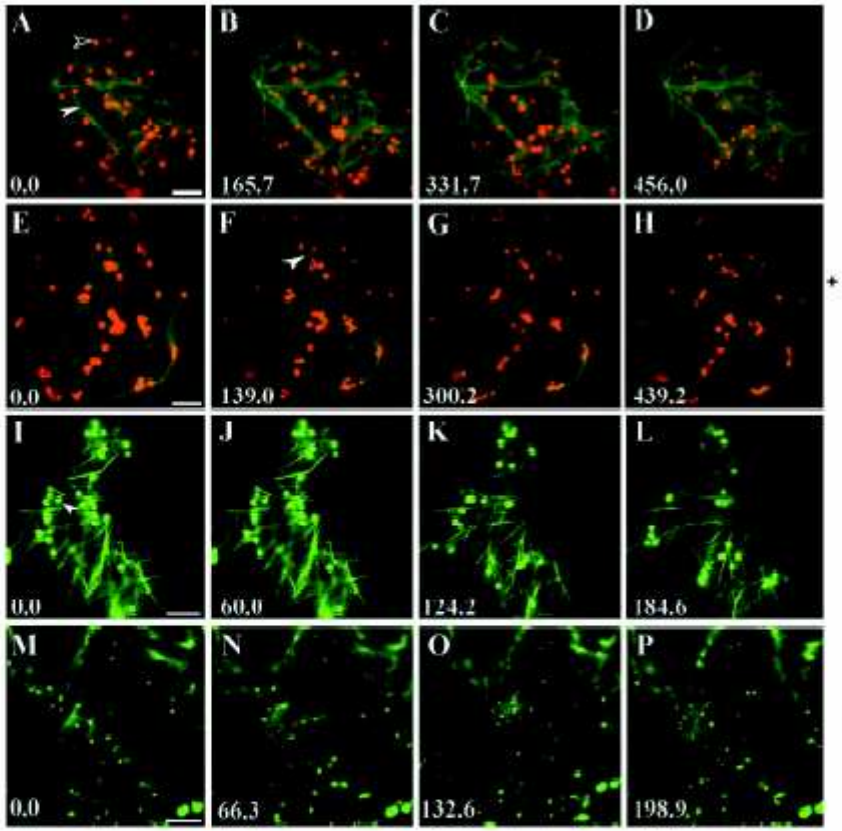
Figure 2 | **The key steps in the formation of COPII-coated vesicles.** Coat assembly is activated by the recruitment of Sar1p–GTP to the membrane. This occurs by the binding of the Sec12p–Sec24p complex and the recruitment of cargo. The Sec13p–Sec31p complex binds next, leading to membrane deformation. When the coat is complete, the vesicle buds. The GTPase activity of Sar1p is enhanced by Sec23p, which acts as a timer, leading to inactivation of Sar1p and uncoating. (GAP, GTPase activating protein.)

This step is well characterized in yeast and animals. The machinery (esp Sar1p) is required for transport in plants, but the vesicles haven't been observed.

1. **Sar1p= Small GTPASE initiates coating in GTP form**
2. **Sec12 = Guanine nucleotide exchange factor (GEF) for Sar1p – marks the spot**
3. **Sec23 = Sar1p GAP (stimulates GTPase)**
4. **Sec24 = Along with Sec23 recruit cargo**
5. **P24 = possible adaptor for cargo recruiting**
6. **Sec31/Sec13=part of the coat**
7. **vSNARE = proteins required for fusion**

GA rostlin se pohybuje po ER/aktinu





Red = Golgi
Green = actin
binding talon

+ lantrunculinn

tubulin GFP
and ST-GFP

+colchicine

Figure 4. Golgi Stack Movement Requires the Actin Cytoskeleton and Occurs Independently of the Presence of Microtubules.

(A) to **(D)** Time lapse of a cell cotransformed with talin-GFP and ST-YFP. Golgi stacks (open arrowhead) align on actin cables (closed arrowhead). The actin network appears to be highly mobile. Bar = 5 μm.

(E) to **(H)** One hour of latrunculin B treatment (25 μM) induces actin depolymerization and cytoplasmic release of the talin-GFP construct. Cytoplasmic organelles are visible in negative contrast (**F**], arrowhead). Golgi movement is inhibited strongly after 1 h of latrunculin B treatment. Compare the time sequence **(A)** to **(D)** with **(E)** to **(F)** and note that the Golgi stacks in **(E)** to **(H)** are relatively immobile compared with those in **(A)** to **(D)** within similar time frames. Bar = 5 μm.

(I) to **(L)** Time lapse of an epidermal cell cotransformed with a tubulin-GFP construct and ST-GFP. Golgi stacks are mostly independent of the microtubule cytoskeleton ([I], arrowhead). Bar = 5 μm.

(M) to **(P)** Depolymerization of microtubules with the drug colchicine does not prevent Golgi stacks from moving. This time series was taken after 1 h of treatment with 1 mM colchicine. Bar = 10 μm.

Time is expressed in seconds at the bottom left of each frame.

Brandizzi et al.
The Plant Cell, Vol. 14,
1293–1309, June 2002,

Jak ER a GA "komunikují" - transportují váčky?

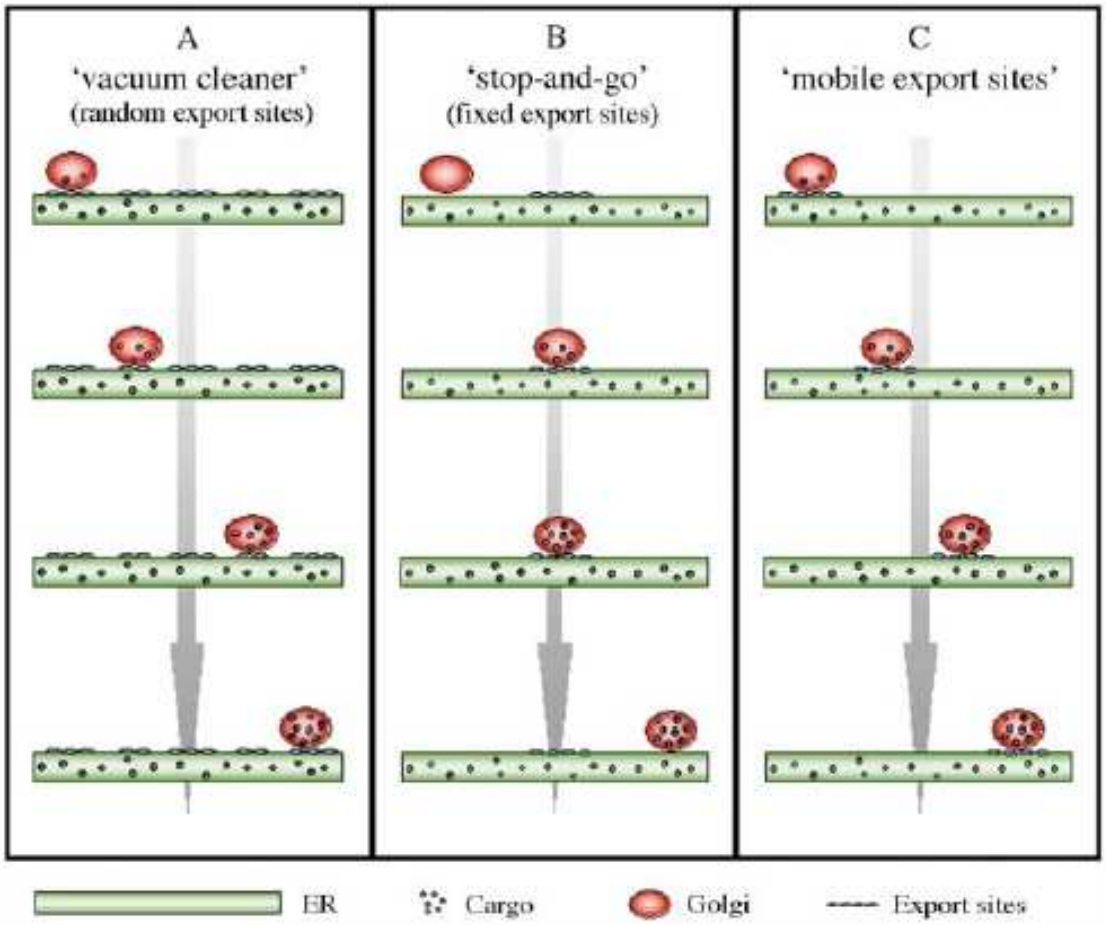


FIG. 3. Models of ER-to-Golgi protein transport. A, The 'vacuum cleaner model' (Boevink *et al.*, 1998) suggests that Golgi stacks move over the ER constantly picking up cargo. According to this model, the whole ER surface is capable of forming export sites, resulting in their random distribution. In contrast, the 'stop-and-go' model (B) hypothesizes that Golgi stacks stop at fixed ER export sites to take up cargo from the ER, before moving onto the next stop. In the more dynamic 'mobile export sites' model (C), Golgi stacks and ER export sites move together as 'secretory units' (Brandizzi *et al.*, 2002*b*) allowing cargo to be transported from the ER towards the Golgi at any time during movement.

FRAP

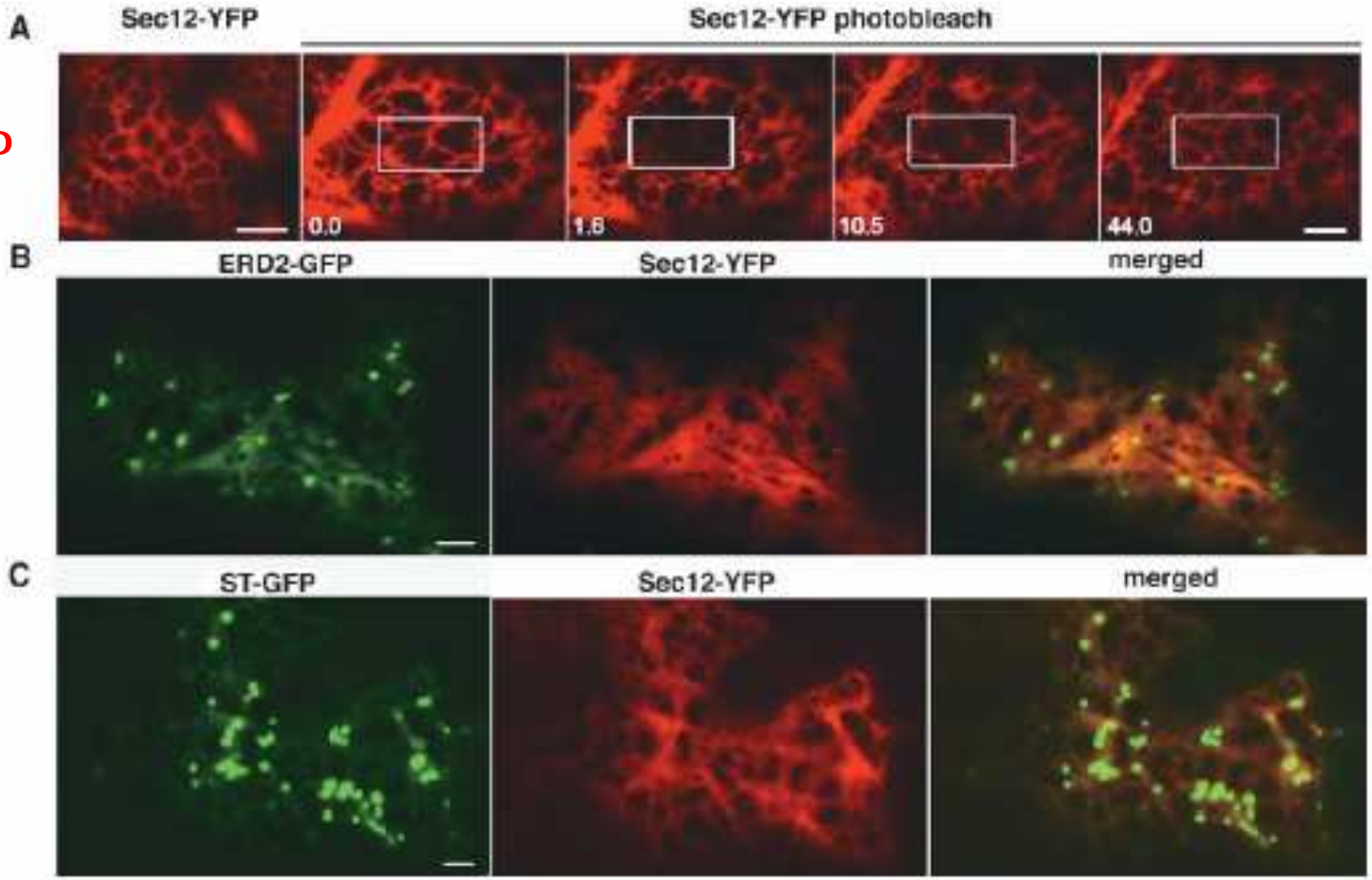


Figure 6. The Sar1p-Specific Guanine Exchange Factor, Sec12p, Distributes in the ER and Moves Freely in the ER Membranes.

(A) Tobacco leaf epidermal cell expressing Sec12-YFP, which labels the ER, and photobleaching of a region of interest (white bordered) of the cortical ER. Time lapse is indicated in seconds at bottom left corner. Note the rapid recovery of fluorescence in the bleached area. Scale bars = 5 μm.

(B) and **(C)** Cell coexpressing Sec12-YFP with either ERD2-GFP **(B)** or ST-GFP **(C)**. Note that Sec12-YFP does not accumulate in discrete punctate structures as Sar1-YFP when coexpressed with a Golgi marker. Scale bars = 5 μm.

AtSar1 kolokalizuje s GA

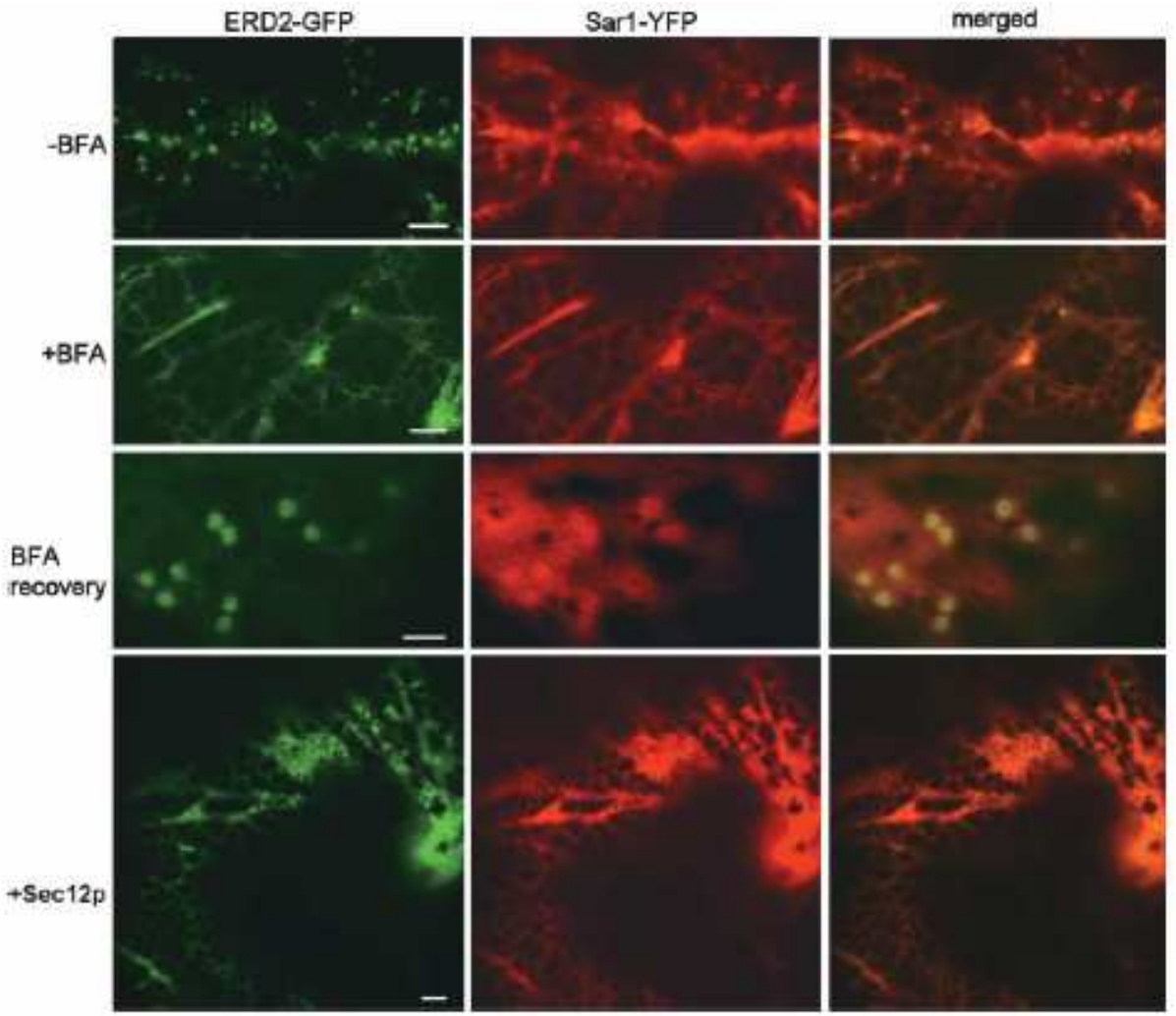


Figure 7. Accumulation of Sar1-YFP at the Golgi Apparatus Vicinity Is Dependent on the Functionality of ER Protein Export.

Cells coexpressing ERD2-GFP and Sar1-YFP show fluorescent Golgi bodies and Sar1-YFP punctate structures (−BFA). Both disappear upon treatment with BFA (100 μg/mL for 1 h; +BFA). This phenomenon is reversible by washing out BFA (BFA recovery). Scale bars = 5 μm. Upon BFA washout, ERD2-GFP redistributes in the Golgi bodies and Sar1-YFP reaccumulates in the vicinity of Golgi bodies. Scale bar = 2 μm. In leaf epidermal cells coexpressing ERD2-GFP, Sar1-YFP and untagged Sec12p (+Sec12p), a reduced accumulation of GFP fluorescence in the Golgi bodies and Sar1-YFP is observed. Scale bar = 5 μm.

