Cytoskelet a buněčné dělení

Cytoskelet – definice a funkce

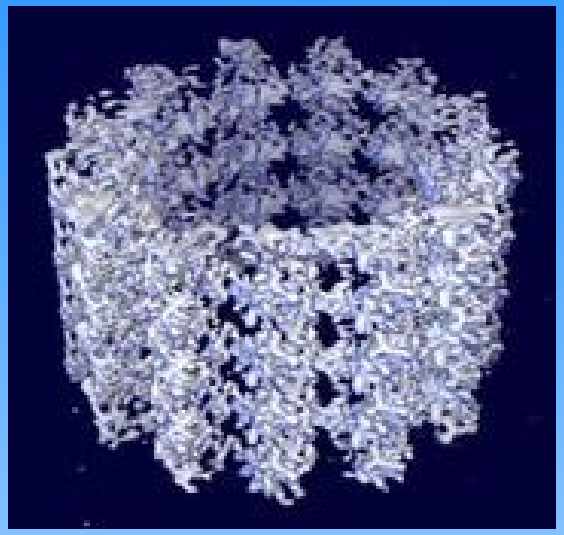
- propojená síť vláknitých proteinů, vyskytuje se v buňkách téměř všech eukaryot

- Funkce:
  - určuje buněčný tvar
  - organizuje cytoplazmu
  - transportuje buněčné struktury (váčky, chromozomy)
  - přispívá k pohyblivosti buňky

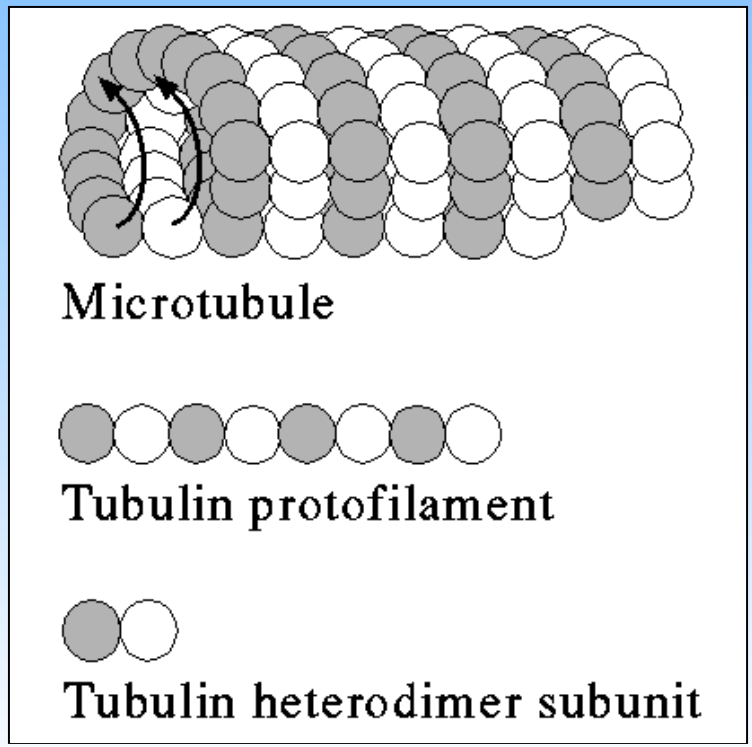
Cytoskelet – složení

- **mikrotubuly** – heterodimery tubulinu
- **mikrofilamenta** – z podjednotek proteinů aktinu
- **intermediální filamenta** – složení nebylo ještě přesně definováno

Mikrotubuly

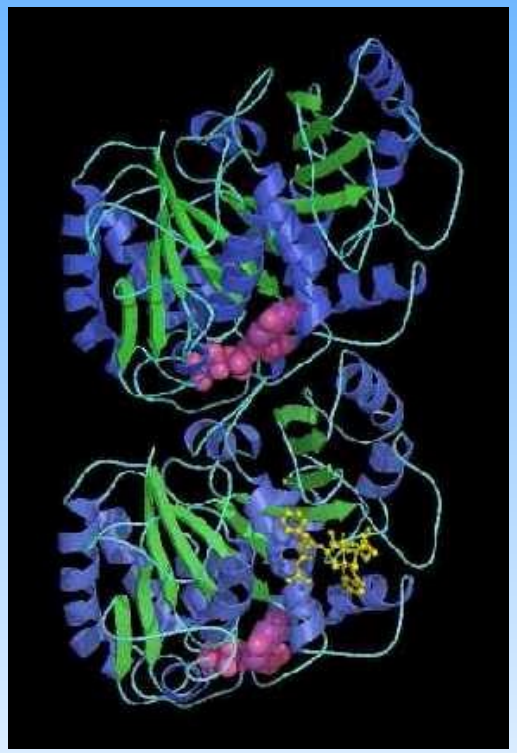


- protein **tubulin** a další proteiny označované jako **MAPs** (microtubule-associated proteins)
- MAPs stabilizují mikrotubuly a specializují jejich funkce, ale nejsou nezbytné pro tvorbu základní struktury tubulinu



