Endomembránový systém

třídění bílkovin – transport váčků – sekretorická dráha – exocytóza – endocytóza

protein sorting – vesicle trafficking – secretory pathway – exocytosis – endocytosis

Endomembránový systém - funkce a dynamika

třídění proteinů – transport váčků – sekretorická dráha – exocytóza – endocytóza
protein sorting – vesicle trafficking – secretory pathway – exocytosis – endocytosis

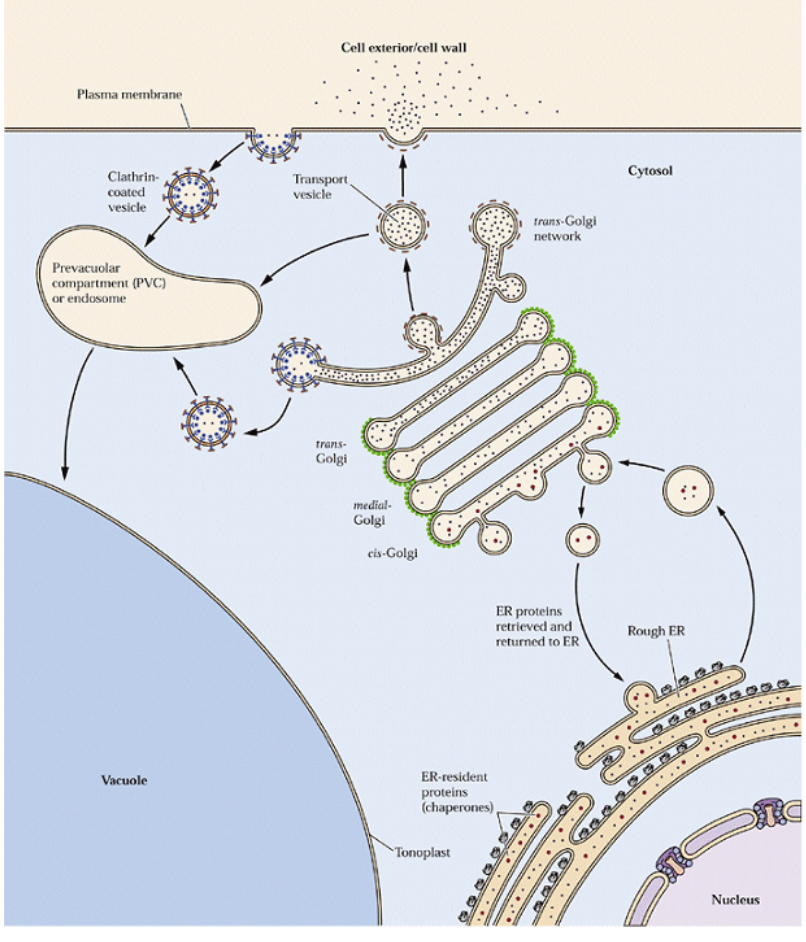
Prokaryotická buňka vystačí s cytopl. membránou, velké eukaryotické buňky vyvinuly endomembr. systém (poměr povrchu/objemu).

Jak se proteiny a lipidy pohybují mezi kompartmenty?

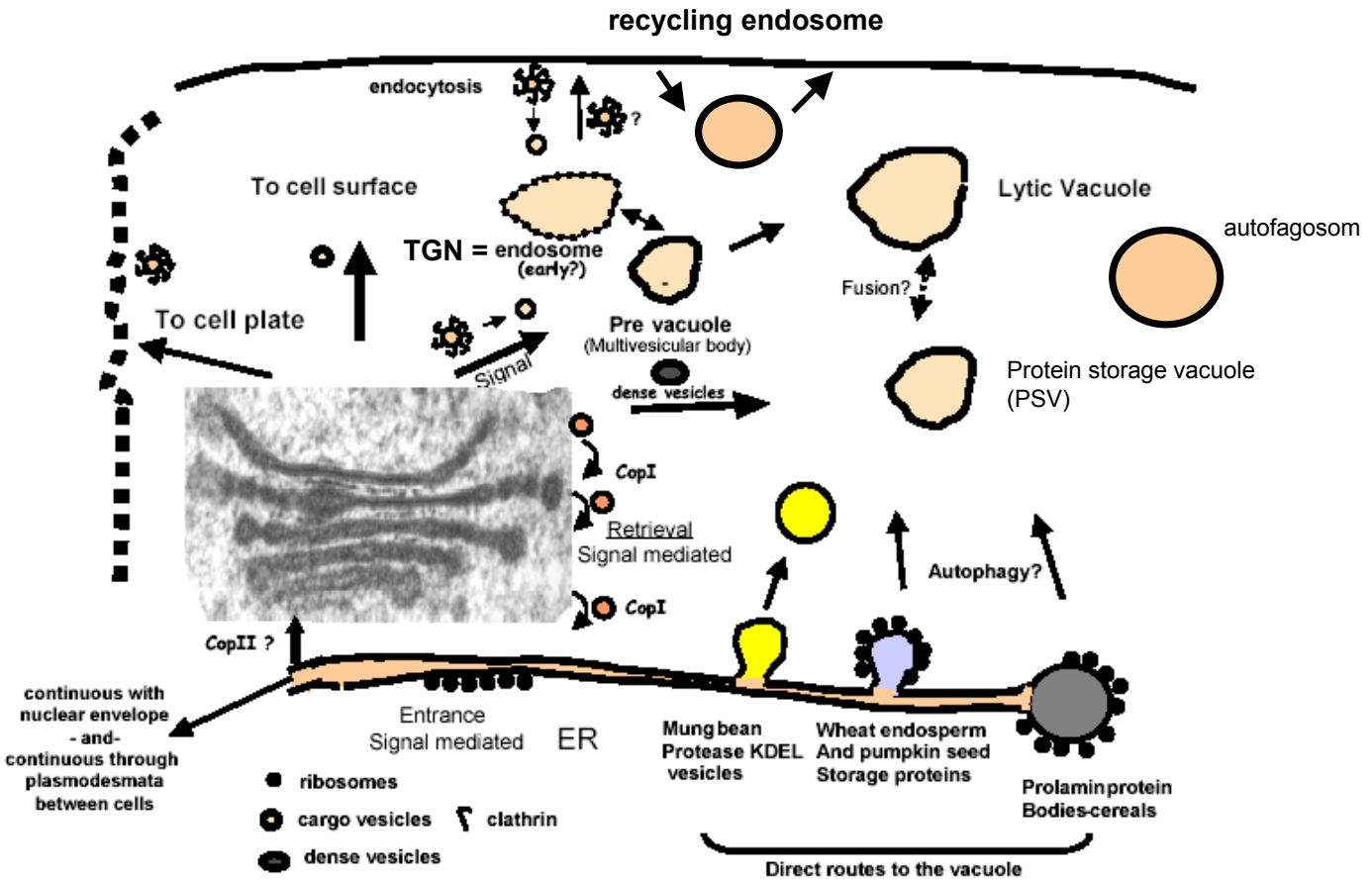
Jak buňka zajistí správnou lokalizaci proteinů?

Molekulární mechanismy transportu váčků.

Součástí endomembránového systému je také jaderná membrána a plasmatická membrána



Součásti endomembránového systému



Transport proteinů v buňce

buňka má 2 *pooly* ribosomů

syntéza proteinů probíhá

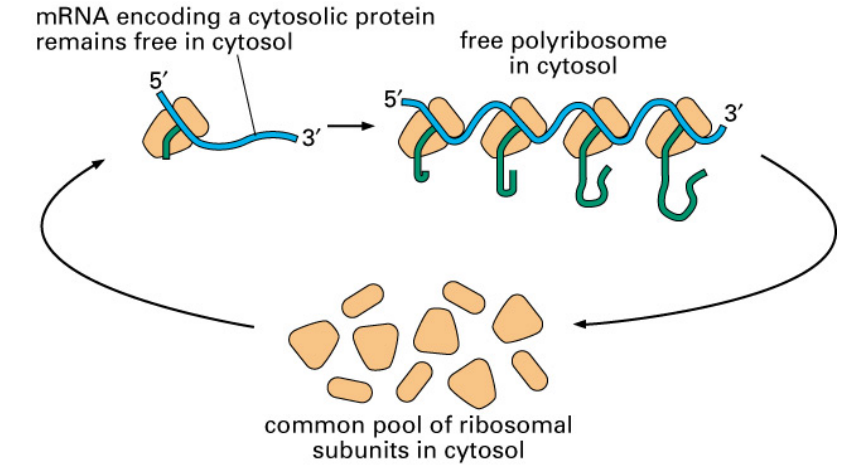
volné

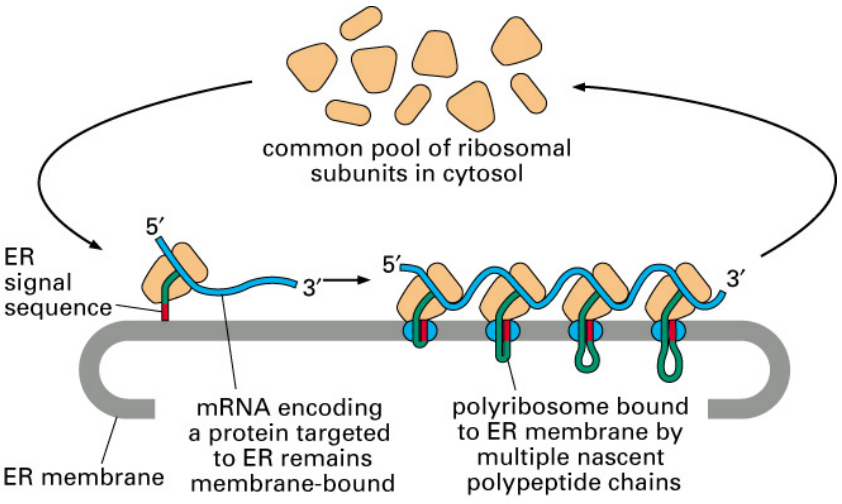
v cytoplazmě

ER vázané

na ER

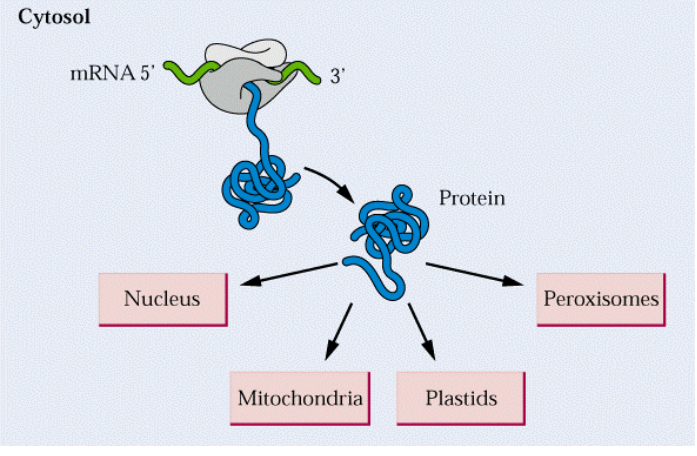
v mitochondriích a plastidech



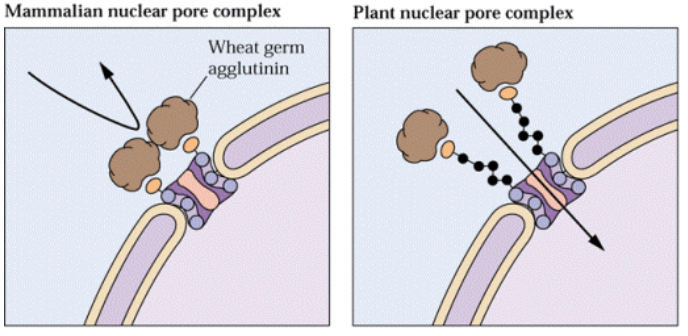


Transport proteinů a lipidů

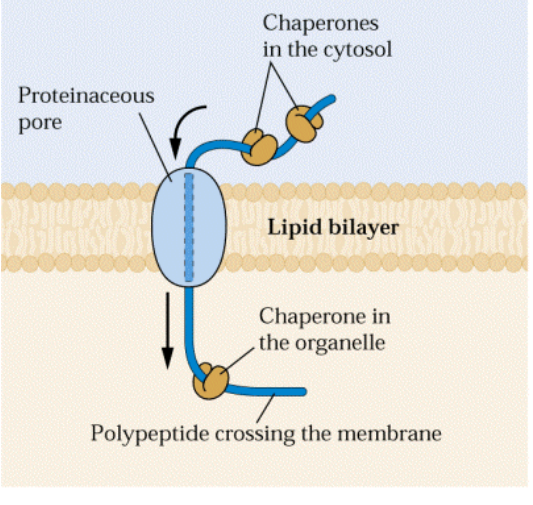
proteiny syntetizované v cytoplasmě se transportují:



1) jadernými póry

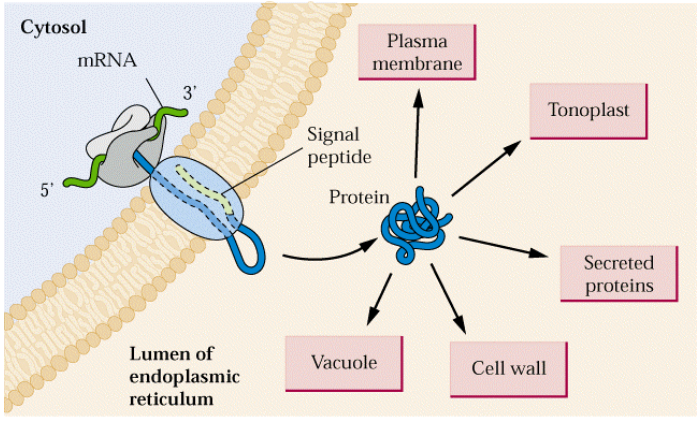


2) translokátory (rozvinutí a opětovné sbalení)

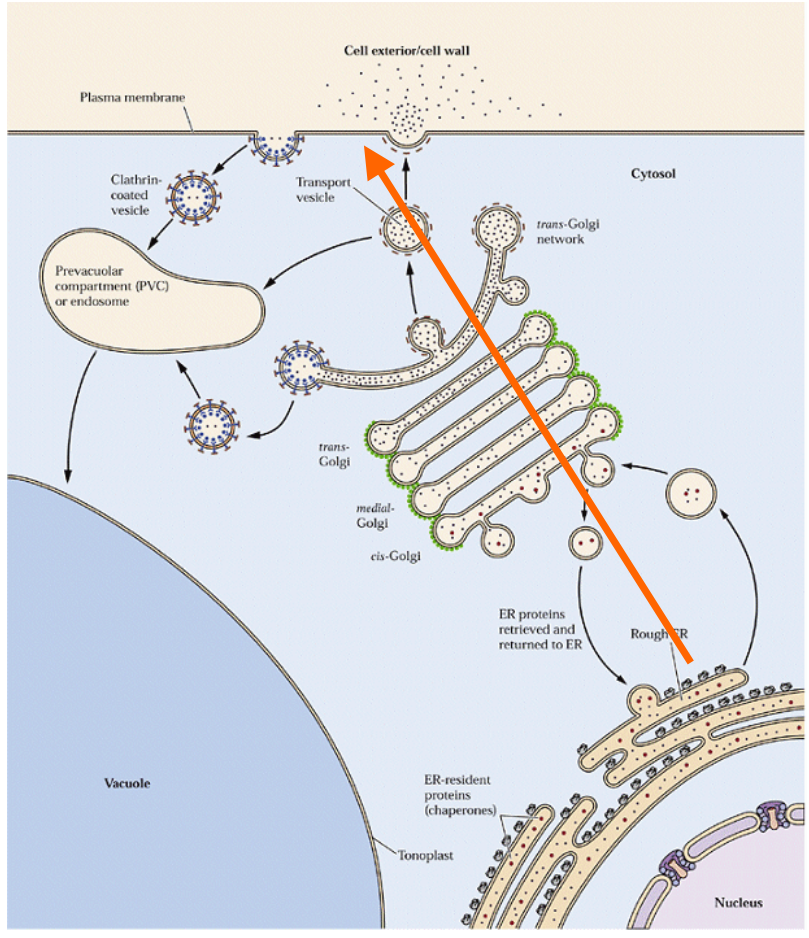


Transport proteinů a lipidů

proteiny endomembránového systému se transportují: 3) vezikulárním transportem



sekretorická dráha



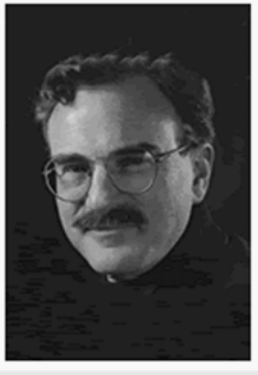
Metody studia sekreční dráhy

2002 Albert Lasker Award for Basic Medical

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.

biochemický přístup

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

genetický přístup

Genetické metody studia sekrece

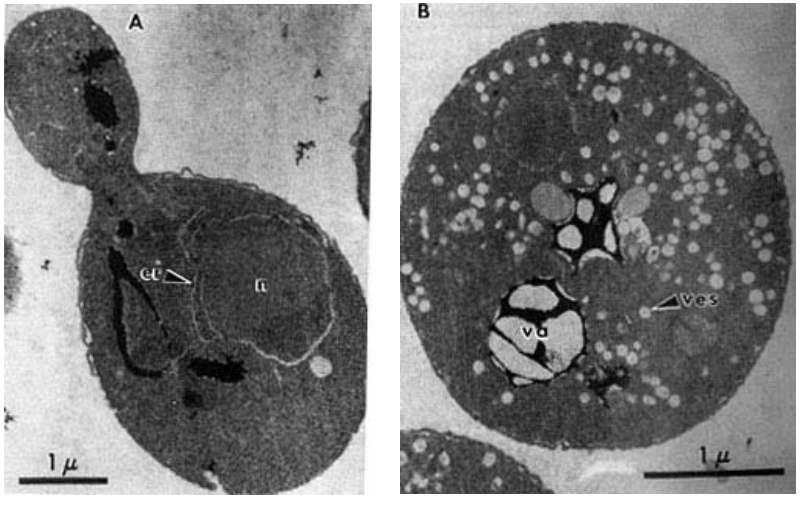
Cell, Vol. 21, 205–215, August, 1980, Copyright ©1980 by Cell Press

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

Cells of a Saccharomyces cerevisiae mutant that is temperature-sensitive for secretion and cell surface growth become dense during incubation at the non-permissive temperature (37 degrees C). This property allows the selection of additional secretory mutants by sedimentation of mutagenized cells on a Ludox density gradient. Colonies derived from dense cells are screened for conditional growth and secretion of invertase and acid phosphatase. The sec mutant strains that accumulate an abnormally large intracellular pool of invertase at 37 degrees C (188 mutant clones) fall into 23 complementation groups, and the distribution of mutant alleles suggests that more complementation groups could be found. Bud emergence and incorporation of a plasma membrane sulfate permease activity stop quickly after a shift to 37 degrees C. Many of the mutants are thermoreversible; upon return to the permissive temperature (25 degrees C) the accumulated invertase is secreted. Electron microscopy of sec mutant cells reveals, with one exception, the temperature-dependent accumulation of membrane-enclosed secretory organelles. We suggest that these structures represent intermediates in a pathway in which secretion and plasma membrane assembly are colinear.

mutageneze
sedimentace denzních buněk
sledování sekrece invertázy
analýza mutantů pomocí elektronové mikroskopie na tenkých řezech



sec15-1 po 2 h @ 37 ºC

akumulace váčků!

Genetické metody studia sekrece

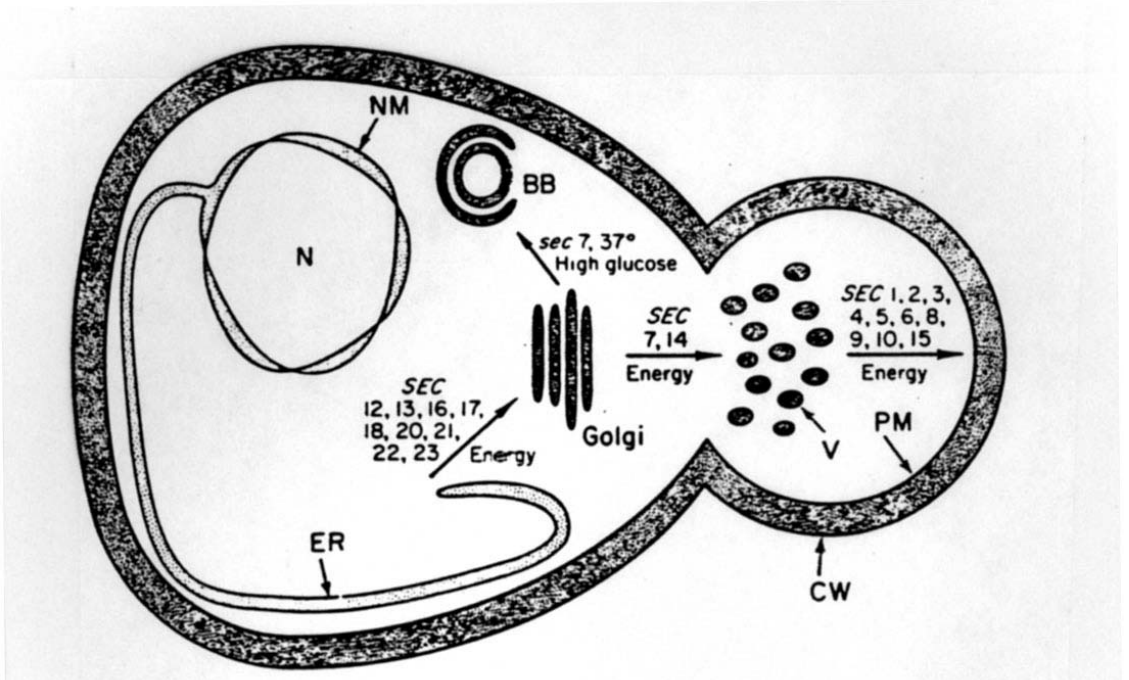


Figure 6. Yeast Secretory Pathway

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.

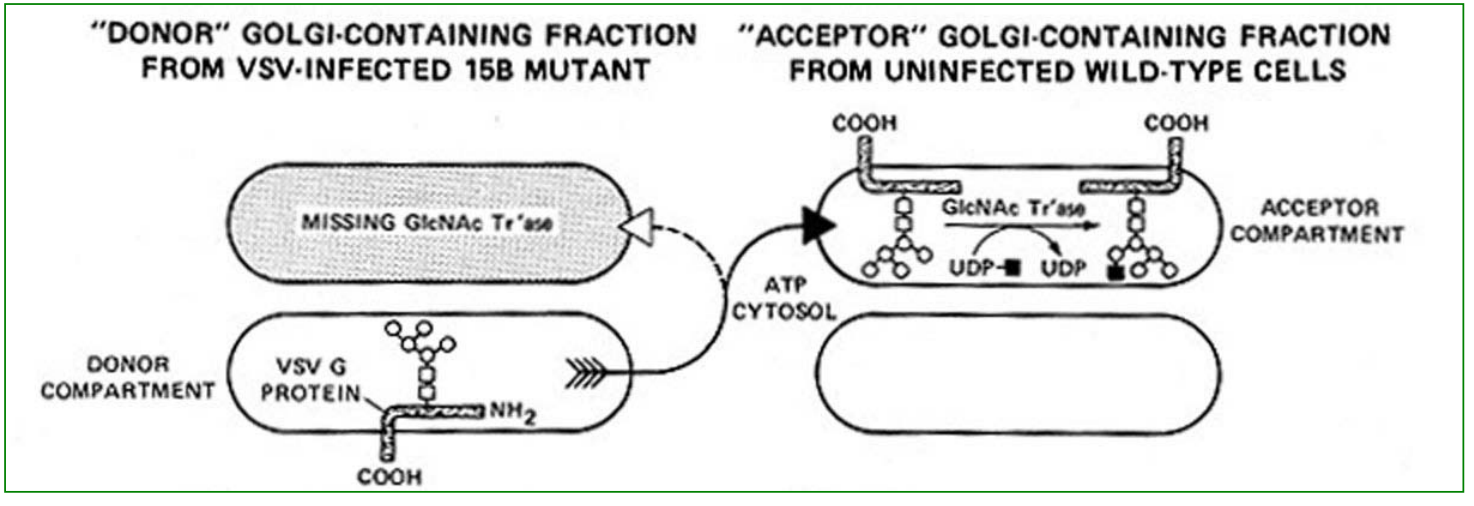
Novick P., Ferro S., and Schekman R. (1981)

Biochemické metody studia sekrece

Cell-free assays of vesicular transport: Reconstitute transport between two organelles *in vitro* (= bezbuněčný systém studia sekrece)

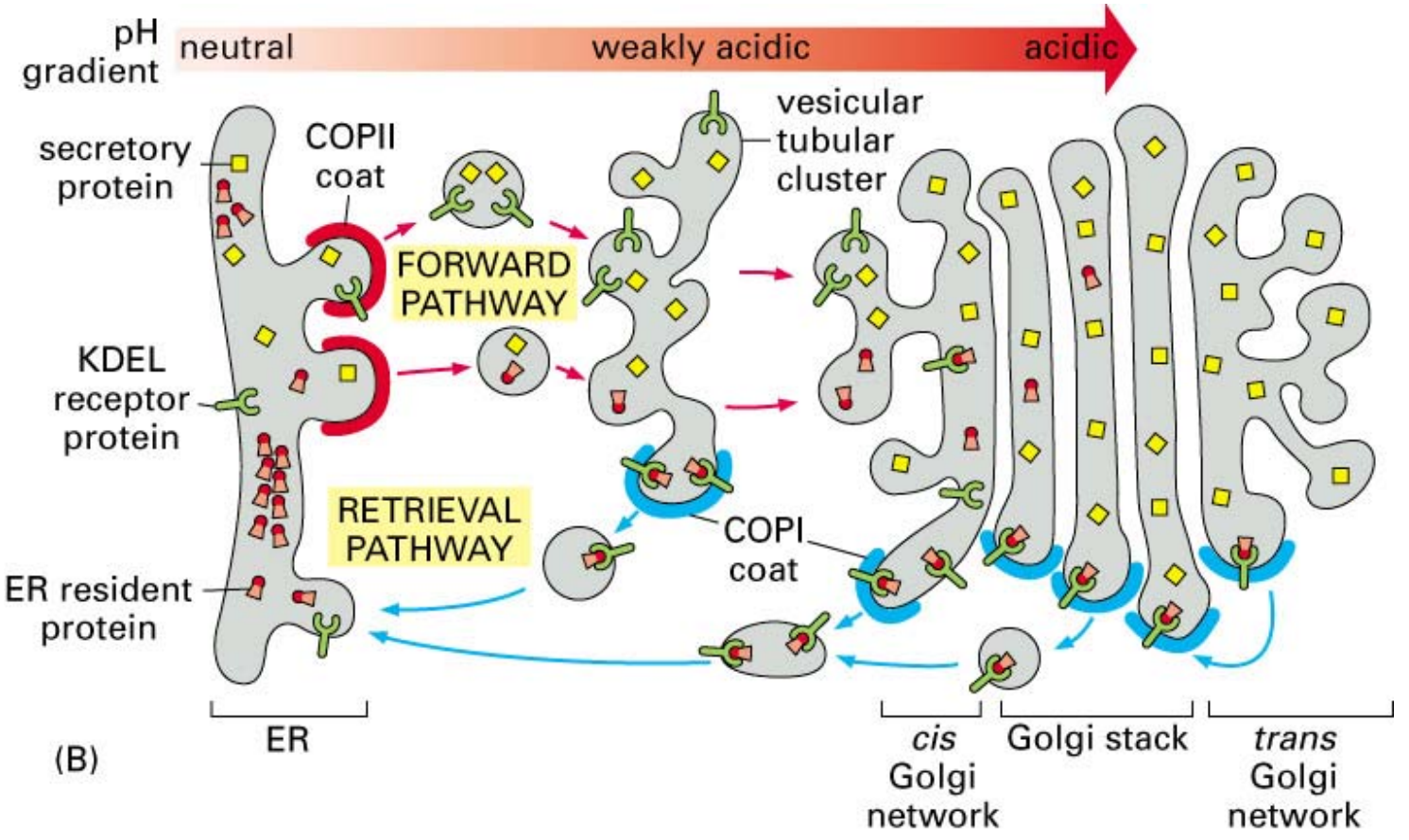
Balch W.E., Dunphy W.G., Braell W.A., and Rothman J.E. (1984)

Reconstitution of the transport of protein between successive compartments of the Golgi measured by the coupled incorporation of N-acetylglucosamine. *Cell* 39, 405-416.