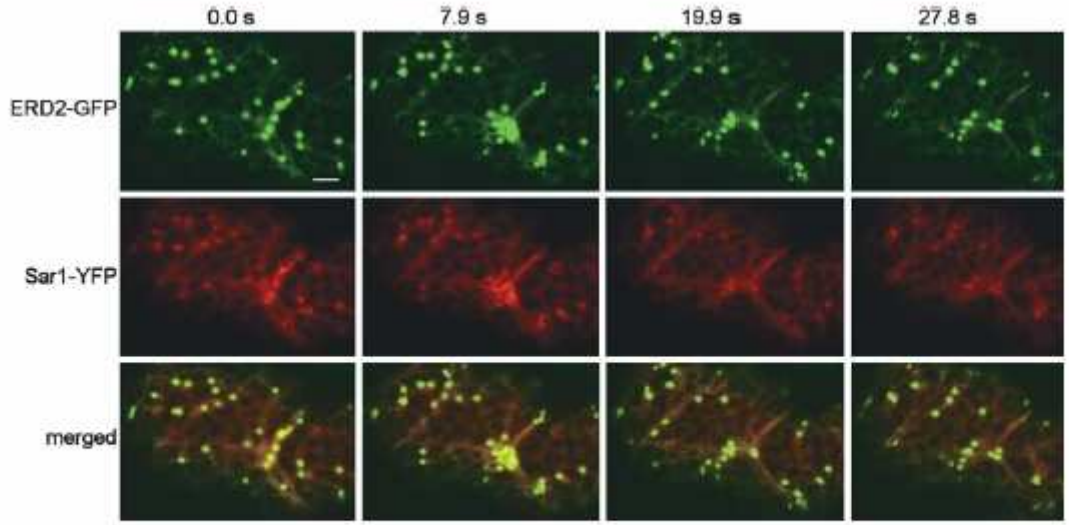
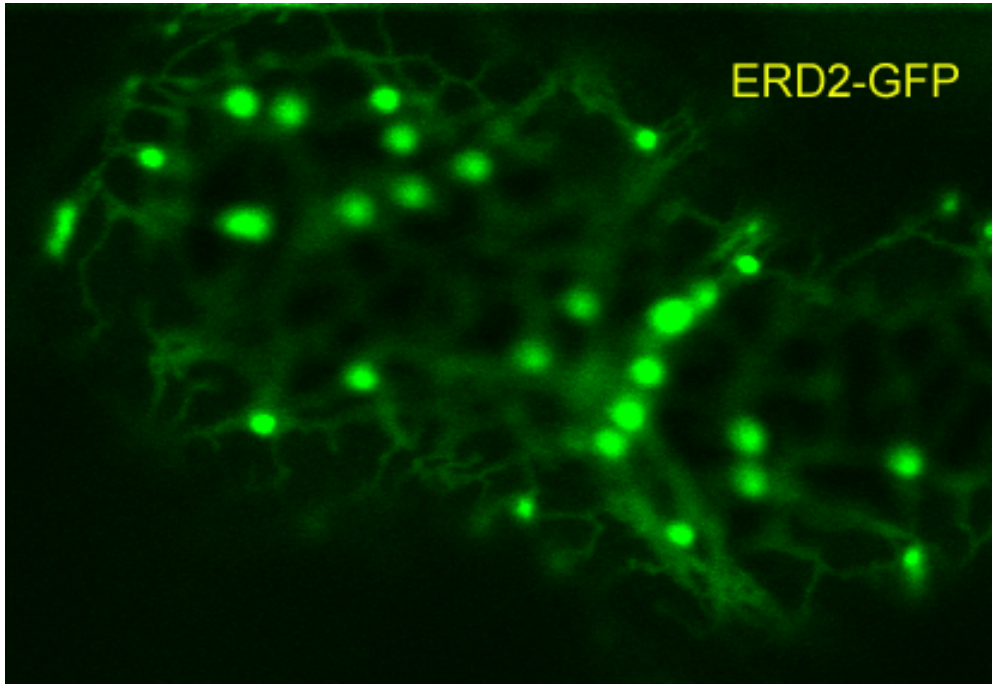
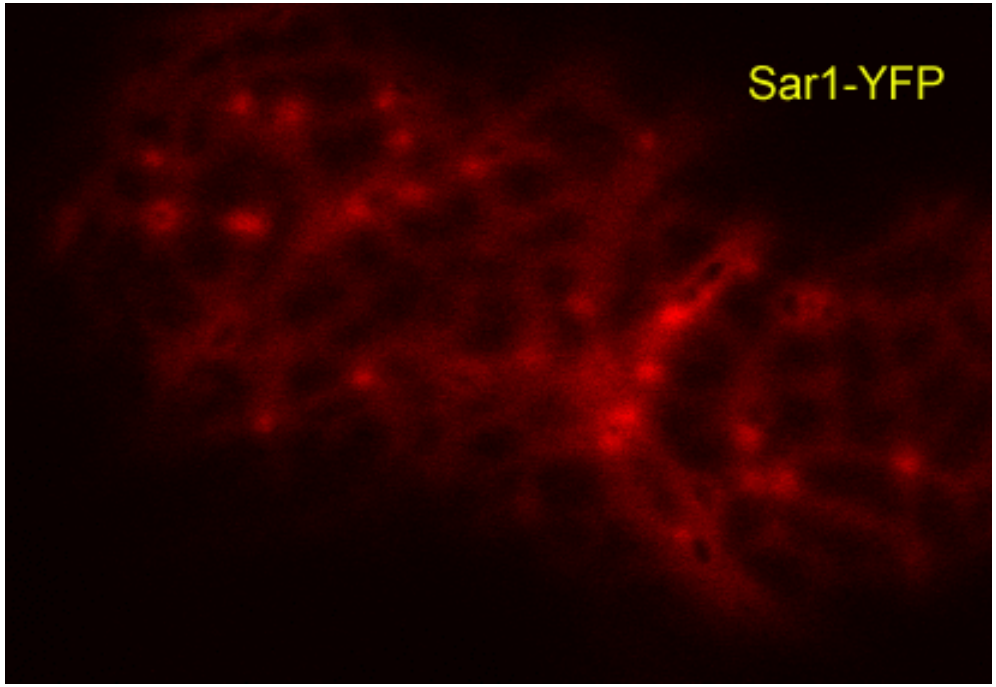
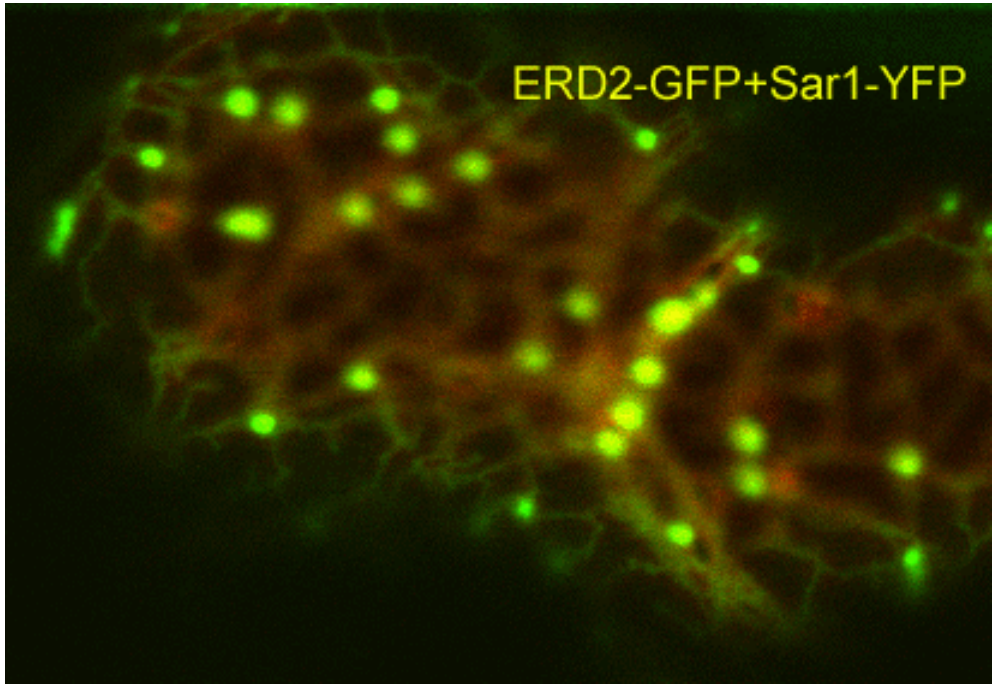


Figure 8. Golgi Stacks and Sar1-YFP Structures Move Together.

Time lapse of a cell cotransformed with ERD2-GFP and Sar1-YFP. Note that Golgi stacks and Sar1-YFP punctate structures move together in the cell at all times. Time of the acquisition of individual frames is indicated on top of ERD2-GFP panels. Scale bar = 5 μm.


ERD2-GFP


Sar1-YFP


ERD2-GFP+Sar1-YFP

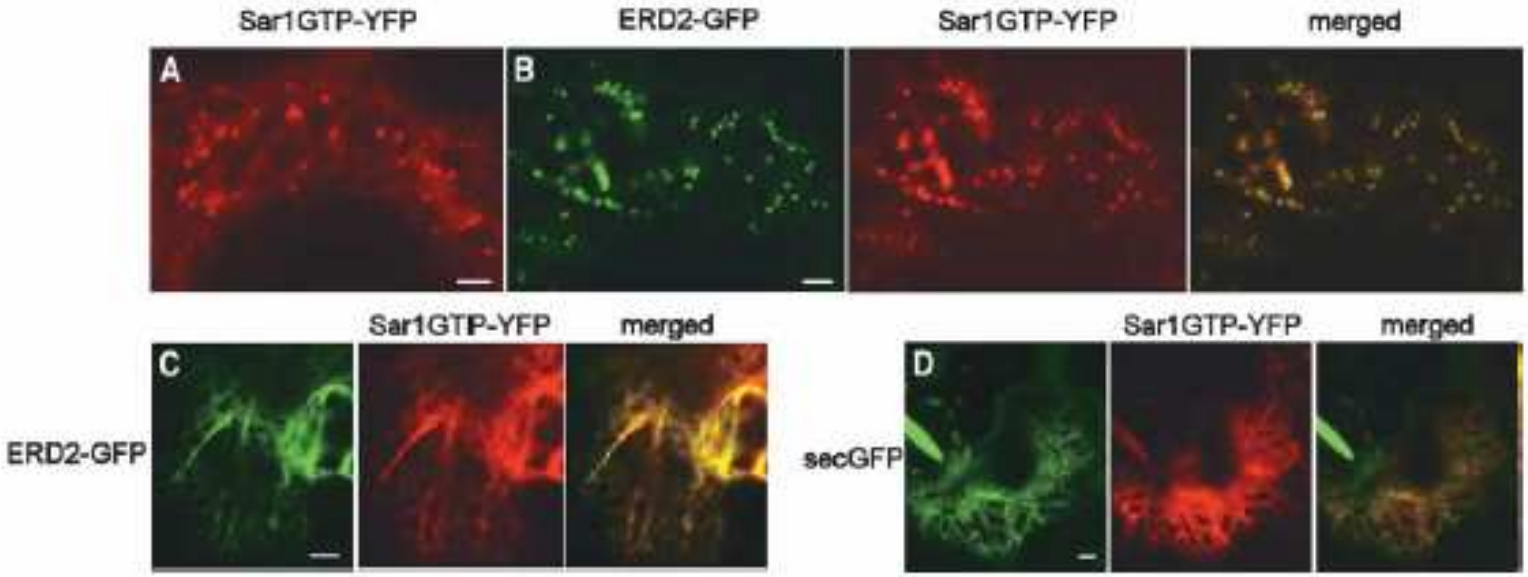


Figure 11. Sar1-GTP-YFP Accumulates in Punctate Structures and Exerts a Dominant Negative Effect on Protein Secretion.

(A) A tobacco leaf epidermal cell expressing low levels of Sar1-GTP-YFP alone (bacterial OD at 600 nm = 0.01) shows punctate structures and cytosolic stain.

(B) Cell coexpressing ERD2GFP and Sar1-GTP-YFP. It is possible to verify that Golgi bodies colocalize with the punctate structures highlighted with Sar1-GTP-YFP (merged).

(C) and **(D)** Cells coexpressing Sar1-GTP-YFP, at higher levels (bacterial OD at 600 nm = 0.05) than cells in **(A)** and **(B)**, and either ERD2-GFP **(C)** or secGFP **(D)**, show GFP accumulation in the ER and a reduced appearance of Sar1-GTP-YFP structures. Note that the confocal microscope settings (laser power and detection gain) for imaging YFP fluorescence in **(C)** and **(D)** are lowered than in **(A)** and **(B)** to reduce oversaturation. Scale bars = 5 μm.

ERES putují s GA.

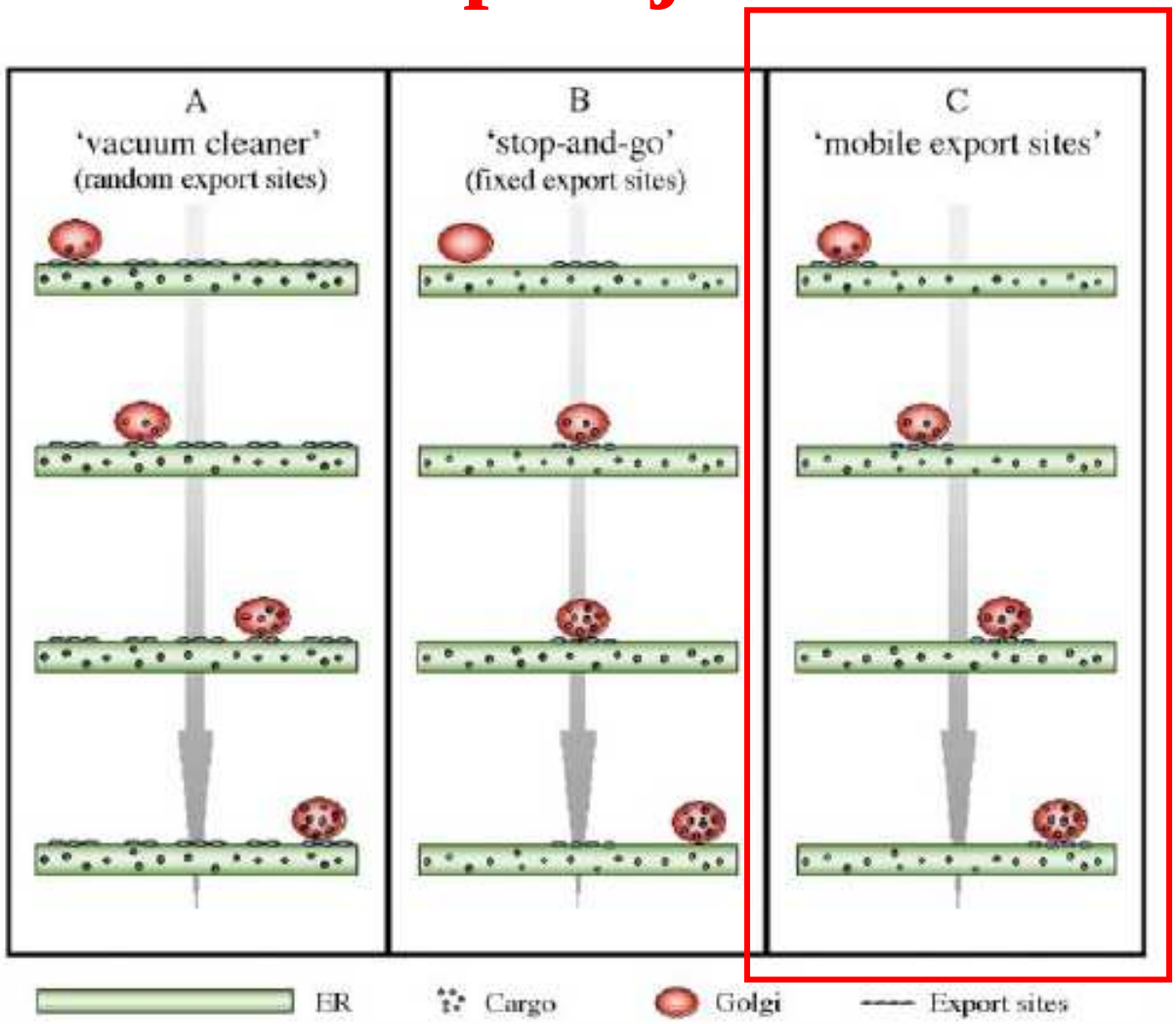
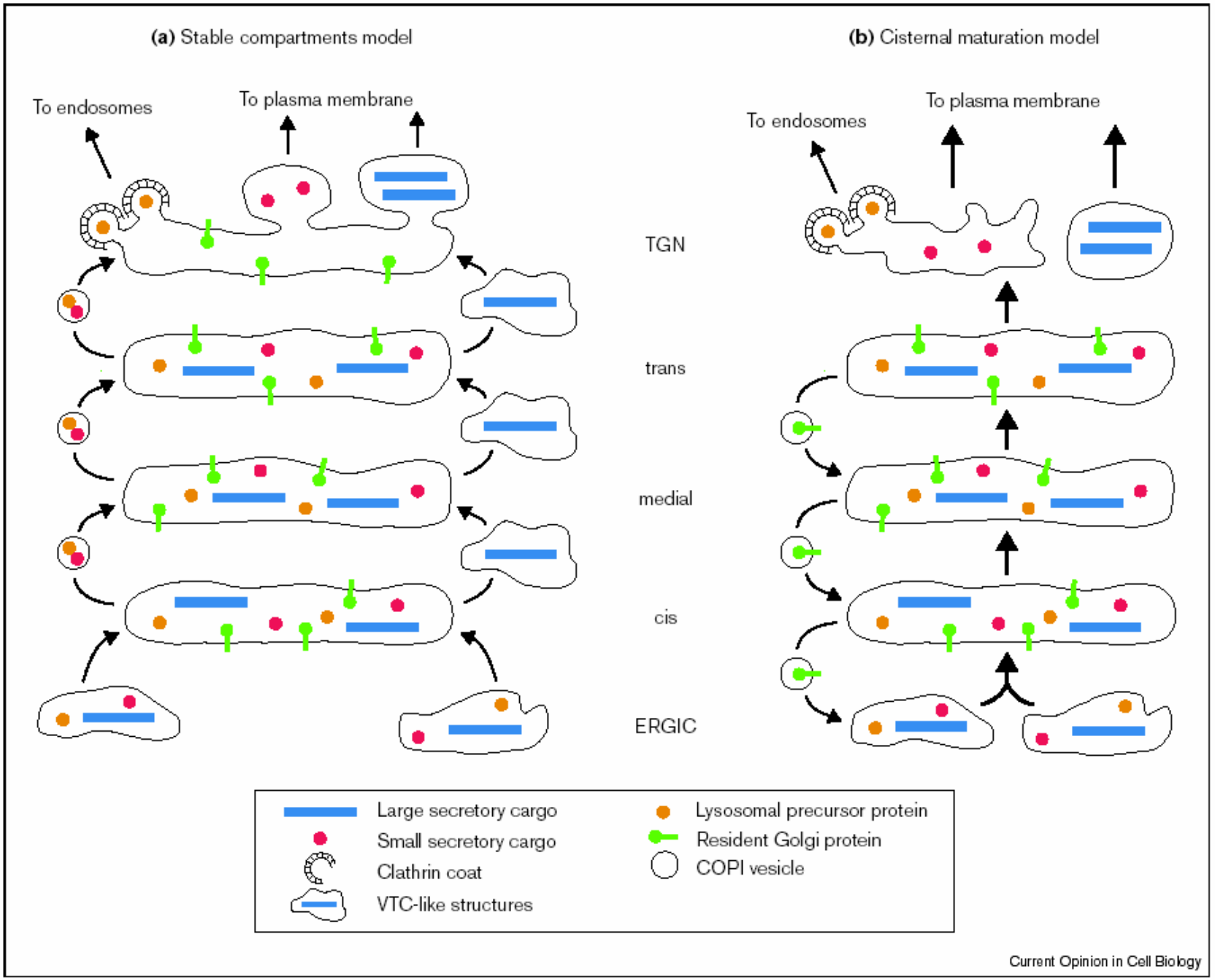


FIG. 3. Models of ER-to-Golgi protein transport. A, The 'vacuum cleaner model' (Boevink *et al.*, 1998) suggests that Golgi stacks move over the ER constantly picking up cargo. According to this model, the whole ER surface is capable of forming export sites, resulting in their random distribution. In contrast, the 'stop-and-go' model (B) hypothesizes that Golgi stacks stop at fixed ER export sites to take up cargo from the ER, before moving onto the next stop. In the more dynamic 'mobile export sites' model (C), Golgi stacks and ER export sites move together as 'secretory units' (Brandizzi *et al.*, 2002*b*) allowing cargo to be transported from the ER towards the Golgi at any time during movement.

Transport GA - Golgi aparátem

- Two Models
  - Stable Compartments: secretory cargo (large and small) moves through by a vesicle-mediated process
  - Cisternal Maturation Model: VTC's fuse into an ERGIC (ER-Golgi intermediate compartment). This matures into a cis-Golgi by removal of proteins found in earlier parts of the secretory pathway. These proteins are sorted into COPI vesicles that move retrograde.


(a) Stable compartments model
(b) Cisternal maturation model
To endosomes
To plasma membrane
To endosomes
To plasma membrane
TGN
trans
medial
cis
ERGIC
Large secretory cargo
Small secretory cargo
Clathrin coat
VTC-like structures
Lysosomal precursor protein
Resident Golgi protein
COPI vesicle
Current Opinion in Cell Biology

Glick 2000

Peri-Golgi vesicles contain retrograde but not anterograde proteins, consistent with the cisternal progression model of intra-Golgi transport

- Use quantitative immuno-EM to localize proteins
- Resident Golgi enzyme - mannosidase II
- Anterograde cargo - VSV-G
- Transport proteins - COPI, KDEL-receptor, giantin, and rBet1

Martinez-Menarguez et al. 2001

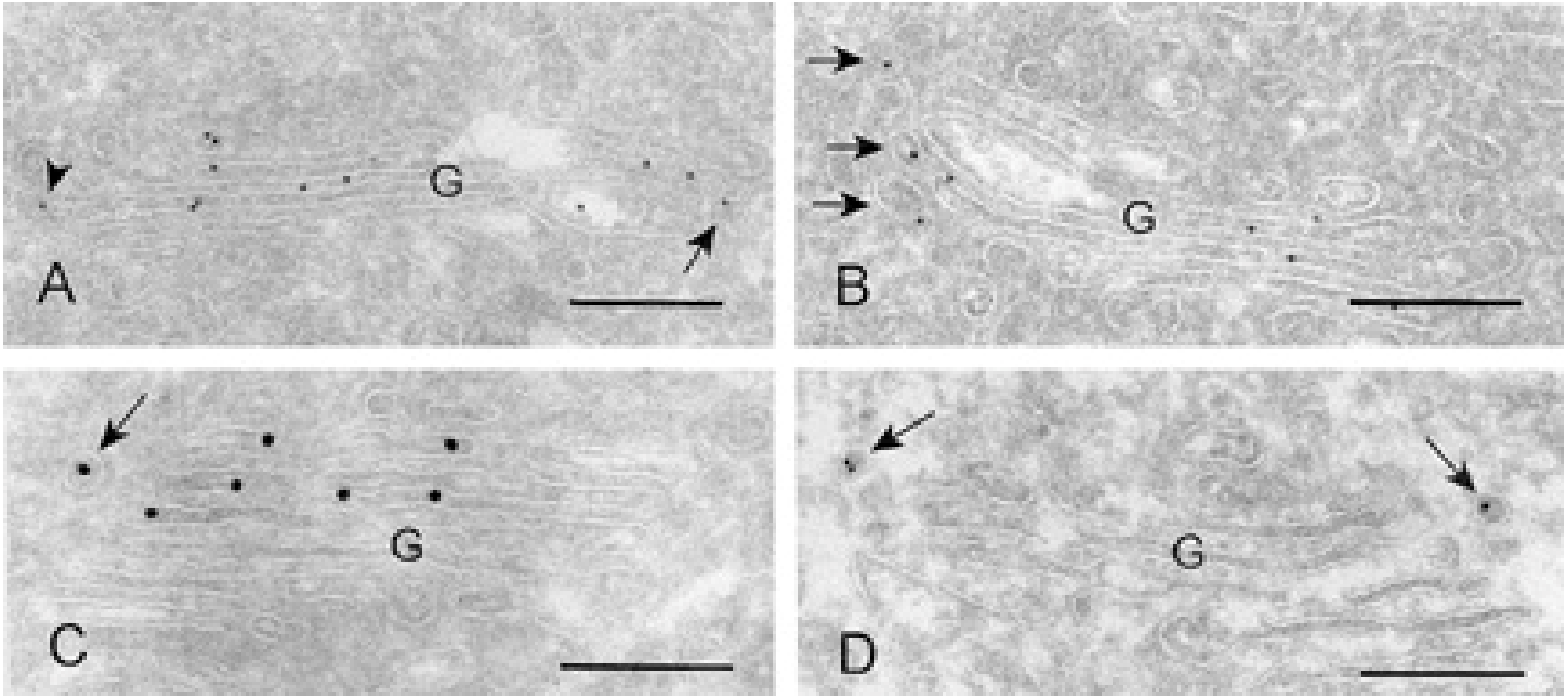


Figure 1. Man II (mannosidase II – GA residentní bílkovina) is present in the cis-medial Golgi, lateral rims of the Golgi, and associated vesicles (arrows)