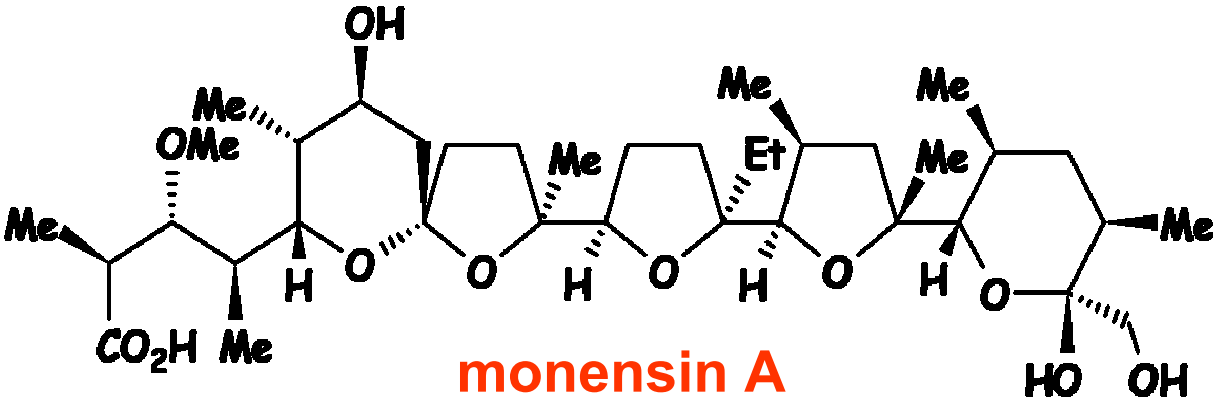
VSV = vesicular stomatitis virus

pH gradient sekretorické dráhy



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

pH gradient sekretorické dráhy



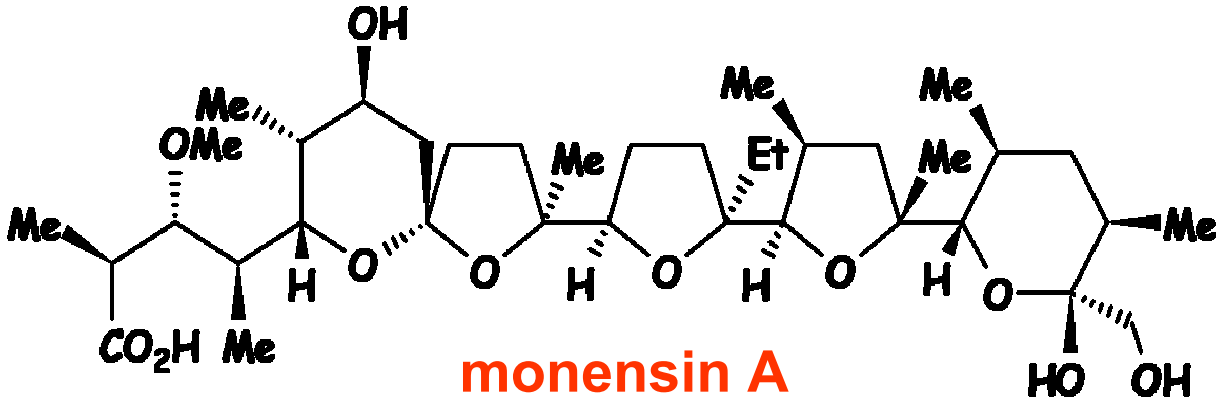
monensin A

IONOPHOR („díry“ v membráně)
blokuje dynamiku transporu Na^+/H^+, a tím regulaci pH v endomembránovém systému

efekt: inhibice sekrece, proteiny se akumulují v ER, trans-GA zvětšuje svůj objem

Monensin isolated from *Streptomyces cinnamonensis* is a well-known representative of naturally polyether ionophore antibiotics. It is able to form pseudomacrocyclic complexes with mono and divalent cations and transport of the cations across cellular membrane. In cells monensin blocks the secretion of glycoproteins. It is soluble in chloroform, ethanol and methanol. Monensin plays an important role as an Na+/H+ antiporter, it blocks intracellular protein transport, and exhibits antibiotic, antimalarial, and other biological activities.

pH gradient sekretorické dráhy



monensin A

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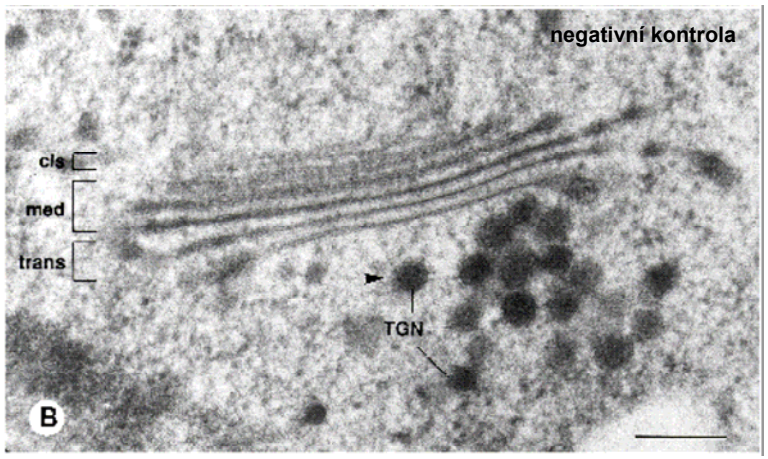
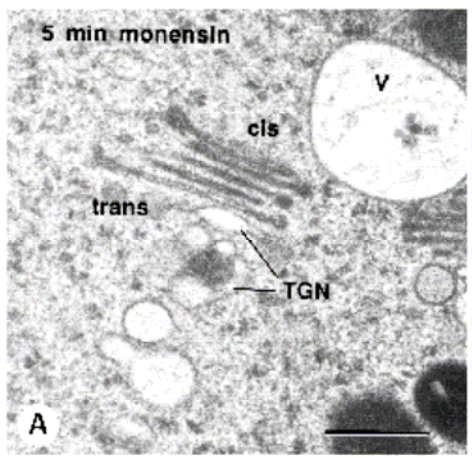
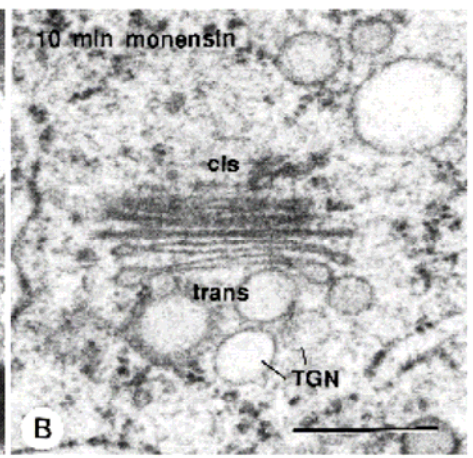
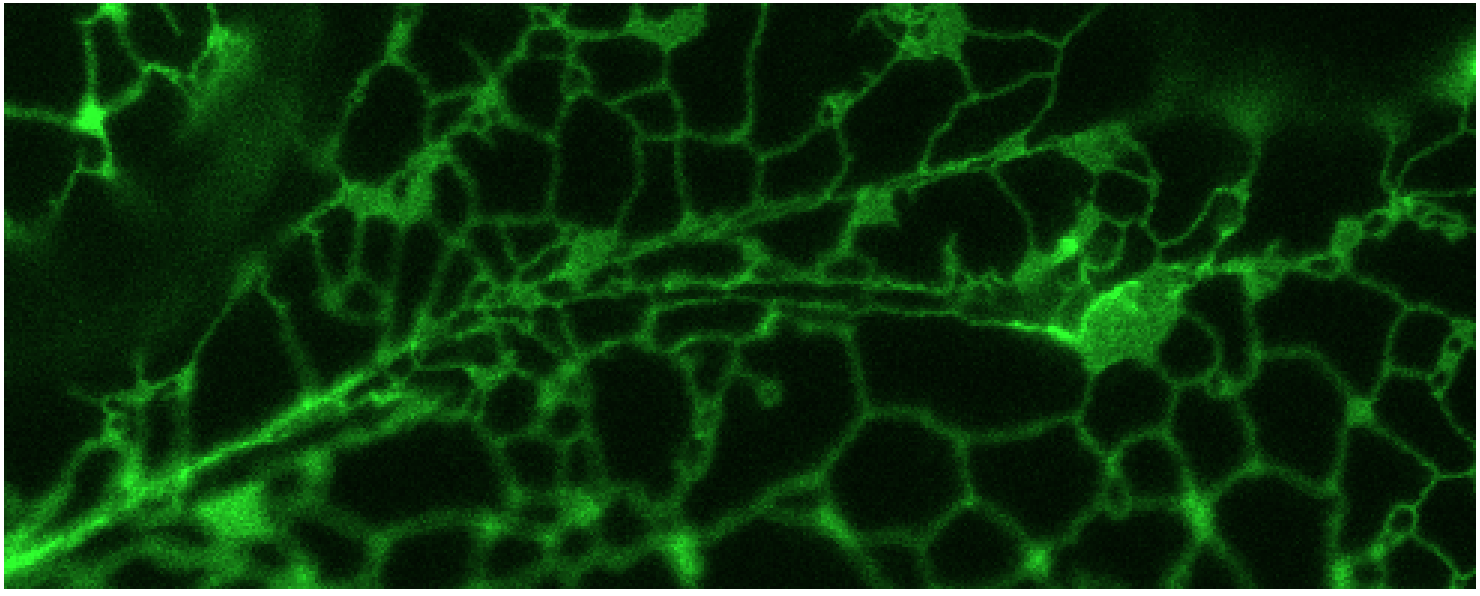






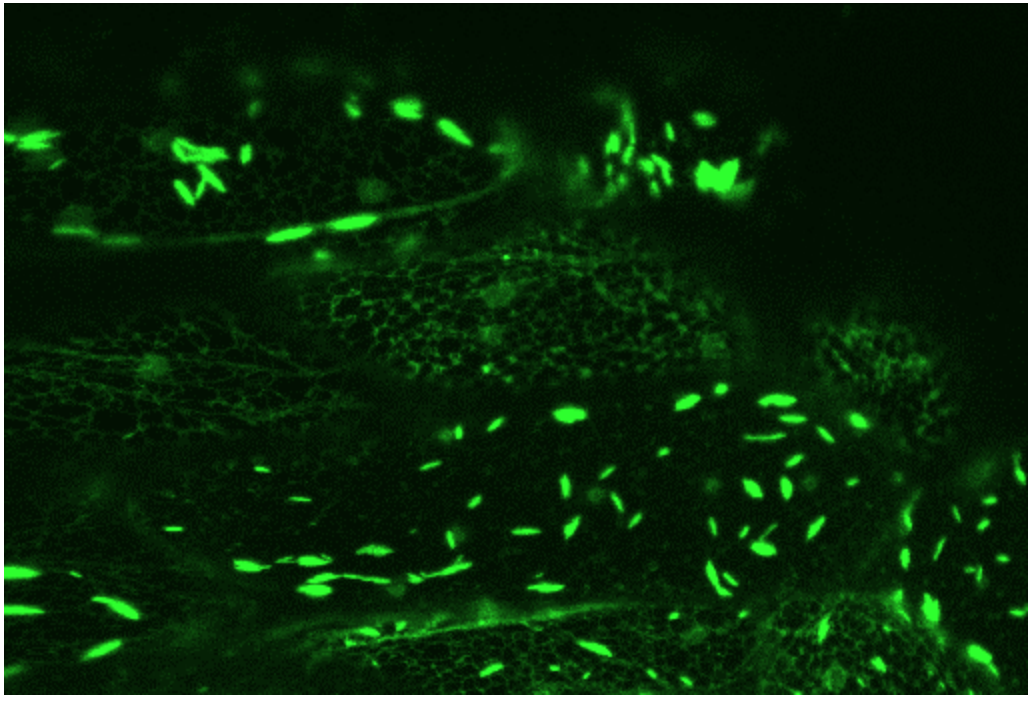
Endoplasmatické retikulum



ER vizualizované pomocí GFP

- syntéza proteinů pro endomembránové kompartmenty, membrány a sekreci
- modifikace proteinů (glykosylace)
- syntéza některých lipidů (růst membrán)
- kontrola kvality proteinů
- signální funkce (regulovaný výtok Ca^{2+})

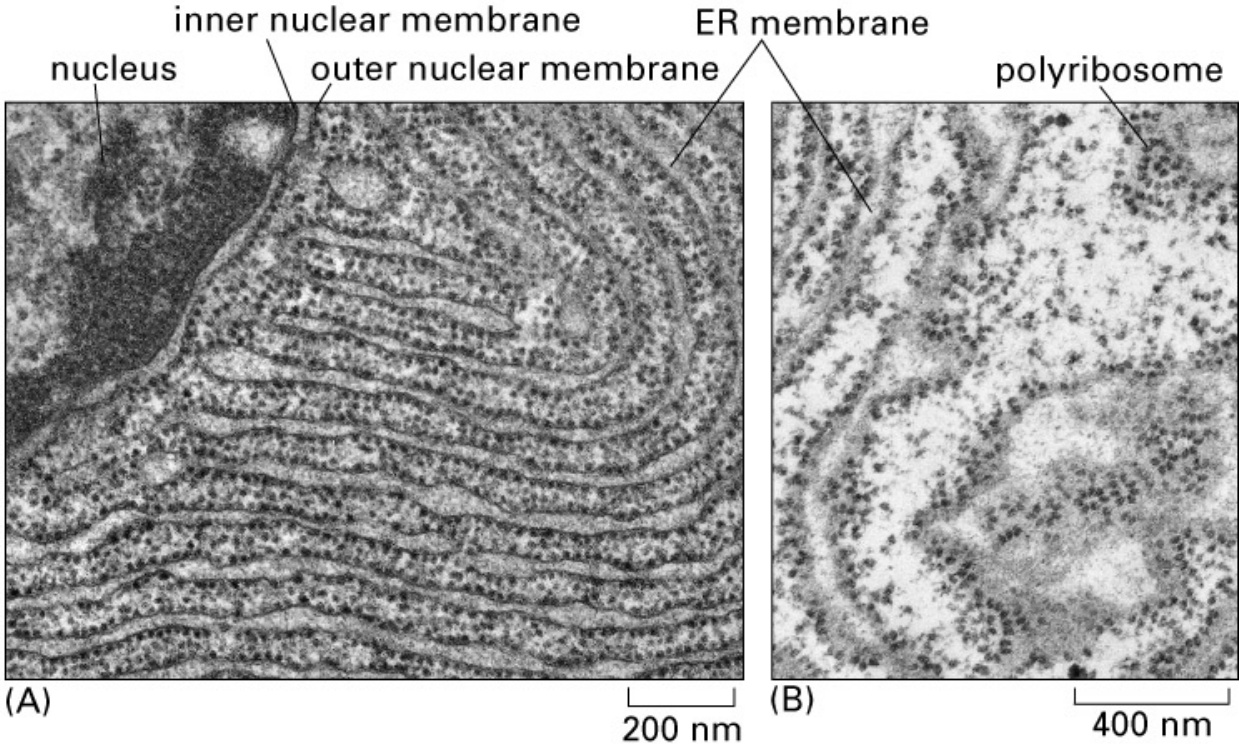
Endoplasmatické retikulum



ER-tělíska (ER bodies)

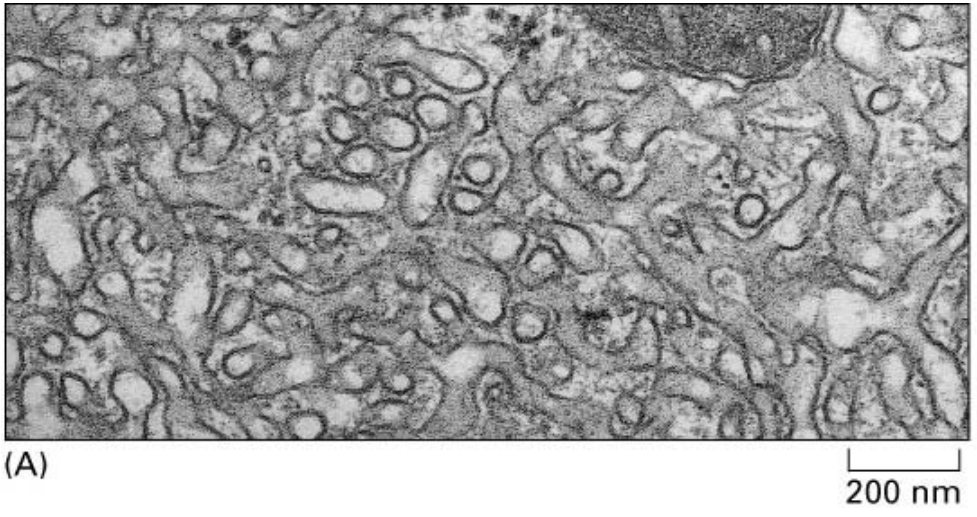
speciální struktury některých rostlinných buněk v listech; obsahují proteázy, které se účastní reakcí na stres a senescence.

Drsné endoplasmatické retikulum



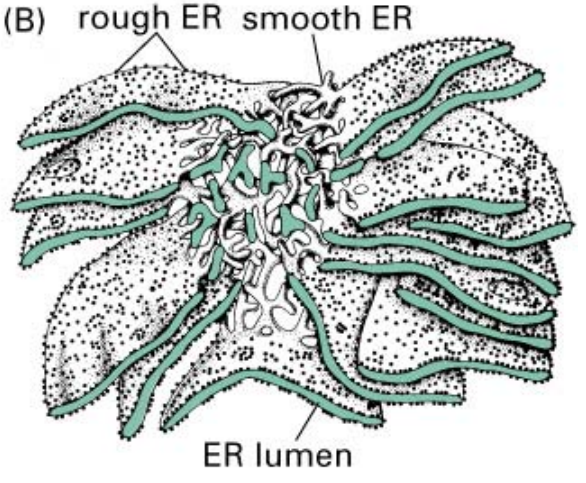
TEM of rough endoplasmic reticulum (rER). Extensive cellular network of membranes. In this case the membranes are involved in protein synthesis and ribosomes are associated with the membranes, giving them a “rough” appearance. The inside of the compartment is called “cisterna”, or “cisternal space”.

Hladké endoplasmatické retikulum

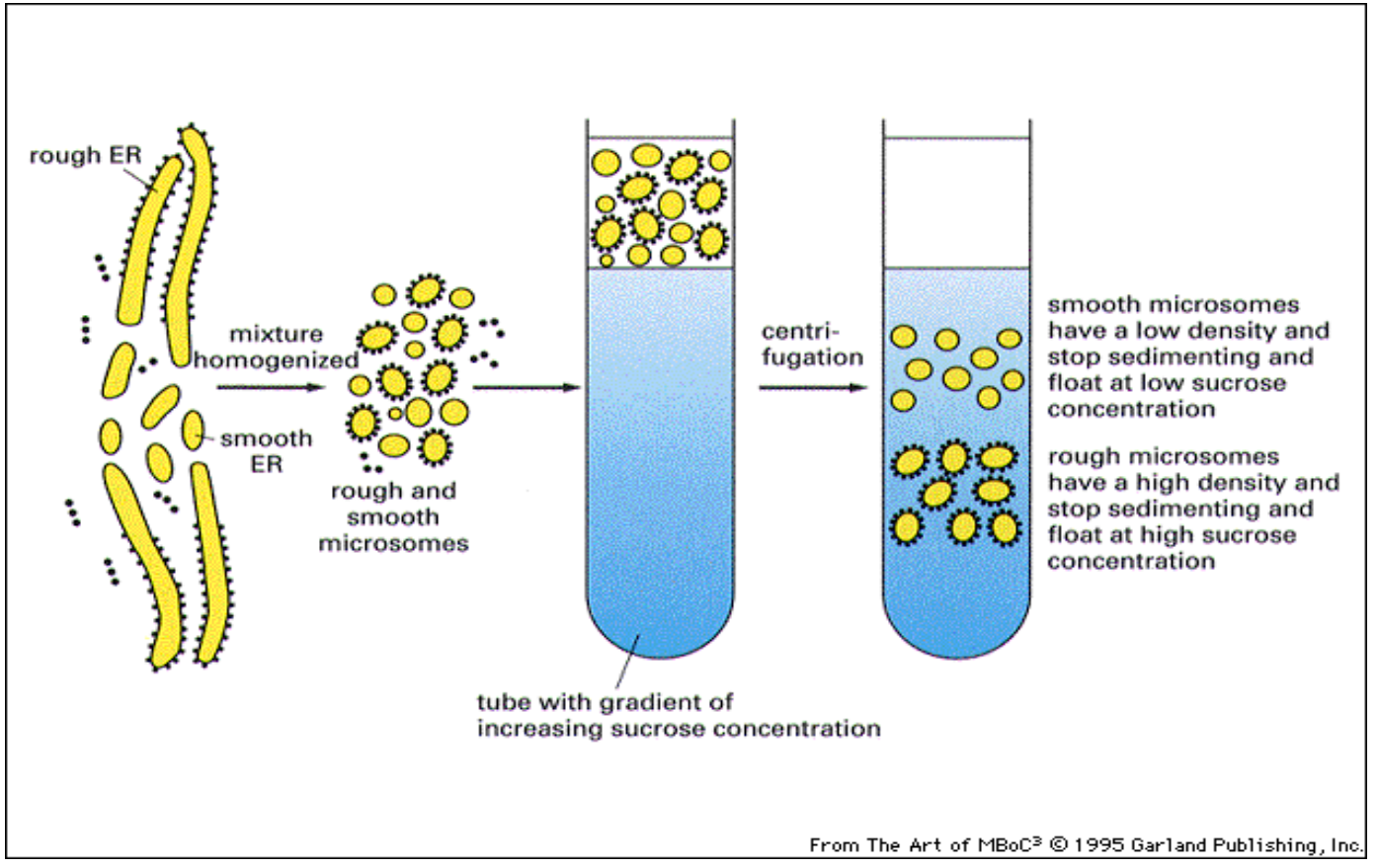


hladké ER (smooth ER, sER)

u rostlin především produkce lipidů a membrán



Endoplasmatické retikulum



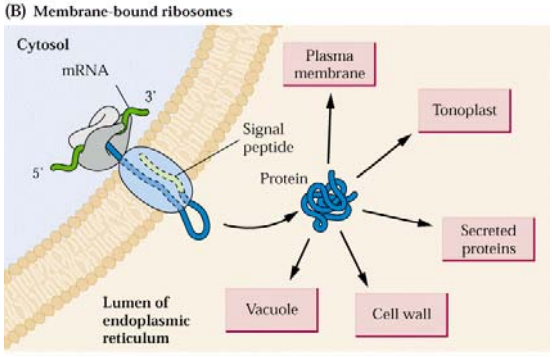
Separation of smooth and rough ER as “microsomes” on sucrose gradient. Different conditions may have to be used to separate peroxisomal microbodies and other vesicles from the smooth microsomes.

Syntéza proteinů na ER

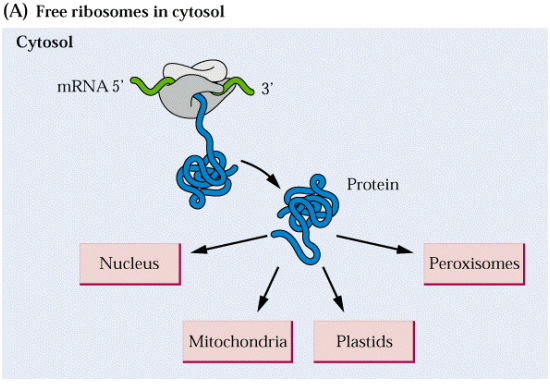
Jak buňka rozliší,
které proteiny syntetizovat v cytoplasmě
a které do ER?

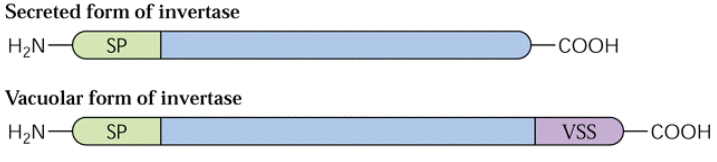
Syntéza proteinů na ER

ER	*signal peptide*
vakuola	*vacuolar sorting signal (VSS)*



chloroplast	*transit peptide*
mitochondrie	*presequence*
jádro	*nuclear localization signal (NLS)*
peroxisome	*p. targeting signal (PTS)*





Syntéza proteinů na ER

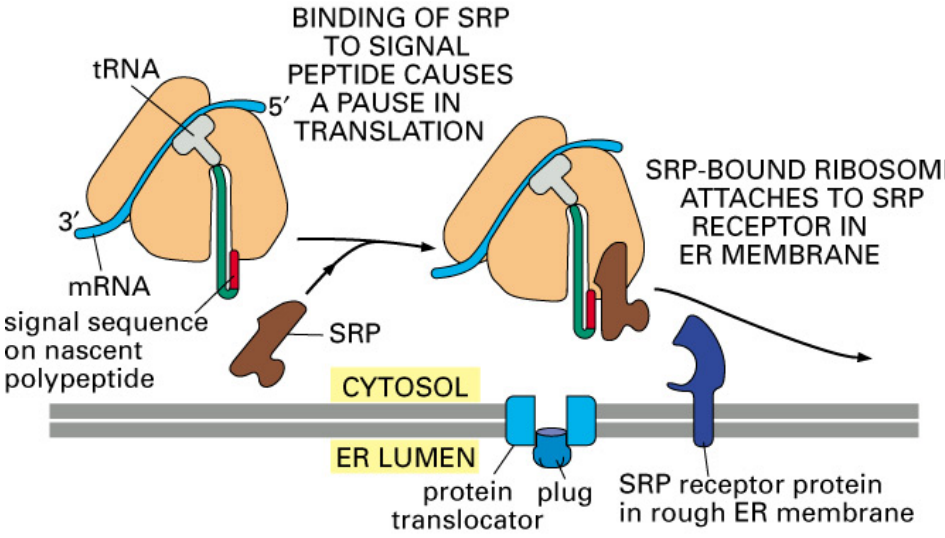
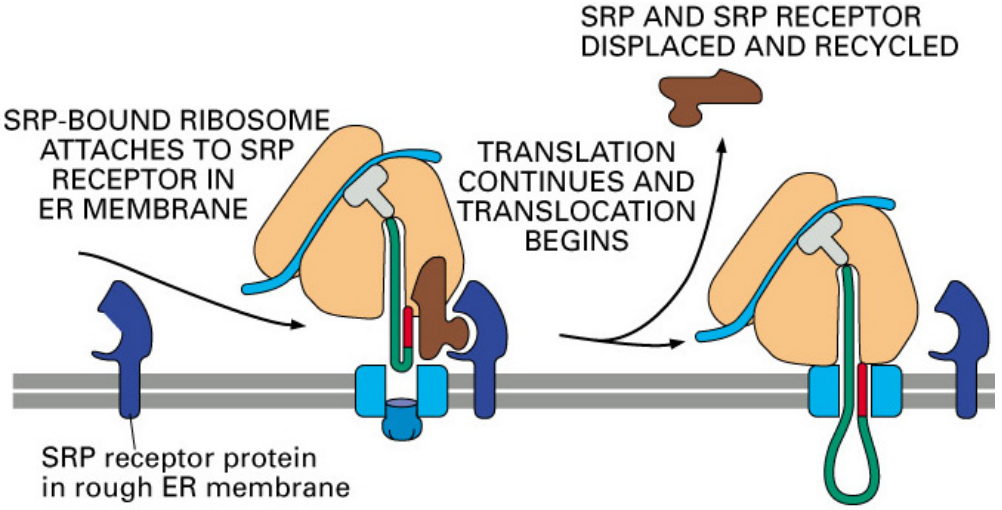


Figure 12–42 part 1 of 2. Molecular Biology of the Cell

signální sekvence

ribosomy jsou associované s drsným ER právě prostřednictvím signální sekvence



Syntéza proteinů na ER

struktura signálního peptidu

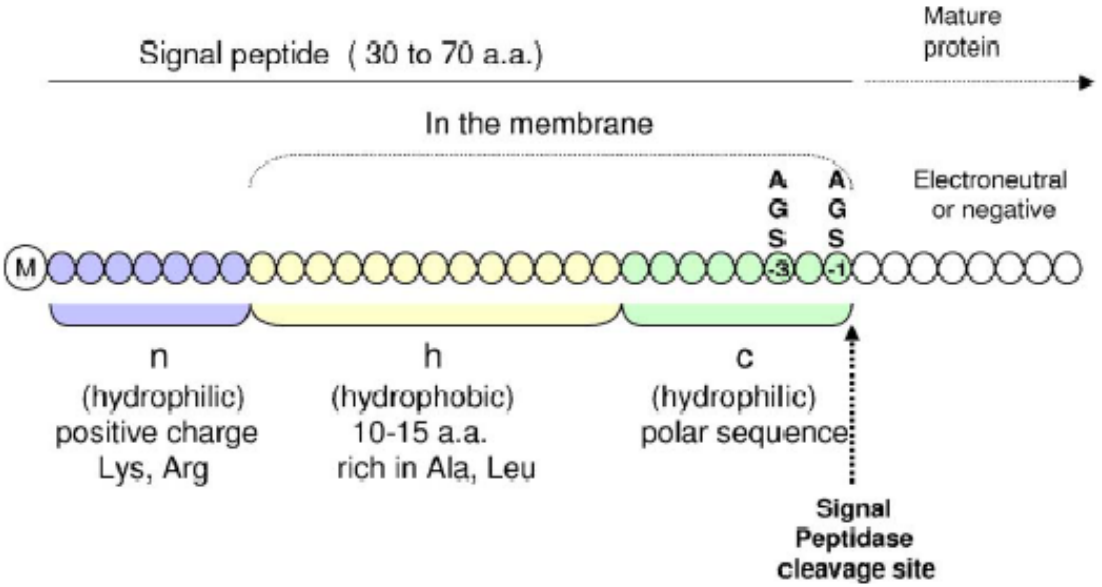
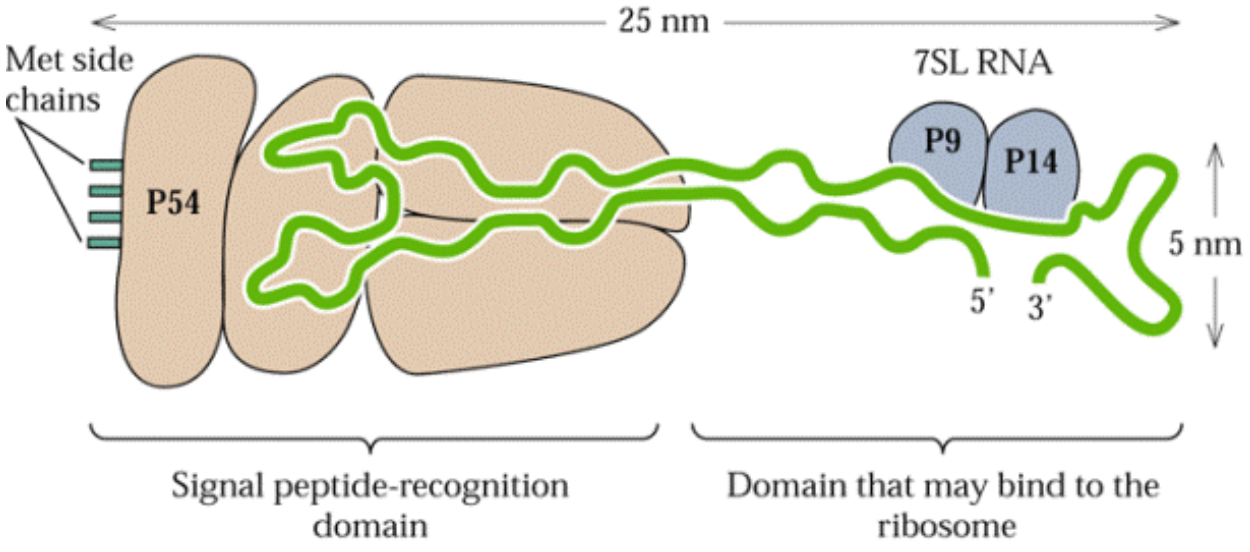


Fig. 3. Typical tripartite structure of the N-terminal signal peptide of preproteins targeted to the ER.