Martinez-Menarguez et al. 2001

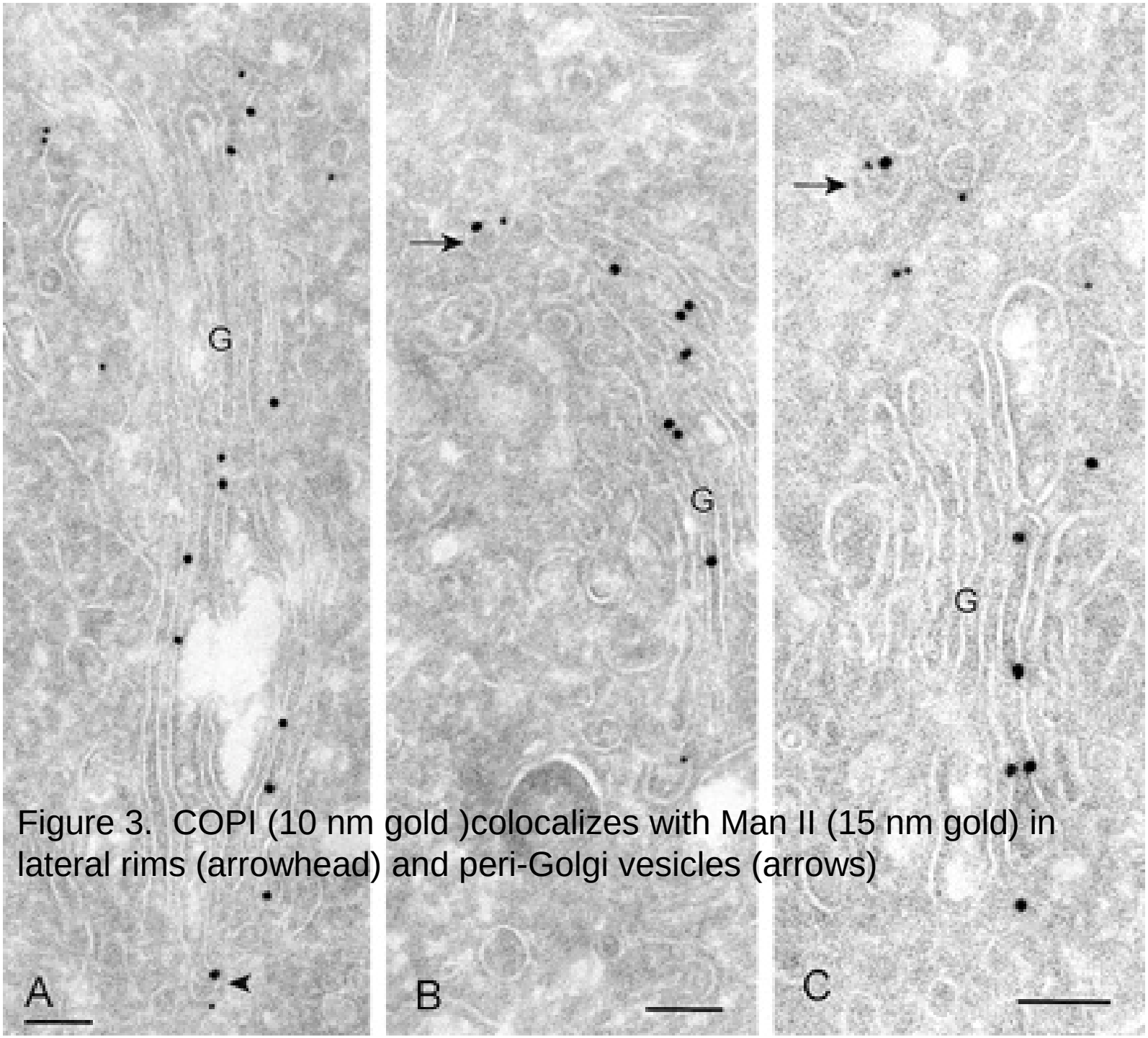


Figure 3. COPI (10 nm gold)colocalizes with Man II (15 nm gold) in lateral rims (arrowhead) and peri-Golgi vesicles (arrows)

Transport through the Golgi

- General agreement that COPI vesicles contain retrograde cargo; whether COPI mediates both retrograde and anterograde transport is still debated
- Mechanisms may depend on cell type and system studied
- Tubule-mediated transport - distinct mechanism or variation on a theme - needs to be studied
- *Tubulární struktury/intermediáty se objevují při nízké teplotě*.

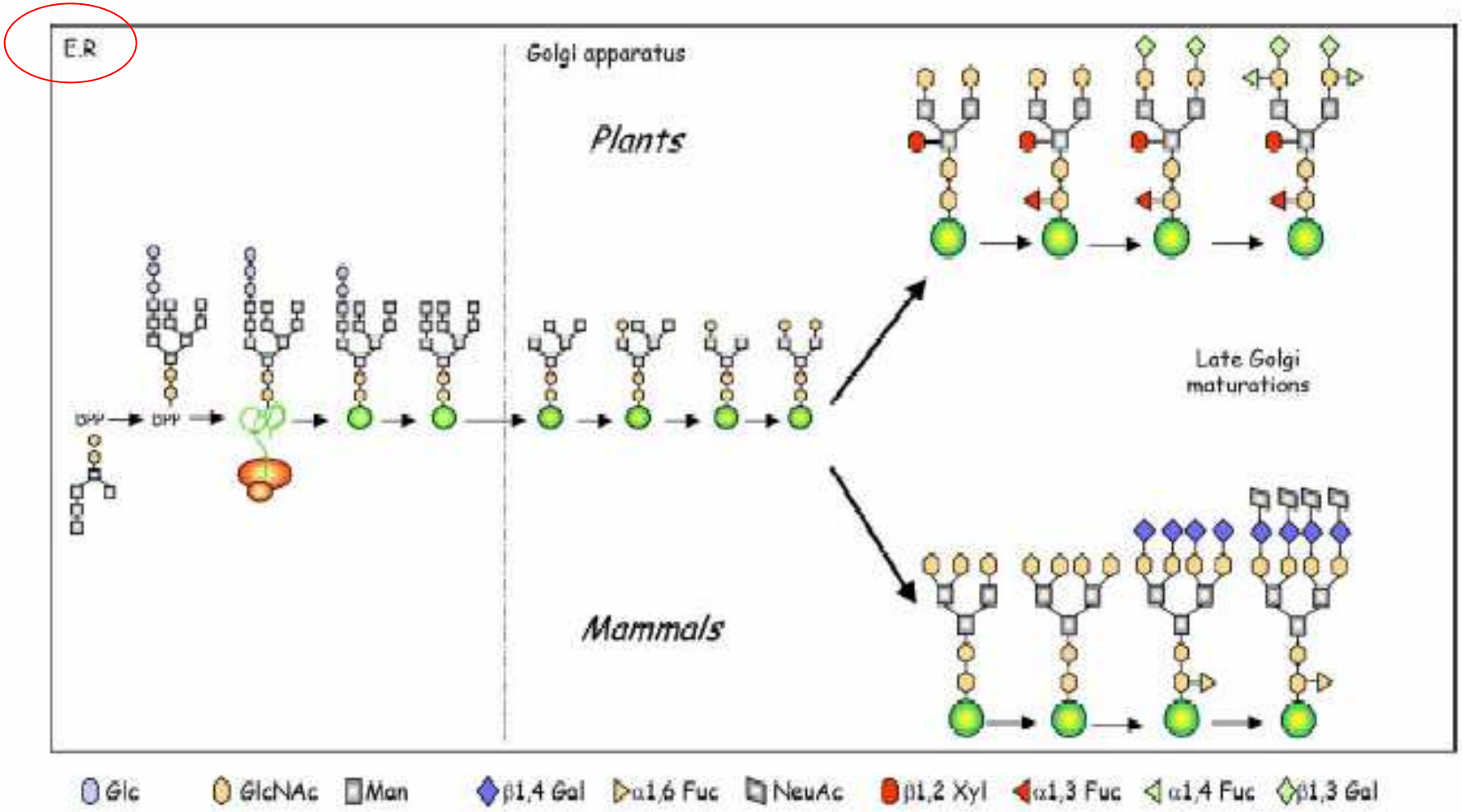


Fig. 1. Addition and processing of N-linked glycans in the endoplasmic reticulum (ER) and Golgi apparatus of plant and mammalian cells. A precursor oligosaccharide assembled onto a lipid carrier is transferred on specific Asn residues of the nascent growing polypeptide. The N-glycan is then trimmed off with removal of glucosyl and most mannosyl residues. Differences in the processing of plant and mammalian complex N-glycans are late Golgi maturation events.

Rozdíly v posttranslačních úpravách komplikují biotechnologické využití.

O-glykos.

na OH- skup.
Ser, Thr či Hyp
Probíhá v GA.

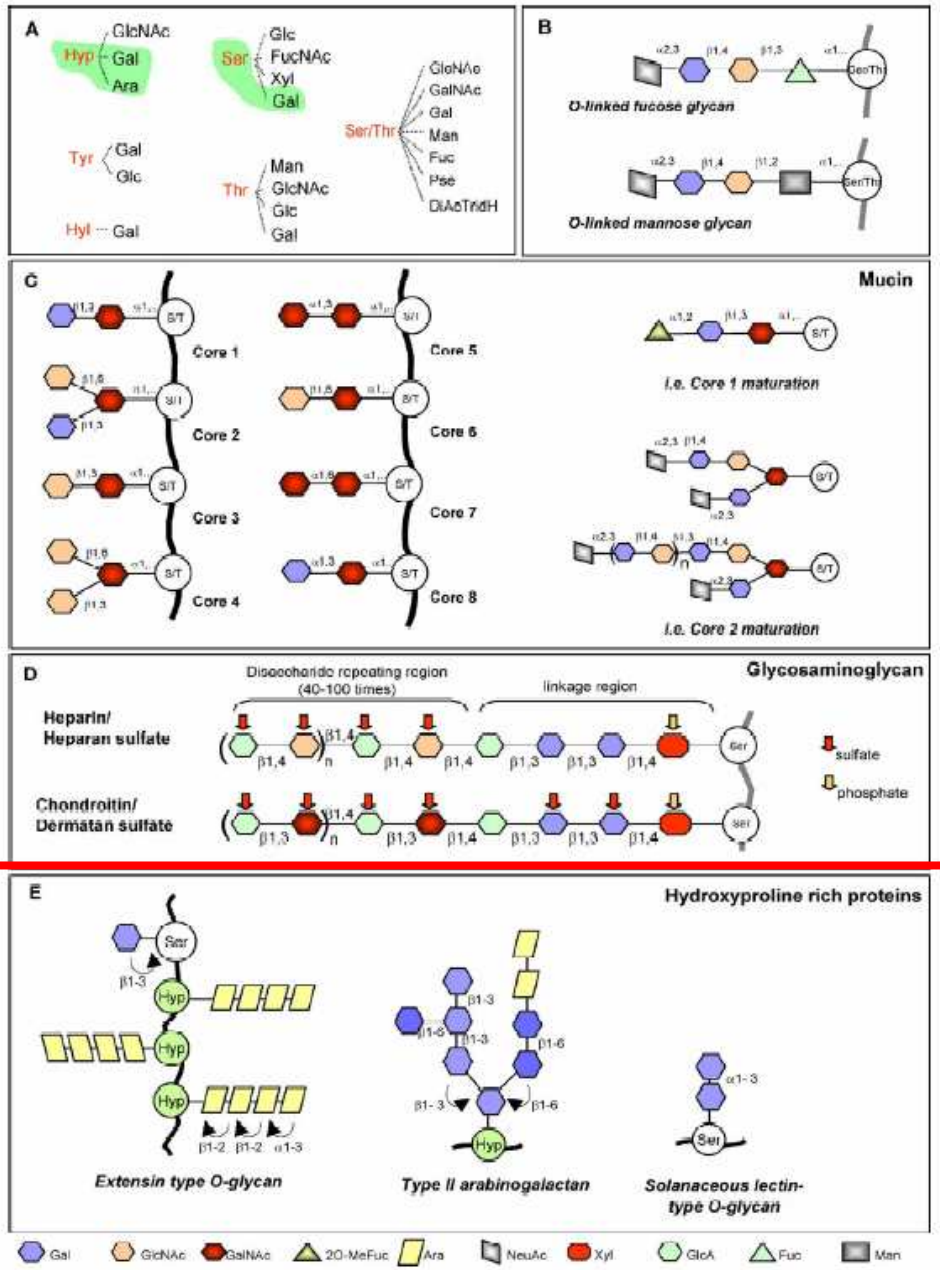
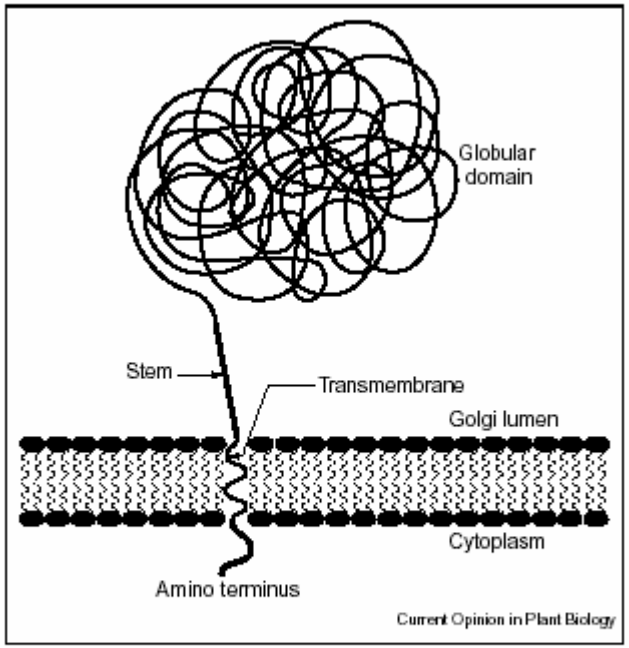


Fig. 2. Some O-glycan structures and linkages commonly found on plant and animal glycoproteins. (A) Five distincts types of sugar-peptide bonds currently identified in animals. Identical linkages now identified in plant are depicted in green (from Spiro [64]). (B) Example of O-linked fucose (Drosophila Notch receptor) or mannose (human α-dystroglycan) type glycan. (C) Structure of eight known core types of mucin-type O-glycans and example of maturation. (D) Structure of sulfated glycosaminoglycans (i.e. chondroitin sulfate, dermatan sulfate, heparin sulfate, heparin) linked to proteoglycans. (E) Example of plant O-linked glycans. Ara: arabinose; FucNac: N-acetylfucosamine; Gal: galactose; GalNAc: N-acetylgalactosamine; Glc: glucose; GlcA: hyaluronic acid; GlcNAc: N-acetylglucosamine; Man: mannose; Pse: pseudaminic acid; DiacTridH: Diactrideoxyhexose; Xyl: xylose.

Velkou část metabolické aktivity GA u rostlin vedle glykosylace bílkovin představuje syntéza pektinů a ruzných "hemiceluloz".

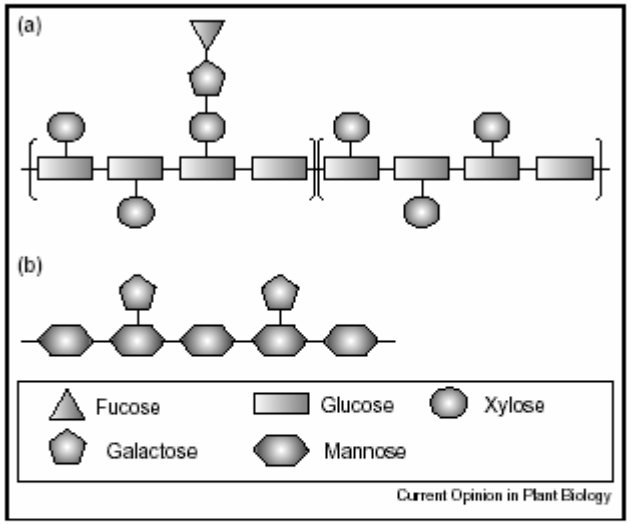
Glykosyl Transferázy v GA

Figure 1



Schematic representation of the topology of most Golgi-localized glycosyltransferases. The amino terminus is located at the cytoplasmic face of the Golgi membrane whereas the globular domain, located near the carboxyl terminus of the protein, is located in the lumen of the Golgi.

Figure 2



Schematic representation of the structure of (a) xyloglucan and (b) galactomannan. Each sugar is shown as a different shape. No attempt is made to depict the position or anomeric configuration of the glycosidic linkages. Parentheses indicate that the enclosed group of sugars are repeated in a regular pattern.

(See also [12] for another recent review that covers this topic.)

- **U rostlin převažuje v dynamice GA maturační mechanismus.** (viz. šupiny řas)

Rab GTPázy jsou regulátory tvorby a pohybu váčků buňkou (některé interagují s cytoskeletálními motory), a při kontaktu s cílovou membránou interagují s poutacími komplexy (tethering c.) např. EXOCYSTEM

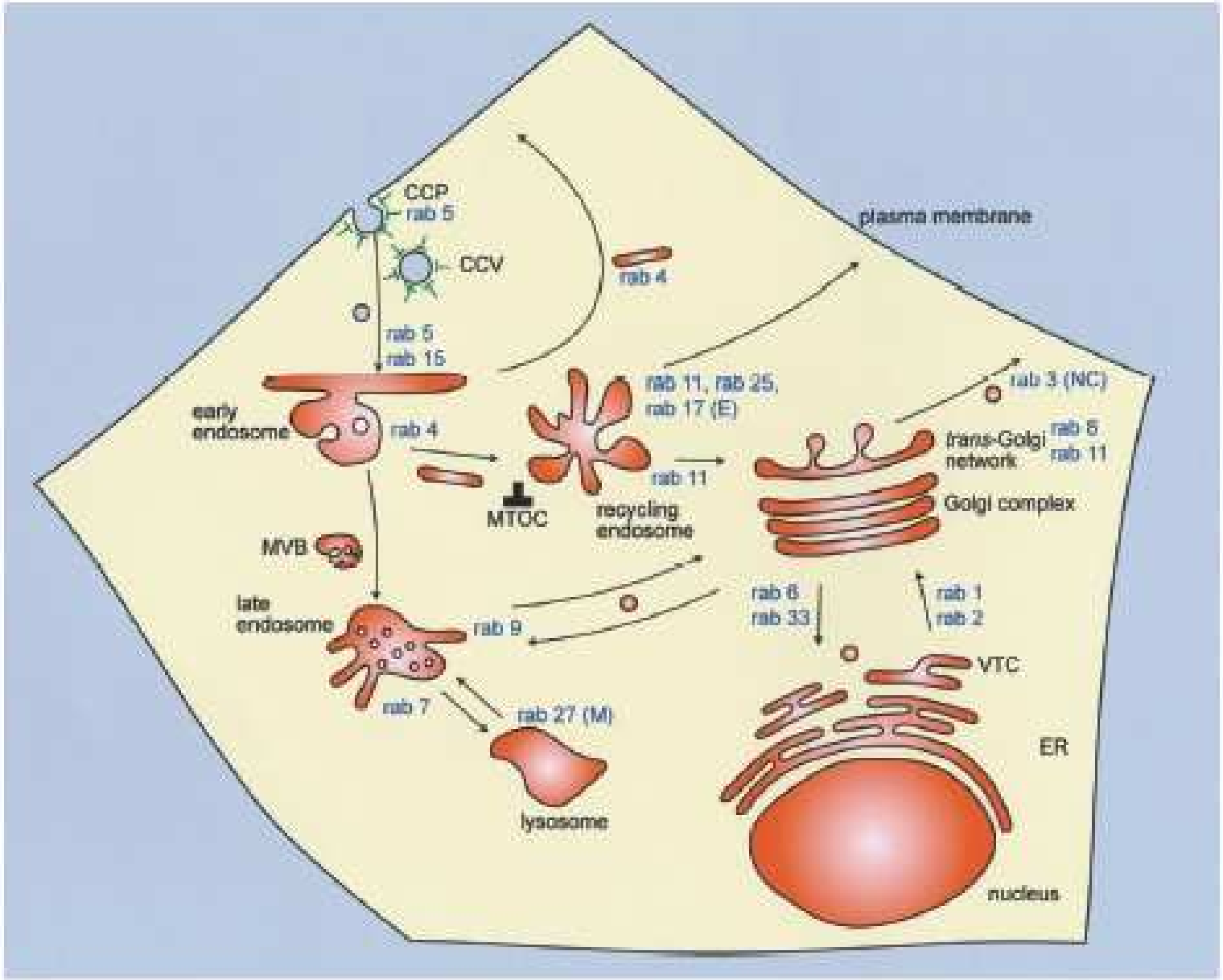
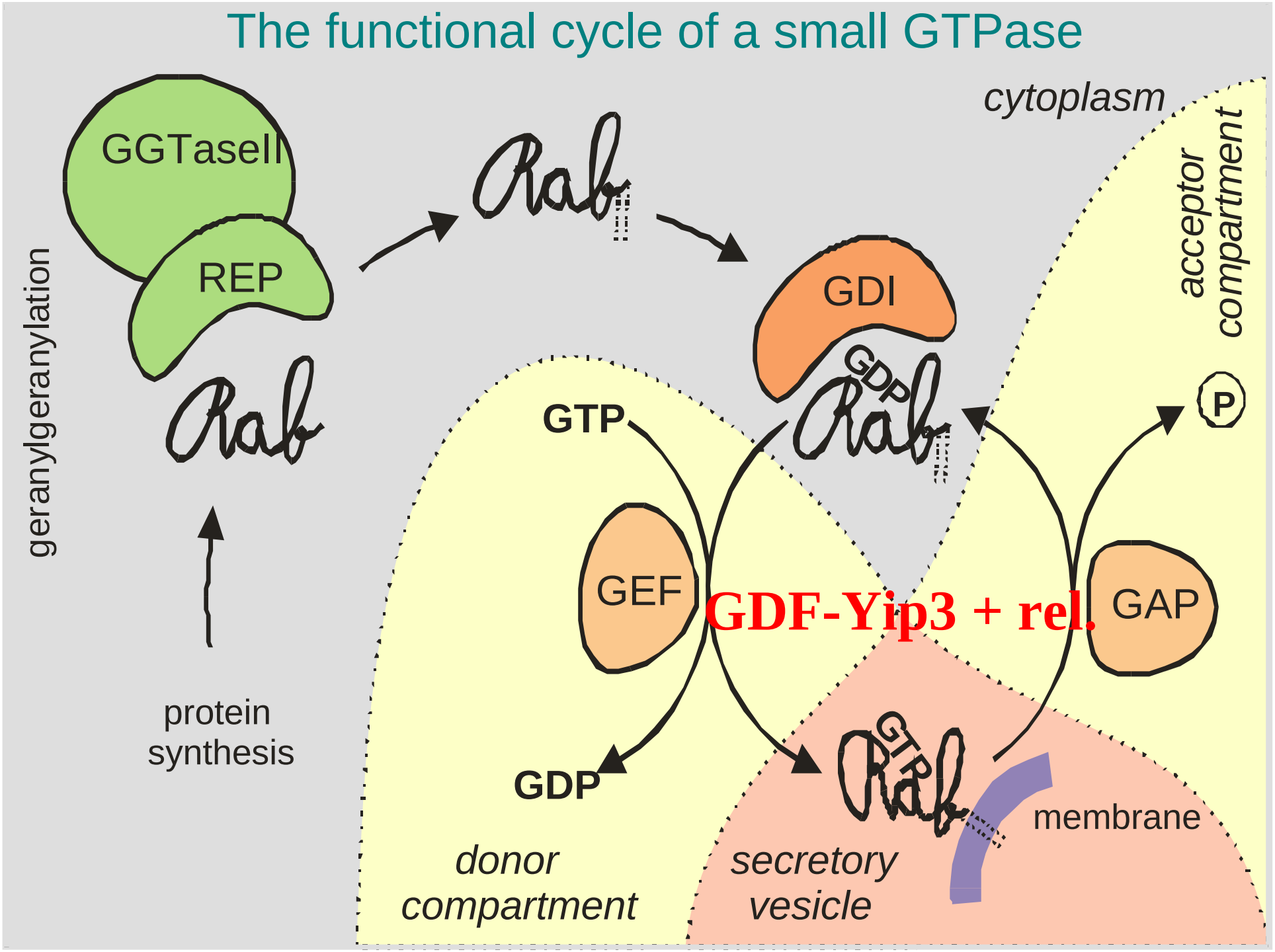
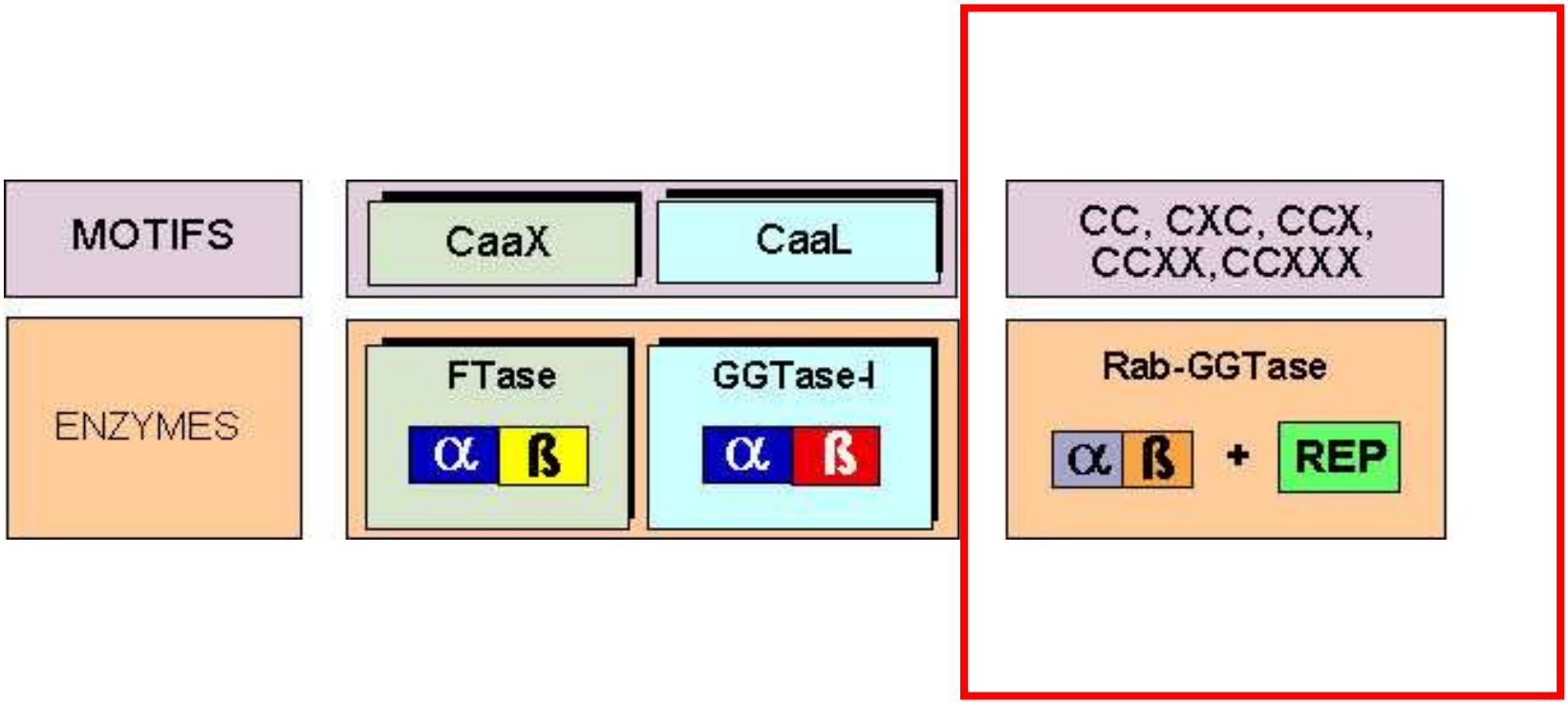


PLATE 1. Intracellular localization of rab proteins. Overview of rab protein localization in mammalian cells. CCP, clathrin coated pit; CCV, clathrin coated vesicle; M, melanosomes; E, epithelial cell type specific expression; NC, neuronal cell specific; VTC, vesiculo-tubular cluster; MVB, multivesicular body; MTOC, microtubule organizing center.


The functional cycle of a small GTPase
GGTaseII
REP
Rab
Rab
GDI
GDP
Rab
cytoplasm
acceptor compartment
P
geranylgeranylation
protein synthesis
GTP
GEF
GDF-Yip3 + rel.
GAP
GDP
GTP
Rab
membrane
donor compartment
secretory vesicle

Protein prenyltransferases



AtRabGDI1 klonován pomocí komplementace.

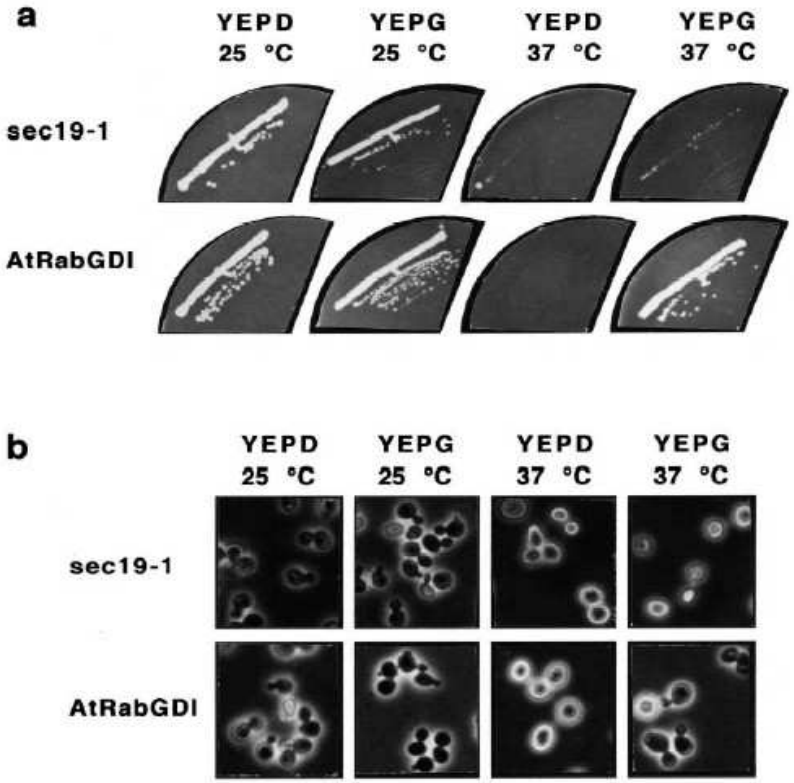
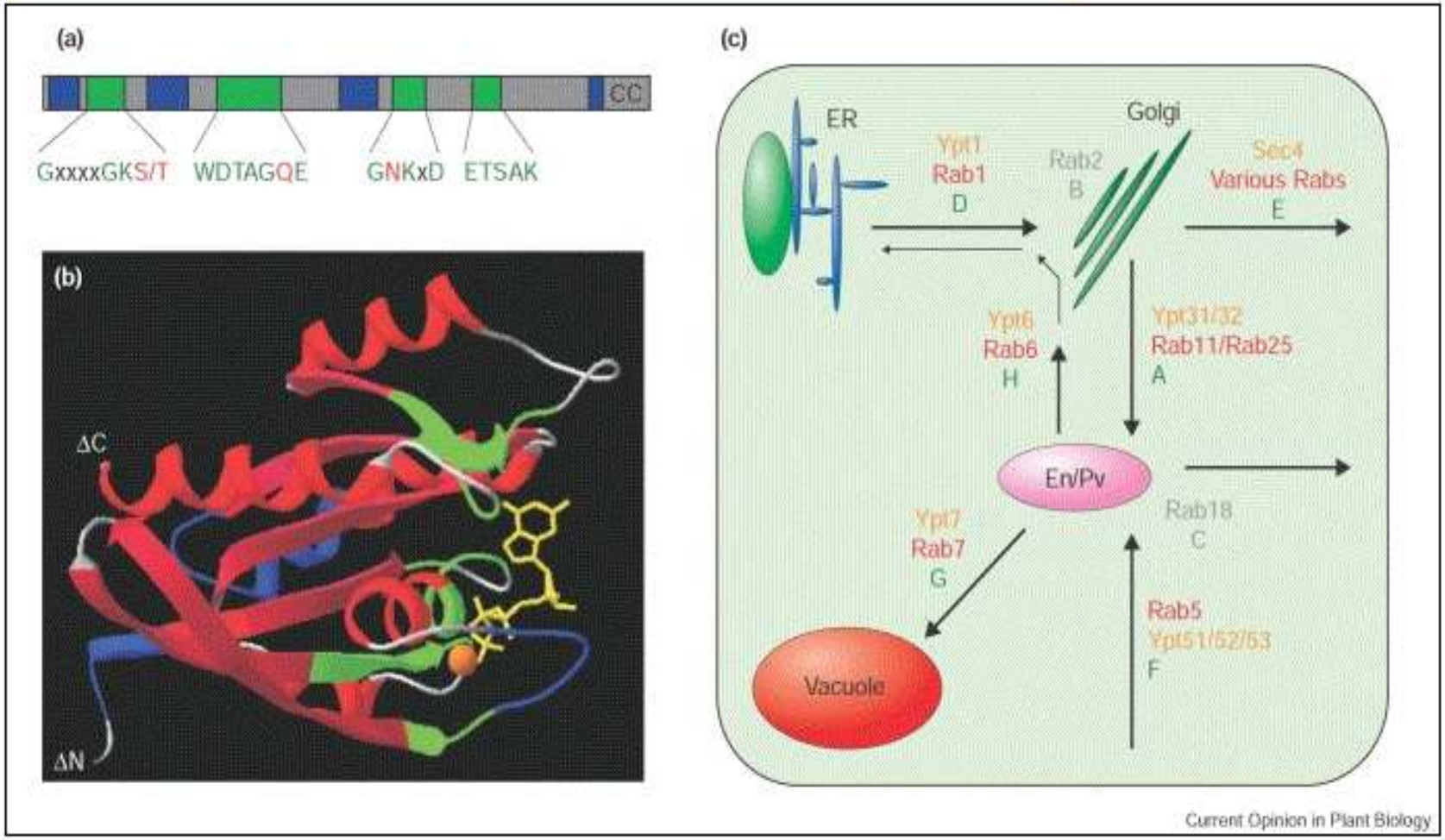
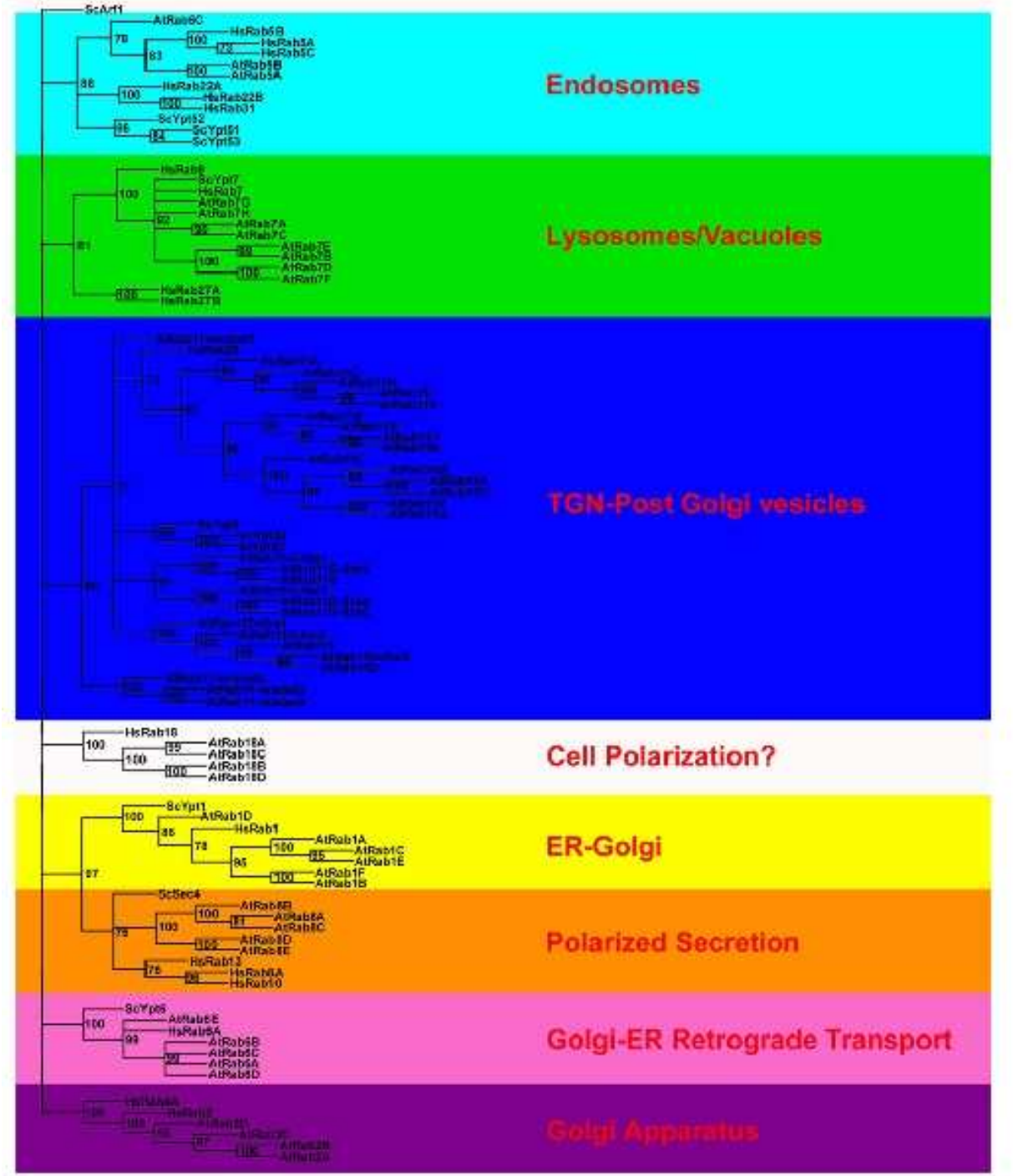


Fig. 1. Complementation of a yeast *sec19-1* mutant by an *A. thaliana* cDNA. The upper row of (a) shows the *sec19-1* yeast strain after growth for 3 days on glucose (YEPD) or galactose (YEPG) media at either the permissive (25°C) or restrictive temperature (37°C). The bottom row shows the growth pattern of a yeast strain transformed with an *Arabidopsis* cDNA (p*ATGDI1*) complementing the *sec19-1* mutant. (b) Phase-contrast microphotographs of cells of the same strains grown in liquid YEPD or YEPG medium for 18 h.

Rab structure and subclassification



RabA is the biggest class in plants (celkem57 všech Rabů u At).



Pleiotropic effects of RabA supression by RNAi

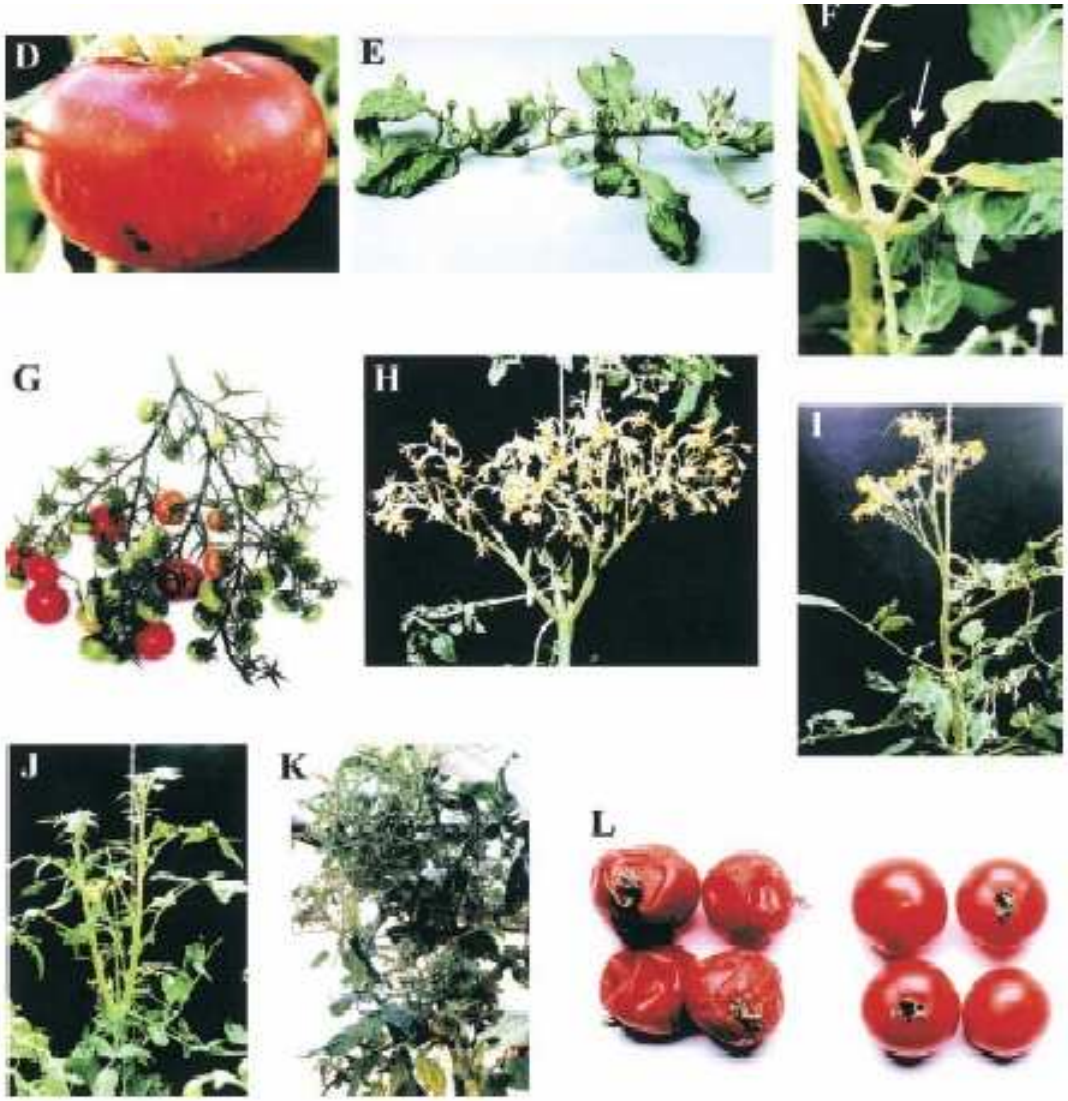


Figure 3. Visual Phenotypes of *Rab* Antisense Transgenic Plants.

(A) Sepals of wild-type flower (left) and transformant flower (right).
(B) Flowers of wild-type (left) and transformant (right) plants.

Transport váčků k plasmalemě.

Jak se váček potká s cílem.

(aneb než přijde ke slovu SNARE)

Obaly doprovázející tvorbu sekretorických váčků na TGN putujících k plasmalemě nejsou dosud známy.

Na EM snímcích rostlinných buněk popsal Staehelin „lace like coat“.

Tethering mechanisms

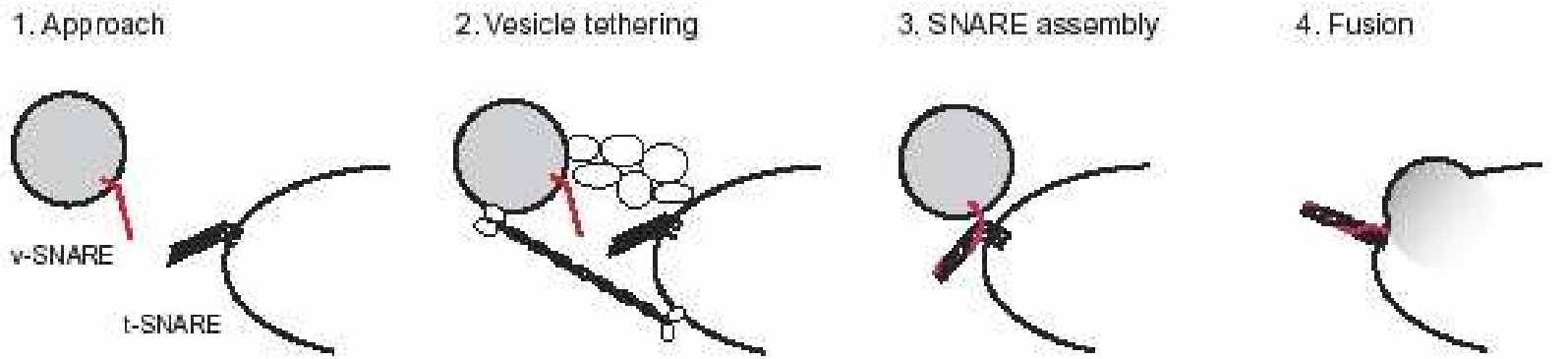


Fig. 1. Steps in the delivery of vesicles to the correct organelle. (1) An intracellular transport vesicle approaches its destination organelle either by diffusion or motor-mediated directed transport. (2) The vesicle is then proposed to be tethered to the organelle by protein complexes and long coiled-coil proteins. (3) A v-SNARE protein on the vesicle then engages a t-SNARE on the target, forming a four-helical bundle whose assembly drives the two bilayers into close proximity, (4) thereby causing membrane fusion. Both vesicle tethering and SNARE assembly have been referred to by others as 'docking', so to avoid confusion we use only the former terms here.