Syntéza proteinů na ER



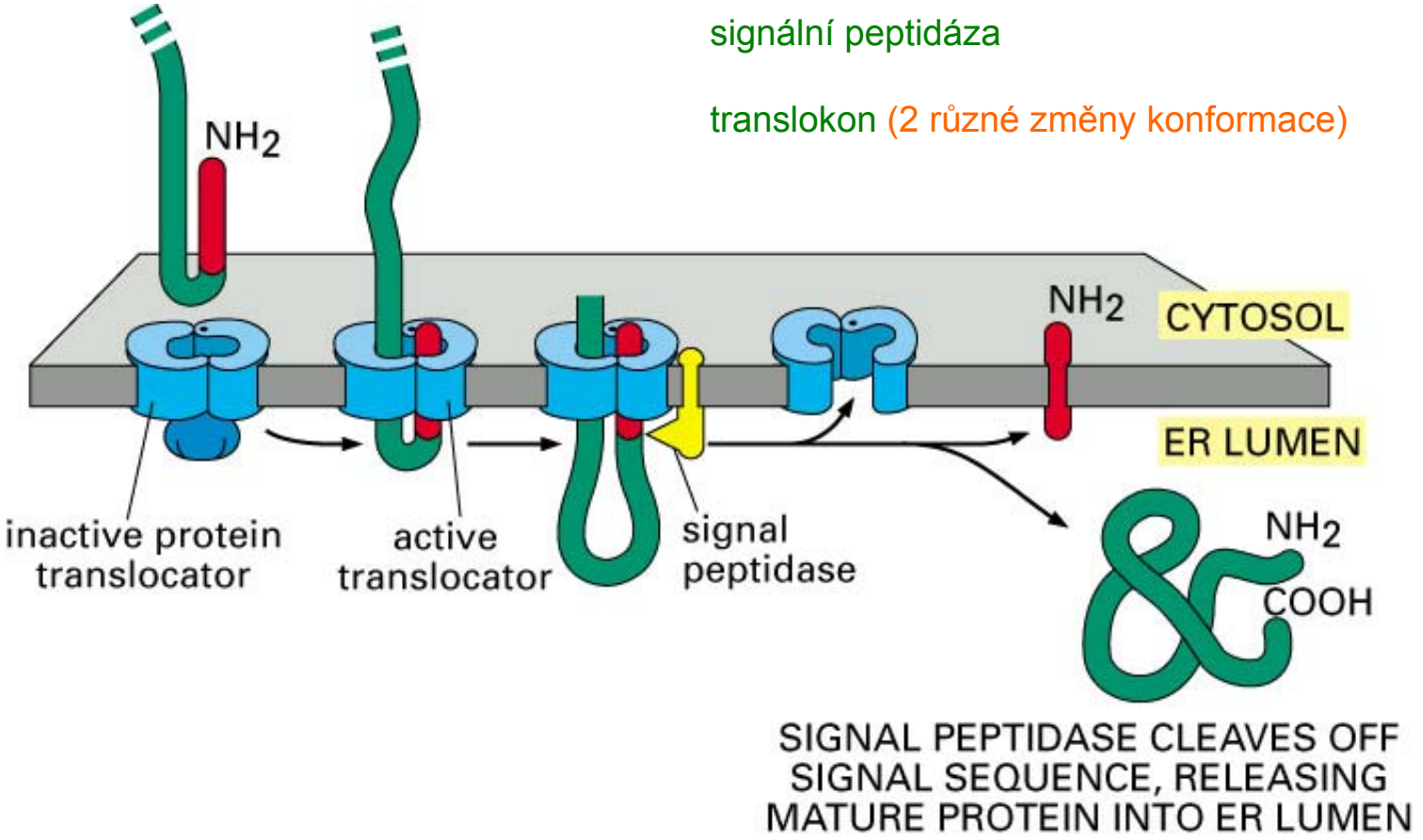
funkční součástí SRP je molekula RNA

Syntéza proteinů na ER

kotranslační import proteinů do ER

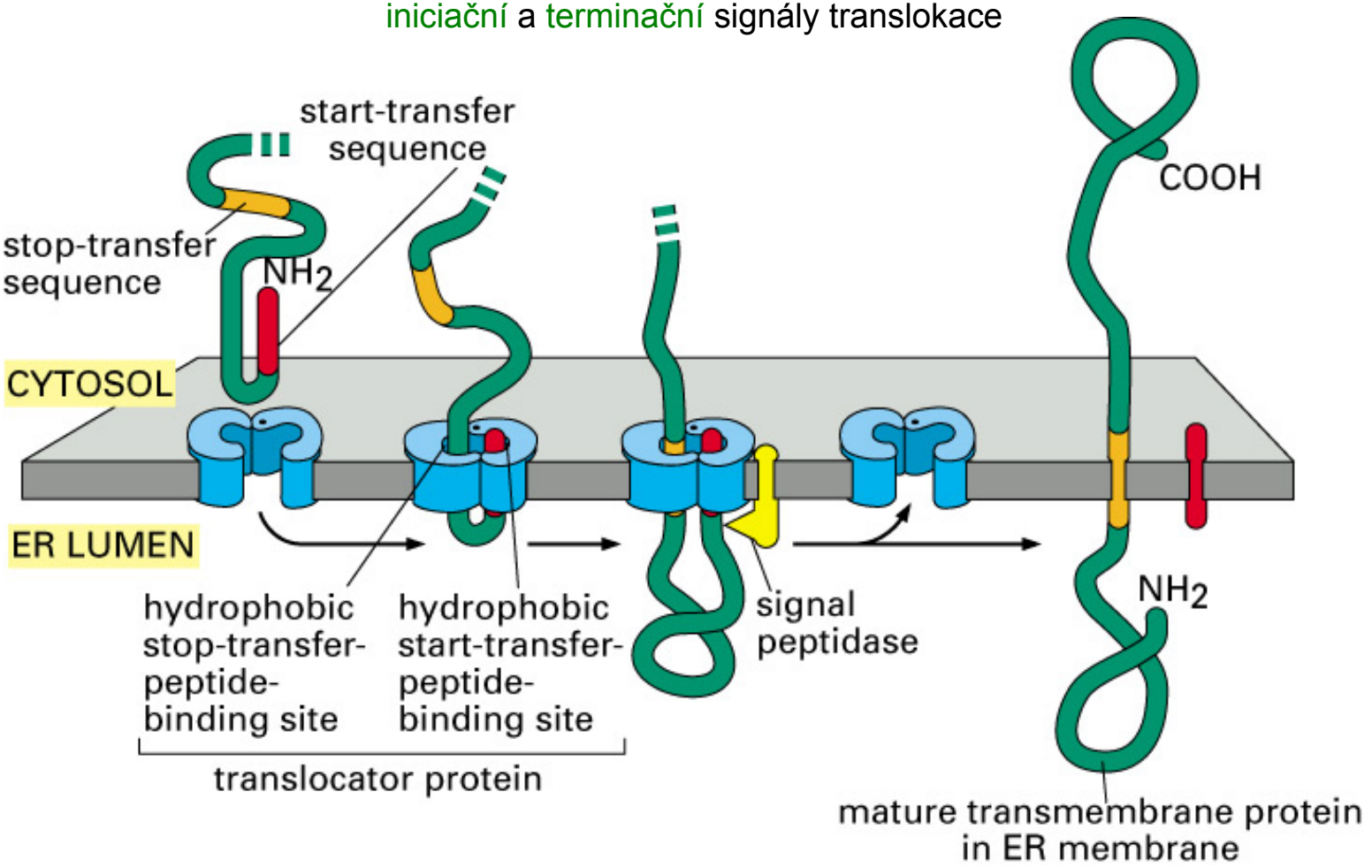
signální peptidáza

translokon (2 různé změny konformace)

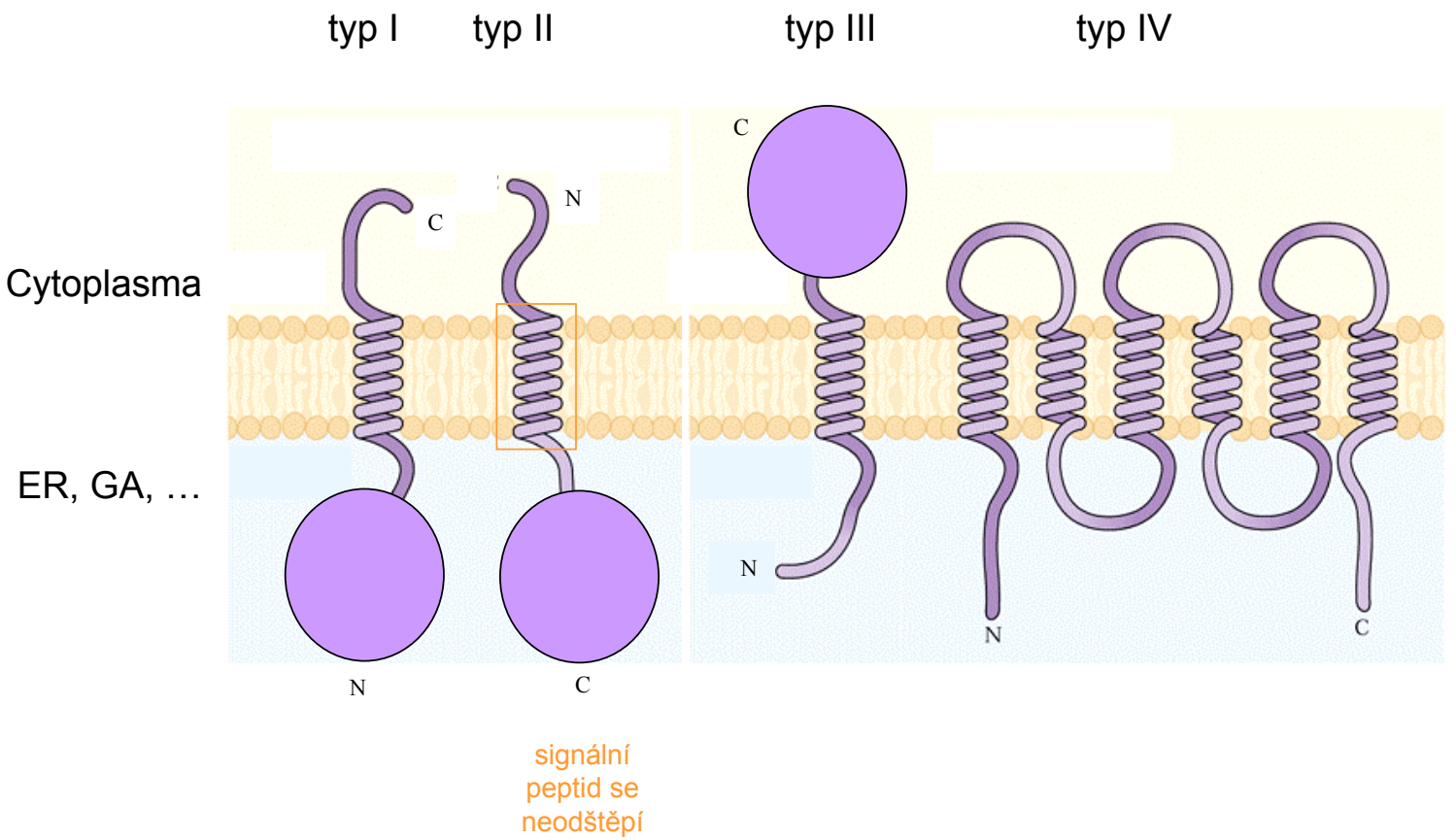


Syntéza proteinů na ER

syntéza transmembránových proteinů:
iniciační a terminační signály translokace



Typy transmembránových proteinů

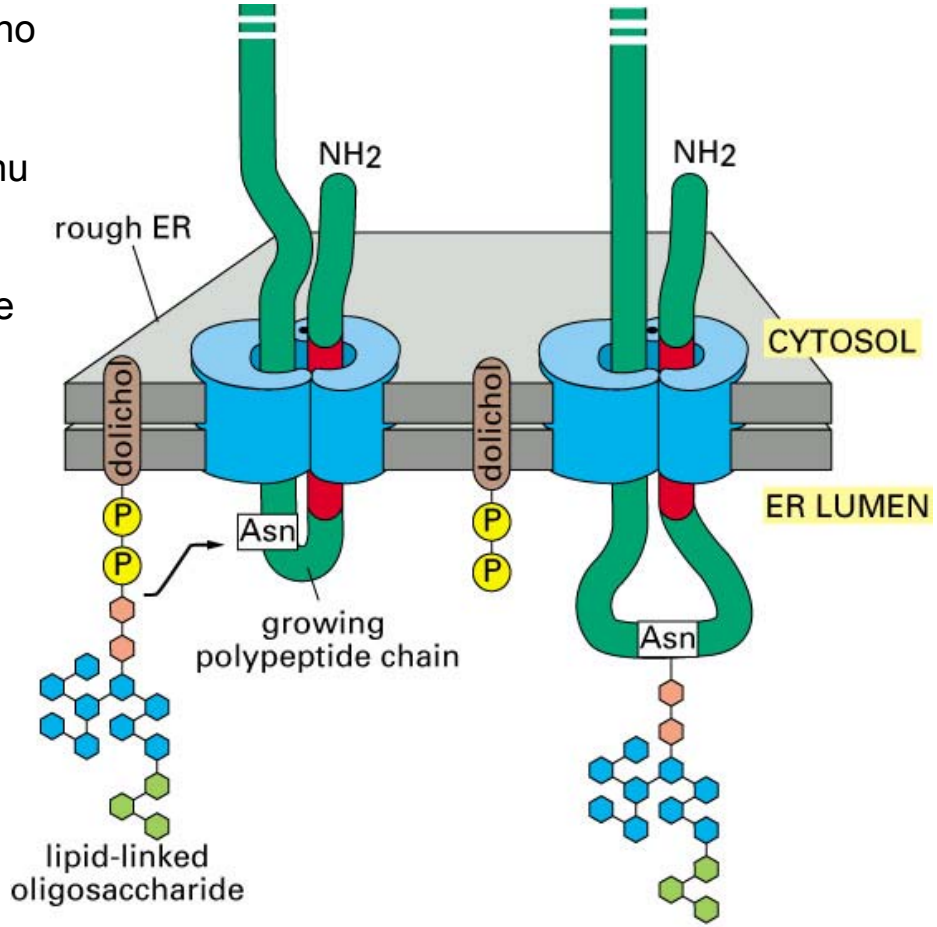


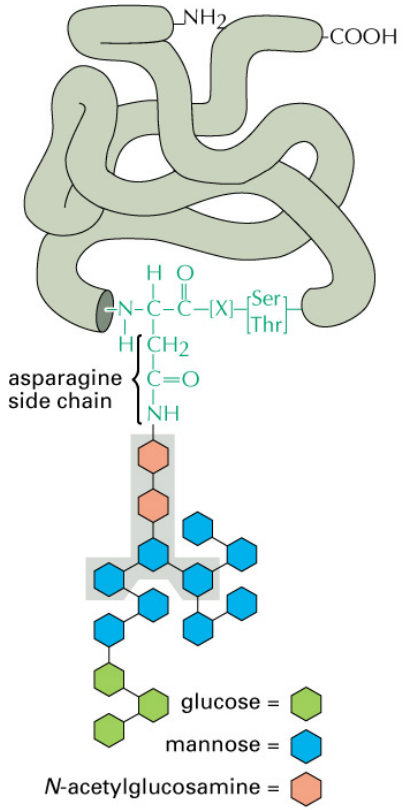
Modifikace proteinů v ER

N-glykosylace

přenesení hotového oligosacharidu z dolicholu na -NH2 skupinu asparaginu (Asn)

takto se modifikuje většina proteinů syntetizovaných do ER





Modifikace proteinů v ER

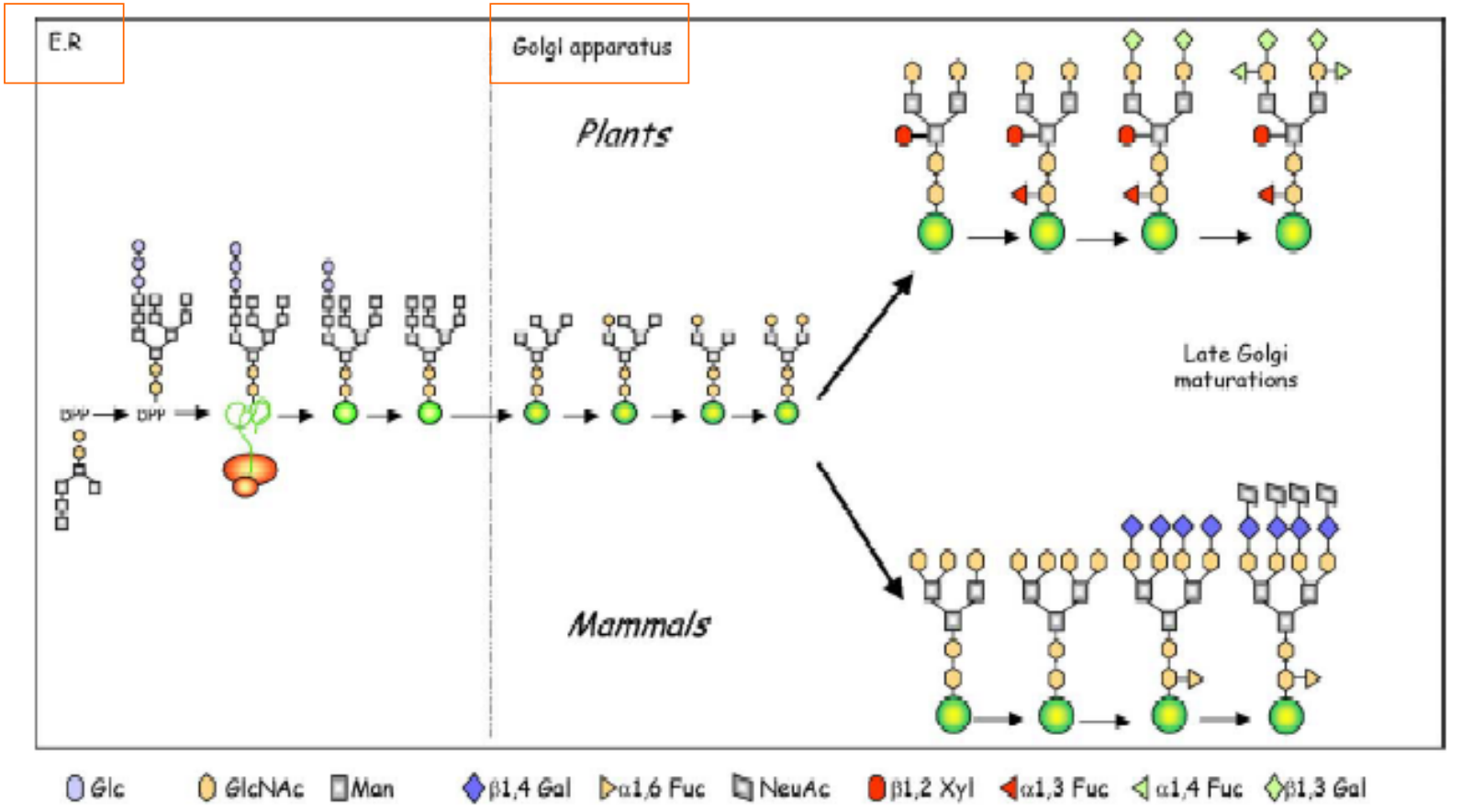


Fig. 1. Addition and processing of N-linked glycans in the endoplasmic reticulum (ER) and Golgi apparatus of plant and mammalian cells. A precursor oligosaccharide assembled onto a lipid carrier is transferred on specific Asn residues of the nascent growing polypeptide. The N-glycan is then trimmed off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

Kontrola kvality proteinů v ER

Retikuloplasminy

skupina proteinů v retikuloplasmě (lumen ER), které se podílejí na regulaci konformace bílkovin uvnitř ER = kontrola kvality bílkovin

např. calnexin, calreticulin, PDI

Calnexin is one of the chaperone molecules, which assist protein folding and quality control in ER, ensuring that only properly folded and assembled proteins proceed further along the secretory pathway. Calnexin retains unfolded or unassembled N-linked glycoproteins in the ER.

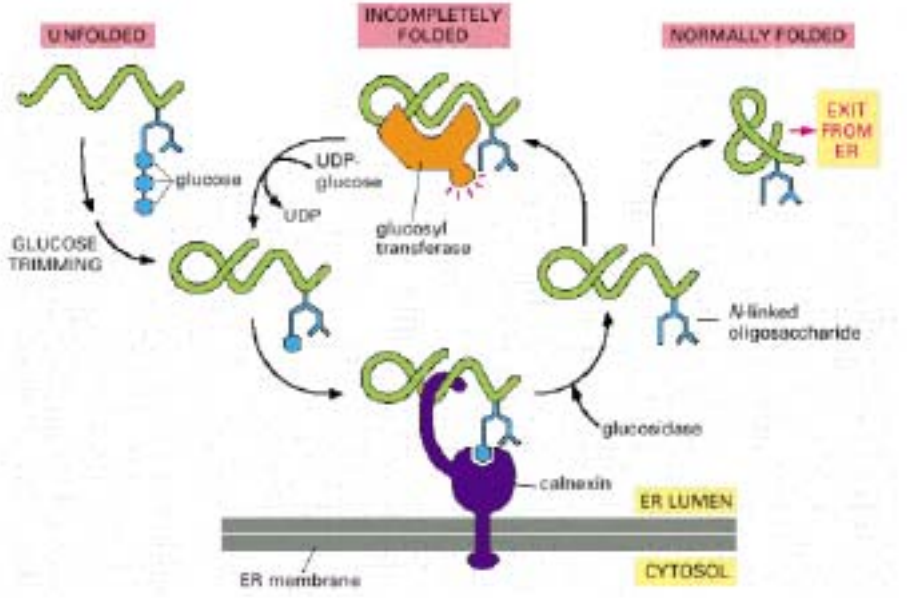
Protein disulfide isomerase (PDI) is an enzyme in the ER in eukaryotes or periplasmic space of prokaryotes that catalyzes the formation and breakage of disulfide bonds between cysteine residues within proteins as they fold. This allows proteins to quickly find the correct arrangement of disulfide bonds, and therefore the enzyme acts to catalyze protein folding.

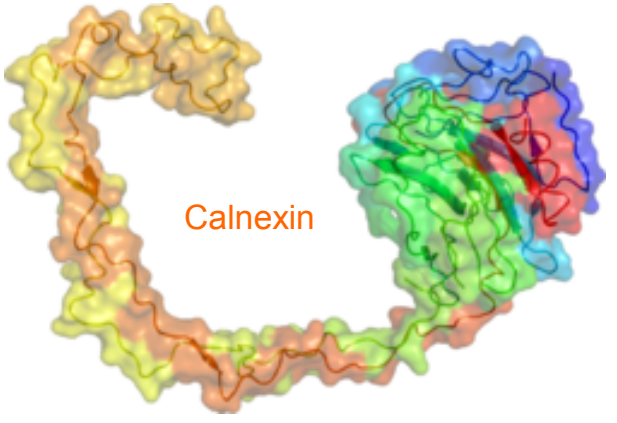
Calreticulin binds to misfolded proteins and prevents them from being exported from the ER to the Golgi apparatus. Calreticulin has the function of binding to oligosaccharides containing terminal glucose residues thereby targeting them for degradation. In normal cellular function, trimming of glucose residues off the core oligosaccharide added during N-linked glycosylation is a part of protein processing.

Kontrola kvality proteinů v ER

Calnexin/Calreticulin

Po deglykosylačních úpravách komplexního polysacharidu vážou substrát, dokud má jednu terminální glukózu – cyklus glykosylace/deglykosylace „drží“ bílkovinu v ER. Po odstranění poslední glukózy se retikuloplasminy „pouštějí“ substrátu a ten uniká z ER.





Řízená degradace proteinů

Nepodařené bílkoviny jsou exportovány ven z ER a po ubikvitinaci jsou degradovány proteasomem.

Řízená degradace proteinů proteasomem je stejně důležitý regulační pochod jako jejich syntéza.

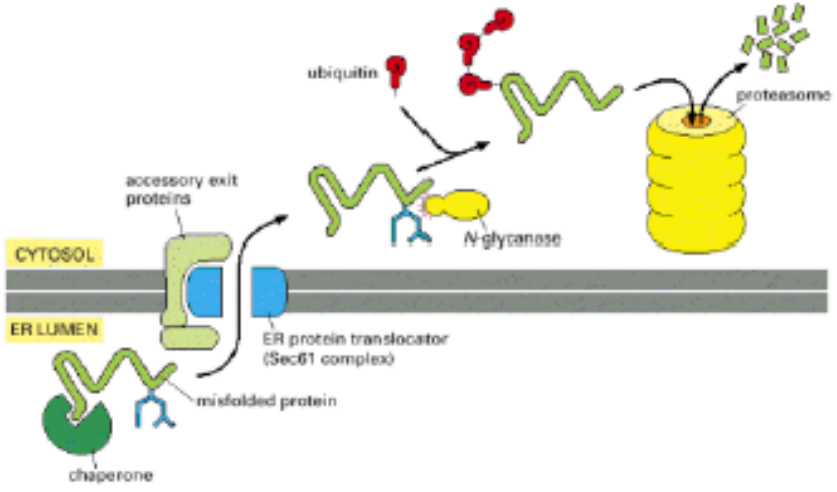
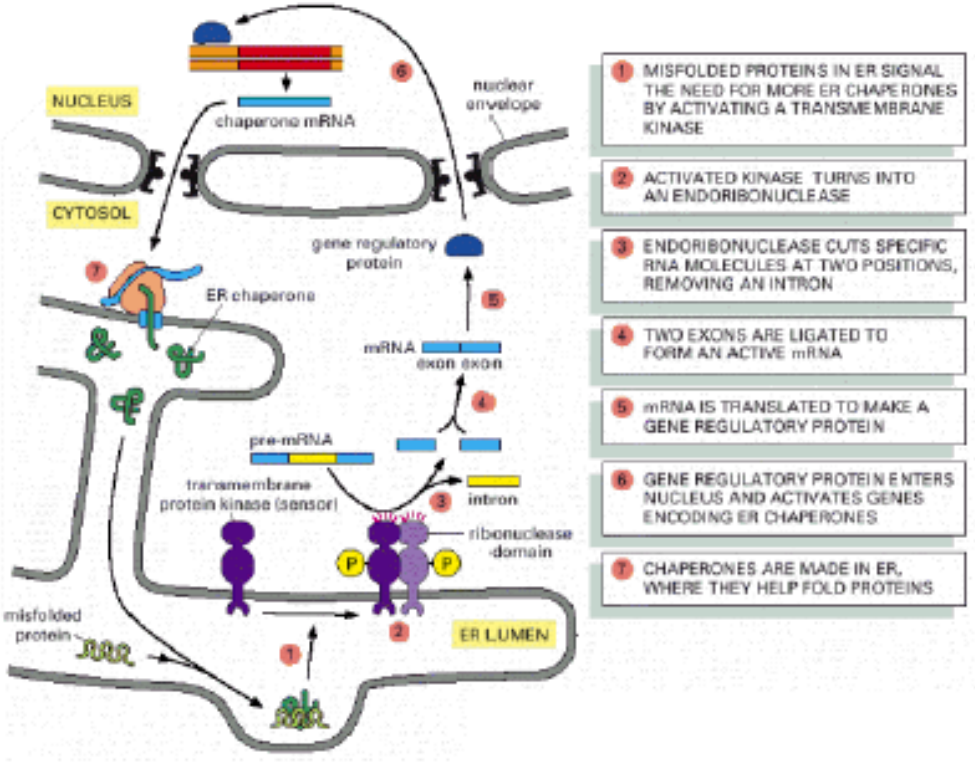


Figure 12-55. The export and degradation of misfolded ER proteins. Misfolded soluble proteins in the ER lumen are translocated back into the cytosol, where they are deglycosylated, ubiquitylated, and degraded in proteasomes. Misfolded membrane proteins follow a similar pathway. Misfolded proteins are 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.

Unfolded Protein Response

uplatňuje se i u rostlin



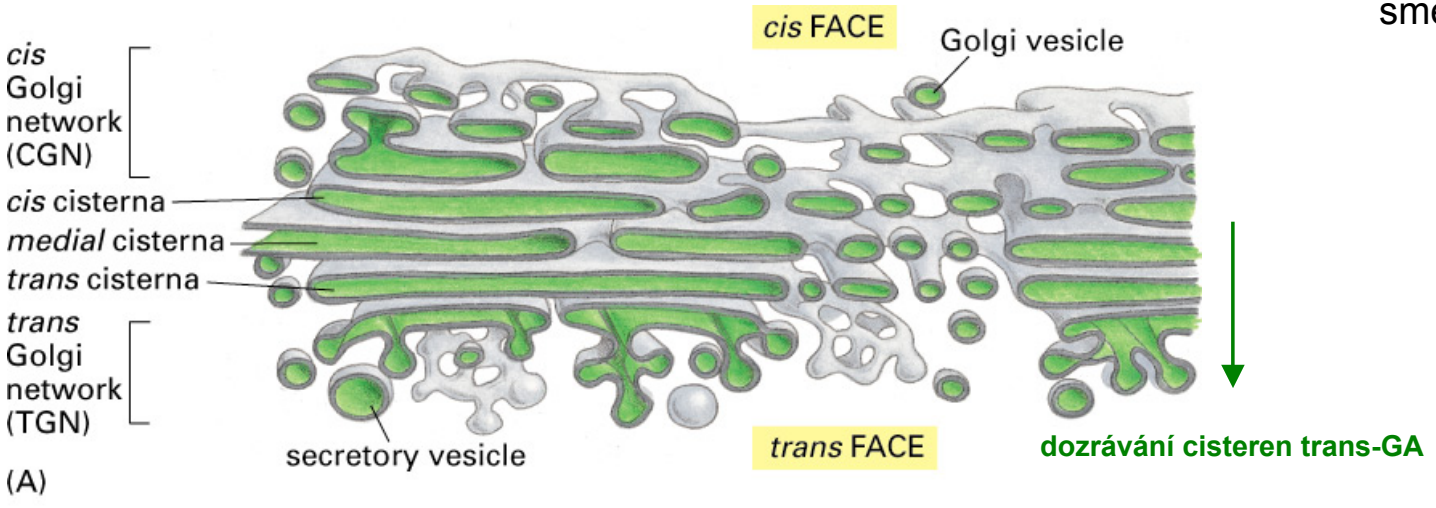
nesbalené proteiny vyvolají pomocí signalizace přes proteinkinázu vyšší expresi chaperonů, které pomohou proteiny správně sbalovat

Denaturované bílkoviny vážou BiP, který normálně drží IRE1 neagregovanou; po agregaci aktivovaná IRE1 vystřihne intron TF XBP1. Vedle toho PERK kináza (ER TM bílk.) inhibuje translaci (fosf. eIF2a). ATF6 je TM TF v membr. ER, který je aktivován také uvolněním z BiPu, transportem do GA a po odštěpení proteázou putuje do jádra.