Tethering complexes

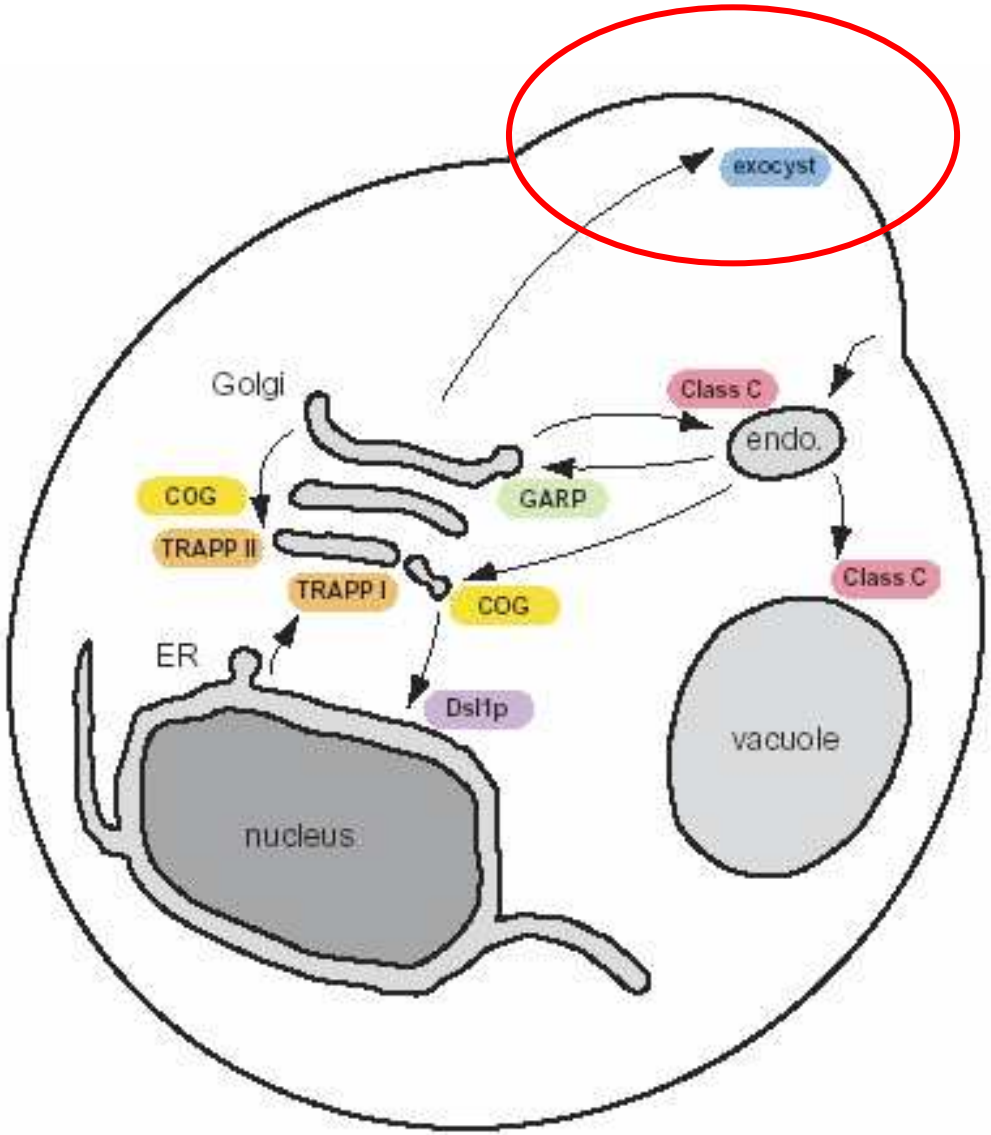


Fig. 2. Putative tethering complexes in the yeast secretory pathway. Protein complexes that have been found to have a role in particular vesicular transport steps are indicated next to those steps. The role of early and late endosomes in yeast is contentious, and so for simplicity this compartment has been shown as a single organelle.

Tethering complexes composition

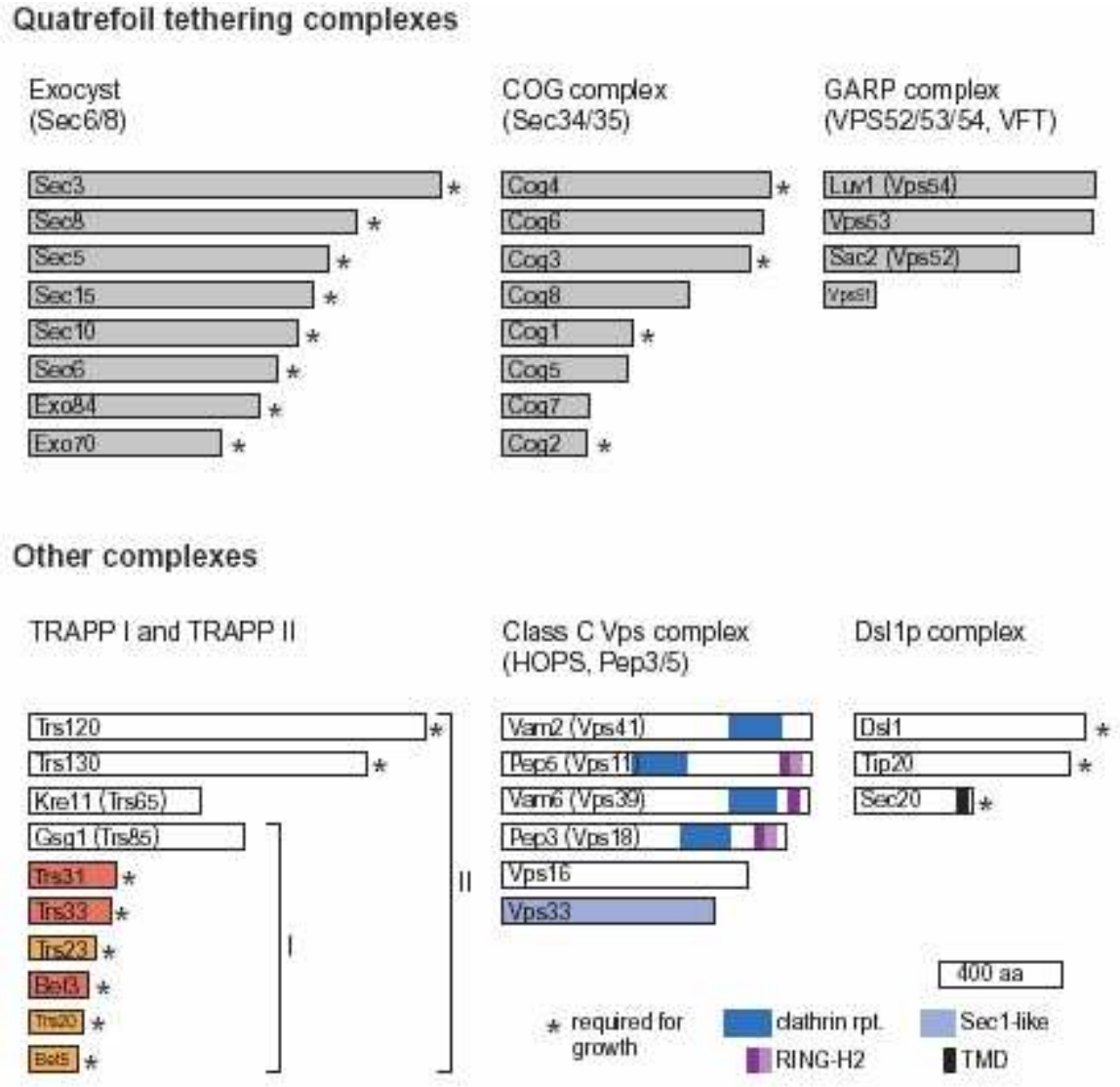


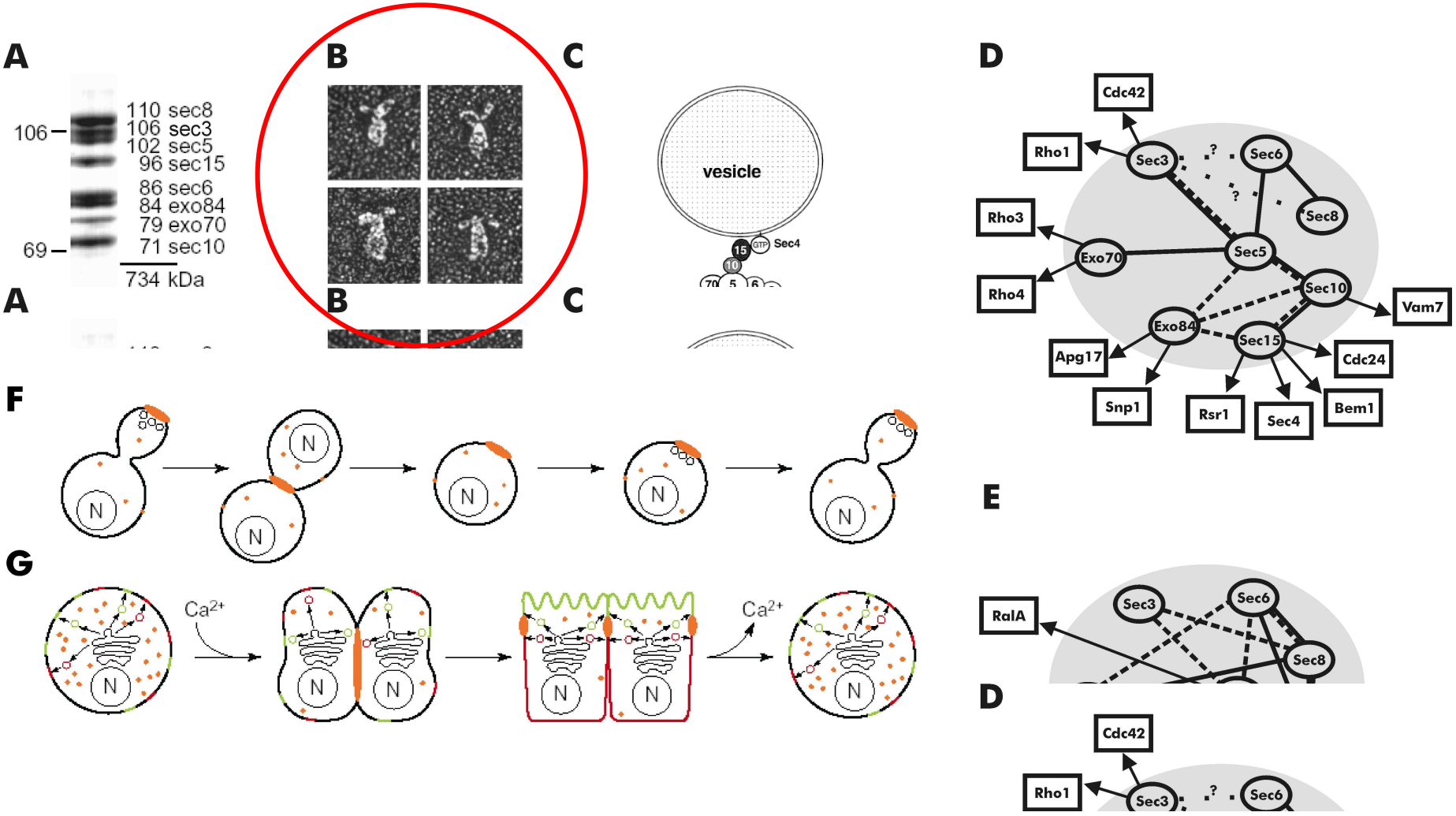
Fig. 3. Composition of proposed tethering complexes. For each complex the known components in the yeast *S. cerevisiae* are shown, arranged by size, and identifiable domains indicated. In each case the standard gene name in the *Saccharomyces* Genome Database is given first, followed by alternative names that have also been used in recent publications. Vps51p is encoded by the open reading frame *YKR020w* (Elizabeth Conibear, personal communication). The two sets of related subunits of the TRAPP complexes are indicated by different colours. Homologues of most of these proteins exist in higher eukaryotes, but in some cases have extra domains. Thus in mammals Sec5 has an N-terminal TIG domain, Exo84 a PH domain, Vam6 a CNH domain (Caplan et al., 2001) and Vam2 a C-terminal RING-H2 domain (Radisky et al., 1997). Vps54 has an N-terminal zinc-finger-like domain in *Drosophila* and *C. elegans*, but not in mammals. Vam6 in both yeast and higher eukaryotes has a conserved half RING domain (C2HC) at its C-terminus. The 'p' has been removed from the yeast protein names for clarity.

RAB- and RHO-interacting protein complexes

Complex	Interacting GTPases	Proposed function	Occurrence
Exocyst (Sec6/8 complex)	Sec4p, Rho1p, Rho3p, Rho4p, Cdc42p, RalA	Tethering of exocytic vesicles to the plasma membrane	fungi, animals, amoebae , plants
COG (Sec34/35 complex)	Ypt1p	Retrograde transport to the cis-Golgi	fungi, animals, amoebae, kinetoplastids, plants
GARP (VFT or Vps52/53/54 complex)	Ypt6p	Retrograde transport to the trans-Golgi	fungi, animals, amoebae, kinetoplastids, apicomplexans, plants
HOPS (Class C VPS complex)	Ypt7p (Vps21p)	Transport to the vacuole (endosome), homotypic vacuolar fusion	generally eukaryotic? (experimental data for yeast, mammals, plants)
TRAPP (TRAPP I and TRAPP II)	Ypt1p, Ypt31/32p	Anterograde transport to the cis-Golgi, intra-Golgi transport	generally eukaryotic? (experimental data for yeast and mammals)
Formin-ass. complex (polarisome)	Cdc42p, Rho-class GTPases	F-actin nucleation, cell polarization	fungi, animals, plants

Jediným z těchto komplexů, který je dobře popsán u rostlin je HOPS komplex (class C, VPS komplex Natasha Raikhel lab)

The exocyst – an effector of small GTPases



Polarita buněk u rostlin

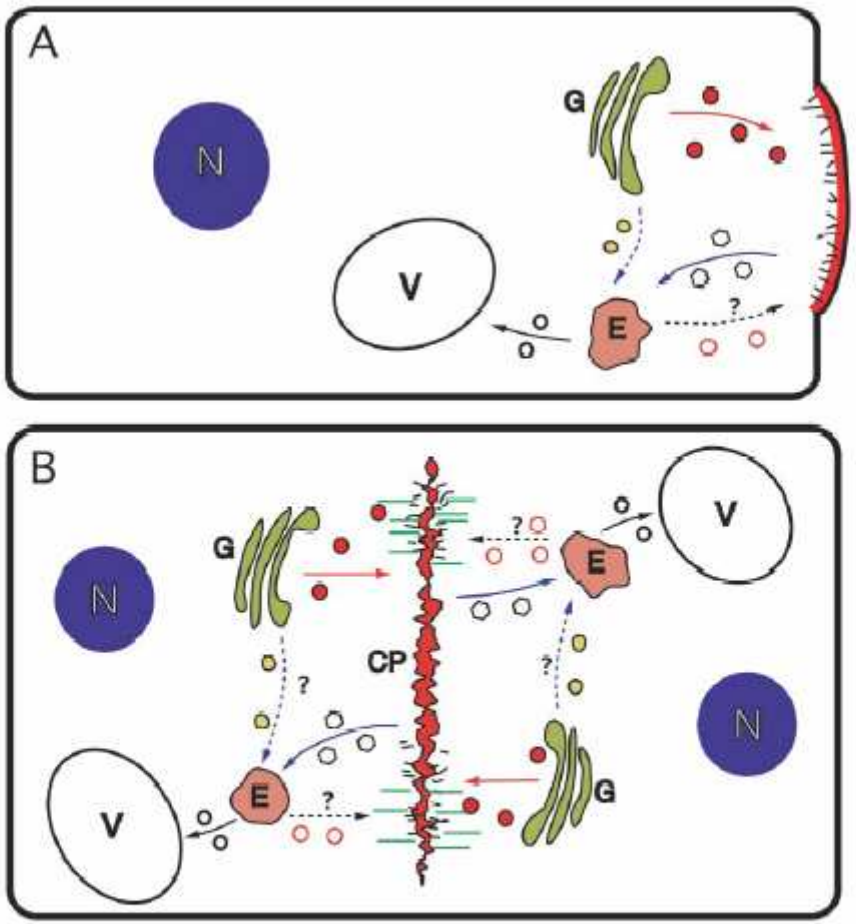
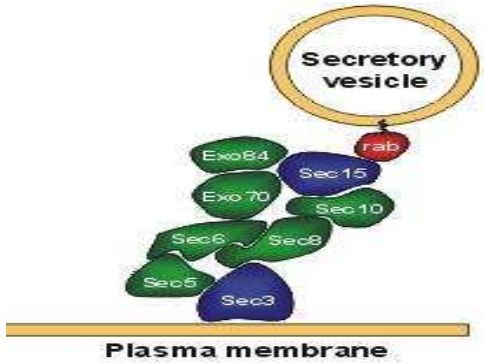


Figure 2: Speculative model for polarized vesicular trafficking during (A) polarized cell-surface expansion and (B) cell-plate formation. A: In polarized growth, vesicles are delivered by multiple exocytic pathways (open and closed red circles) toward sites of expansion on the plasma membrane (red). Targeting of these vesicles may be guided by short MFs (black). B: During cytokinesis, exocytic vesicles are directed toward the cell plate (red). Targeting of these vesicles is guided by phragmoplast MTs (green) and short MFs (black). Cell-plate transport vesicles may originate directly from the Golgi apparatus or from an intermediate compartment (e.g. an early and/or late endocytic/prevacuolar endocytic compartment). Membrane is recycled via clathrin-coated vesicles (white hexagons) from the plasma membrane and cell plate (blue arrows) and delivered to an endocytic compartment (pink). See text for additional details. CP: cell plate, E: endocytic compartment, G: Golgi, N: nuclei, V: vacuole. The ER is not shown.

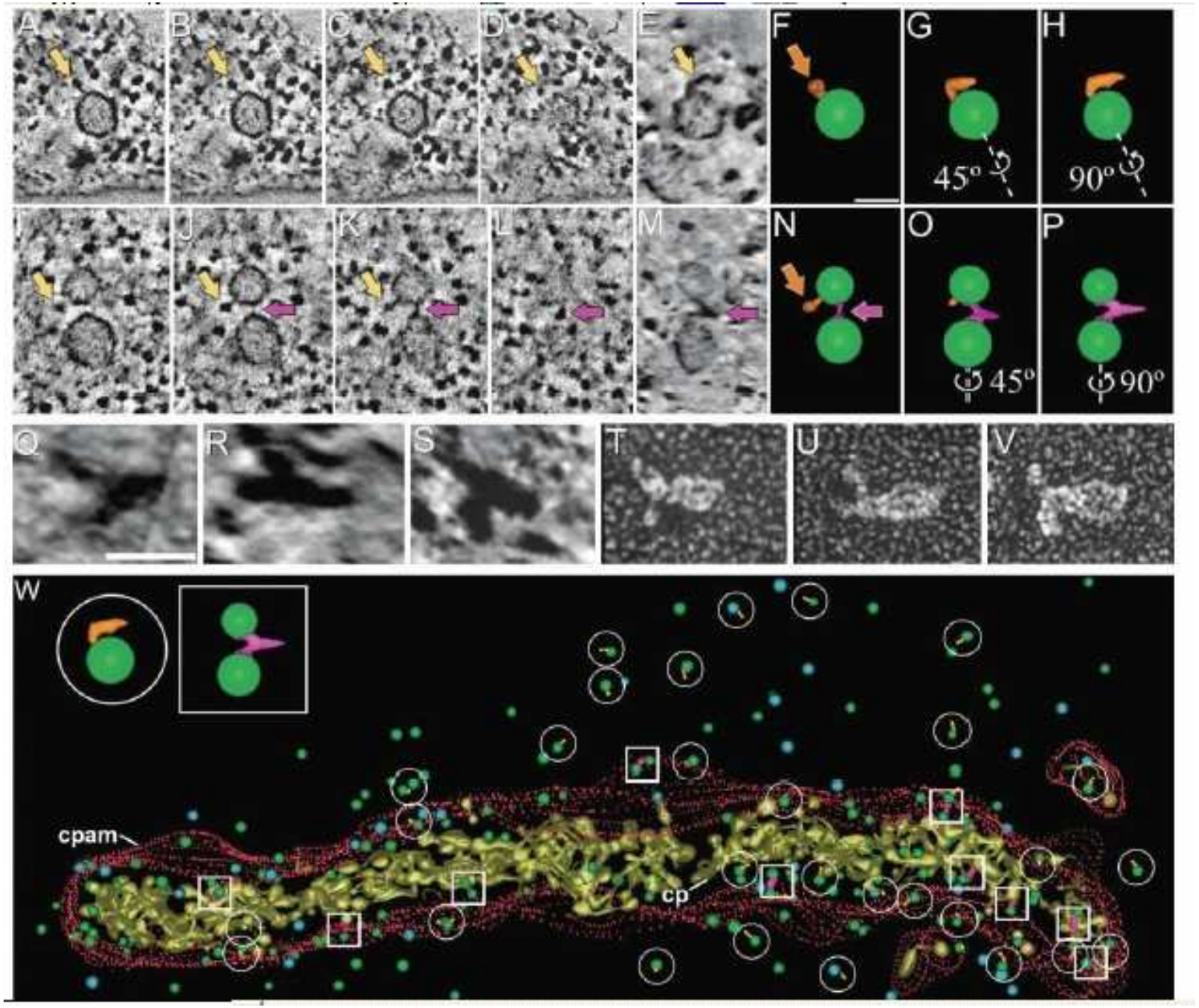
The Exocyst: effector of Rab and Rho GTPases

- a multisubunit complex required for exocytosis (TerBush et al., EMBO J. 15:6483-6494, 1996)
- interacting with the translocon on ER
- conserved in yeast, fly and mammals
- required for polarity in epithelia, neurons and for budding in yeast cells (Grindstaff et al., Cell 93:731-740, 1998, Finger et al., Cell 92:559-581, 1998)
- does it exist outside the Apicomplexa?
- **… do plants have Exocyst?**

Putative orthologs of the eight exocyst components are identified in *Arabidopsis thaliana* genome.