Golgiho aparát

ER

směr sekrece

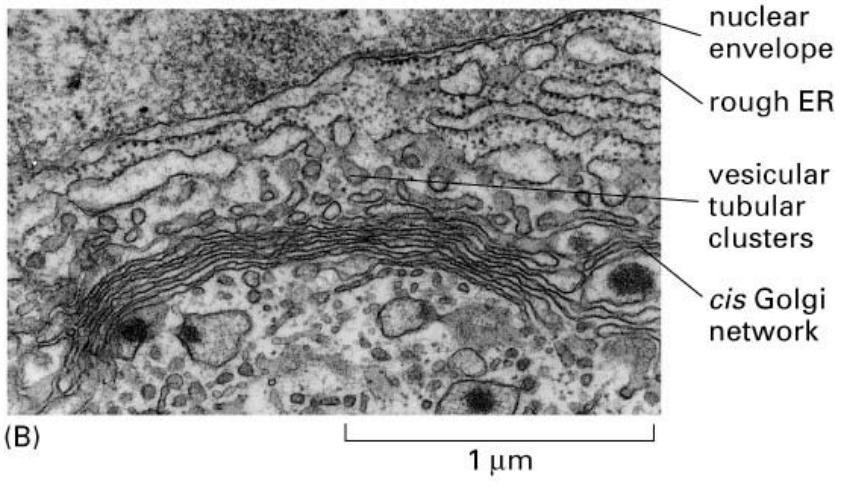


CM

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

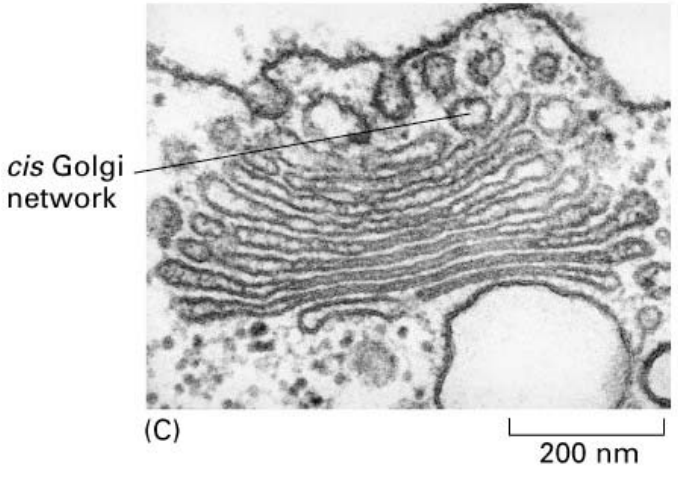
Velkou část metabolické aktivity GA u rostlin představuje vedle glykosylace proteinů především syntéza pektinů a různých "hemicelulóz„ - stavebních látek buněčné stěny.

Golgiho aparát



živočišné buňky:

přechodová zóna mezi ER a GA

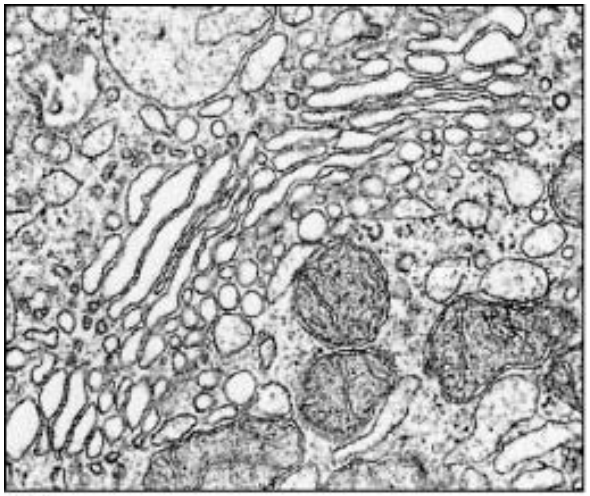


rostlinné buňky:

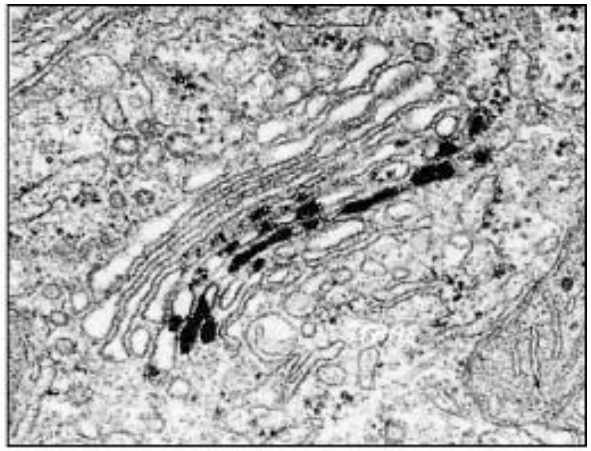
bez přechodové zóny mezi ER a GA

Golgiho aparát

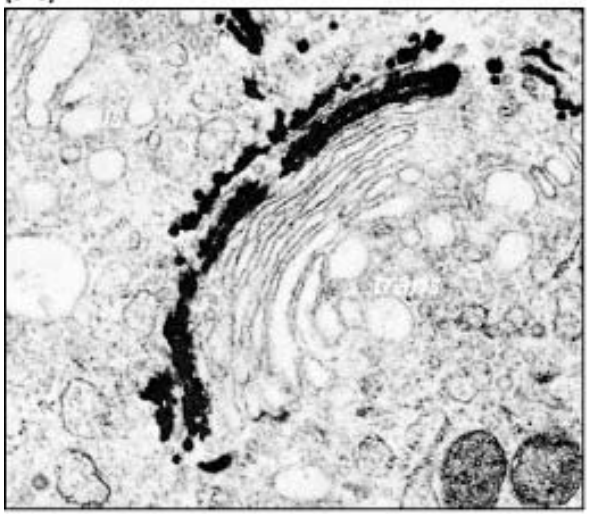
biochemické kompartmenty GA



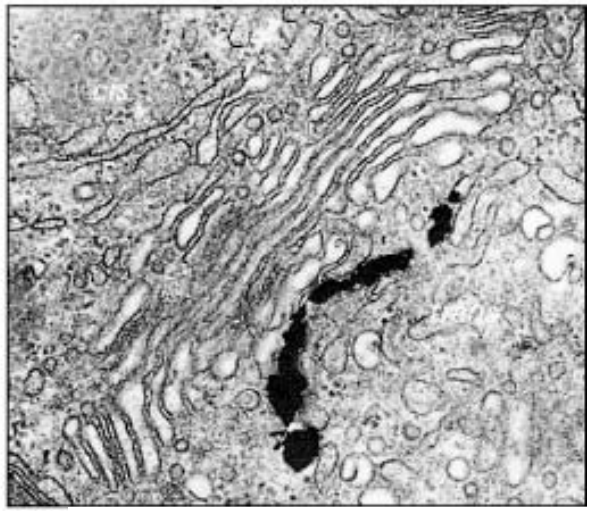
(A) Unstained



(C) Nucleoside diphosphatase activity = trans-Golgi cisternae



(B) Stained cis-Golgi cisternae



(D) Acid phosphatase activity = trans-Golgi network (TGN)

Modifikace proteinů v GA

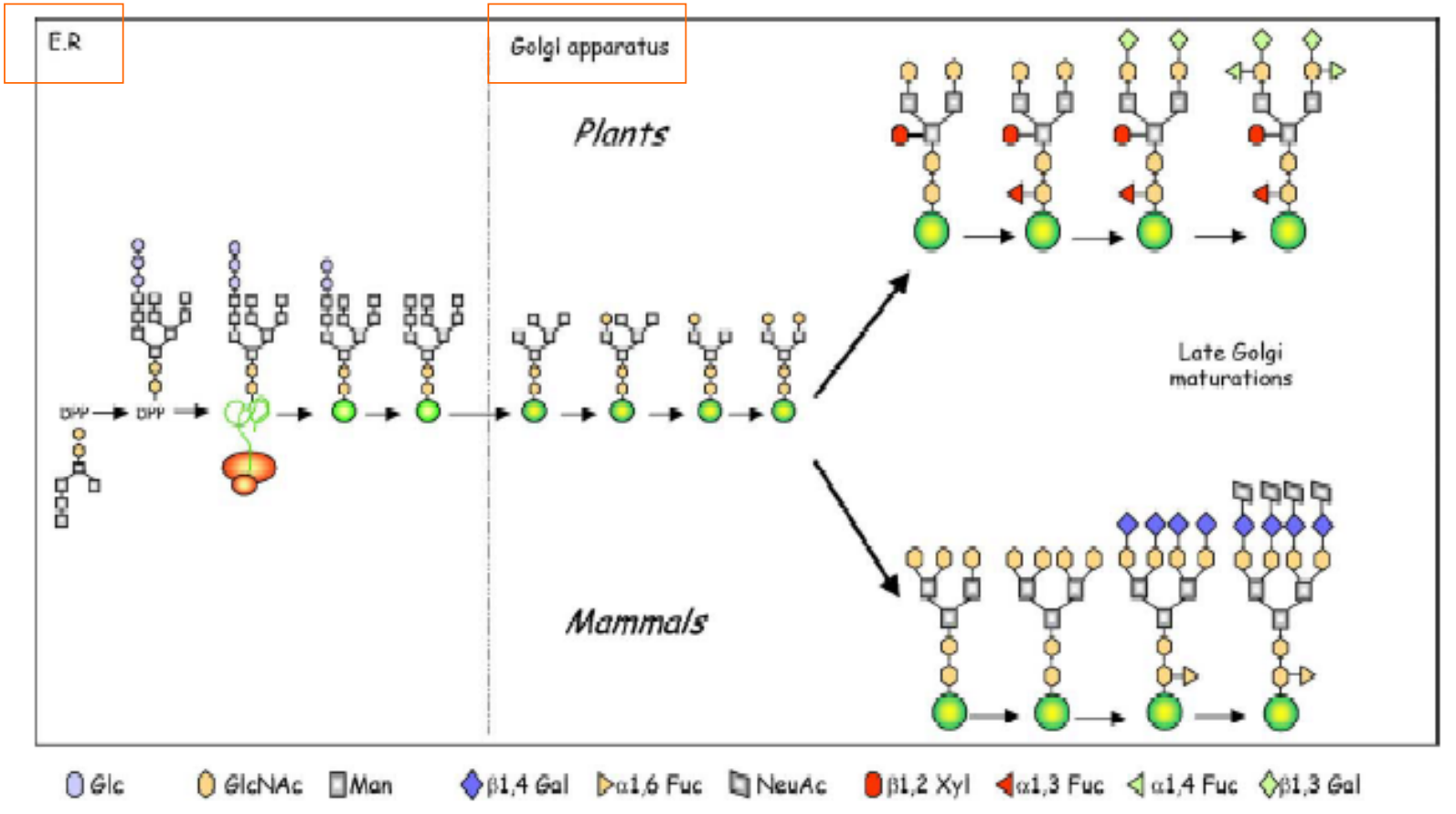
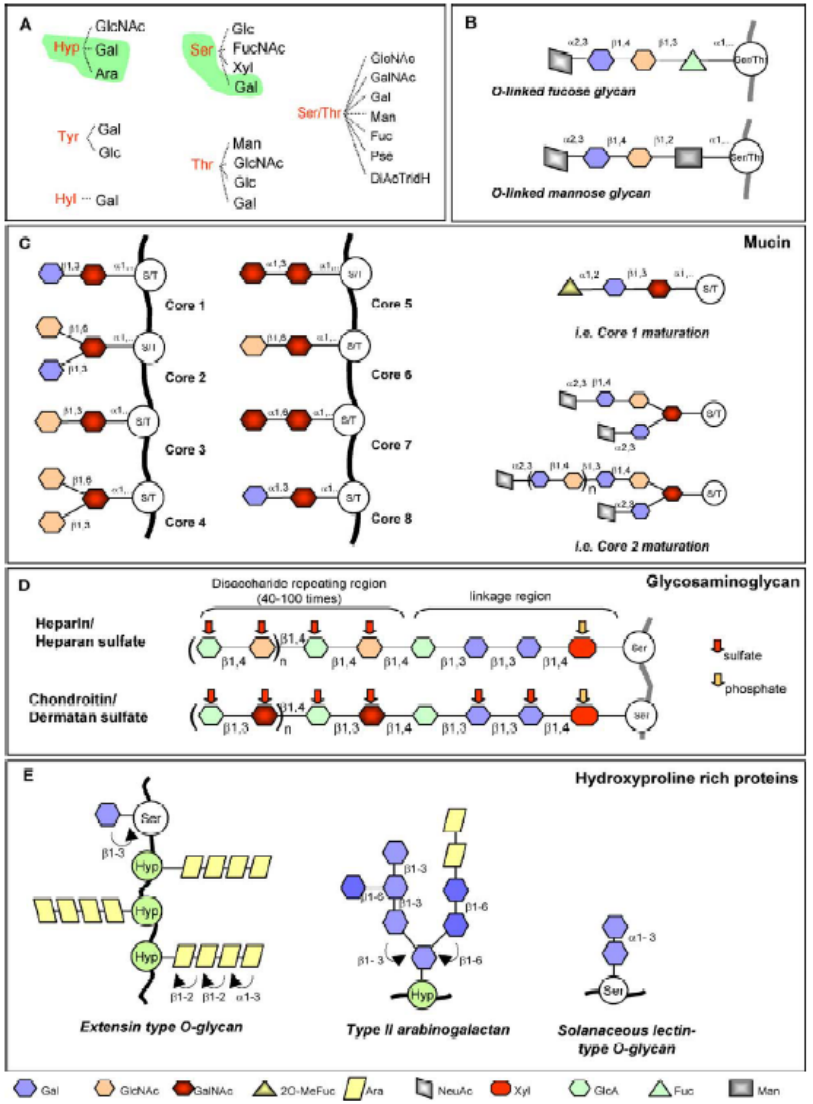


Fig. 1. Addition and processing of N-linked glycans in the endoplasmic reticulum (ER) and Golgi apparatus of plant and mammalian cells. A precursor oligosaccharide assembled onto a lipid carrier is transferred on specific Asn residues of the nascent growing polypeptide. The N-glycan is then trimmed off 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 modifikacích komplikují biotechnologické využití.

Modifikace proteinů v GA

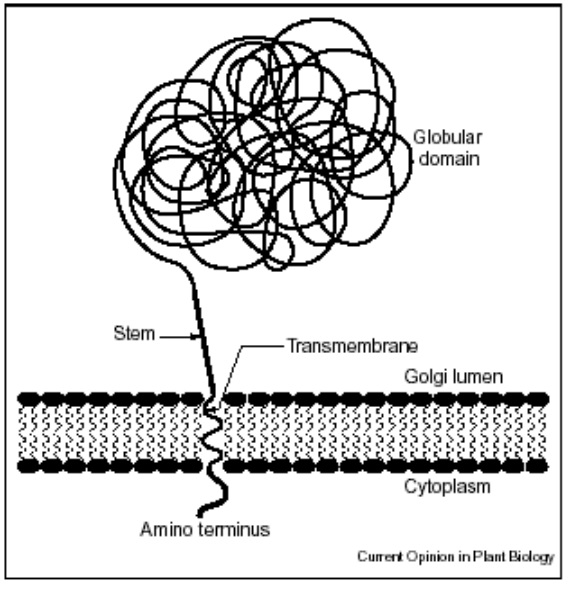


O-glykosylace

na -OH skupině
Ser, Thr nebo hydroxyprolinu (Hyp)

cukerné zbytky se připojují obvykle po jednom

Modifikace proteinů v GA

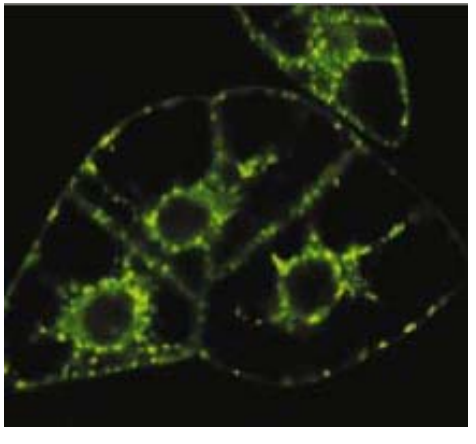


Schematic representation of the topology of most Golgi-localized glycosyltransferases. The amino terminus is located at the cytoplasmic face of the Golgi membrane whereas the globular domain, located near the carboxyl terminus of the protein, is located in the lumen of the Golgi.

glykosyltransferázy v GA

transmembránové proteiny, které připojují cukerné zbytky

ve fúzi s GFP slouží často jako markery GA - např. živoč. sialyltransferáza (ST)



Endomembránový systém 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.

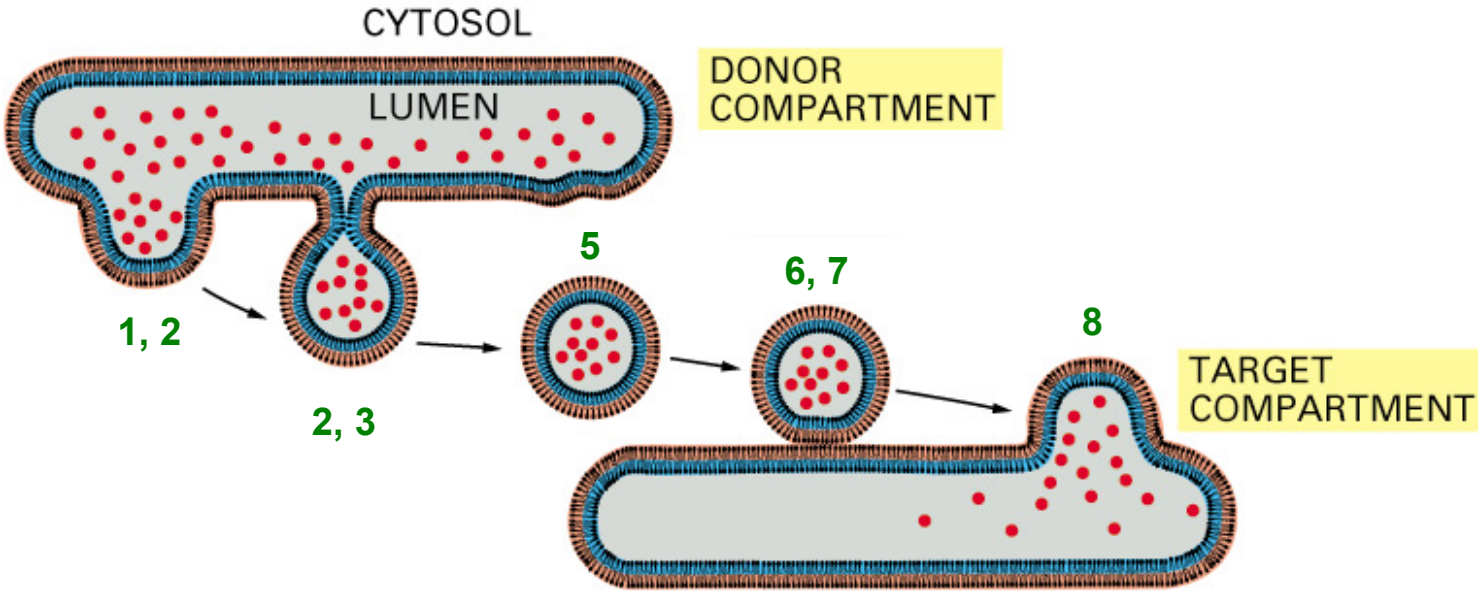
Universal Features

*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.

Vesicle trafficking

Vesicle trafficking



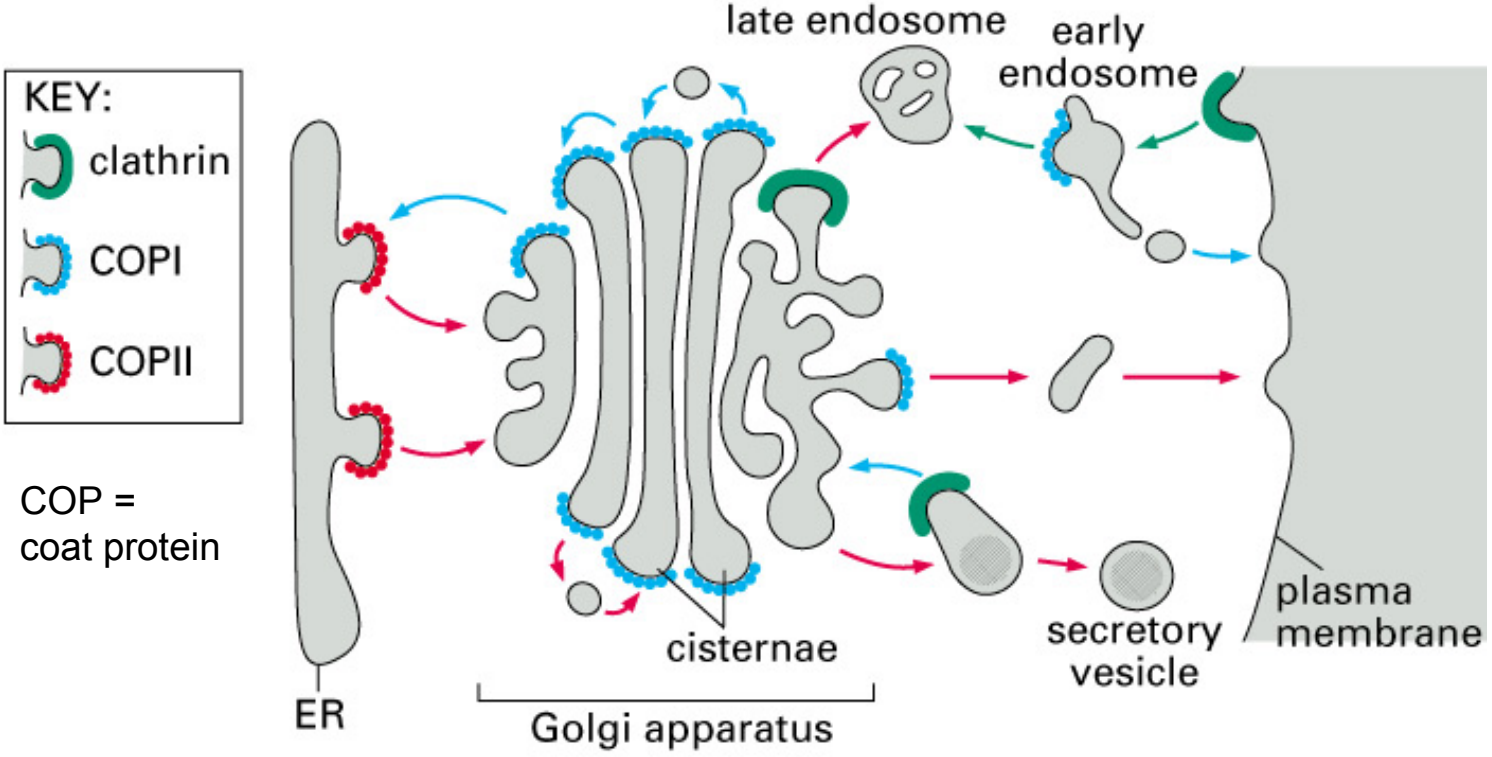
1) specifikace místa pučení váčku
2) pučení váčku + naložení nákladu
3) odstřižení váčku od membrány
4) *uncoating* váčku
5) transport váčku
6) *tethering* váčku (25 nm)
7) *docking* váčku (5-10 nm)
8) fúze váčku s cílovou memebránou

Transportní váčky

COPII mediates ER to Golgi transport

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

CCV (clathrin coated vesicles) mediates GA to plasmalema transport



Retrográdní transport

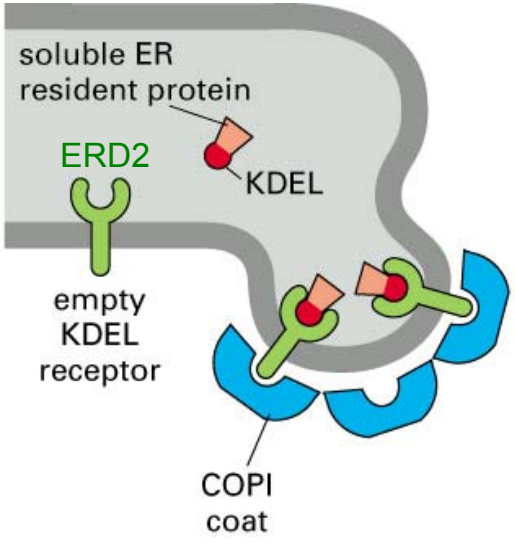
vrací proteiny specifické pro ER zpět z GA do ER, udržování spec. obsahu ER

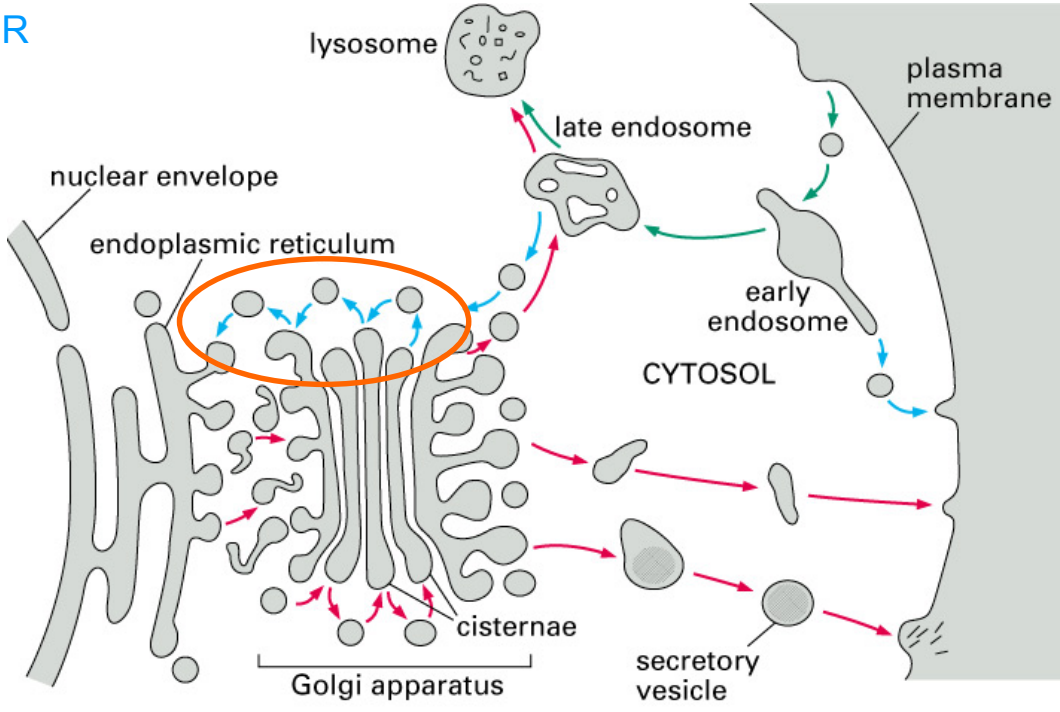
adresová sekvence (tzv. retenční signál) KDEL (HDEL u kvasinky *S. cerevisiae*) na C-konci proteinů lumen ER (retikuloplasminů)

KDEL = Lys-Asp-Glu-Leu

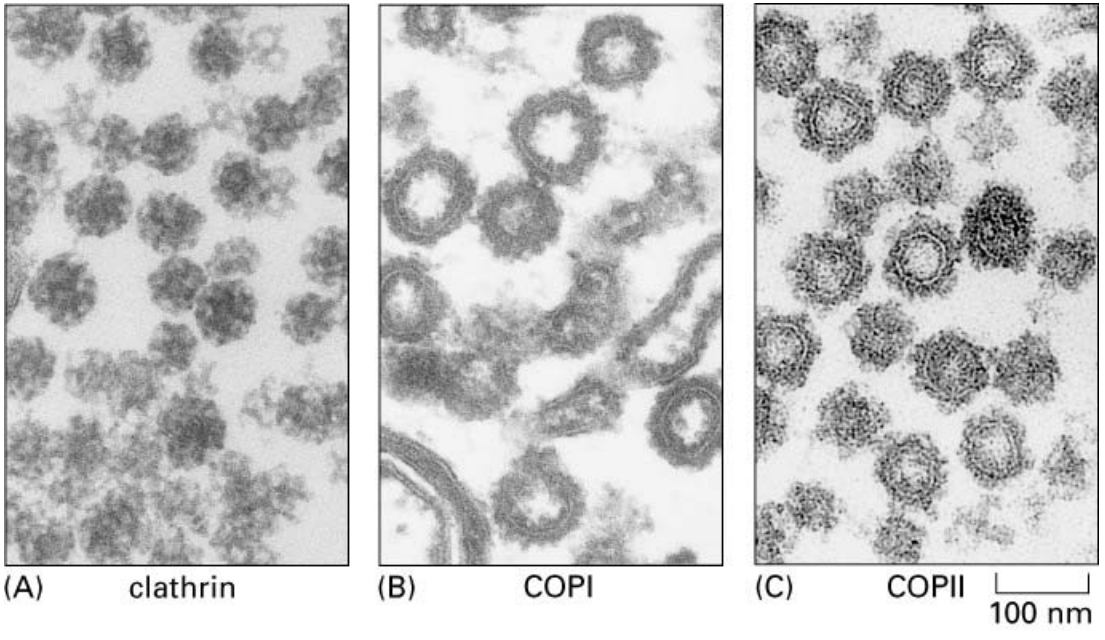
rozpoznání receptorem ERD2

doprava pomocí COPI váčků do ER





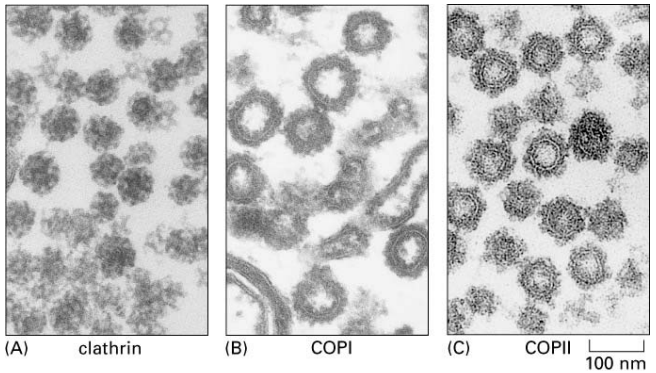
Transportní váčky

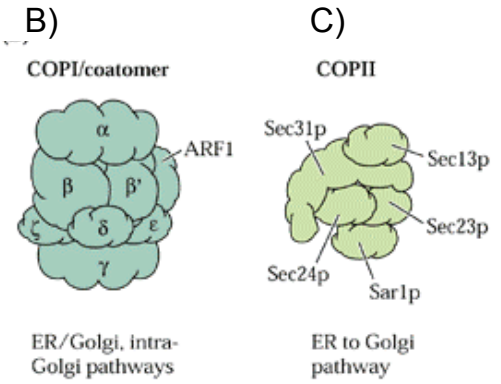


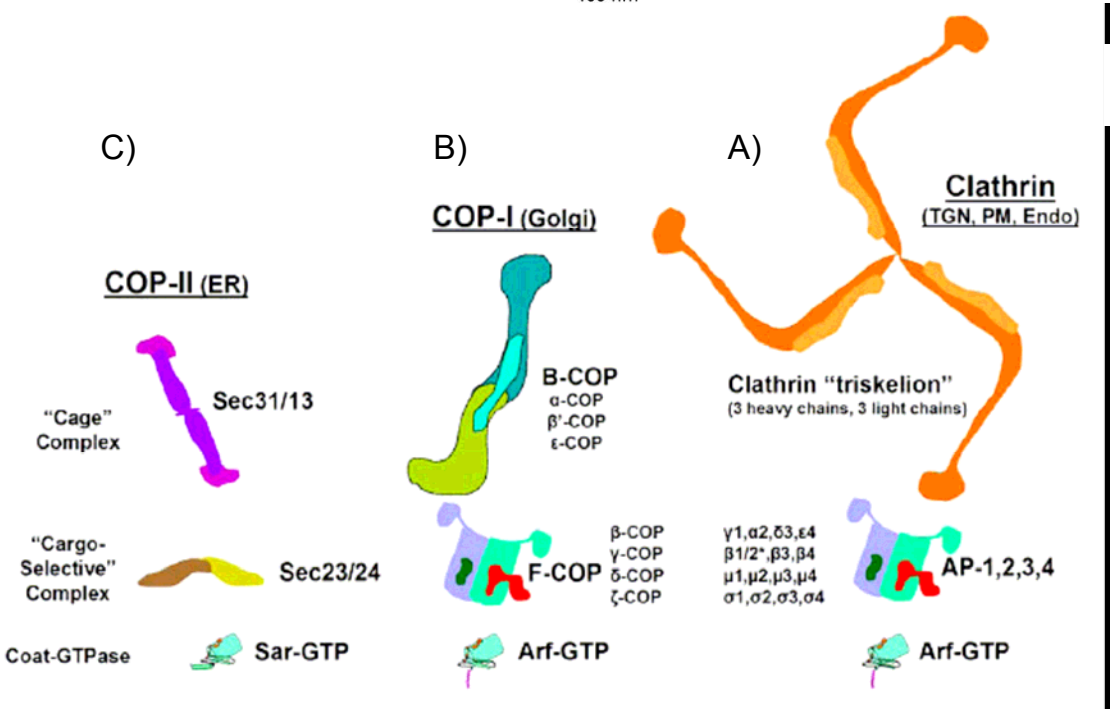
Tvorbu obalů/váčků regulují malé GTPázy z rodin ARF a SAR

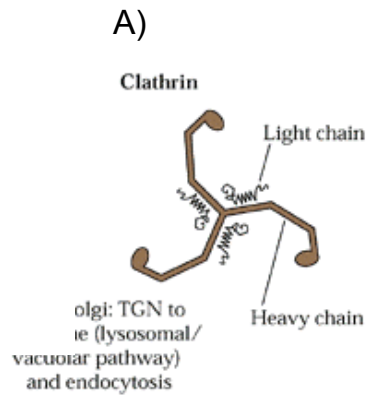
- Sar1 – COPII
- ARF1 a homology ARF1 – COPI, CCV (clathrin coated vesicles)
- neprostudované typy obalů mohou využívat jiné GTPázy

Transportní váčky

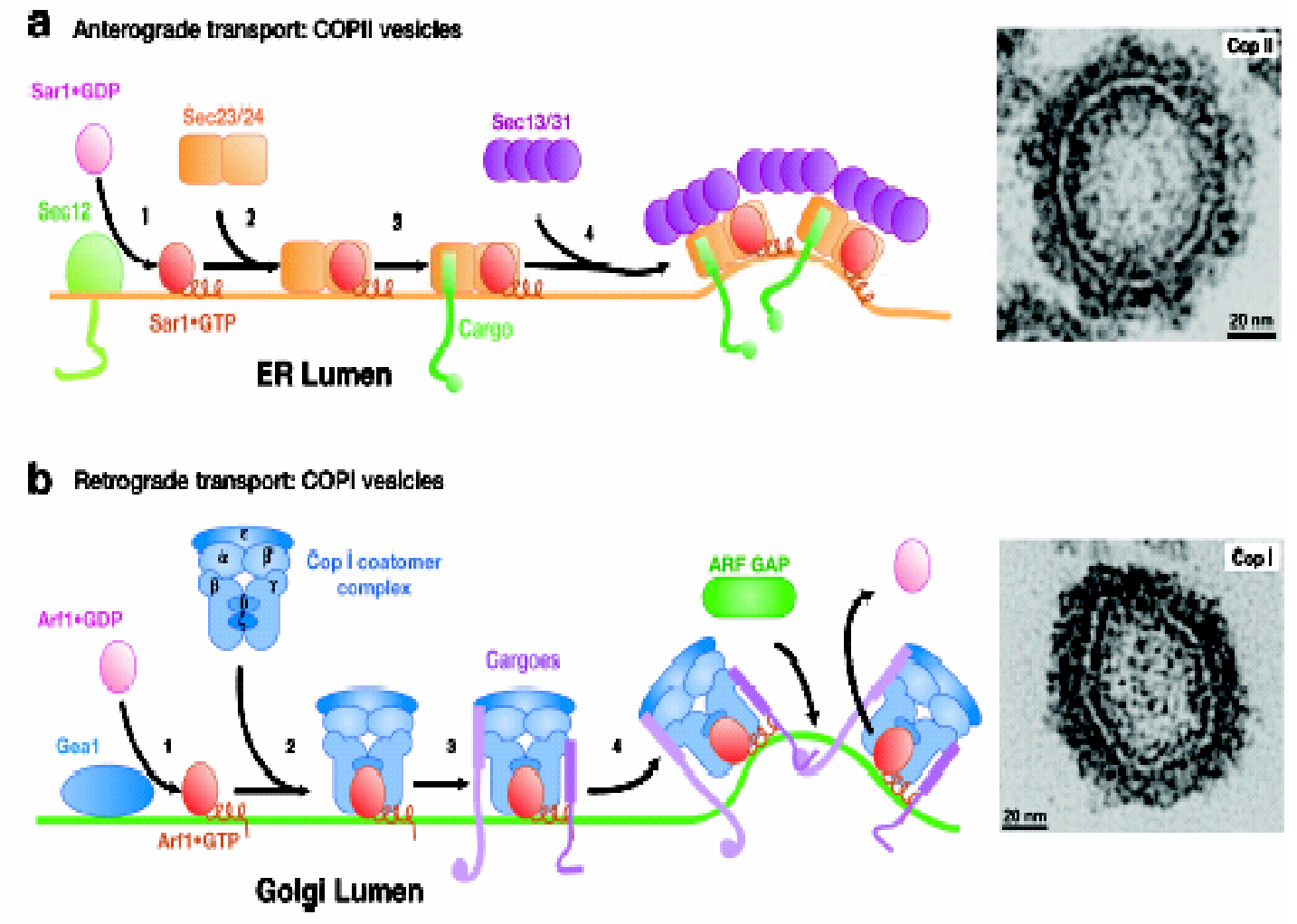






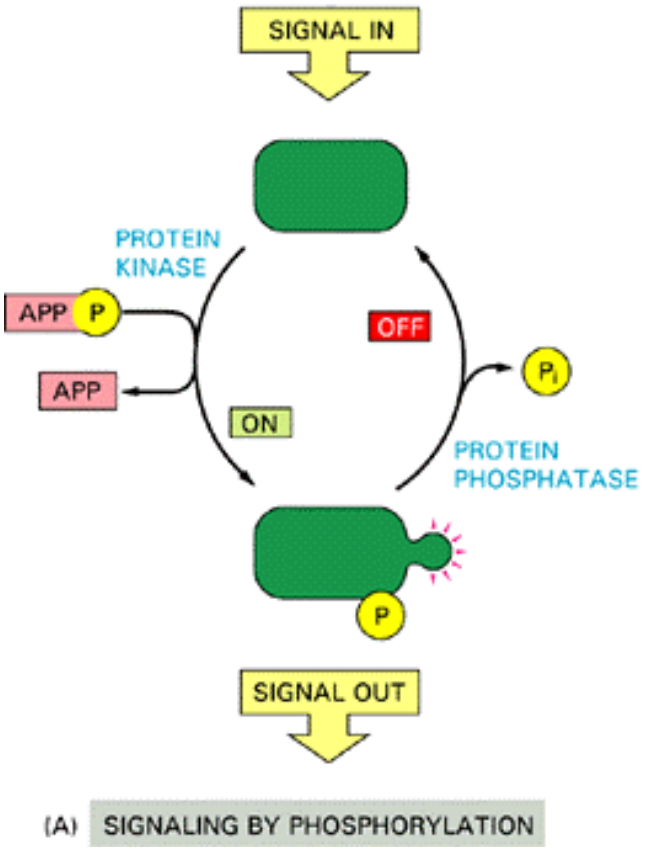


Tvorbu váčků regulují malé GTPázy



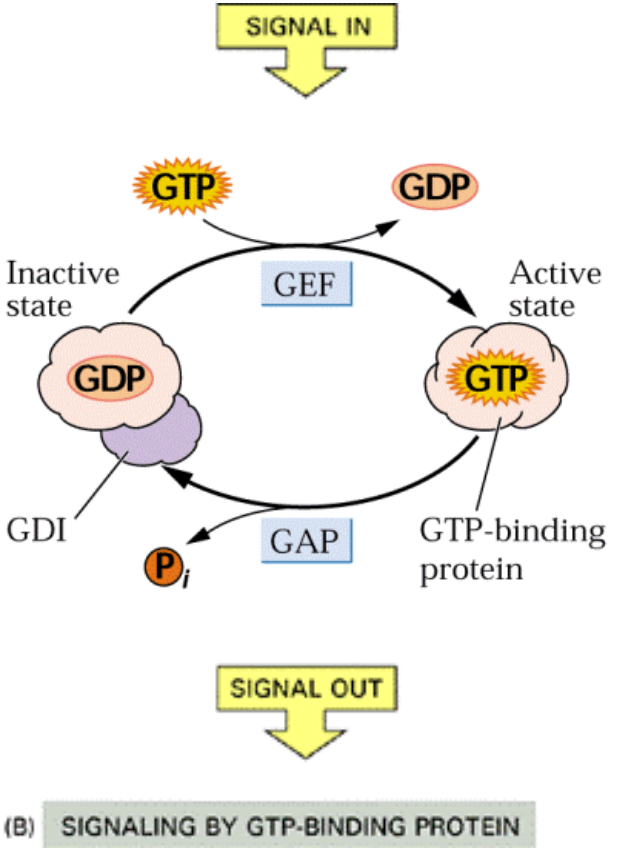
Dva hlavní mechanizmy signalizace u eukaryot

1) fosforylace



(A) SIGNALING BY PHOSPHORYLATION

2) GTP-vazebné proteiny



(B) SIGNALING BY GTP-BINDING PROTEIN

Tvorbu váčků regulují malé GTPázy

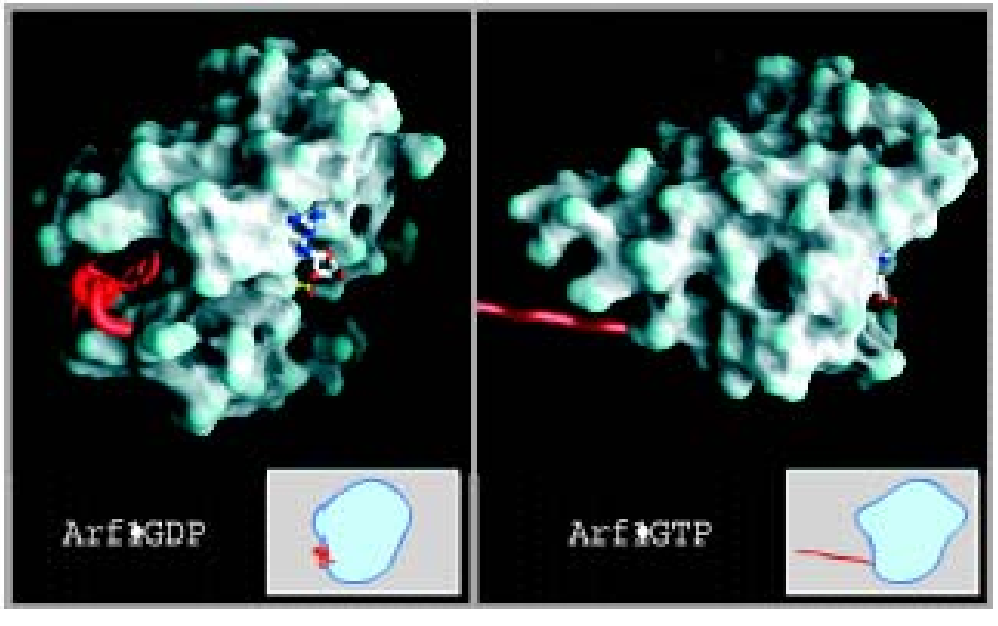
Nucleotide-dependent (GTP/GDP) confomational changes in ARF1

ARF = ADP ribosylation factor

GTPase switch

GDP forma - neaktivní

GTP forma - aktivní



Lee et al. 2004

ARF1 cykluje mezi cytoplasmou a membránou

- na N-konci navázaný hydrofobní myristyl
- v GDP formě ukrytý v molekule
- v GTP formě vystrčený ven, interakce s membránou

Váčky COPII – anterográdní transport

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

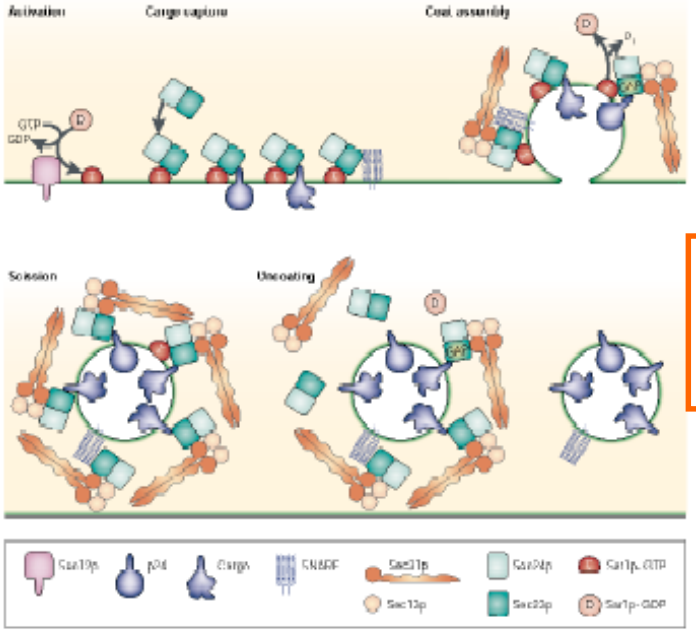


Figure 2 | **The key steps in the formation of COPII-coated vesicles.** Coat assembly is activated by the recruitment of Sar1p–GTP to the membrane. This allows the binding of the Sec23p–Sec24p complex and the recruitment of cargo. The Sec13p–Sec31p complex binds next, leading to membrane deformation. 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

Váčky COPI – retrográdní transport

CopI
cis Golgi to ER
and intra Golgi

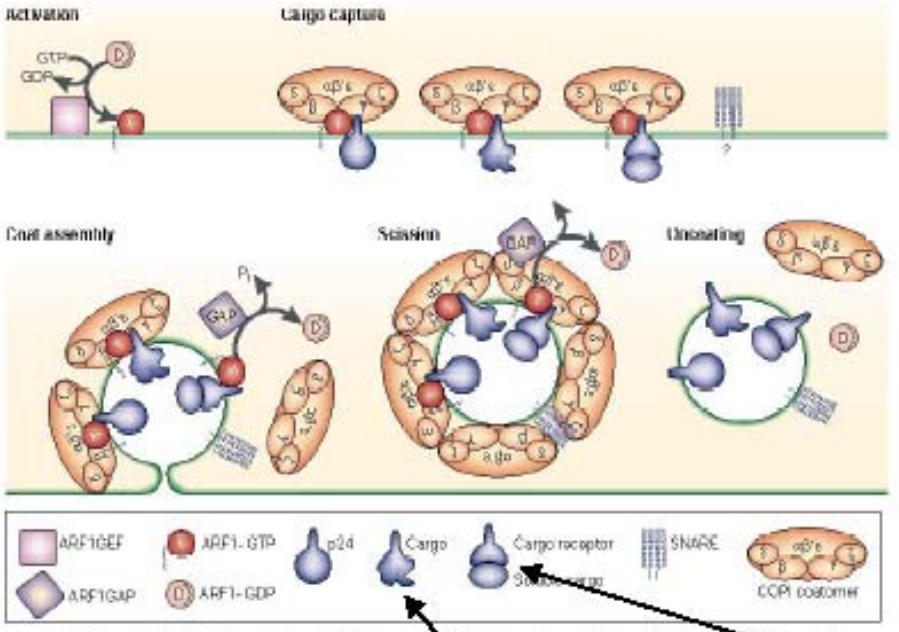
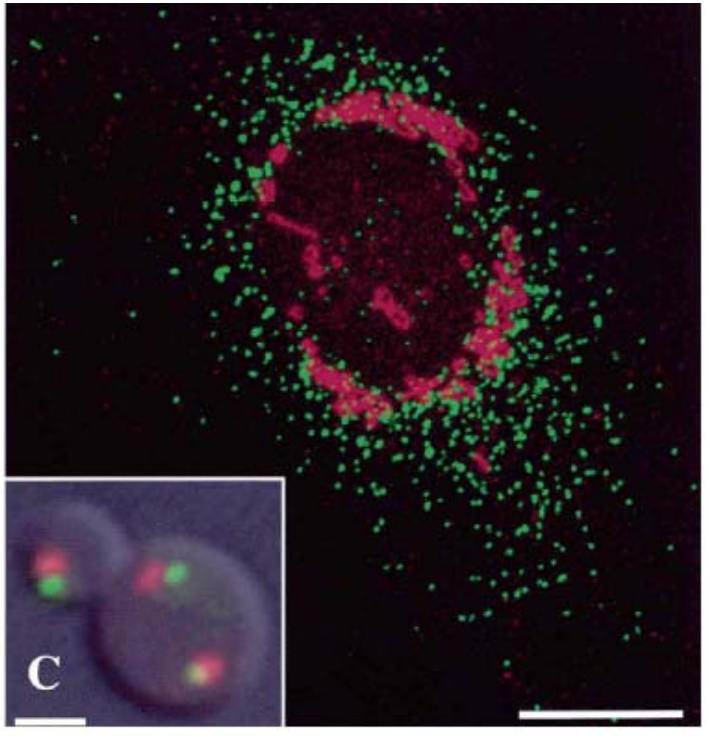


Figure 3 | **The key steps in the formation of COPI-coated vesicles.** Coat assembly is activated by the recruitment of ARF1-GTP to the membrane. This allows the binding of the COPI coatomer and the recruitment of cargo. GTP hydrolysis is slow when ARH-1 is bound to its preferred cargo, allowing kinetic 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 ARH-1 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

Transport z ER do GA

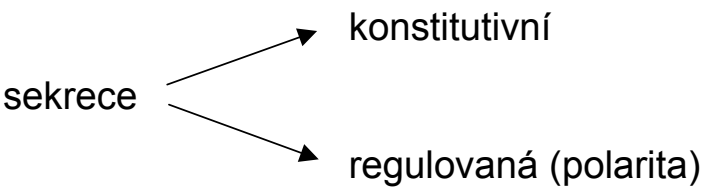


- Mammalian NRK cell stained for ER exit sites (COPII component mSec13) and cis-Golgi marker (Giantin).
- Yeast cell stained for transitional ER (COPII = Sec13p) and cis-Golgi marker Sec7p.
- Note differences in physical distances

Jak to funfuje u rostlin?

Také mají Sar1 GTPázu.

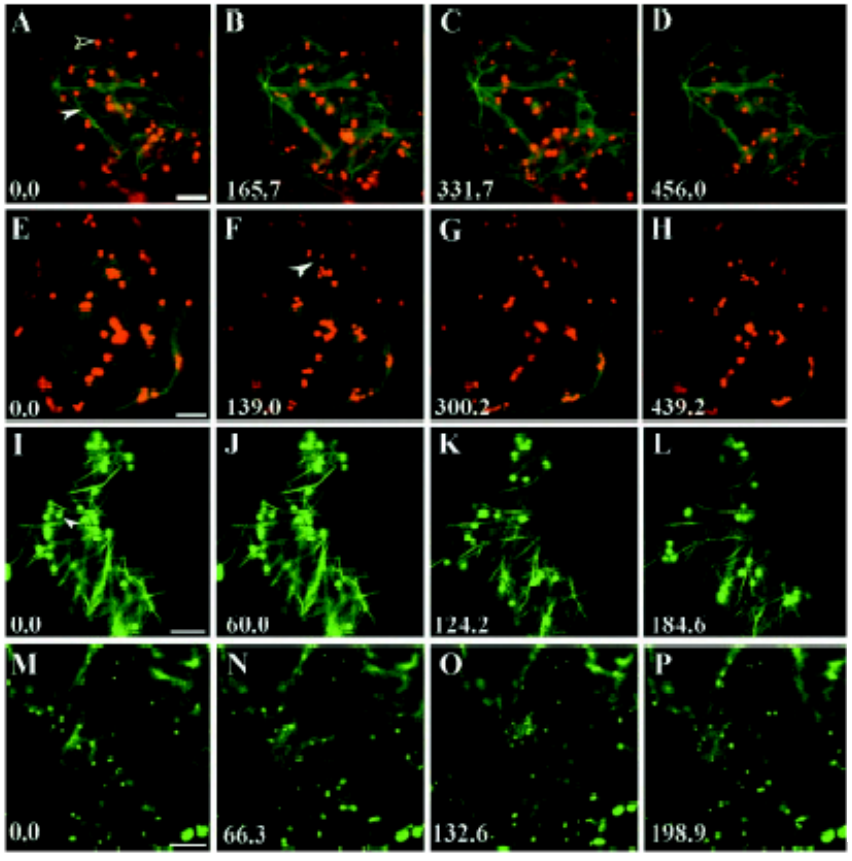
Transport mezi GA a plasmalemou



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

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

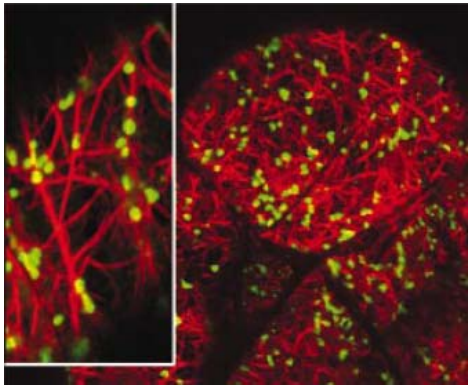
GA rostlin se pohybuje po ER/aktinu



Golgi

aktin

zničení aktinového cytoskeletu latrunculinem



Golgi

tubulin

narušení mikrotubulárního cytoskeletu colchicinem

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 GT-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 (36 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,

Mechanismus transportu váčků

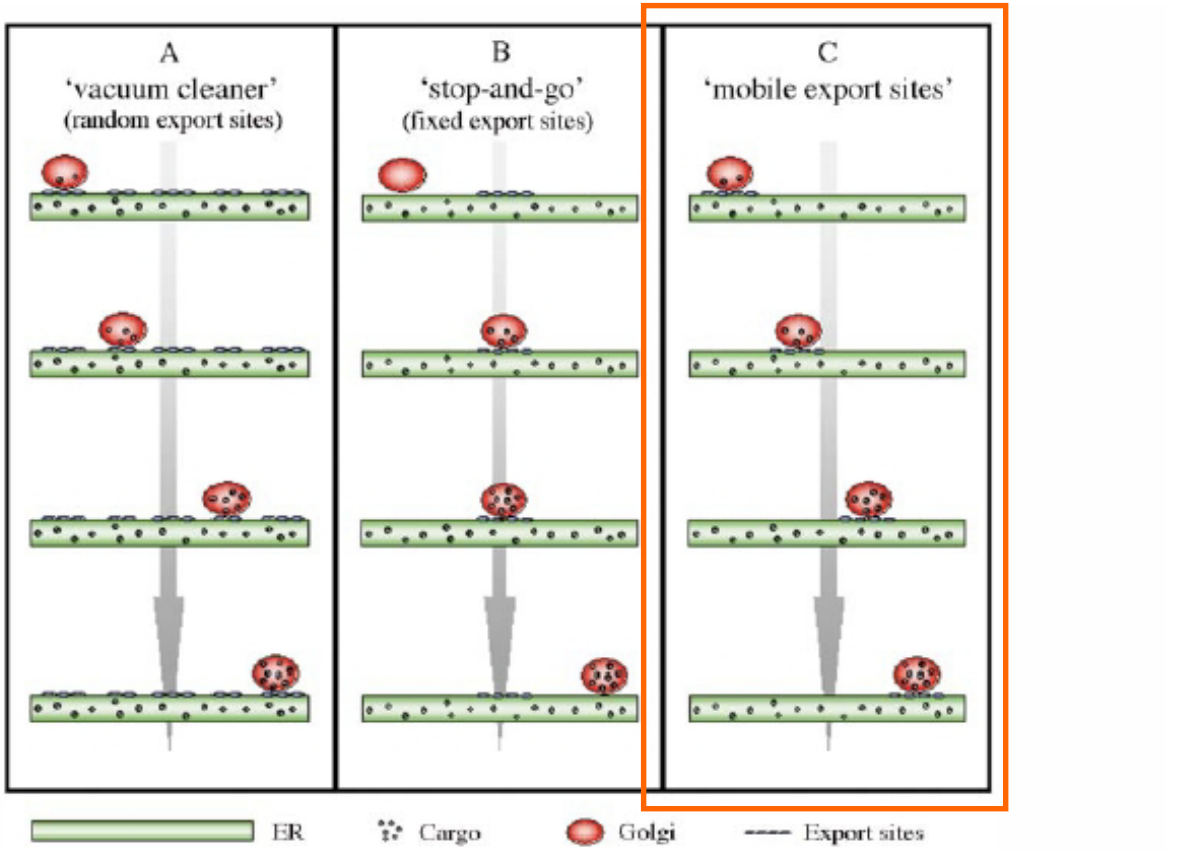
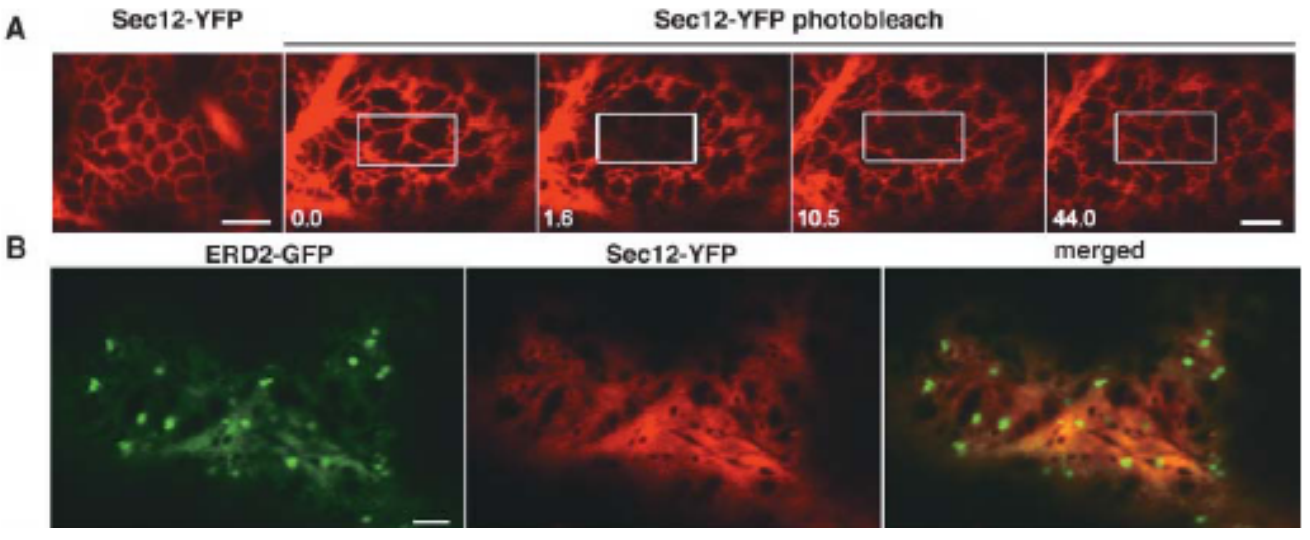


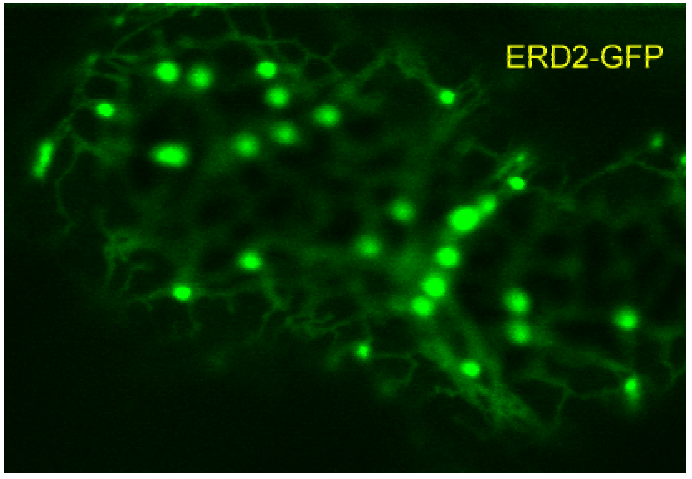
FIG. 3. Models of ER-to-Golgi protein transport. A, The 'vacuum cleaner model' (Boevink *et al.*, 1998) suggests that Golgi stacks move over the ER constantly picking up cargo. According to this model, the whole ER surface is capable of forming export sites, resulting in their random distribution. In 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.