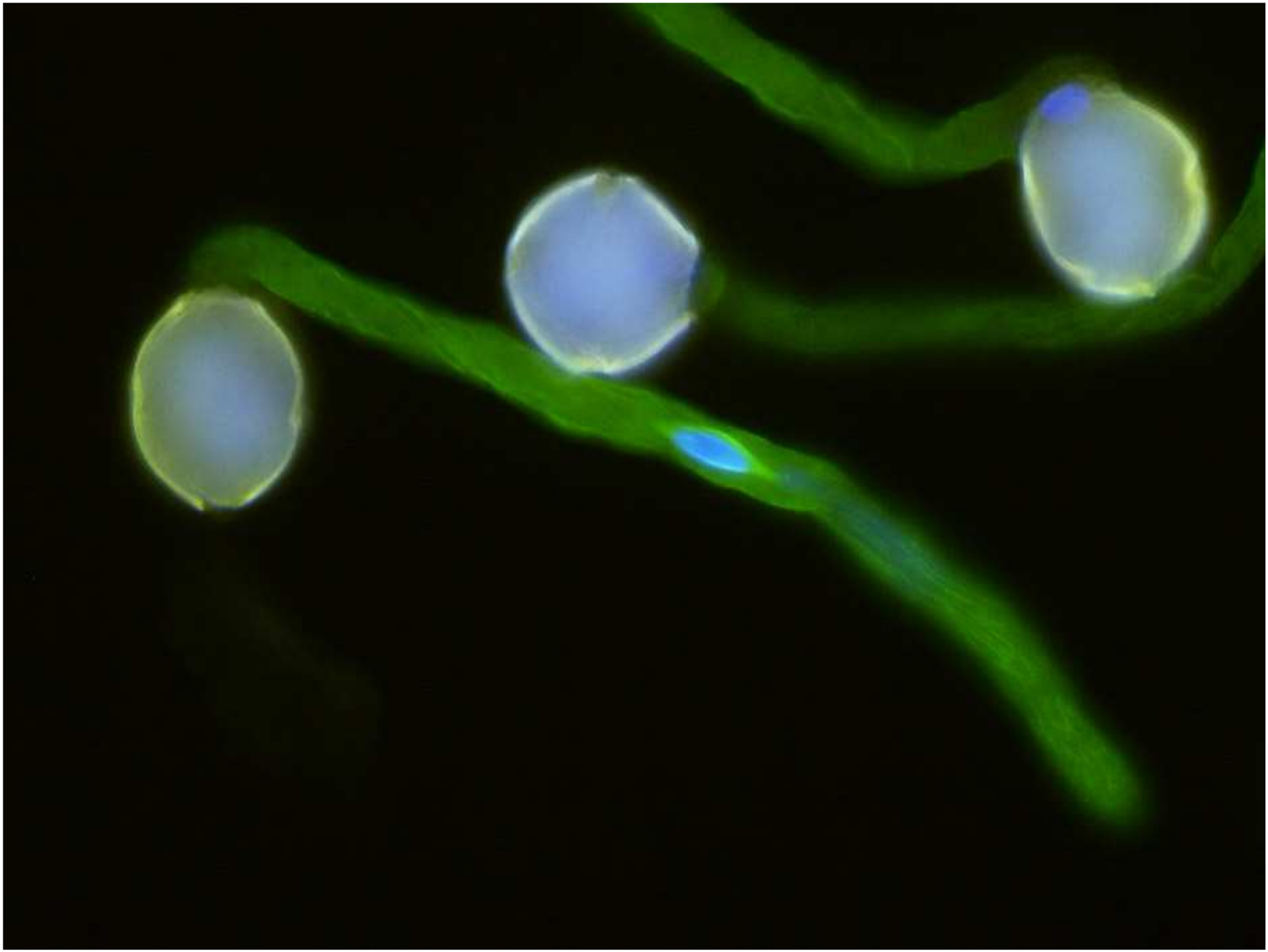


Organism	Exocyst Component							
	Sec3	Sec5	Sec6	Sec8	Sec10	Sec15	Exo70	Exo84
Arabidopsis thaliana	2	2	1	1	1	2	23	3
S. cerevisiae	1	1	1	1	1	1	1	1
Caenorhabditis elegans	1	1	1	1	1	1	1	1
Anopheles gambiae	1	1	1	1	1	1	1	1
Drosophila melanogaster	1	1	1	1	1	1	1	1
Homo sapiens	1	1	1	1	1	2	1	1
Mus musculus	1	1	1	1	1	2	1	1
Ratus norvegicus	1	1	1	1	1	1	1	1

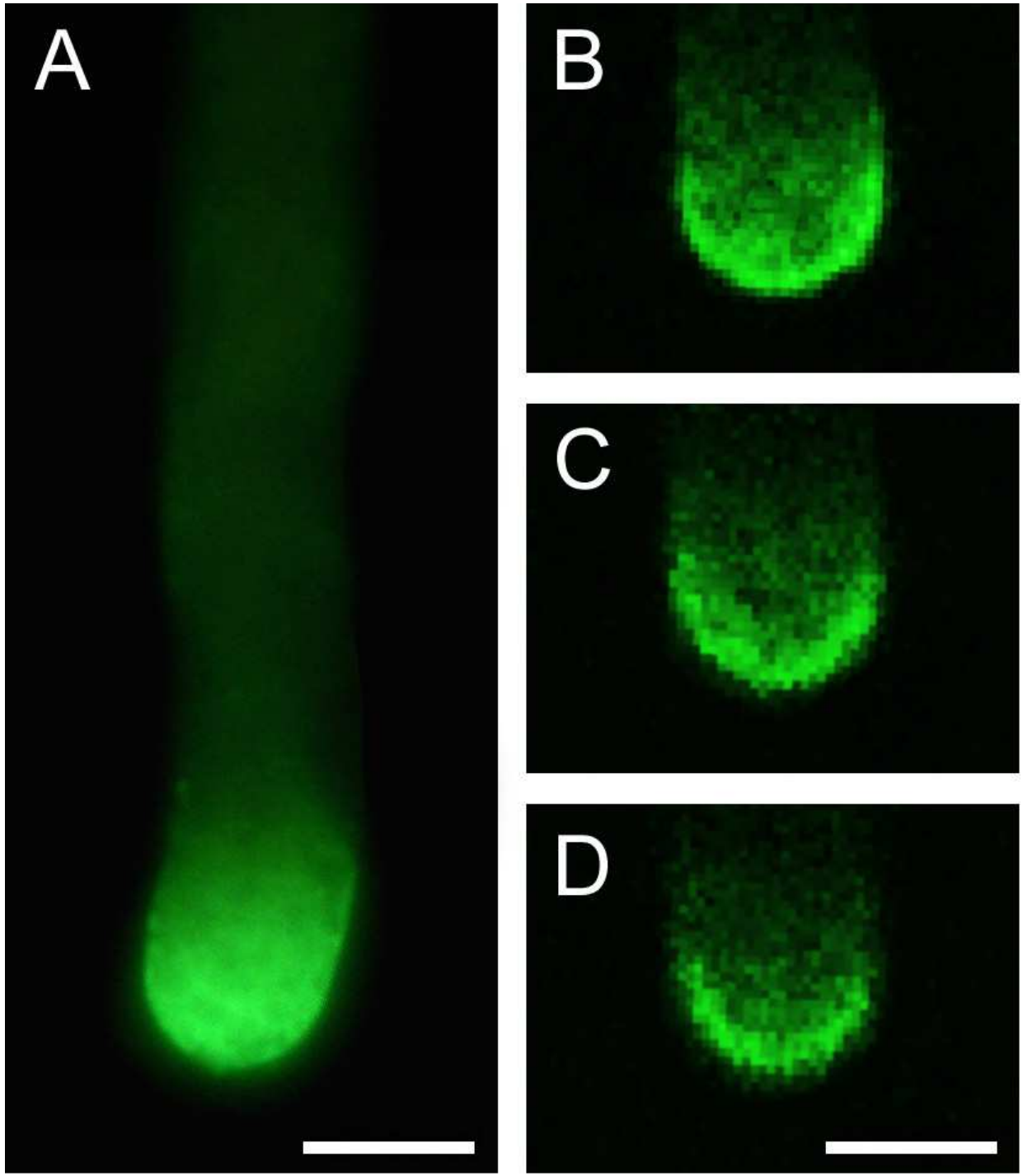


Tomographic analysis of putative vesicle tethering complexes

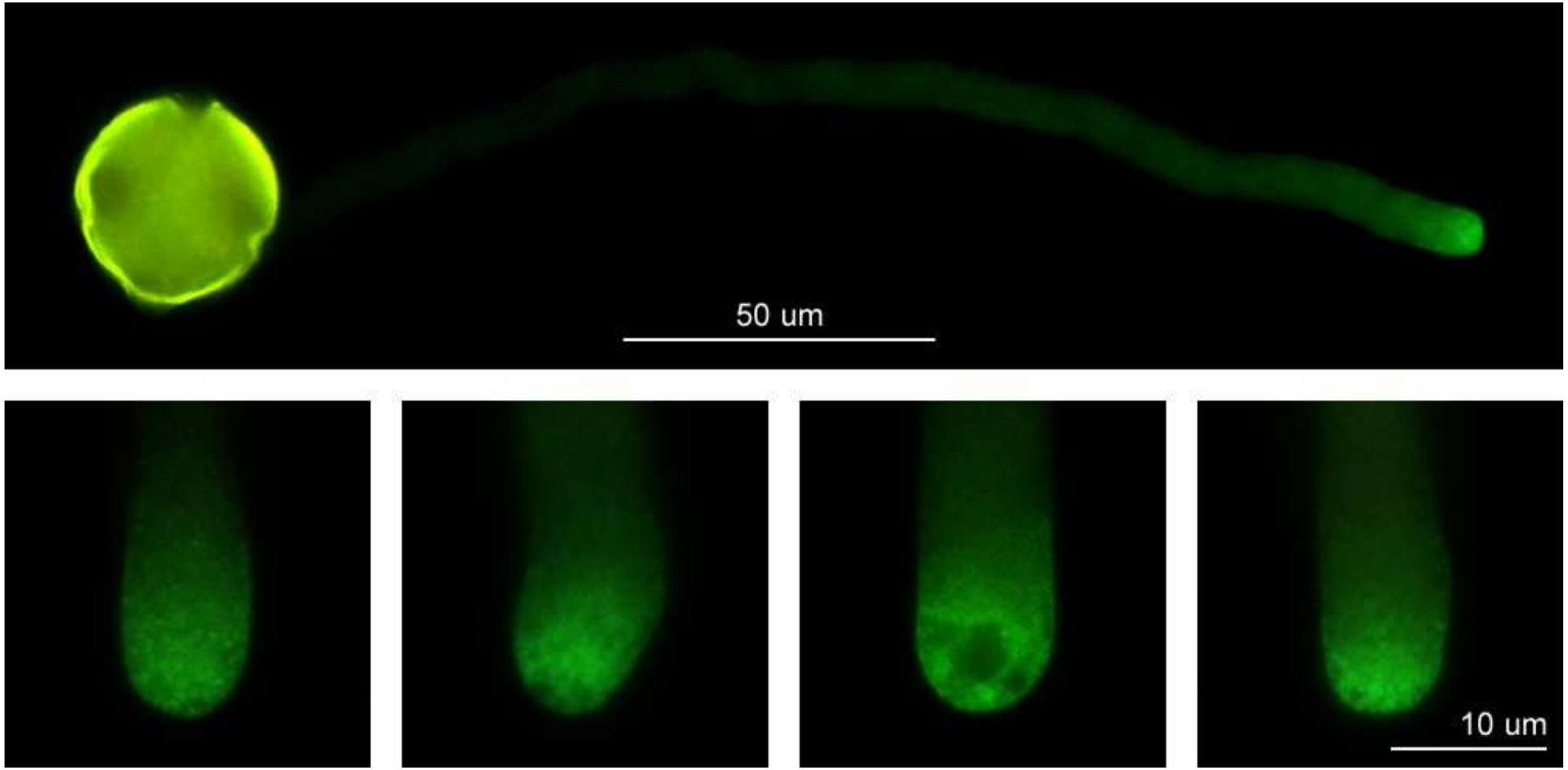
(Segui-Simarro, Staehelin A. et al. 2004. Plant Cell 16: 836.)



RabA in pollen tubes

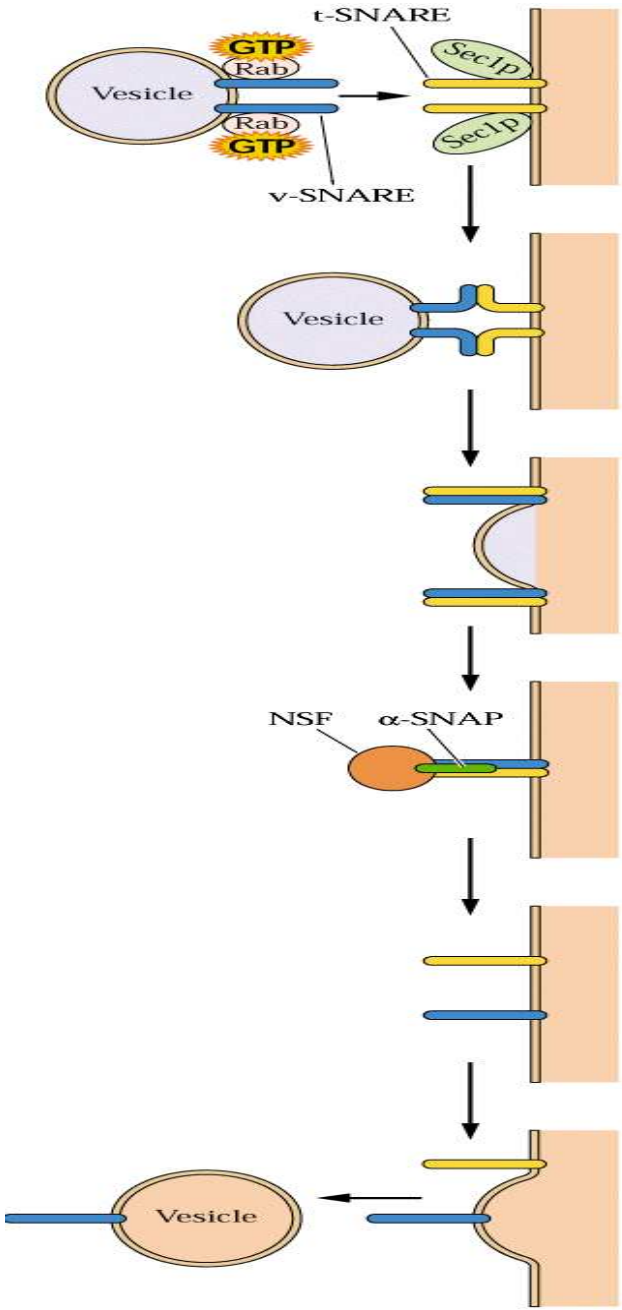


Sec6 in pollen tubes



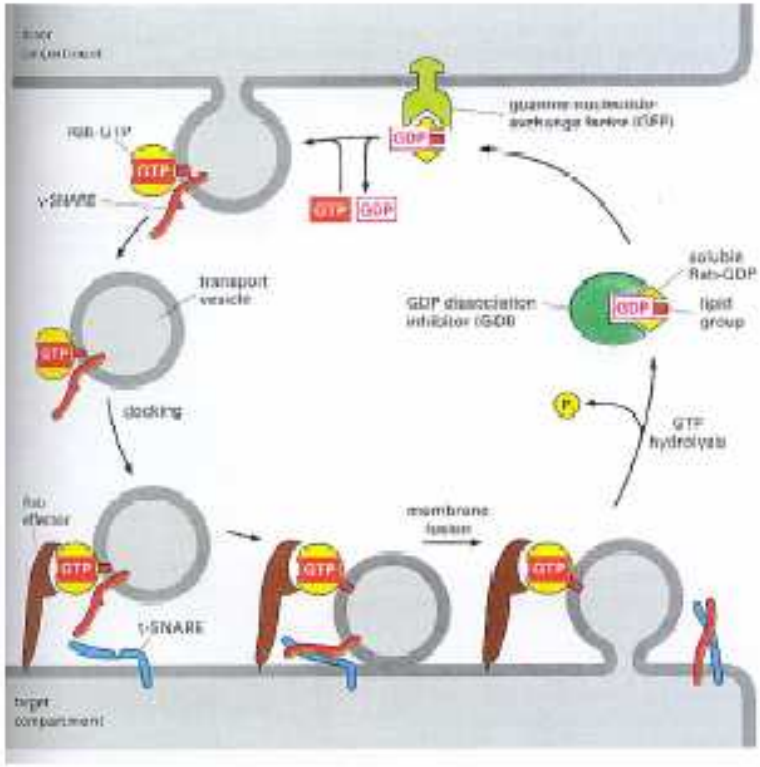
Podařilo se nám ukázat, že exocyst existuje také u rostlin a účastní se regulace buněčné polarity.

Specifita fůze váčku s cílovou membránou je zajišťována interakcí membránových SNARE bílkovin na váčku (v-SNARE) a cílové membráně (t-SNARE).



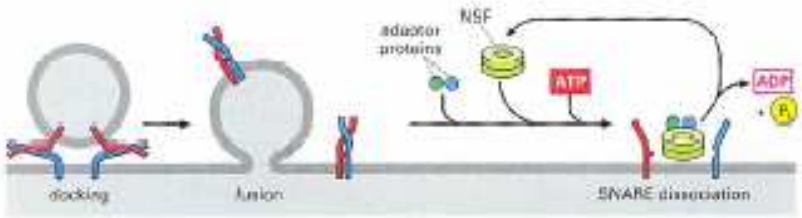
U Arabidopsis
je celkem 54 SNARE
bílkovin.

Selecting and fusing with the target membrane



Rabs and SNARES

Rab proteins in their GDP form are cytosolic and only associate with the budding vesicle membrane when activated by their corresponding GEF to bind GTP. Rab-GTP interacts with specific Rab effector proteins on the target membrane and brings the v-SNARE in contact with tSNAREs. The SNAREs promote fusion and can do so in vitro. The SNARE complex consists of one vSNARE and two or three tSNAREs, making up a four helix bundle.



NSF

NSF is an *AAA* ATPase that helps pry the SNAREs apart after fusion. It was the first identified component of the fusion machinery, named after its sensitivity to N-ethyl maleimide.

FUSE in the regulated (to co vypadá u rostlin jako konst. sekrece je většinou asi nějak regulováno!) pathway is mediated SNAREs and controlled by elevations in cytoplasmic Ca^{2+}

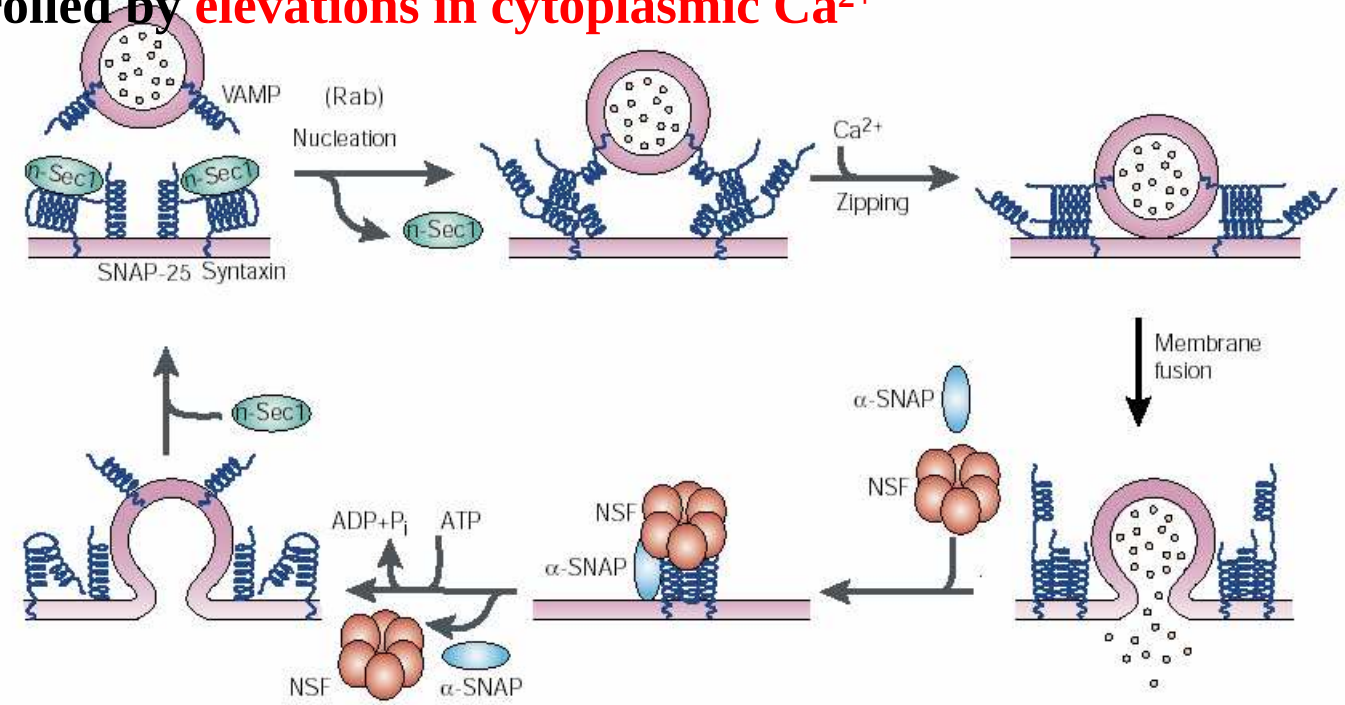
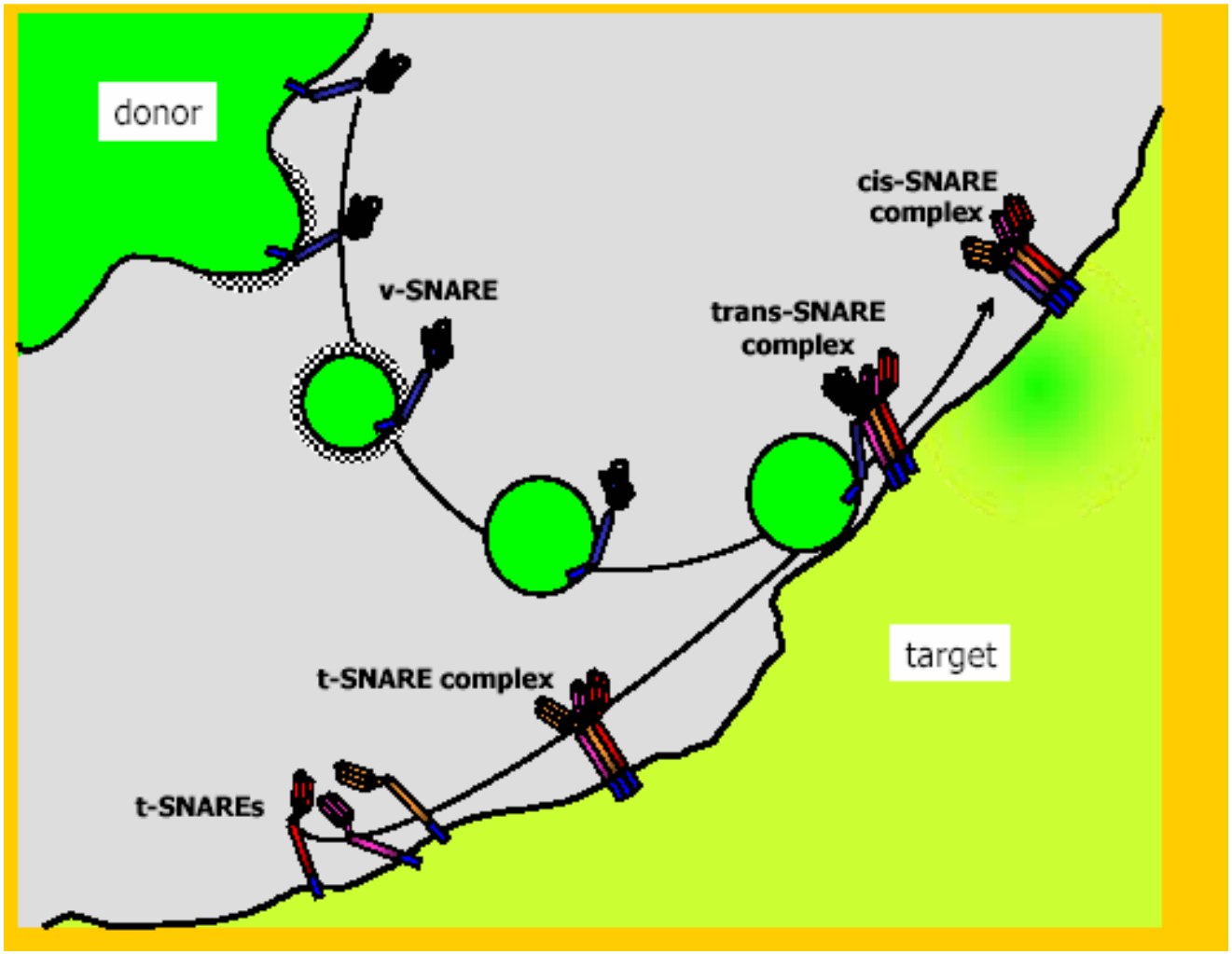