Mechanismus transportu váčků

FRAP



Mechanismus transportu váčků

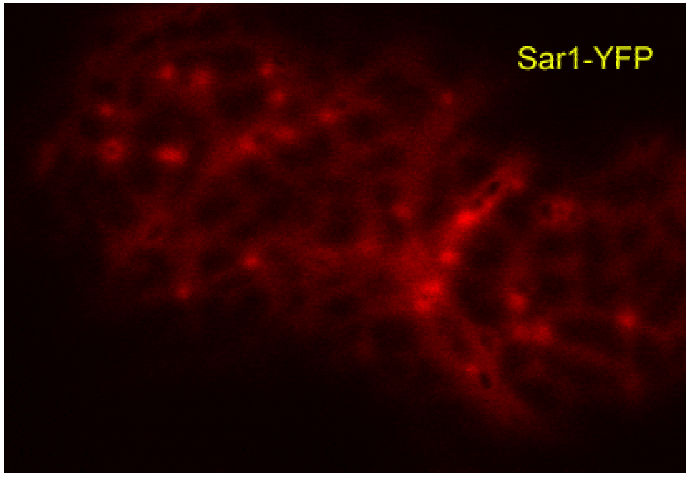


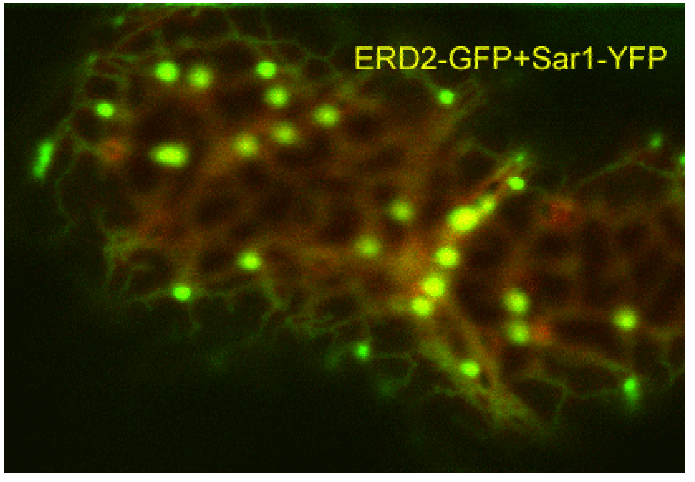
ERD2 – marker cis-Golgi

SAR1 – marker exit sites

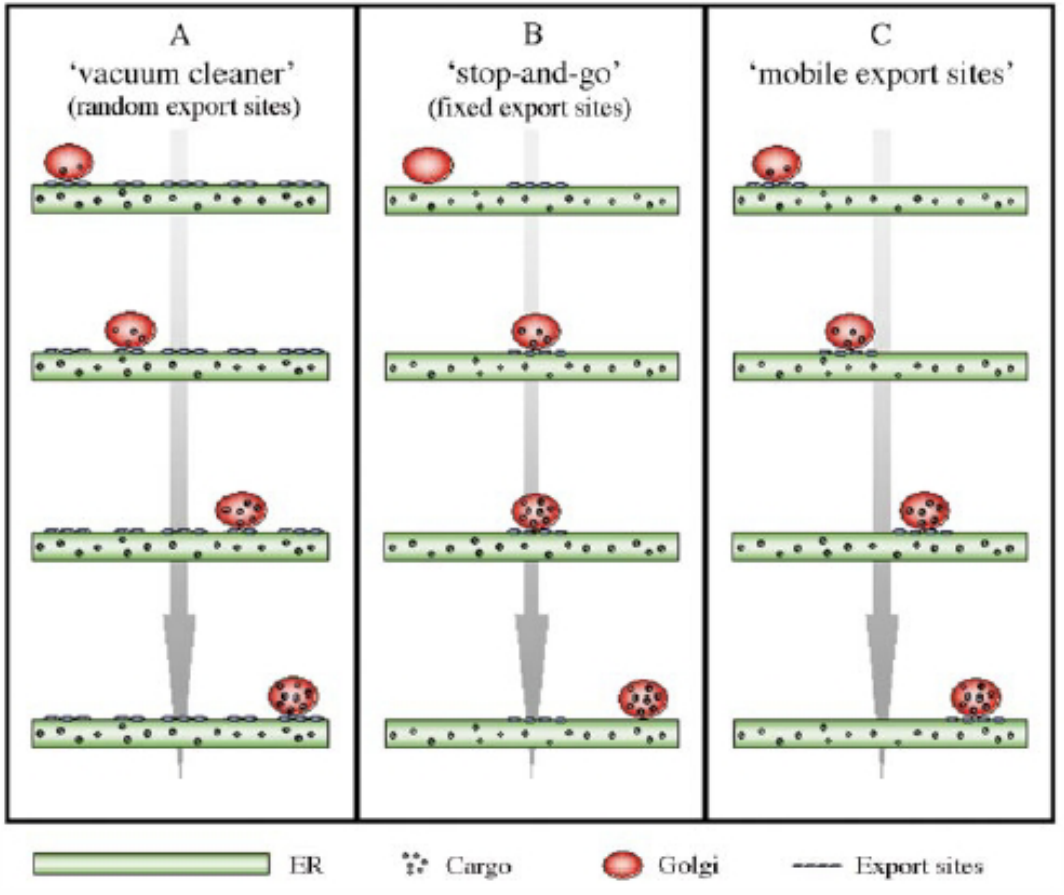
ERD2-GFP

SAR1-YFP merge





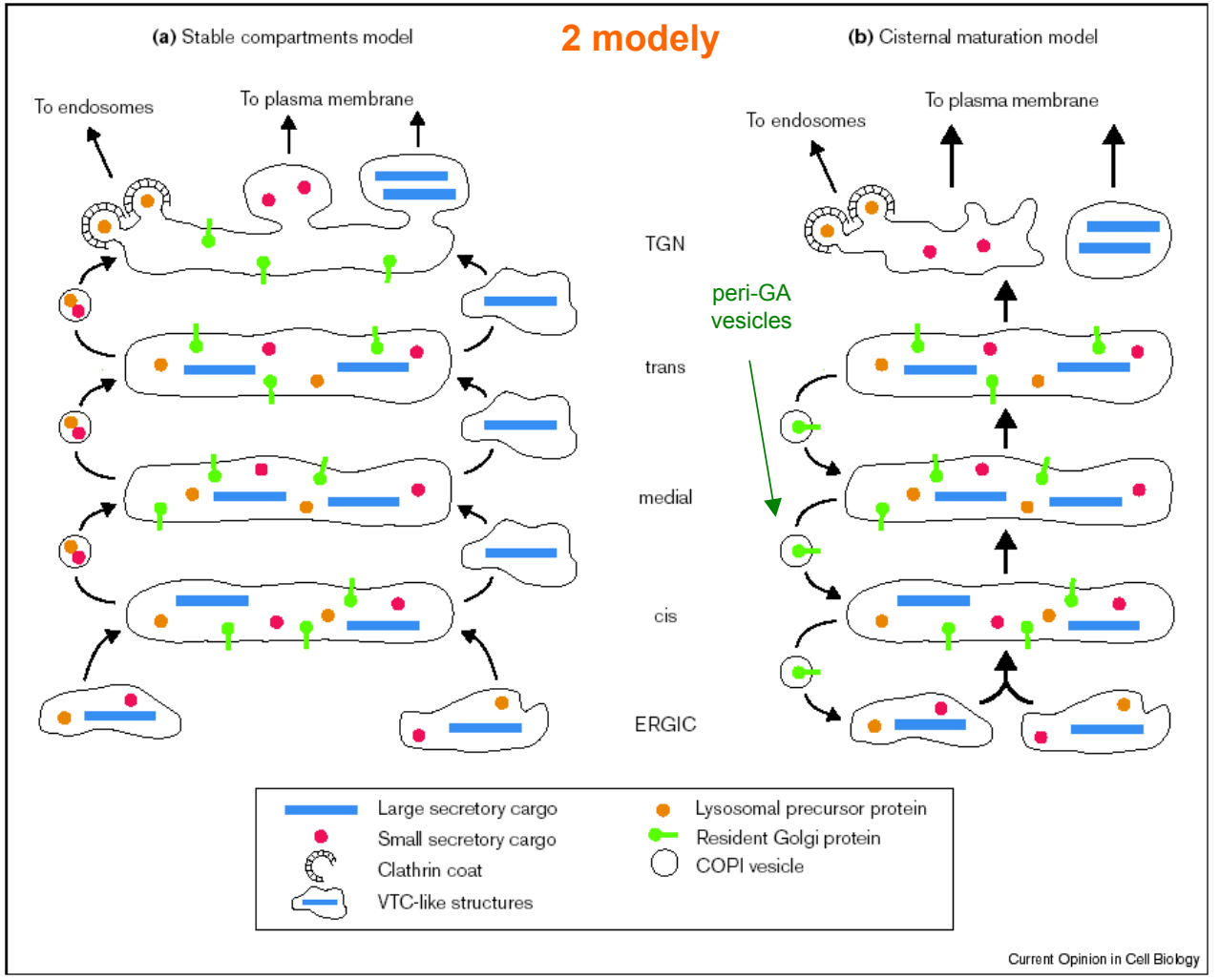
Mechanismus transportu váčků



ER exit sites (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 Golgiho aparátem



a) movement trough a vesicle-mediated process

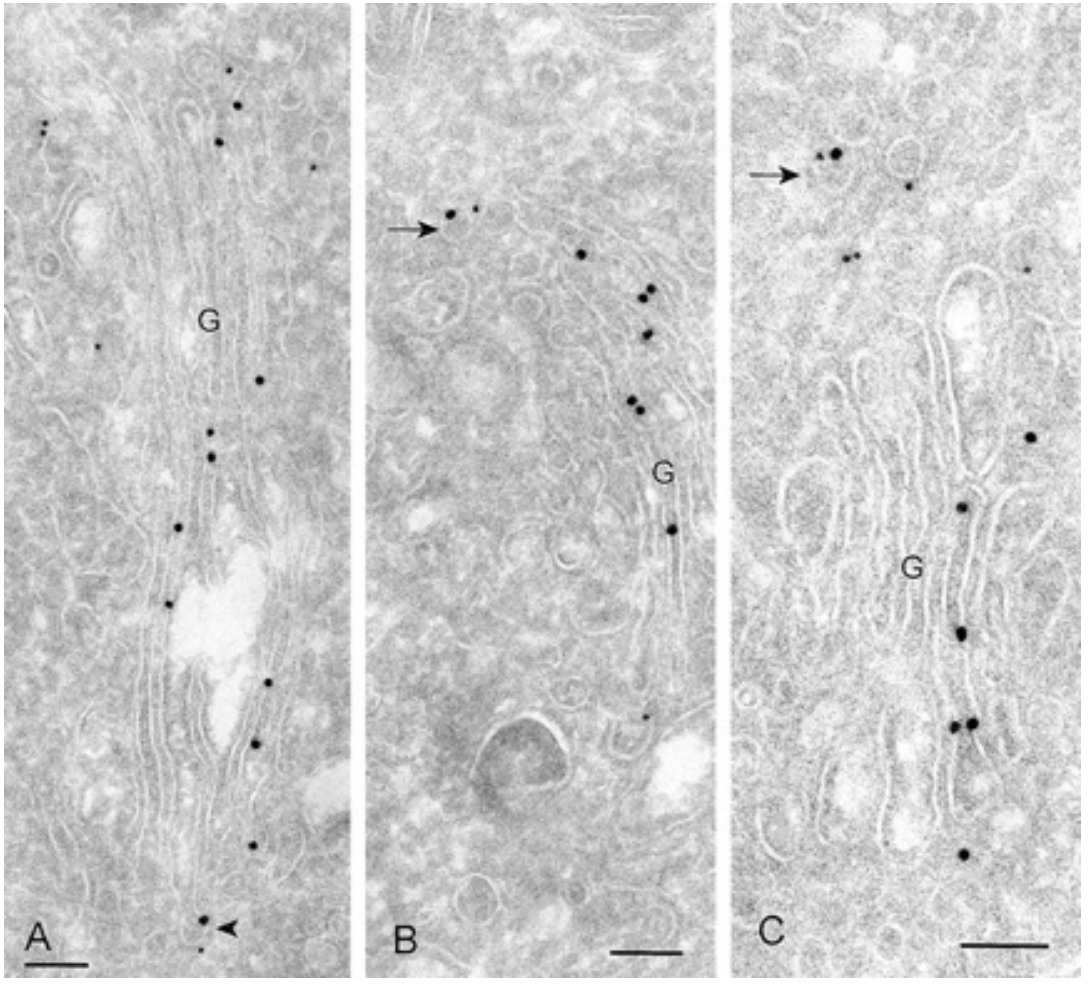
b) cisternal maturation model:

vesicles fuse to 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

Transport Golgiho aparátem

a) movement trough a vesicle-mediated process	peri-GA váčky by obsahovaly sekretované proteiny	např. experimentálně produkovaný protein G viru VSV
b) cisternal maturation model:	peri-GA váčky by obsahovaly proteiny rezidentní v GA	např. mannosidase II, giantin, KDEL-receptor, rBet1

Transport Golgiho aparátem

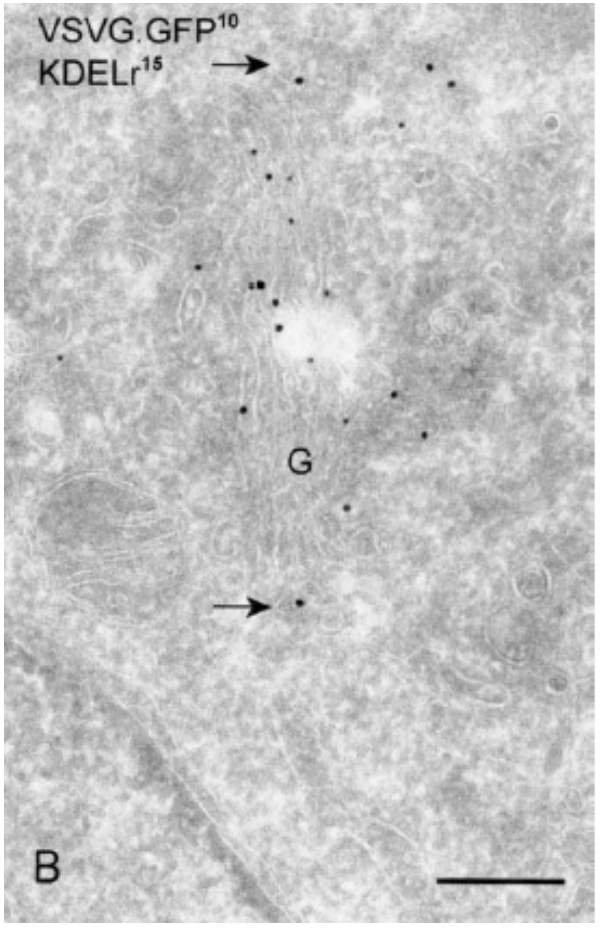


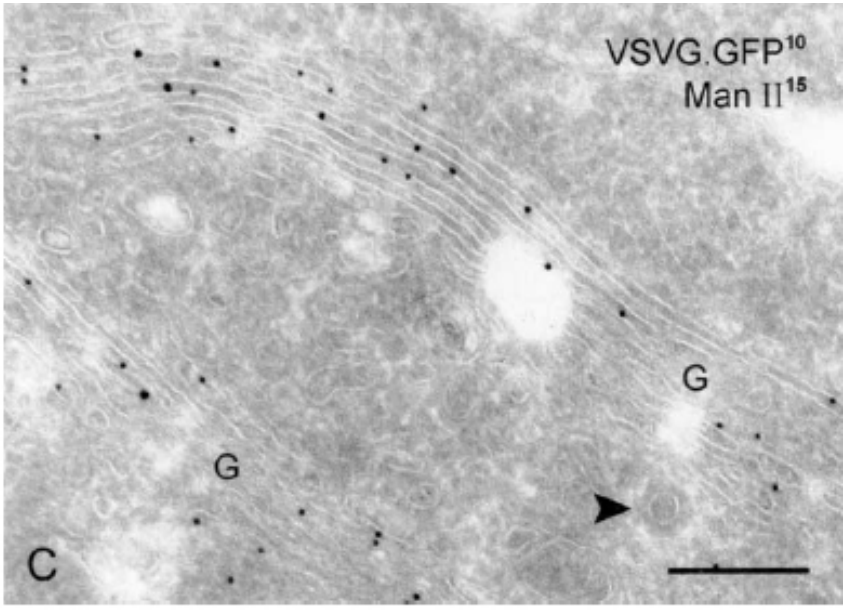
COPI (10 nm gold) colocalizes with mannosidase II (15 nm gold) in lateral rims (arrowhead) and peri-Golgi vesicles (arrows)

Martínez-Menárguez et al. 2001

immuno-elektronová mikroskopie

Transport Golgiho aparátem





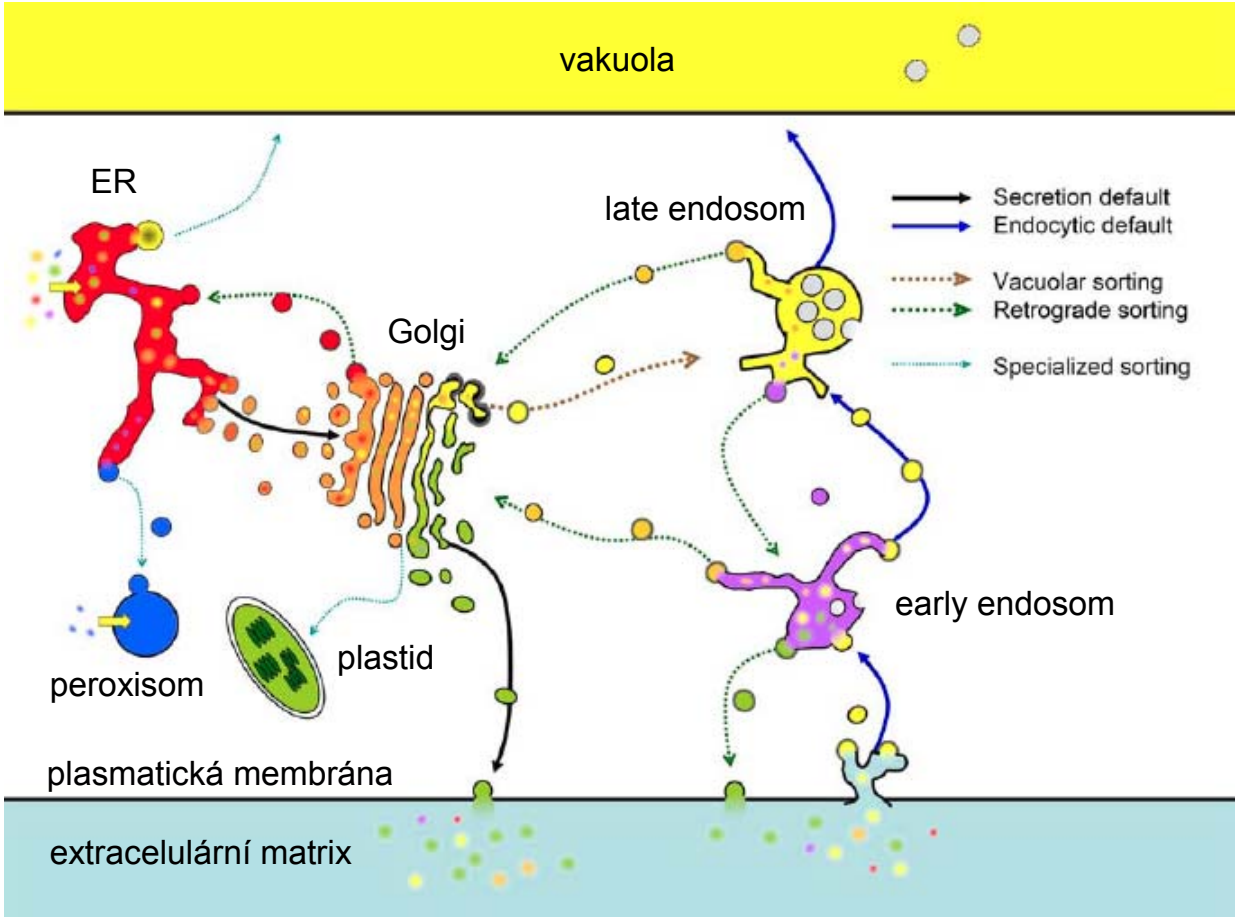
VSVG is absent from peri-GA vesicles, but present in GA cisternae

u rostlin převažuje maturace cisteren GA (model B)

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

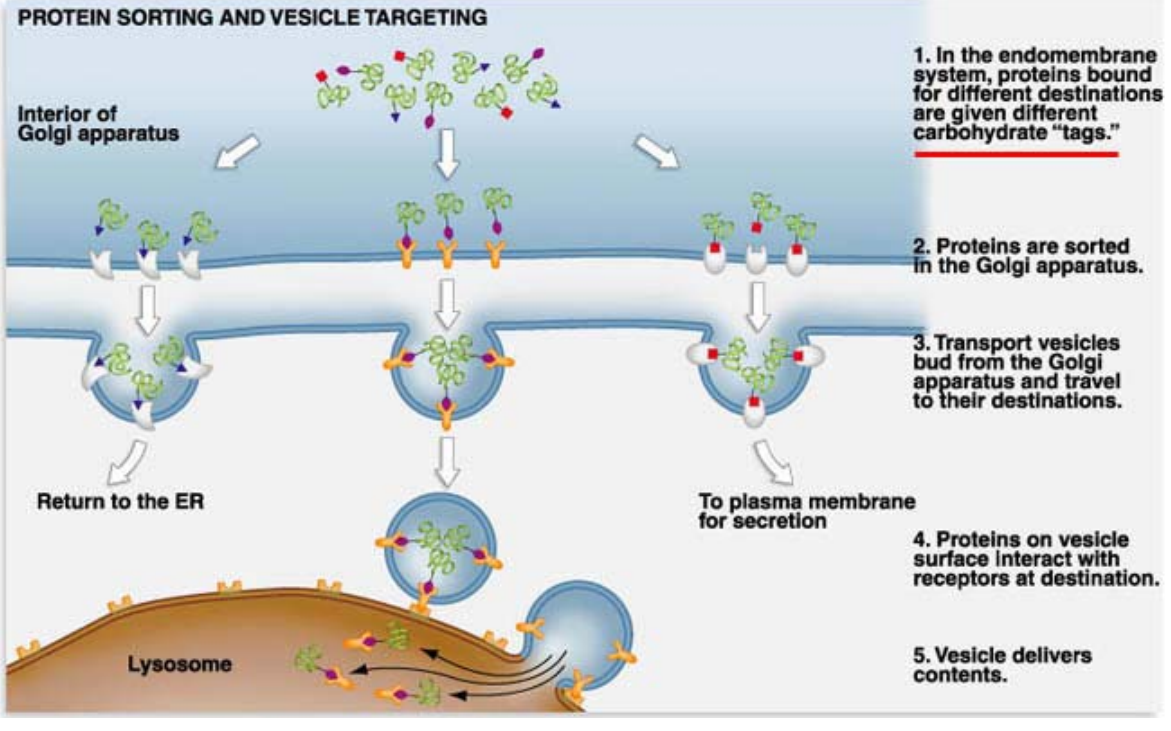
Martínez-Menárguez et al. 2001

Třídění proteinů



Třídění proteinů

bilaterální interace na několika úrovních zajišťují specifitu transportu váčků



Exocytóza

Adresování váčků
k plazmatické membráně

Rab GTPases regulate vesicle trafficking

Rab GTPázy regulují tvorbu a pohyb váčků (některé interagují s cytoskeletálními motory) a při kontaktu s cílovou membránou interagují s poutacími komplexy (*tethering complexes*).

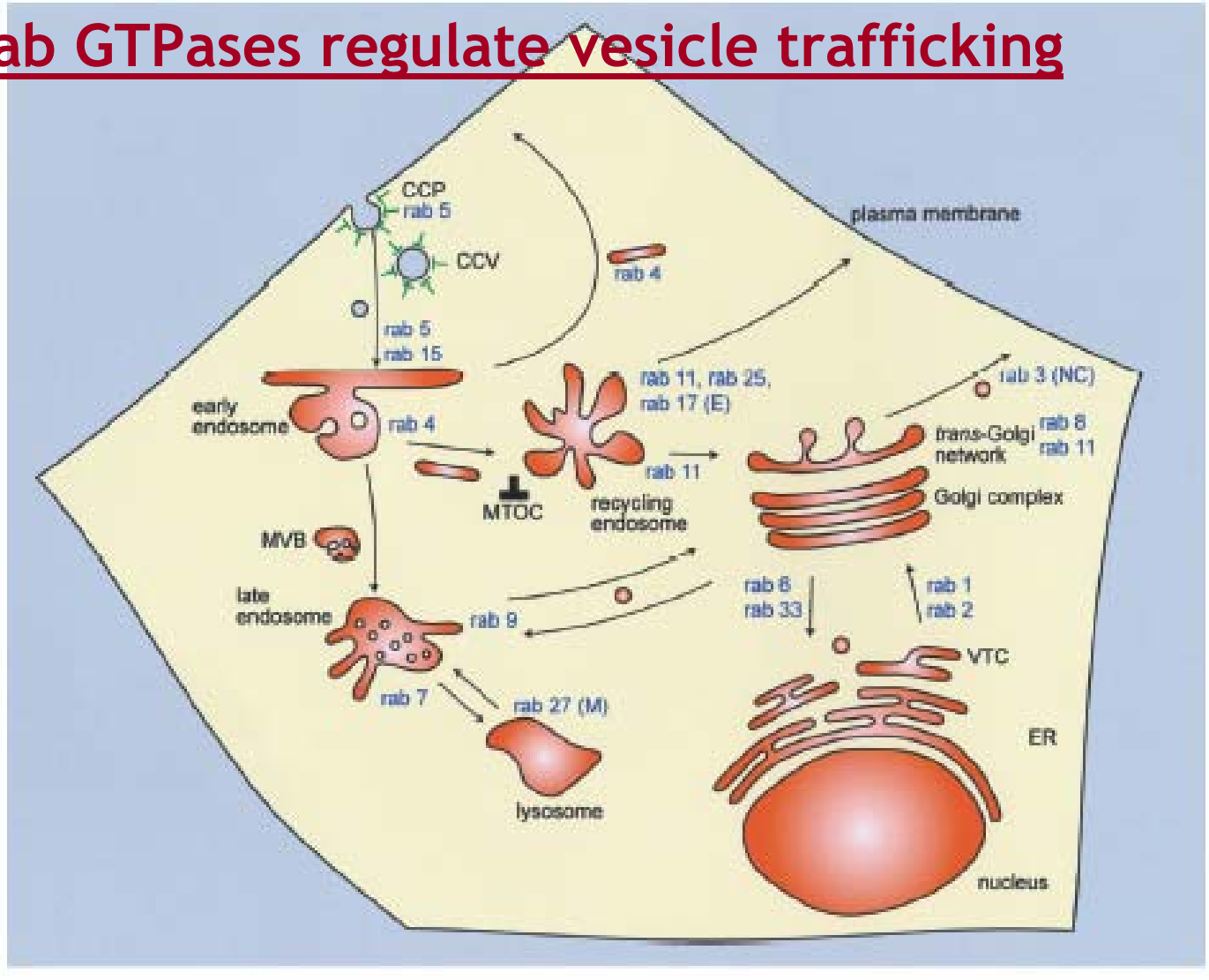
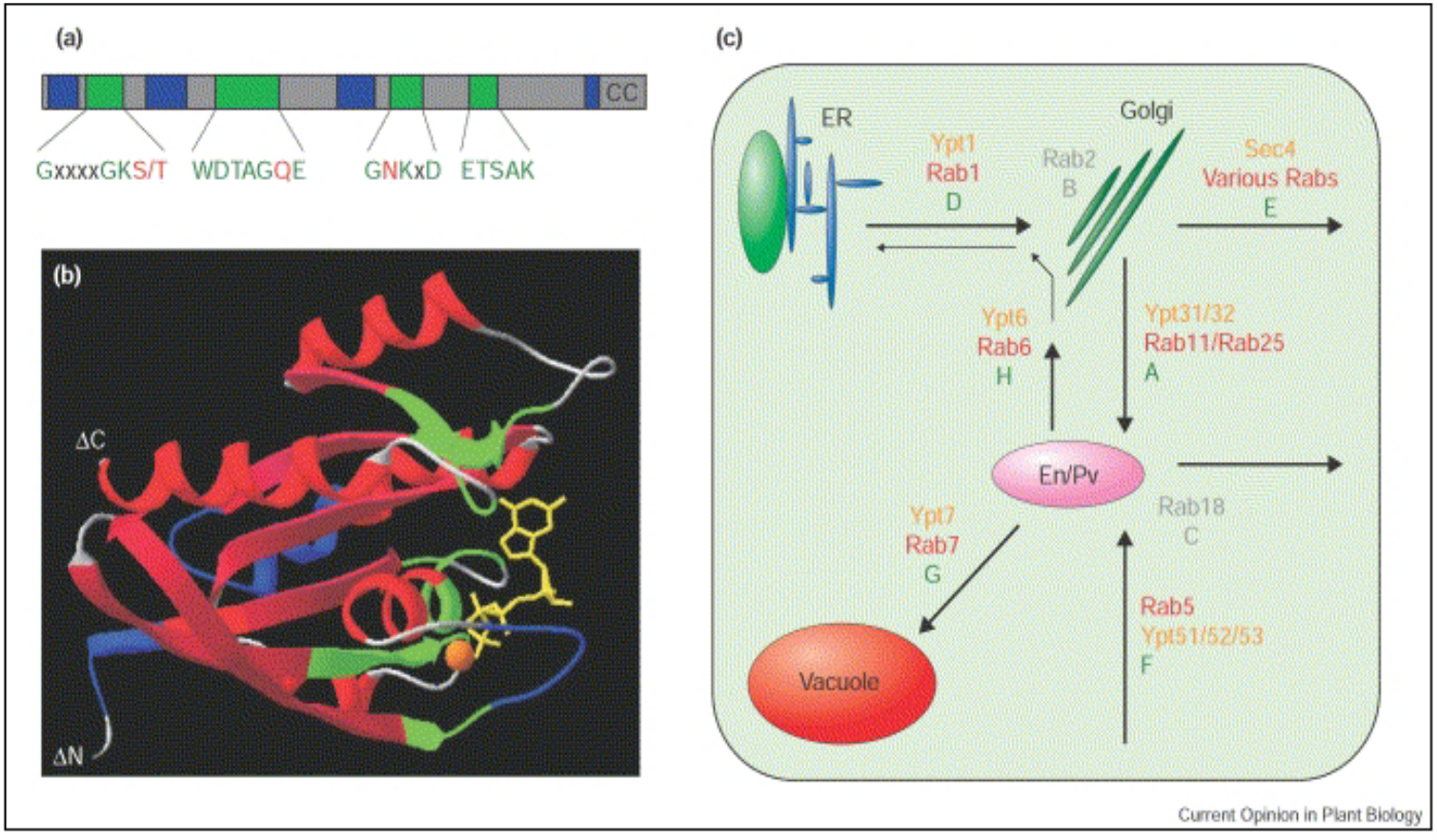