Figure 4 | **Molecular model of vesicle exocytosis**. Syntaxin is bound to n-Sec1 before formation of the core complex. Rab proteins might facilitate the dissociation of n-Sec1 from syntaxin, allowing subsequent binding (nucleation) between the three neuronal SNAREs, syntaxin, SNAP-25 and VAMP (for simplicity, only one coil is drawn for SNAP-25). Ca^{2+} triggers the full zipping of the coiled-coil complex, which results in membrane fusion and release of vesicle contents. After the fusion event, recruitment of α-SNAP and NSF from the cytoplasm and subsequent hydrolysis of ATP by NSF causes dissociation of the SNARE complex. Syntaxin, VAMP and SNAP-25 are then free for recycling and another round of exocytosis. (NSF; *N*-ethyl-maleimide-sensitive fusion protein; SNAP-25, 25 kDa synaptosome-associated protein; SNARE, soluble NSF attachment protein receptor; VAMP, vesicle-associated membrane protein.)

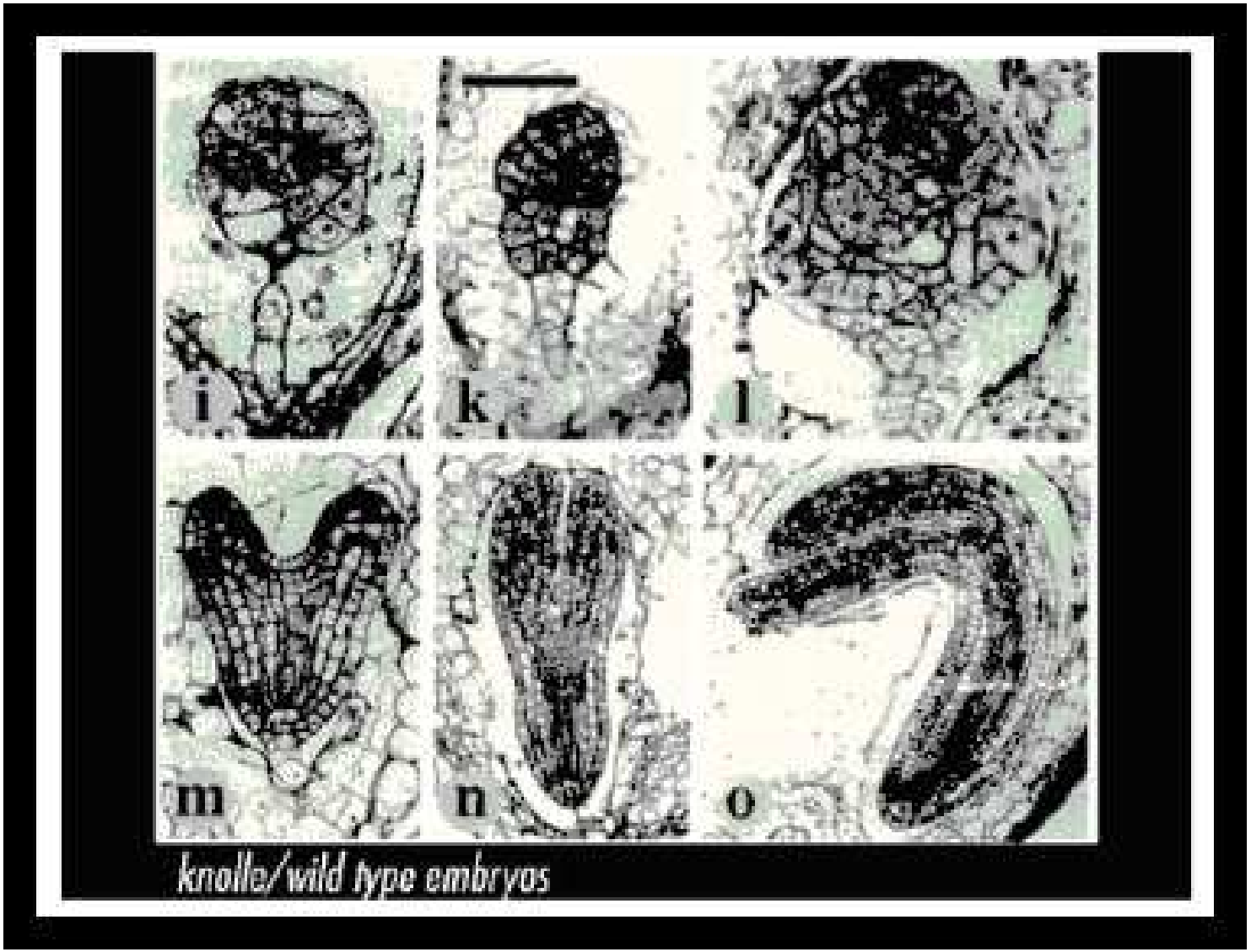
inhibition of regulated secretion: prevent elevations in Ca^{2+} through use of BAPTA-AM etc.; in neurons, use of botulinum neurotoxins, which are selective proteases for neuronal SNARE proteins, to inhibit regulated but not constitutive secretory pathway



SNARE komplex je het-erotetramer - R (váček) + QaQbQc (cílová membrána)

V řízení ranných stádií embryogeneze rostlin hrají důležitou úlohu regulátory buněčné morfogeneze – sekretorická dráha a cytoskelet

KNOLLE – *knolle (kn)* má narušenou tvorbu buněčných přepážek a v důsledku toho orientaci buněčných dělení

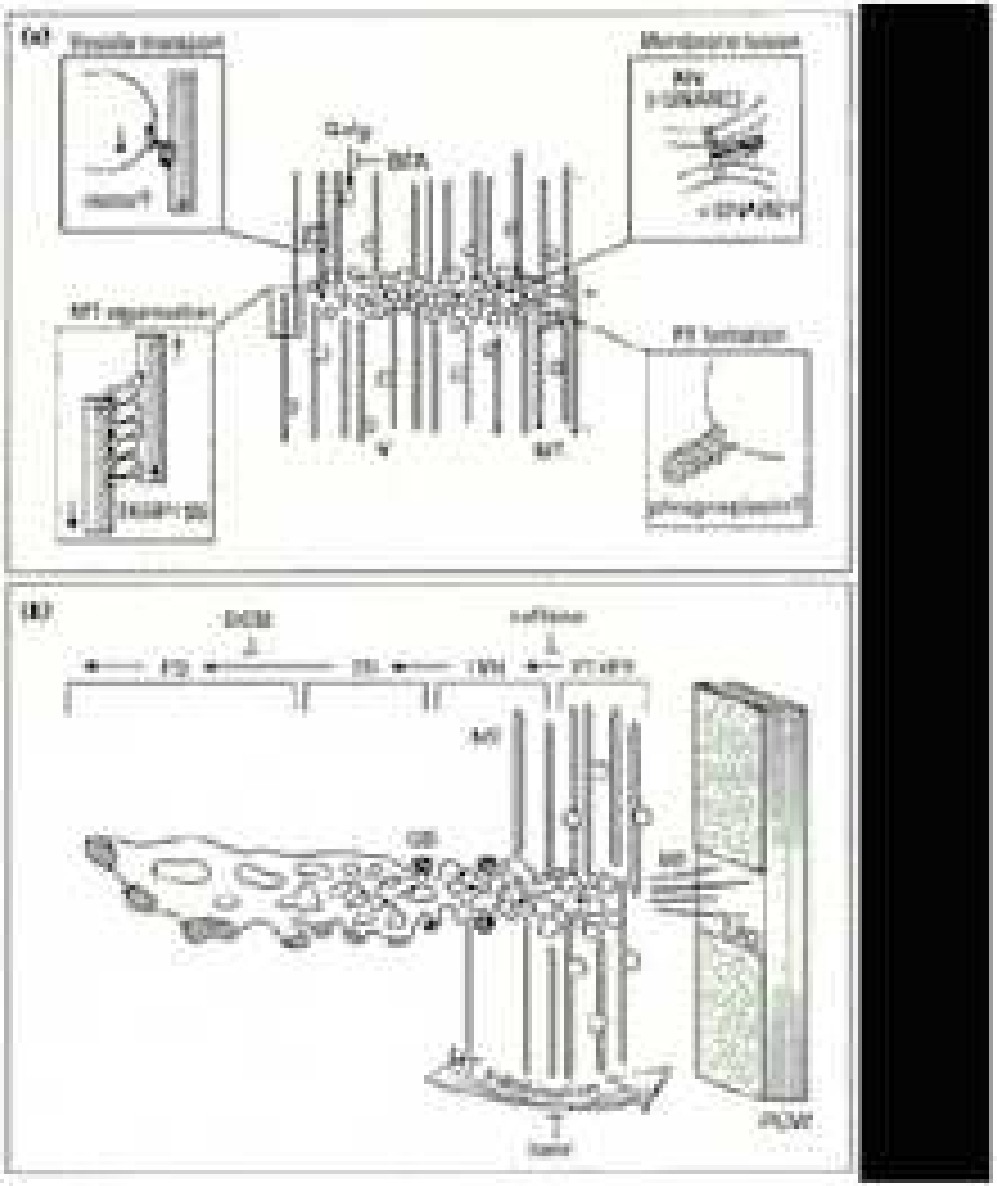


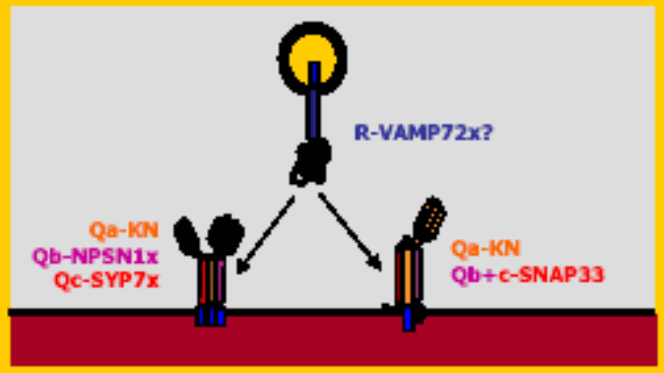
Podobný fenotyp má Sec1 homolog ***keule*** a

gnom
= GEF pro Arf GTPázy

model for the role of KNOLLE during cell wall formation

Knolle je
t-SNARE
bílkovina

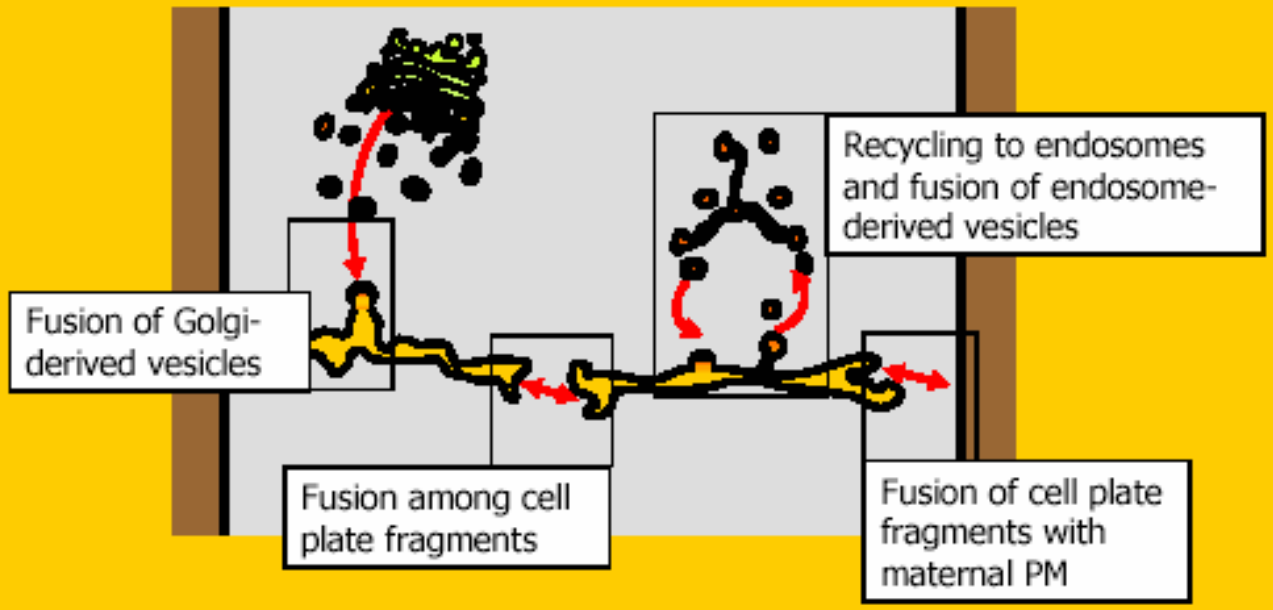




Why have 2 complexes at the Cell Plate?

- Redundancy?
- Specialized cargo?
- Specialized events/organelles?

Potential roles for the SNARE complexes at the Cell Plate:



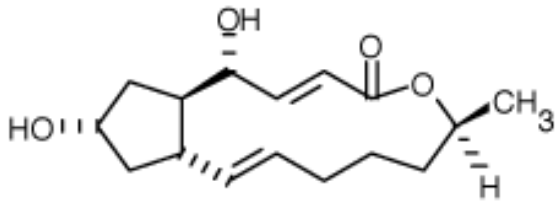
Důležitým nástrojem studia sekreční dráhy je brefeldin A

brefeldin A

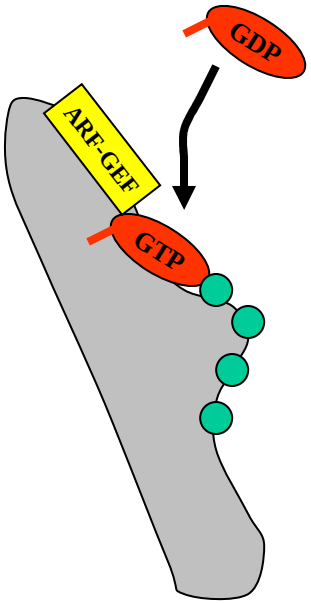
Molecular Formula: $C_{16}H_{24}O_4$

Molecular Weight: 280.36

CAS Number/Name: 20350-15-6 / 1,6,7,8,9,11a,12,13,14,14a-Decahydro-1,13-dihydroxy-6-methyl-4H-cyclopent[f]oxacyclotridecin-4-one



Research tool for membrane trafficking: BFA



Vesicle budding in Golgi is ARF1-dependent; ARF1 undergoes recruitment to Golgi membrane by ARF-GEF-promoted GTP for GDP exchange; ARF1 recruits protein coats (green) needed for vesicle budding; brefeldin A binds at ARF1-GDP /ARF1-GEF interface and inhibits GTP exchange/ membrane recruitment of ARF1 and coats; Golgi stack is disrupted and protein trafficking through the Golgi is inhibited, ALE I DALŠÍ KOMP. Arf-GEFy působí na několika „stanicích“ endom. Syst.

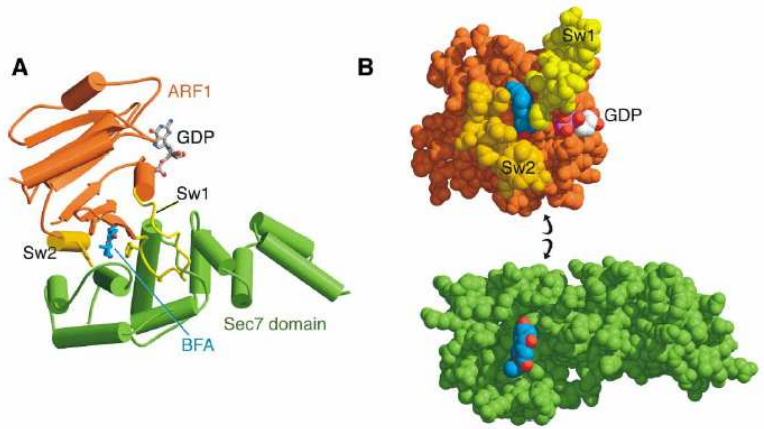


Figure 1. Overall Structure of the ARF•GDP•Sec7•BFA Complex

(A) Ribbon diagram with the Sec7 domain of Gea1 colored green and BFA colored blue with oxygen atoms red. Human ARF1 is orange, with its switch I and 2 regions in lighter hues.

(B) "Open book" view of the interfacial surfaces, with space-filling models of the Sec7 domain and ARF1 rotated apart by 180°. Molecules are colored as in (A). Bound BFA is included on both proteins.