PLATE 1. Intracellular localization of rab proteins. Overview of rab protein localization in mammalian cells. CCP, clathrin coated pit; CCV, clathrin coated vesicle; M, melanosomes; E, epithelial cell type specific expression; NC, neuronal cell specific; VTC, vesiculo-tubular cluster; MVB, multivesicular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

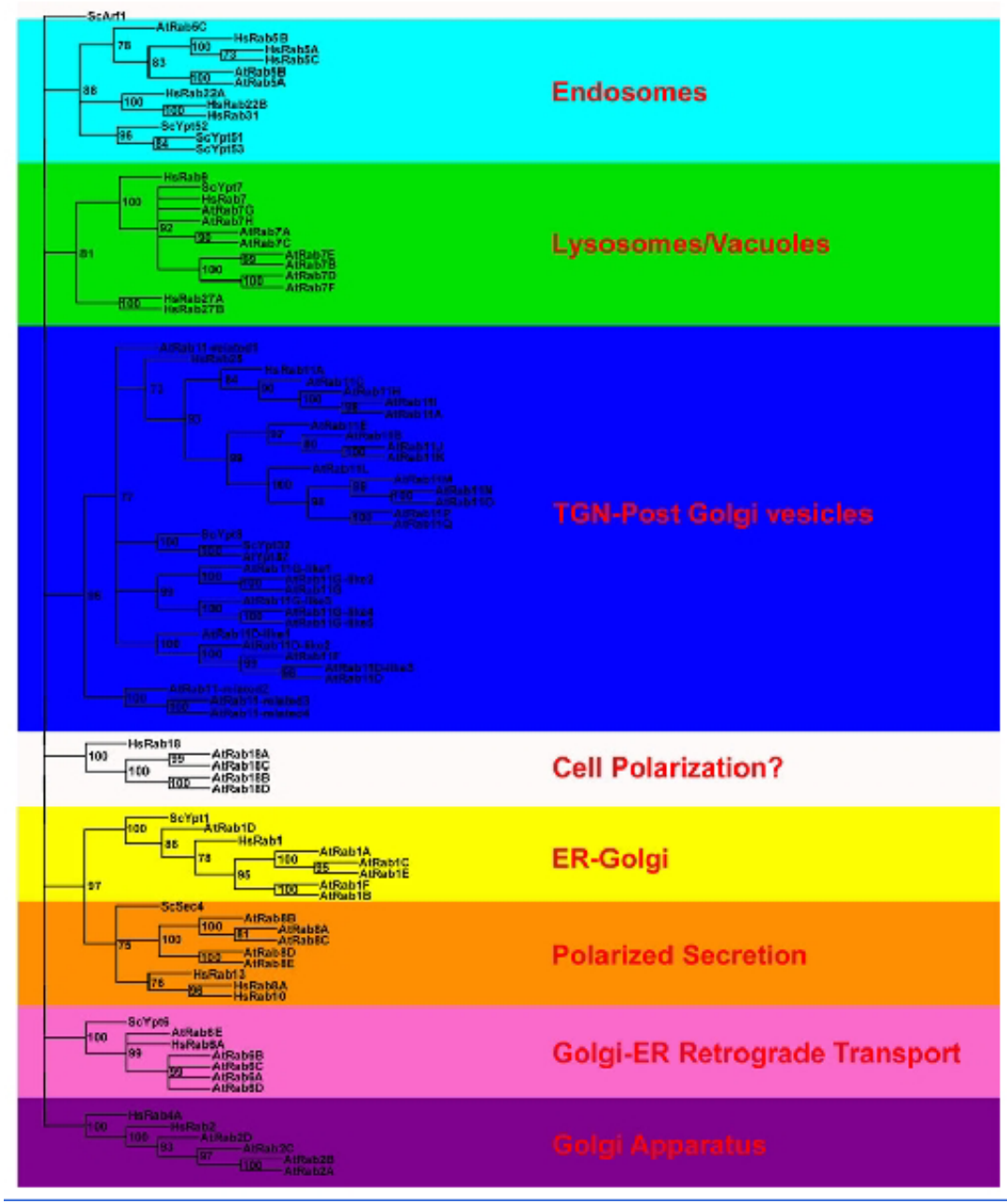
Struktura a klasifikace Rab GTPáz



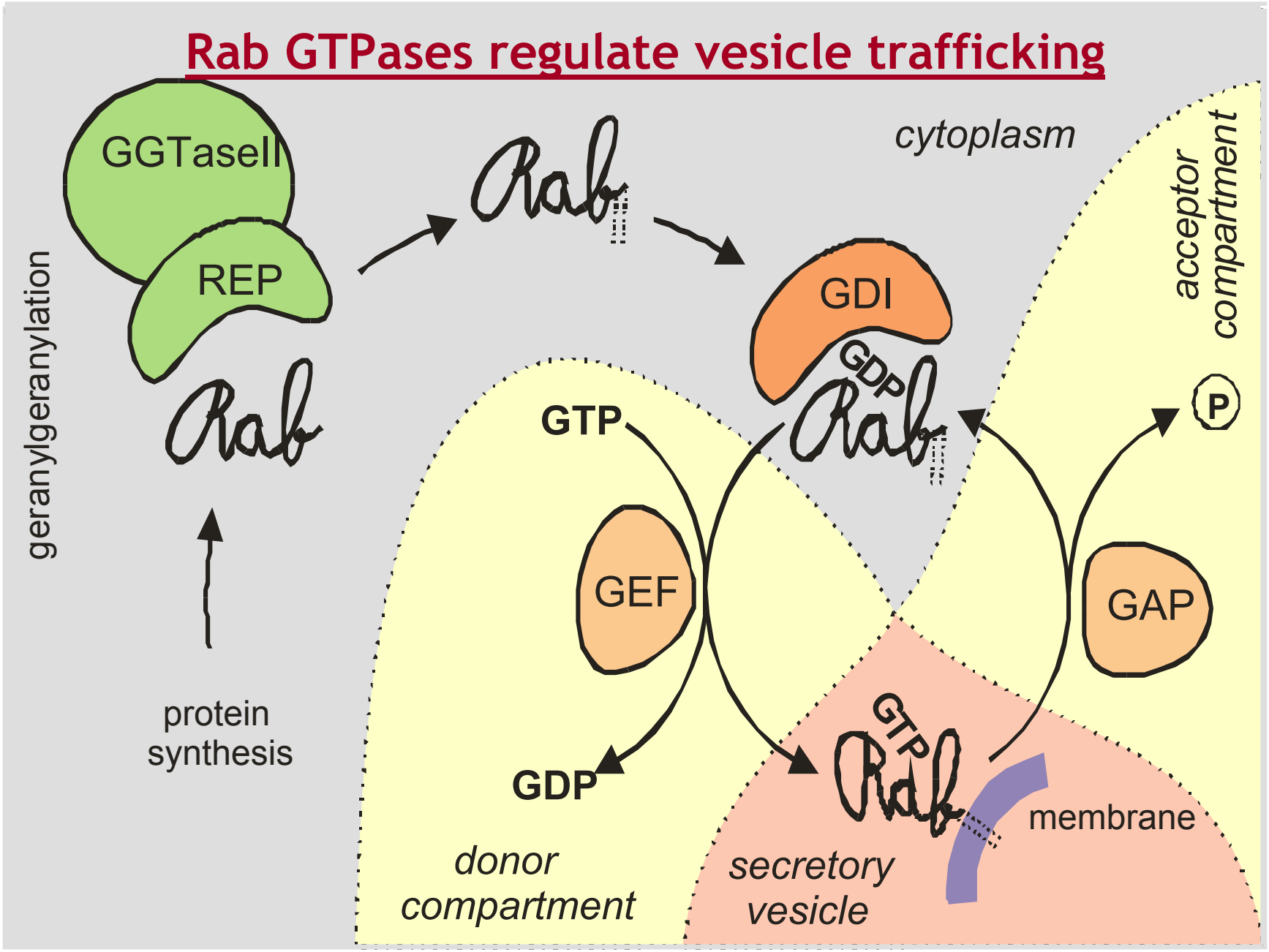
kvasinkové živočišné rostlinné

Struktura a klasifikace Rab GTPáz

Arabidopsis má 57 Rab GTPáz
polovinů představuje skupina RabA (homology Rab11 a Rab 25)
RabA regulují export z Golgi a recyklaci membrán (endosom).



Rab GTPases regulate vesicle trafficking



Rab GTPázy nezbytné pro správnou morfogenezi

Pleiotropic effects of RabA supression by RNAi

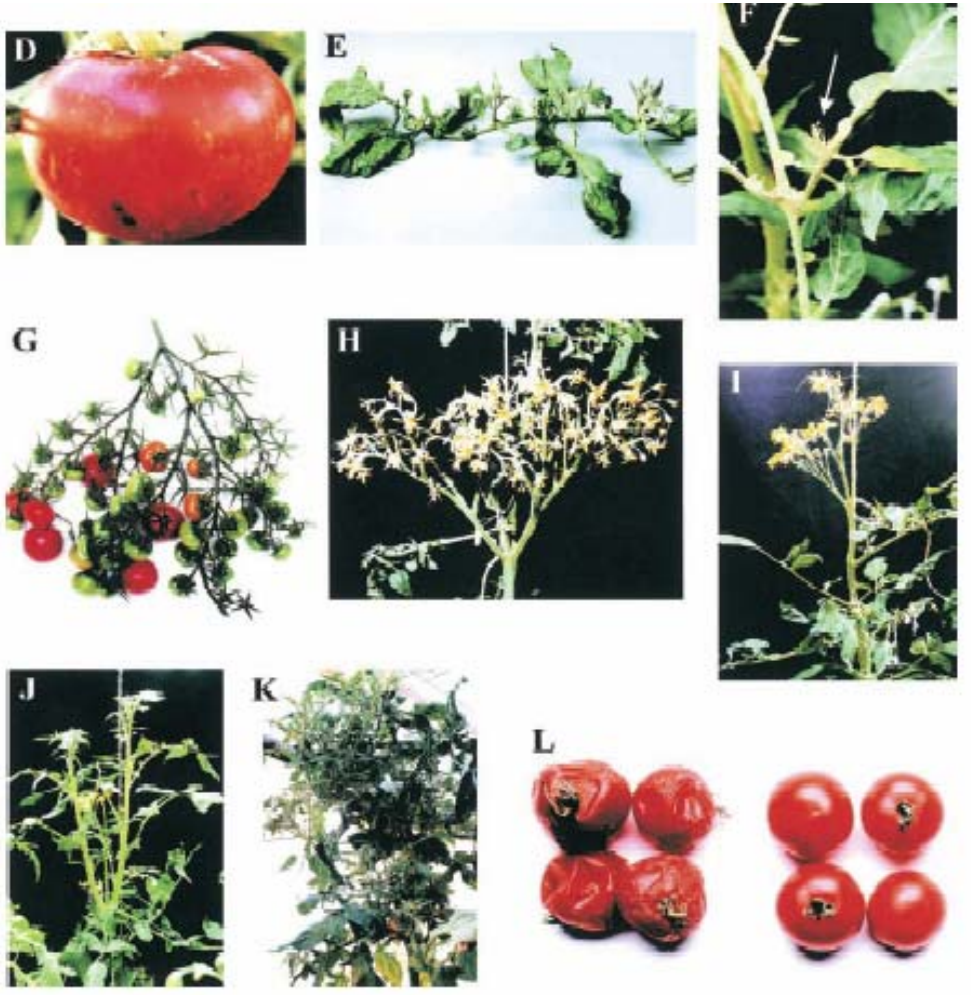


Figure 3. Visual Phenotypes of *Rab* Antisense Transgenic Plants.

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

Poutací komplexy

Tethering mechanism

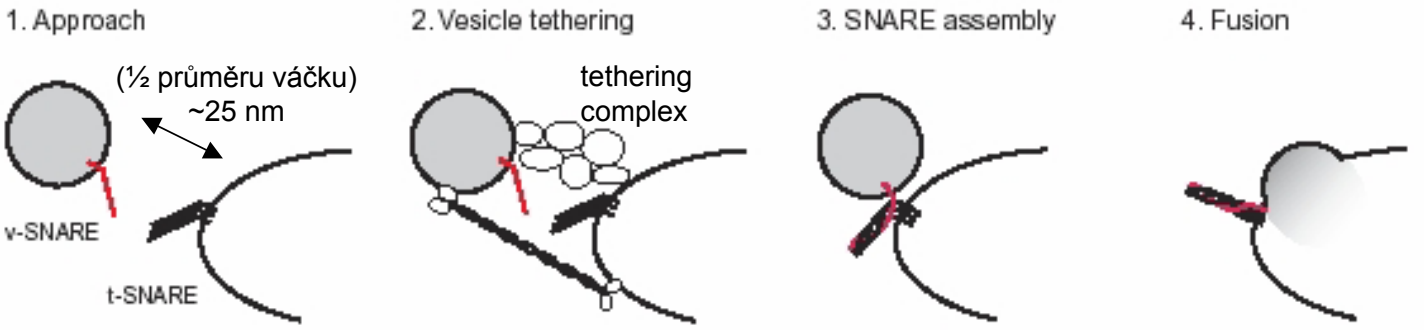


Fig. 1. Steps in the delivery of vesicles to the correct organelle. (1) An intracellular transport vesicle approaches its destination organelle either by diffusion or motor-mediated directed transport. (2) The vesicle is then proposed to be tethered to the organelle by protein complexes and long coiled-coil 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.

Polarized secretion requires proper targeting of secretory vesicles to specific sites on the plasma membrane.

Tethering complexes hold vesicles in proximity to the plasma membrane and facilitate, thus, the formation of SNARE complexes that mediate fusion.

Cílový kompartment bývá určen kombinací specifického „landmarku“ (důležitou roli hrají Rho/Rop GTPázy) a lipidového složení membrány.

Poutací komplexy

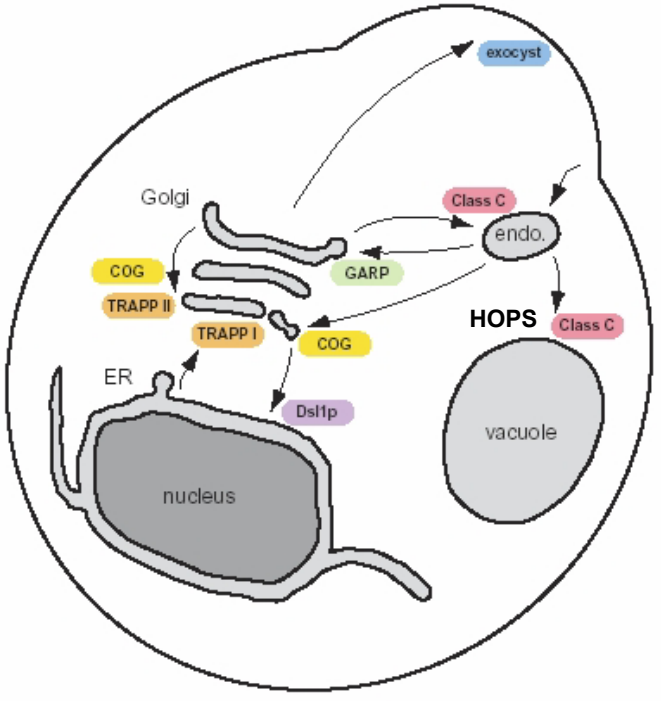
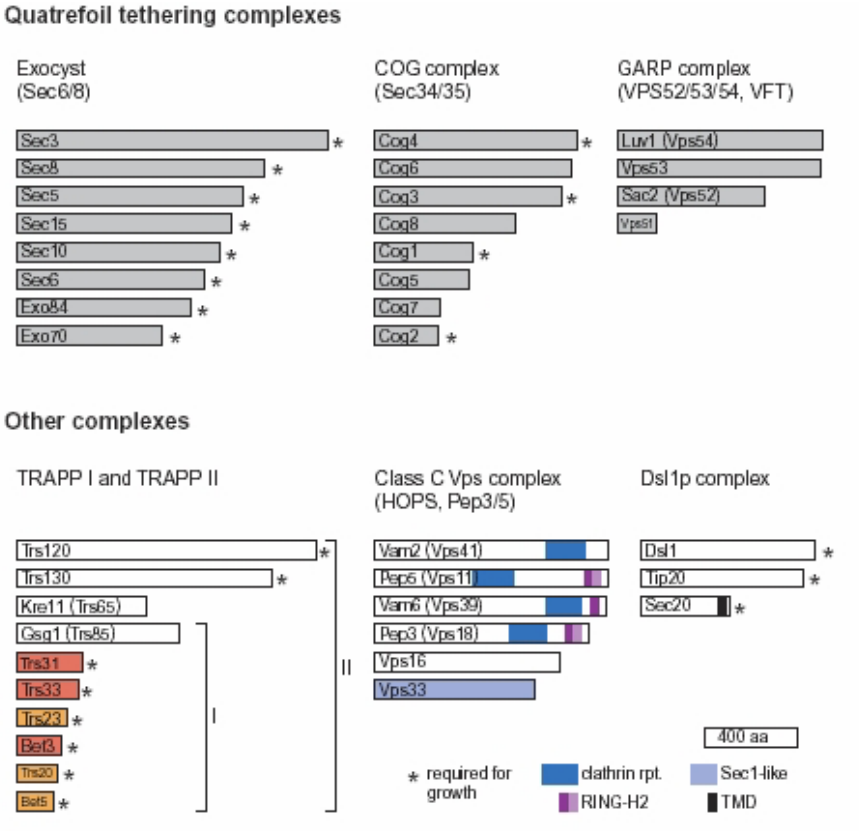
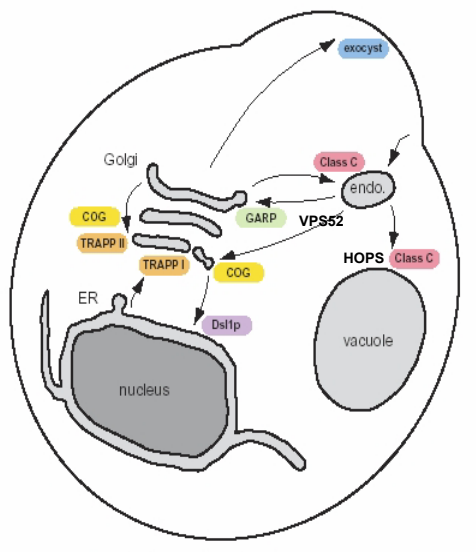


Fig. 2. Putative tethering complexes in the yeast secretory pathway. Protein complexes that have been found to have a role in particular vesicular transport steps are indicated next to those steps. The role of early and late endosomes in yeast is contentious, and so for simplicity this compartment has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



Poutací komplexy – efektory malých GTPáz

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)

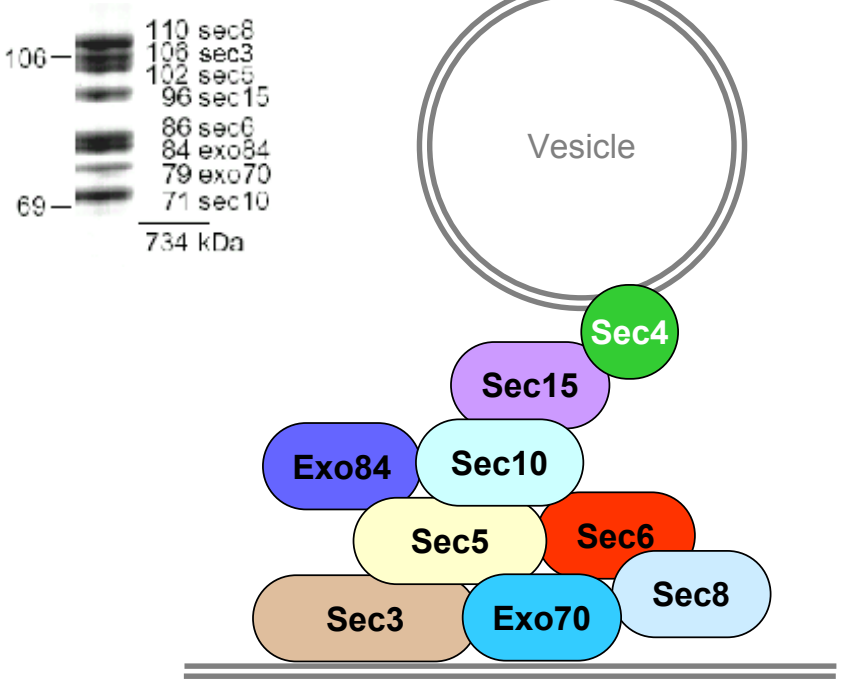


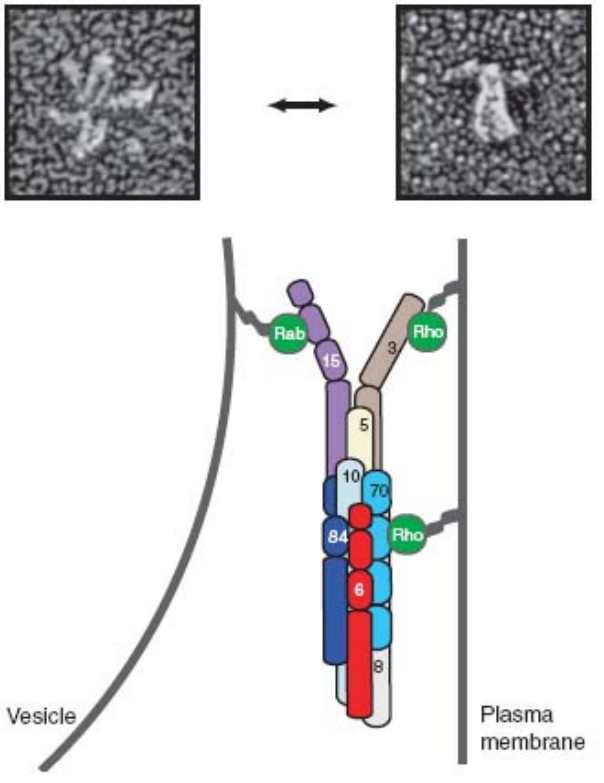
u rostlin experimentálně charakterizován pouze **HOPS komplex** (Natasha Raikhel's lab) a **exocyst** (naše lab)

Komplex exocyst

Exocyst is a multisubunit complex required for exocytosis (TerBush et al., 1996), polarity in epithelia, neurons and for budding in yeast cells.

(Hsu et al. 1998)



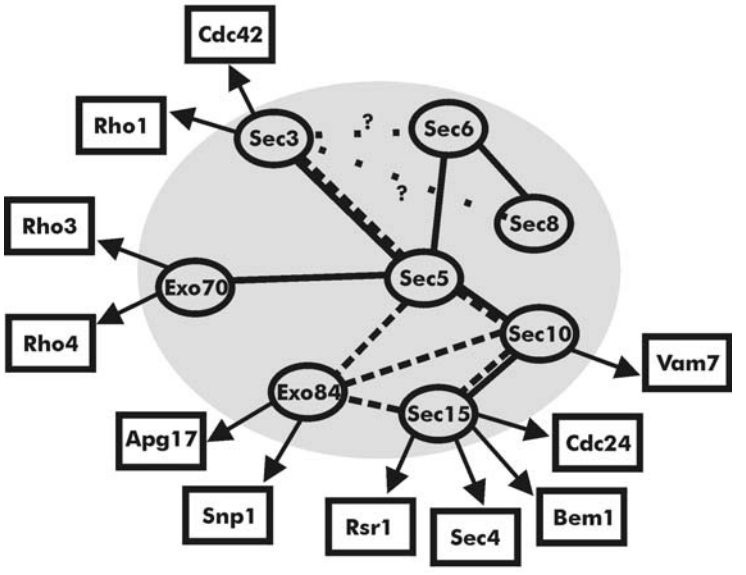


(Munson and Novick 2006)

Komplex exocyst

In Yeast

- Sec4p (Rab GTPase) controls the final step of the exocytic pathway; the exocyst is an effector for Sec4p
- Rho3p is involved in regulation of actin polarity, transport of exocytic vesicles, and docking and fusion of vesicles with the plasma membrane



Exocyst a polarita

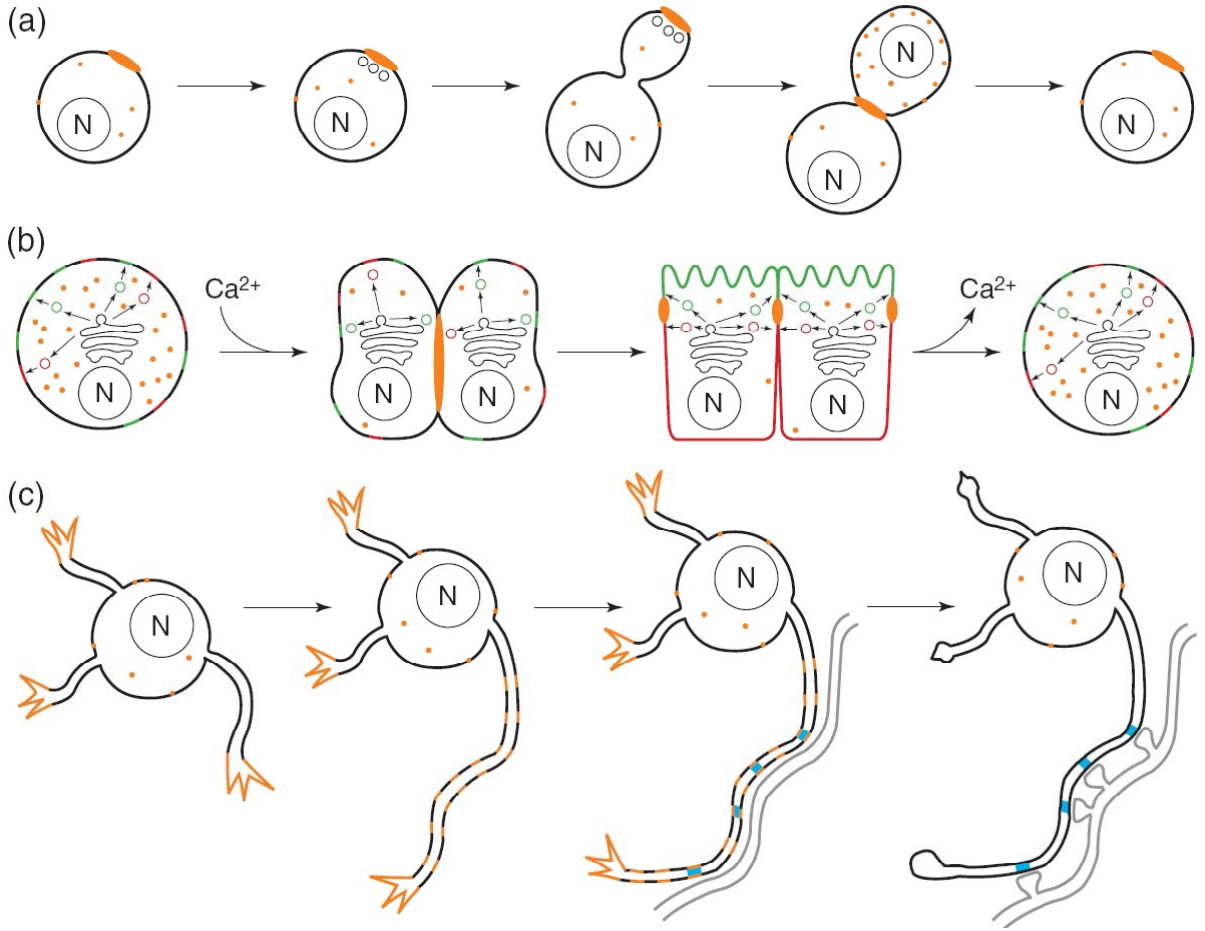
exocyst se podílí na určení a udržení polarity buněk

lokalizuje se do míst intenzivní sekrece

yeast

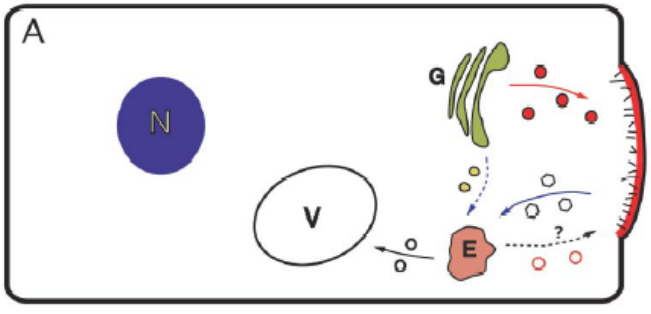
MDCK epithelial cells

neurons

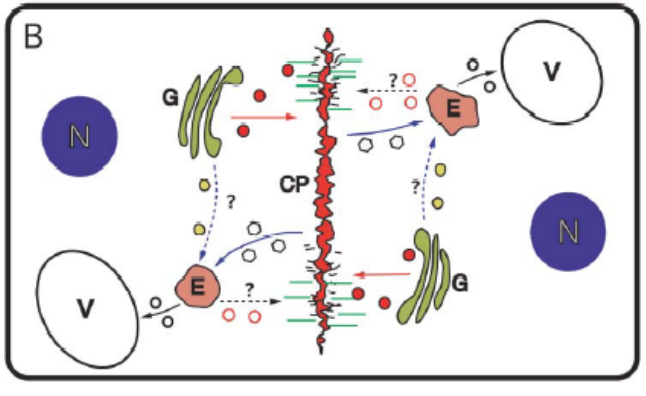


Exocyst a polarita

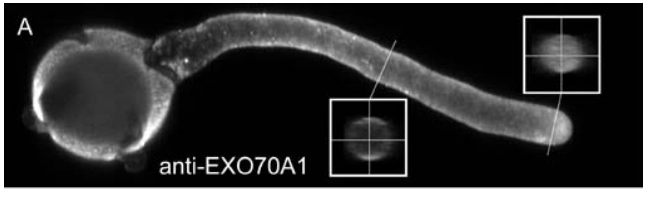
příklady vysoce polarizované sekrece/růstu



rostoucí kořenový vlásek

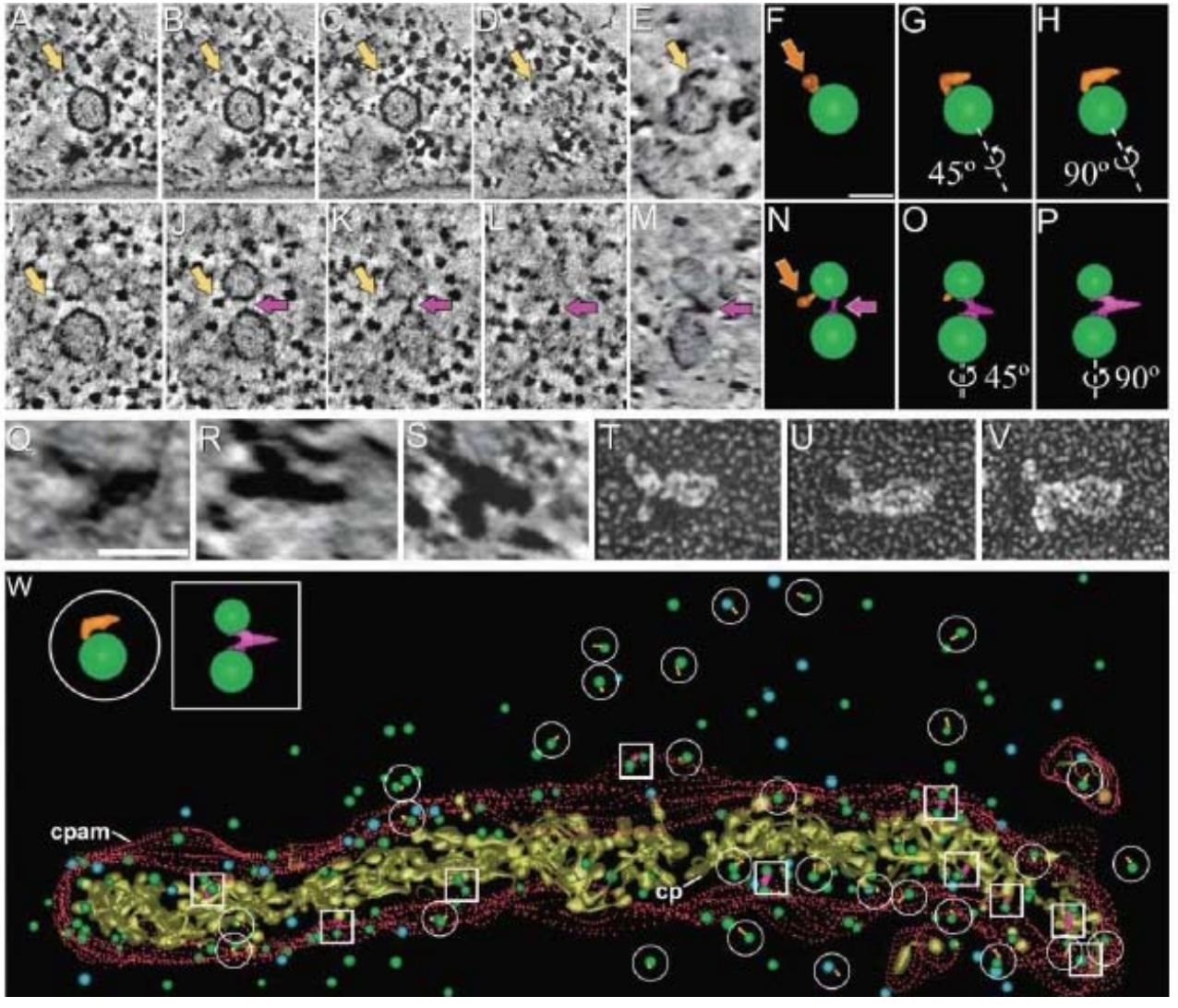


vznikající buněčná přepážka



rostoucí pylová láčka

Mají rostliny exocyst?