CopI
cis Golgi to ER
and intra Golgi

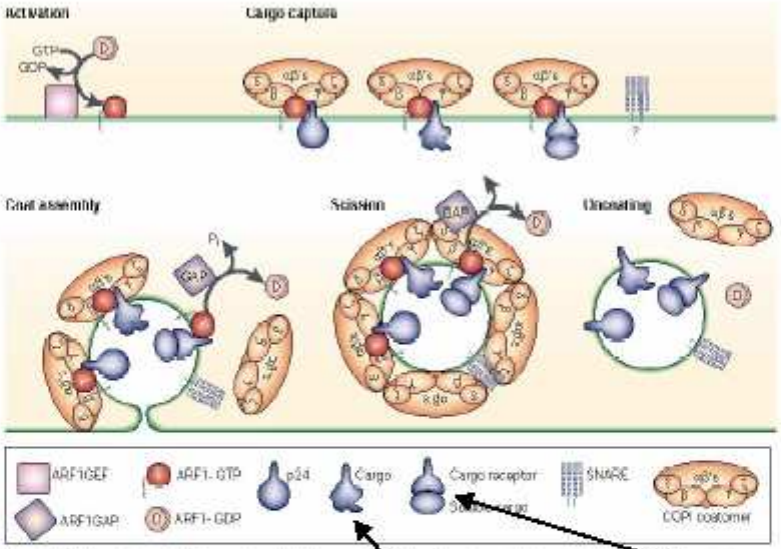


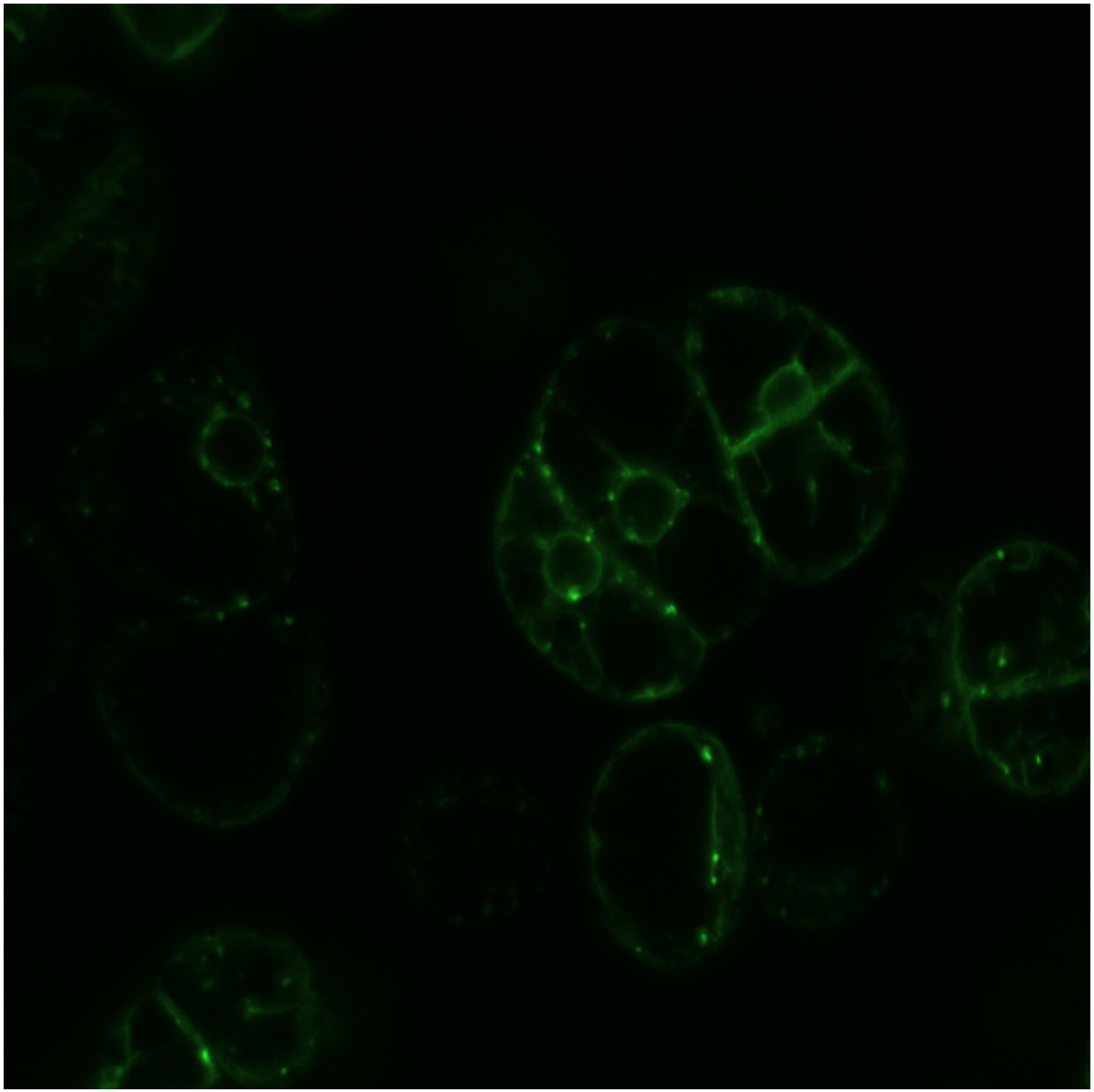
Figure 3 | **The key steps in the formation of COPI-coated vesicles.** Coat assembly is activated by the recruitment of ARF1-GTP to the membrane. This allows the binding of the COPI coatomer and the recruitment of cargo. GTP hydrolysis is slow when ARF1 is bound to its preferred cargo, allowing kinetic regulation of coat recruitment. Membrane deformation occurs at the same time as coat recruitment. When the coat is complete, the vesicle buds. The GTPase activity of ARF1 is enhanced by ARF1GAP, which acts as a timer, leading to inactivation of ARF1 and uncoating. (ARF1, ADP-ribosylation factor 1; ARF1GAP, ADP-ribosylation factor 1 GTPase activating protein; ARF1GEF, ADP-ribosylation factor 1 guanine exchange factor.)

e.g., ERD2
and KDEL protein

1. ARF1=small GTPASE, initiates coating in GTP form (myristoylated)
2. ARF1-GEF=marks the spot This is a brefeldin A target
3. ARF1-GAP=stimulates ARF1 GTPase

4. Cargo membrane protein = KKXX motif in C-terminus
5. Cargo receptor, e.g. KDEL receptor
6. Coatamer = coat recruited by ARF1-GTP and cargo membrane protein. γ subunit recognizes KKXX

BFA
on
GA



V rostlinných buňkách se po působení BFA objevují min.dva nové kompartmenty (v různých buňkách různě):

1. Hybridní cisGA-ER (podobně jako u živočichů).
2. Tzv. „BFA-compartment“ – transGA-endosom.

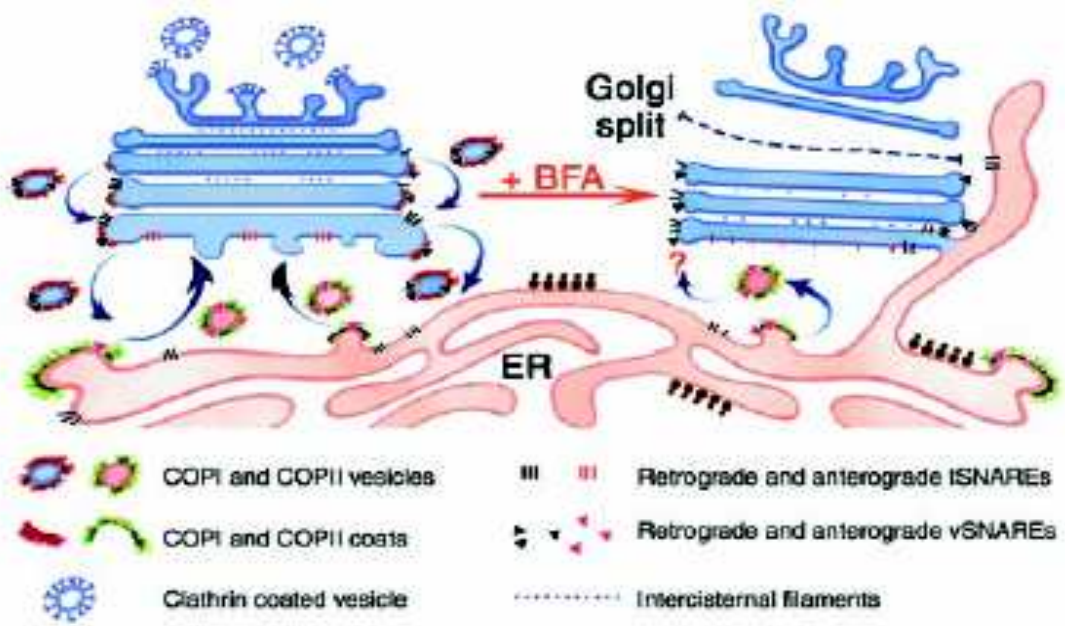


Figure 1. Effects of BFA on membrane trafficking between ER and Golgi. In untreated cells (left), export from the ER (pink) is mediated by COPII-coated vesicles (green). Fusion of these anterograde vesicles depends on anterograde v-SNAREs on the vesicles (red triangles) and t-SNAREs on the cis Golgi (red lines). Anterograde transport (at least to the PVC) continues on the trans side of the Golgi with clathrin-coated vesicles (crossed blue lines). Retrograde transport within the Golgi and from the Golgi to the ER depends on COPI coats (red) and retrograde v- and t-SNAREs (black triangles and lines). In BFA-treated cells (right), all vesiculation at the Golgi stops due to the inhibition of Arf1. As a result, retrograde v-SNAREs remain exposed on the Golgi and the cisternae fuse directly with the ER. Cisternal maturation continues in the presence of BFA so that early Golgi compartments assume a more trans-like morphology. At the same time, the later cisternae and the TGN are lost to the cytoplasm and, eventually, to the BFA compartment. COPII vesicle formation at the ER is initially not inhibited by BFA, but anterograde vesicles may no longer be able to fuse with the maturing cis cisterna, thus effectively blocking ER-to-Golgi transport.

Problém interpretace vlivu BFA

1. V buňce je (podle stavu diferenciace) několik GEFů (8x u At) pro několik ARFů(12x u At), různě silněexprimovaných a různě lokalizovaných.
2. **Některé ARF-GEFy (u At 3x) nejsou! inhibovány BFA.**

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The *Arabidopsis* GNOM ARF-GEF Mediates Endosomal Recycling, Auxin Transport, and Auxin-Dependent Plant Growth

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Jutta Keicher,[1] Wolfgang Kornberger,[1]
Philippe Muller,[2] Alain Delbarre,[2]
Takashi Ueda,[3] Akihiko Nakano,[3]
and Gerd Jürgens[1,4]

1. Polar auxin transport depends on the PIN1 (a putative auxin carrier) and its asymmetrical localization to the plasma membrane
2. PIN1 continuously cycles between PM and endomembrane compartments.
3. The Gnome gene encodes an ARF-GEF and its thought to play a role in PIN1 localization
   - a. *gnome* mutant plants exhibit loss of cell to cell alignment
   - b. Similar effects have been observed by flooding plants with auxin or by using certain auxin inhibitors
   - c. *gnome* mutants are defective in PIN1 localization but are not affected in localization of other proteins via the secretory pathway.
   - d. PIN1 is rapidly internalized in response to brefeldin A; Gnome is a BFA sensitive ARF-GEF.

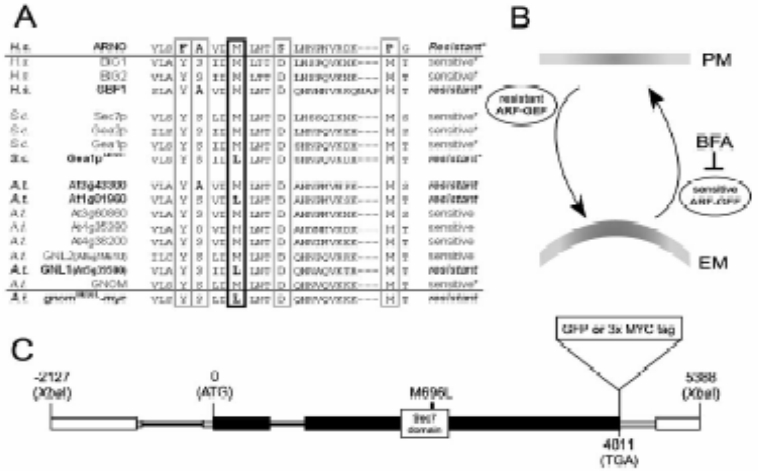

Figure 1. Engineering of a BFA-Resistant GNOM Variant

(A) Sequence alignment of the region determining BFA resistance of ARF-GEFs. All large ARF-GEFs from human (H.s.), yeast (S.c.), and *Arabidopsis* (A.t.) are shown. At the top, ARNO, as an example of a BFA-resistant, small, mammalian ARF-GEF. Residues known to be involved in BFA sensitivity are boxed. The ones determining resistance are written in bold. Asterisks indicate that BFA sensitivity/resistance was determined experimentally, and unmarked ones are predictions inferred from this data. The residue boxed in black indicates the amino acid exchange chosen to be introduced into GNOM (bottom line).

(B) Schematic model to explain internalization of PM markers upon BFA treatment. BFA is thought to block a sensitive ARF-GEF responsible for recycling, while ongoing endocytosis might be mediated by a resistant ARF-GEF or, alternatively, by ARF-independent endocytosis.

(C) Overview of the BFA-resistant GN^{M696L}-myc construct used. Positions indicated are relative to the translational start. Black boxes indicate translated regions, light gray boxes UTRs, lines indicate introns, and white boxes intergenic regions (promoter). The box marked "Sec7 domain" indicates the region of the central catalytic domain. A 3× myc-tag was translationally fused to the 3′ end of the ORF of the complementing genomic *Xba*I fragment. The resulting construct was then mutated.

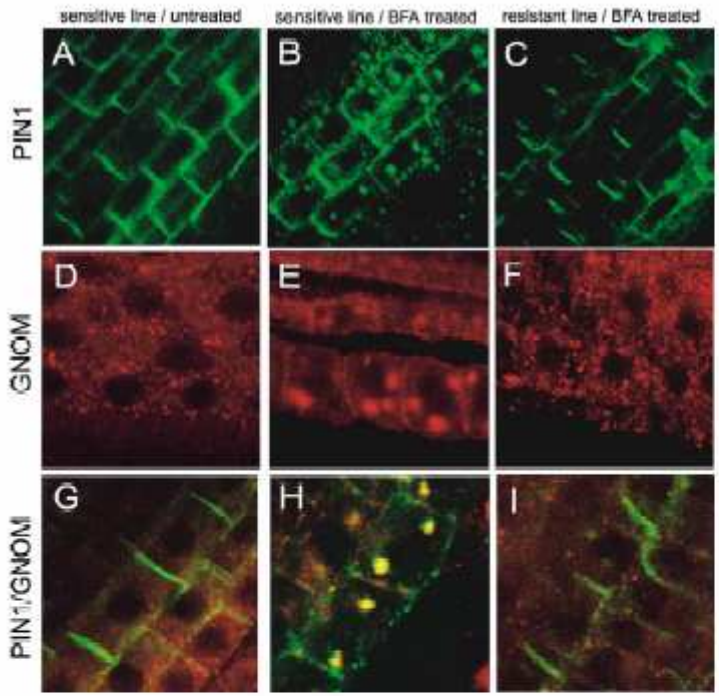

Figure 2. Brefeldin A Responses of PIN1 and GNOM Are Altered in Engineered GNOM Lines

Confocal images of seedling root tips stained with PIN1 antibody (green) and monoclonal myc antibody (red) in GNOM-myc transgenic lines. (A–C) PIN1, (D–F) GNOM-myc, and (G–I) PIN1 and GNOM-myc. (A, D, and G) Control treatment on GN^{wt}-myc line, (B, E, and H) BFA 50 μM for 60 min on GN^{wt}-myc line, and (C, F, and I) BFA 50 μM 60 min on $GN^{R5/6}$-myc line. Note yellow intracellular dots in (H), indicating colocalization of PIN1 and GNOM.

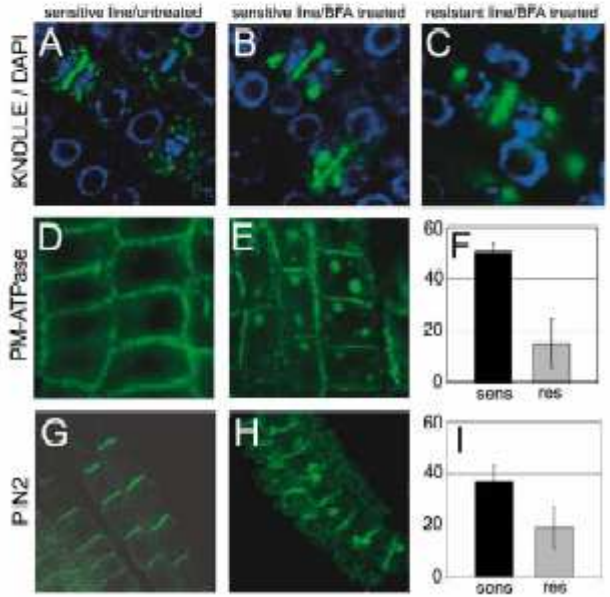

Figure 3. BFA Resistance of Other Plasma Membrane Markers in BFA-Resistant Lines

(A–C) KNOLLE, (D and E) PM-ATPase, and (G and H) PIN2. (A, D, and G) Untreated GN^{wt}-myc line, (B, E, and H) BFA 50 μM for 60 min GN^{wt}-myc, and (C) BFA 50 μM for 60 min $GN^{R5/6}$-myc line. (F and I) Percentage of cells showing intracellular accumulation of label after BFA treatment in sensitive versus resistant lines. "Sens" is GN^{wt}-myc line (black) and "res" is $GN^{R5/6}$-myc (gray). Each bar is an average of five root tips, representing about 1000 cells in total.

Polární transport IAA je závislý na polární lokalizaci PIN auxinových výtokových přenašečů.
Jejich lokalizace je závislá na polarizované sekreci a aktinovém cytoskeletu.
Auxin inhibuje endocytózu
(J. Friml lab - Tübingen)

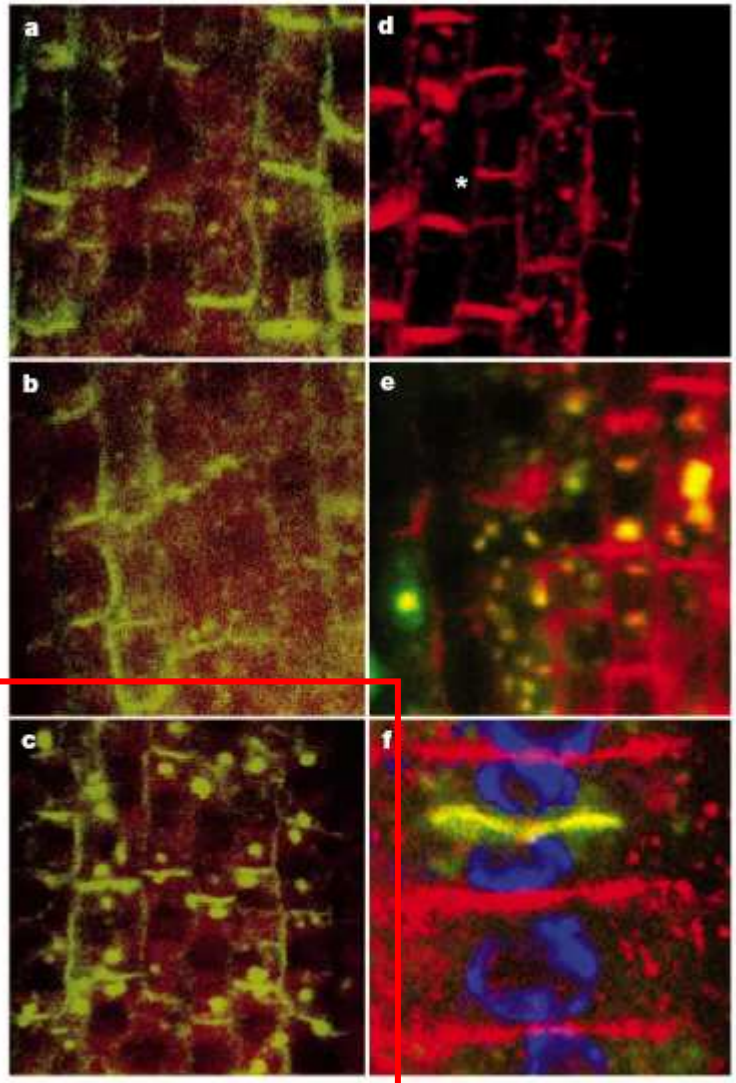

Figure 2 PIN1 localization affected by cytoskeleton-depolymerizing drugs. **a**–**c**, Cytochalasin D (cytD) effect on BFA inhibition of PIN1 (green) cycling. **a**, Treatment with 20 μM cytD for 2 h. **b**, Pre-treatment with 20 μM cytD for 15 min, then 50 μM BFA and 20 μM CytD for 45 min. **c**, Treatment with 50 μM BFA for 45 min followed by 90 min washing out BFA with 20 μM CytD. **d**, PIN1 staining after 10 μM oryzalin for 2 h—note patches in cell marked with asterisk as compared with normal localization in cell below. **e**, **f**, Colocalization of PIN1 (red) and KNOLLE (green) in small patches (yellow) after treatment with 10 μM oryzalin for 2 h (**e**), and in plane of cell division (yellow) of untreated control (**f**). DAPI staining of nuclei is blue.

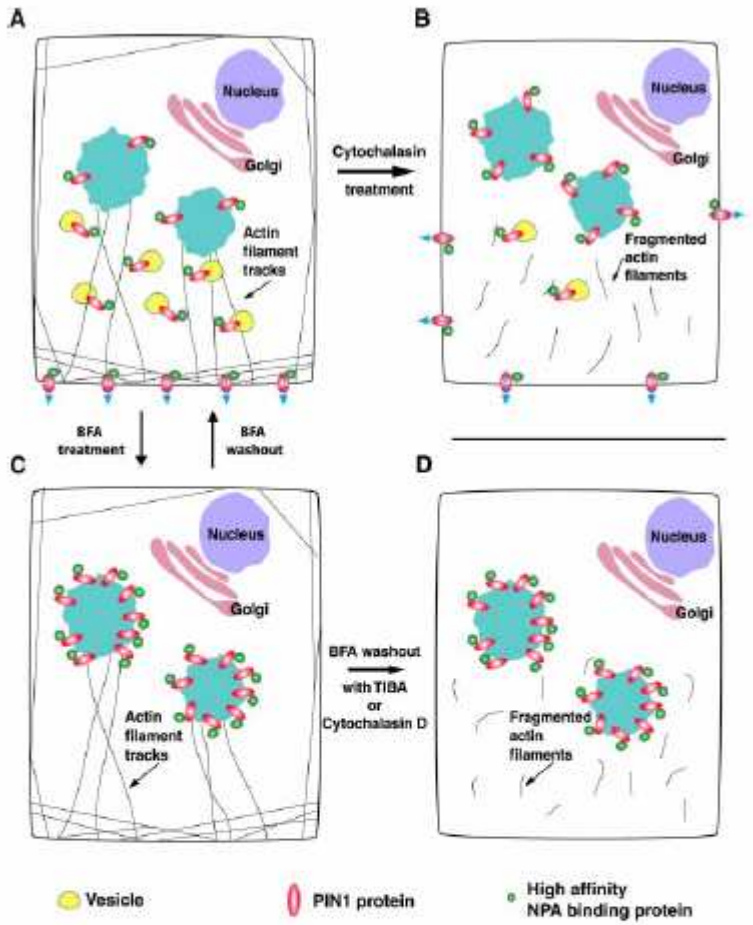

Figure 1. Model of Experiments That Have Examined the Mechanisms of the Control of PIN1 Protein Localization.

(A) Vesicle-dependent transport of the PIN1 protein to the basal membrane appears to be along actin tracks. A high-affinity NPA binding protein has been found to interact with actin and may act as a bridge between these transport vesicles and the actin tracks and/or may serve to localize PIN1-containing IAA efflux complexes to the basal membrane.

(B) Treatment with cytochalasin leads to a random PIN1 protein distribution and reduces polar auxin transport.

(C) Treatment with BFA leads to a loss of PIN1 protein on the membrane and an accumulation of PIN1 protein at two undefined internal membrane structures. The effect is reversible upon removal of the BFA.

(D) Treatment with either cytochalasin or the IAA efflux inhibitor TIBA during the removal of BFA prevents the restoration of asymmetric PIN1 distribution. Treatment with either cytochalasin or TIBA before BFA treatment prevents the BFA-induced PIN1 internalization, and treatment with TIBA alone has no effect on PIN1 localization (data not shown).

(This figure was modified from Mudsy and DeLong [2001]).

Dynamiky cytoskeletu a endomembránového systému jsou neoddělitelně recipročně provázány.