Tomographic analysis of nascent cell plate in cytokinesis (putative vesicle tethering complexes).

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

Mají rostliny exocyst?

Lab of Cell Biology - Institute of Experimental Botany
&
Lab of Plant Cell Morphogenesis - Charles University

Collaboration

Rex Cole and John Fowler (Oregon State University, Corvallis, USA)

Nicole Schlager and Marie-Theres Hauser (BOKU, Vienna, Austria)

Frank Hochholdinger (University of Tuebingen, Germany)

www.ueb.cas.cz – Laboratoř biologie buňky

Mají rostliny exocyst?

(Eliáš et al. 2003)

	SEC3	*SEC5*	*SEC6*	*SEC8*	*SEC10*	*SEC15*	*EXO70*	*EXO84*
yeast	1	1	1	1	1	1	1	1
mammals	1	1	2	1	1	2	1	1
Oryza	2	1	1	1	1	3	39	3
Arabidopsis	2	2	1	1	2	2	23	3

Populus 26

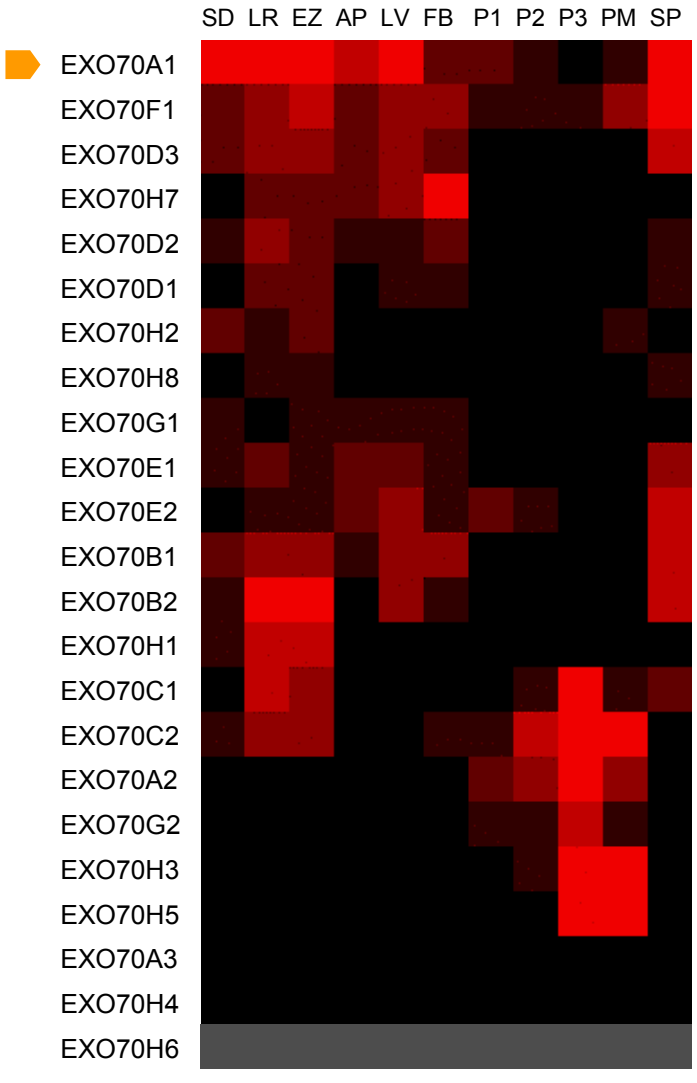
Physcomitrella 13

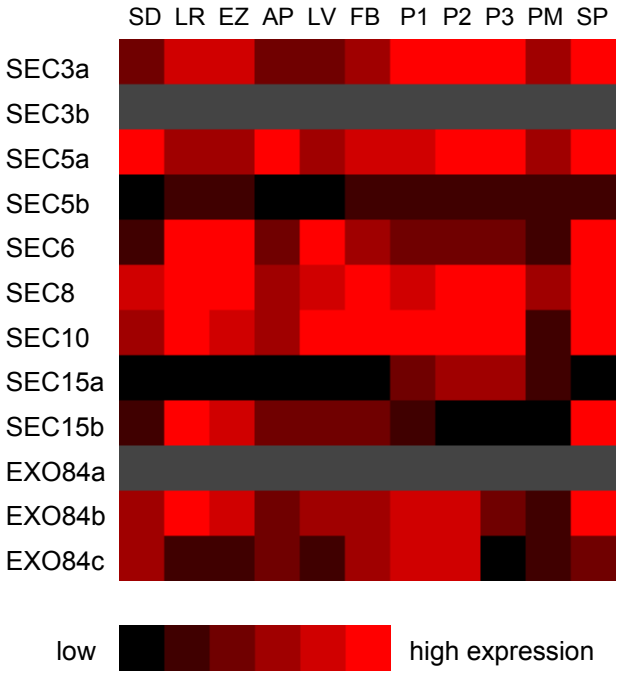
Selaginella 5

Chlamydomonas 1

- homologs of all eight exocyst subunits have been identified *in silico* in all tested plant genomes
- plant homologs of exocyst subunits are often encoded by multiple genes in contrast to yeast and animals
- the *EXO70* gene proliferated into a large gene family in land plants

Analýza exprese genů exocystu u Arabidopsis





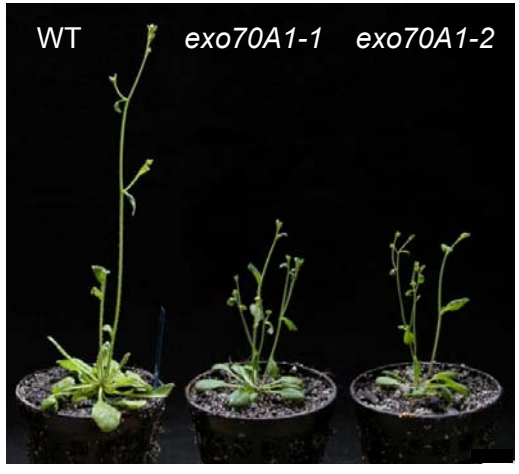
SD – seedlings
LR – lateral roots
EZ – elongation zone
AP – aerial parts
LV – leaves
FB – flower buds

P1 – unicellular pollen
P2 – bicellular pollen
P3 – tricellular pollen
PM – mature pollen
SP – suspension

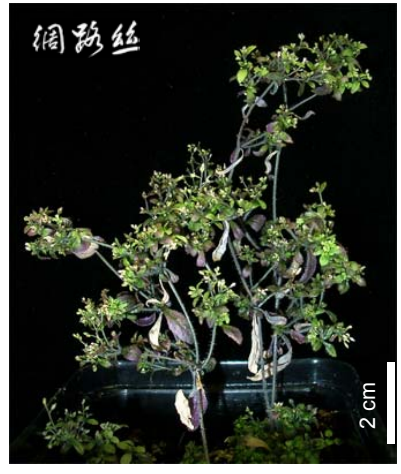
Affymetrix ATH1 DNA chip
Genevestigator (www.genevestigator.ethz.ch)

Mutace genů exocystu

3 weeks



6 weeks

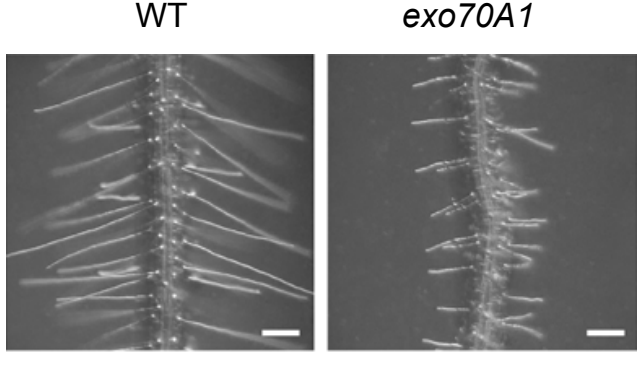


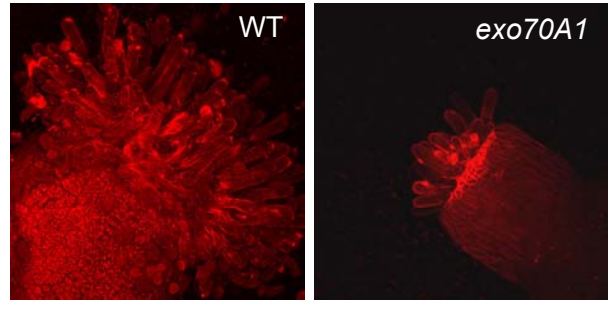
4 months

Mutant homozygotes exhibit:

- dwarfish growth
- reduced apical dominance
- indeterminate growth
- delayed senescence
- short root hairs

mutant *exo70A1*





Mutace genů exocystu

Rex Cole and John Fowler (Oregon State University)

Natural self-crosses of hetegozygotes (%)

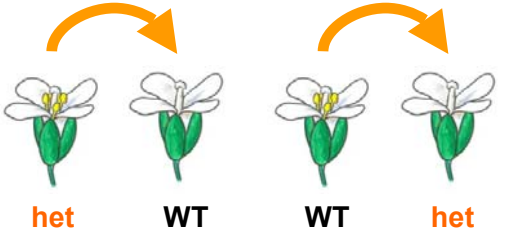
	WT	het	hom
expected	25	50	25
SEC6/sec6-1	**48**	**52**	**0**
SEC6/sec6-2	**54**	**46**	**0**
SEC8/sec8-1	**52**	**48**	**0**
SEC8/sec8-2	**50**	**50**	**0**
SEC8/sec8-3	**47**	**53**	**0**
SEC8/sec8-4	28	49	23
SEC8/sec8-5	24	47	29
SEC8/sec8-6	19	58	23
SEC8/sec8-7	**49**	**51**	**0**

Manual backcrosses to WT (%)

WT	het
50	50
100	**0**
100	**0**
100	**0**
100	**0**
100	**0**
82	18
78	22
69	31
100	**0**

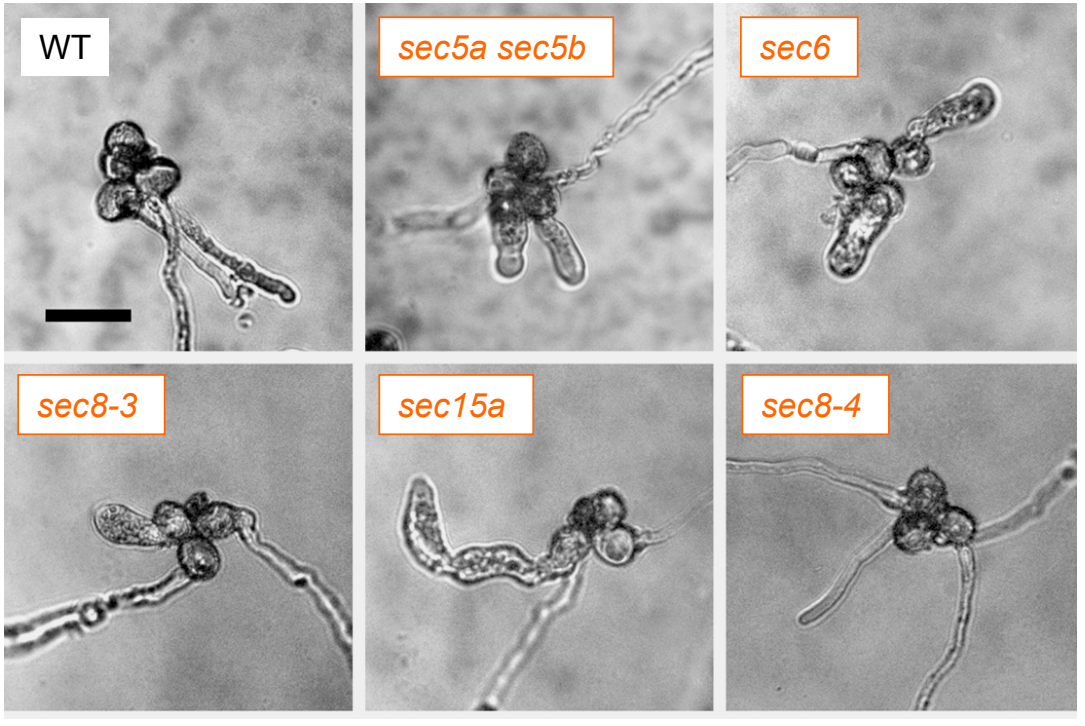
WT	het
50	50
50	50
52	48
47	53
54	46
50	50
51	49
52	48
52	48
49	51

mutants in *SEC6* and *SEC8* show a pollen-specific transmission defect



Mutace genů exocystu

zatímco mutantní pylová zrna *exo70A1* klíčí normálně (aktivní jsou zde jiné isoformy exo70), mutanti *sec5, sec6, sec8, sec15* vykazují defekt v polárním růstu pylové láčky



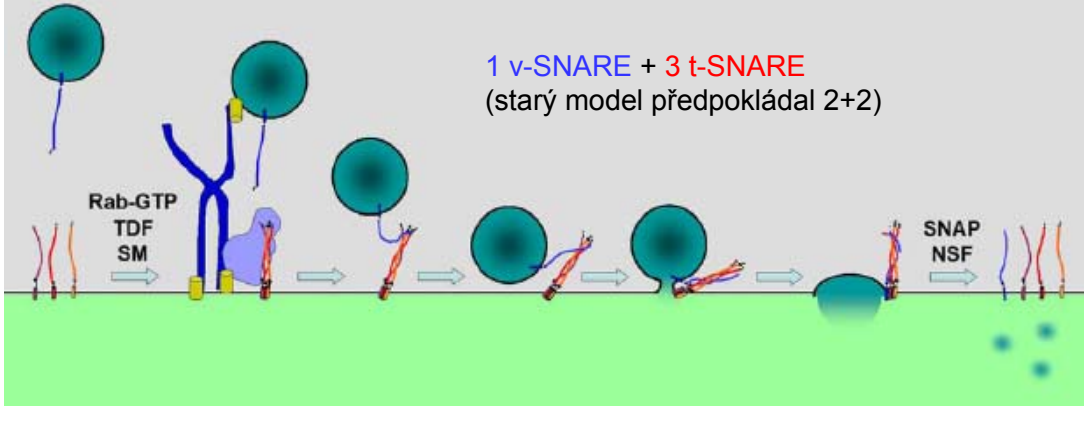
Fúze váčků s membránou

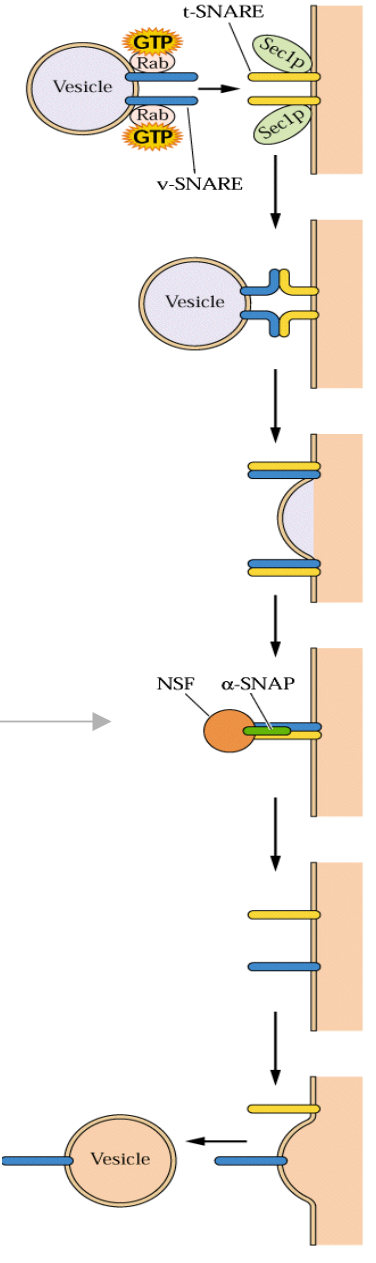
Specifita konečné fúze váčku s cílovou membránou je zajišťována interakcí membránových SNARE proteinů na váčku (v-SNARE) i na cílové membráně (t-SNARE).

Arabidopsis má celkem 54 SNARE proteinů.

SNARE = **S**oluble **N**SF **A**ttachment **Re**ceptor

NSF = N-ethyl maleimid Sensitive Factor

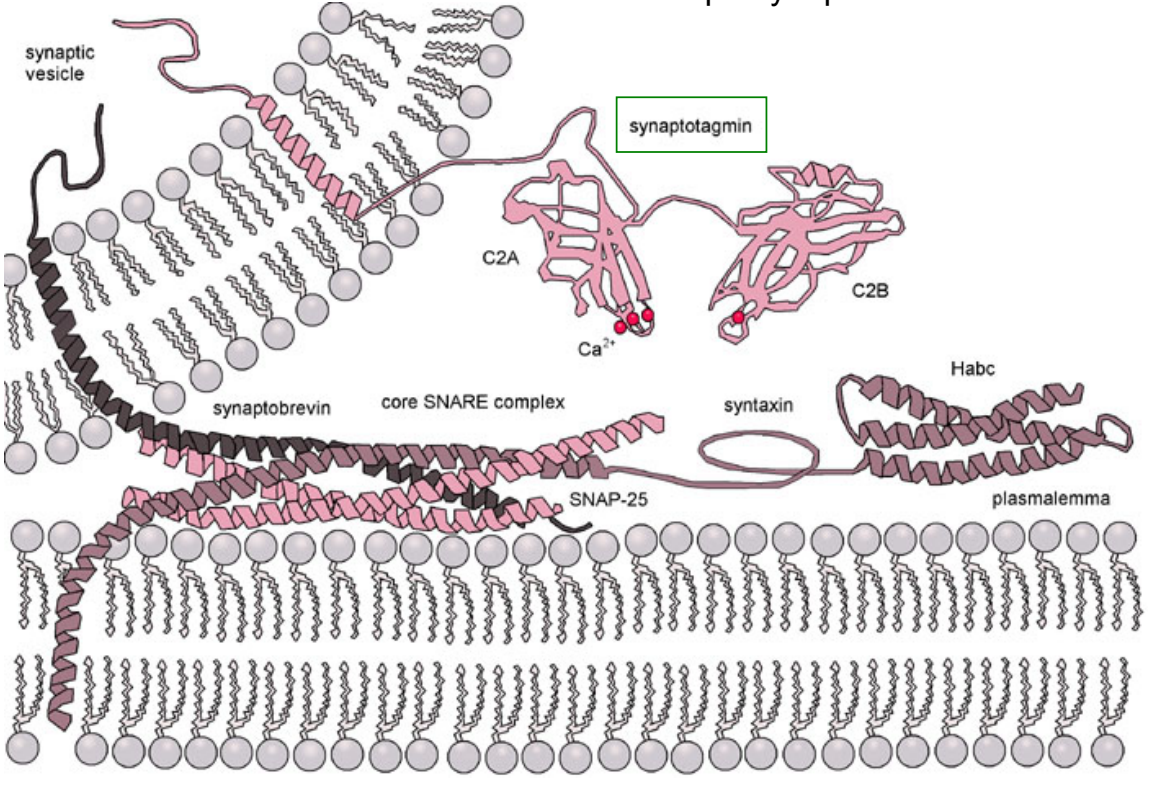


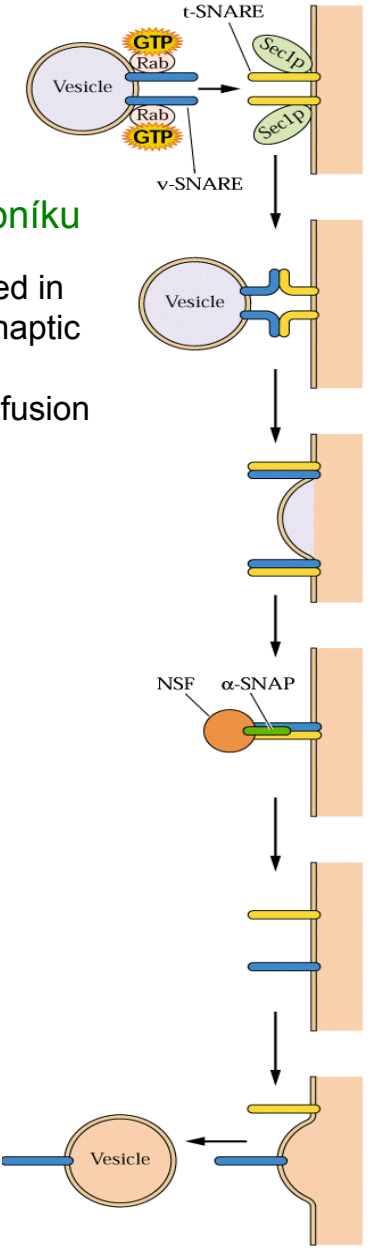


Fúze váčků s membránou

synaptotagminy jsou klíčové regulátory fúze váčků citlivé k vápníku

Synaptotagmin is a Ca^{2+} sensor and is involved in
(i) early synaptic vesicle docking to the presynaptic membrane via interaction with SNAP-25
(ii) late steps of Ca^{2+} evoked synaptic vesicle fusion with the presynaptic membrane.





Exocytóza

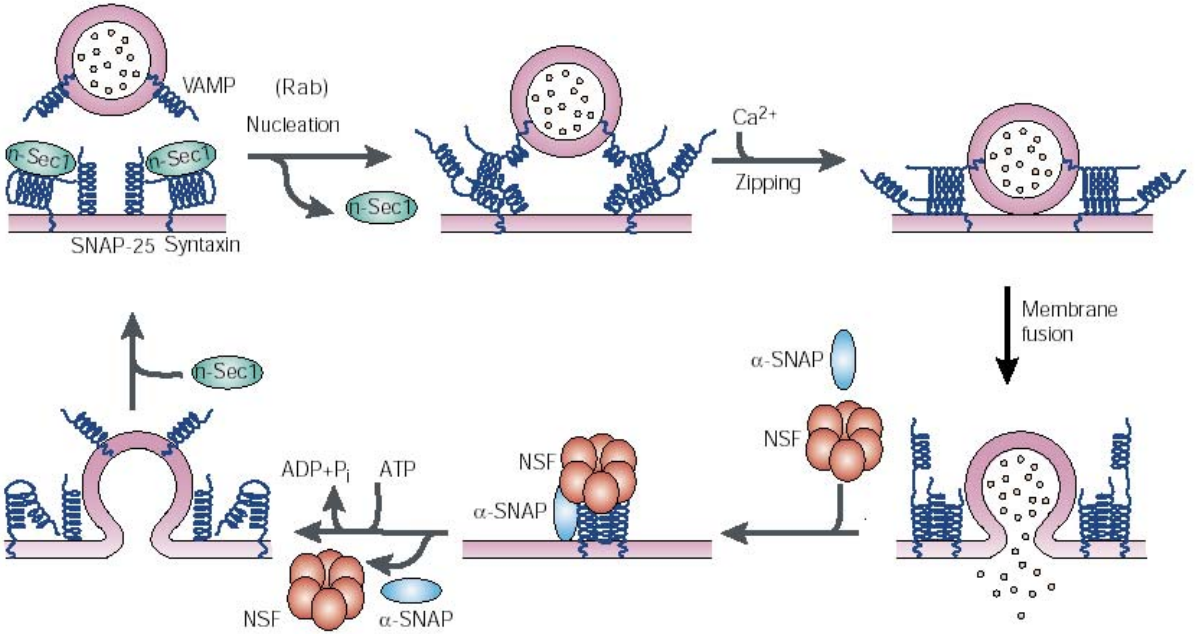


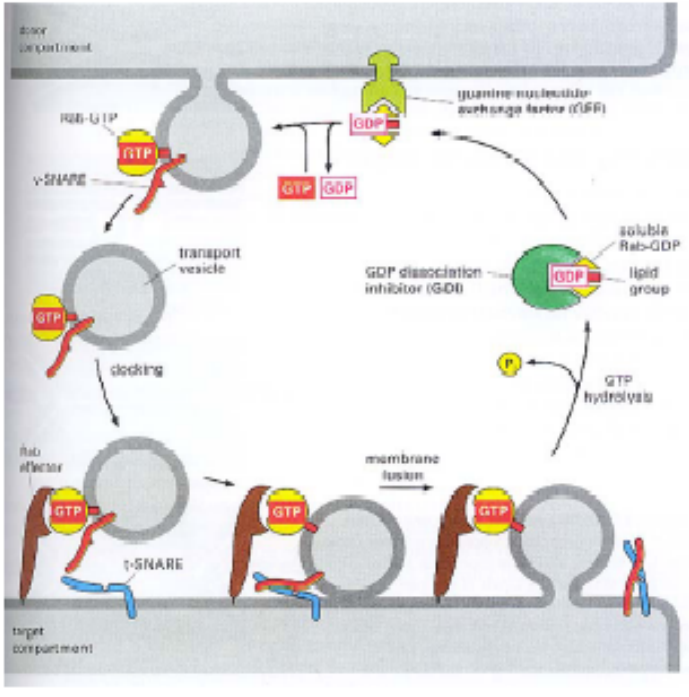
Figure 4 | **Molecular model of vesicle exocytosis.** Syntaxin is bound to n-Sec1 before formation of the core complex. Rab proteins might facilitate the dissociation of n-Sec1 from syntaxin, allowing subsequent binding (nucleation) between the three neuronal SNAREs, syntaxin, SNAP-25 and VAMP (for simplicity, 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.)

Fuse in the regulated secretory pathway is mediated by SNAREs and controlled by elevations in cytoplasmic Ca^{2+}.

inhibition of regulated secretion only: botulinum toxins cleave (through its protease activity) SNAP-25, leading to paralysis in clinically developed botulism

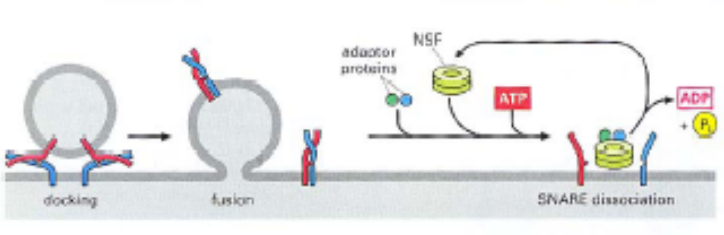
Exocytóza

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.

Když něco nefunguje

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

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

KEULE (At homolg Sec1) – mutant *keule* má podobný fenotyp

GNOM (GEF pro Arf GTPázy) – mutant *gnom* má postižen polární transport auxinových transportérů (PINy)