Tubulin

- **heterodimer** složený z polypeptidů označovaných jako α- a β-tubulin. Jsou podobné, pokud jde o složení aminokyselin, ale natolik rozdílné, že protilátky proti α-tubulinu nereagují na β-tubulin a naopak
- Mw tubulinu je asi 110 kDa zatímco u monomerů je asi 55 kDa



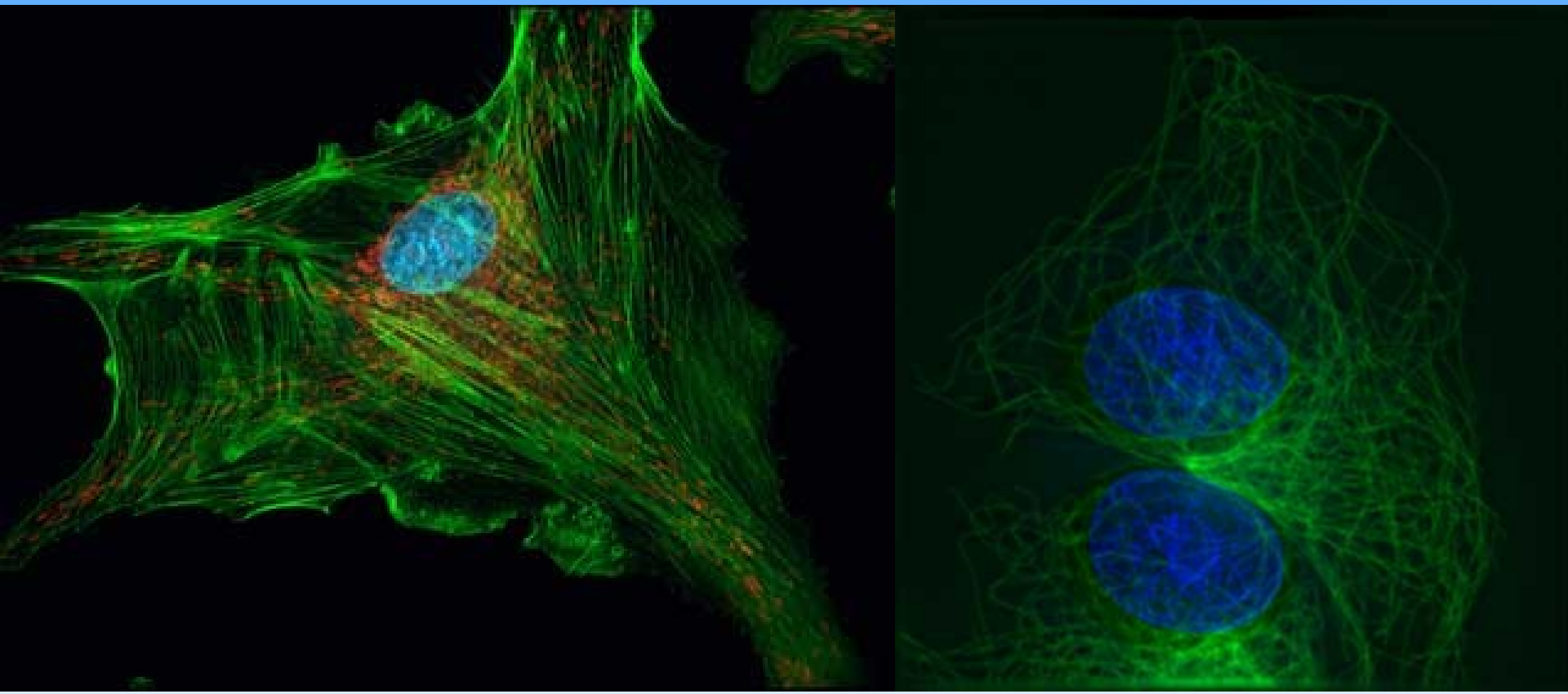
Podmínky dimerace tubulinu

- přítomnost — GTP, Mg^{2+}
- nízké koncentrace — Ca^{2+}
- pH optimum — 6,9
- teplota — 20 – 37°C

Disagregace tubulinu

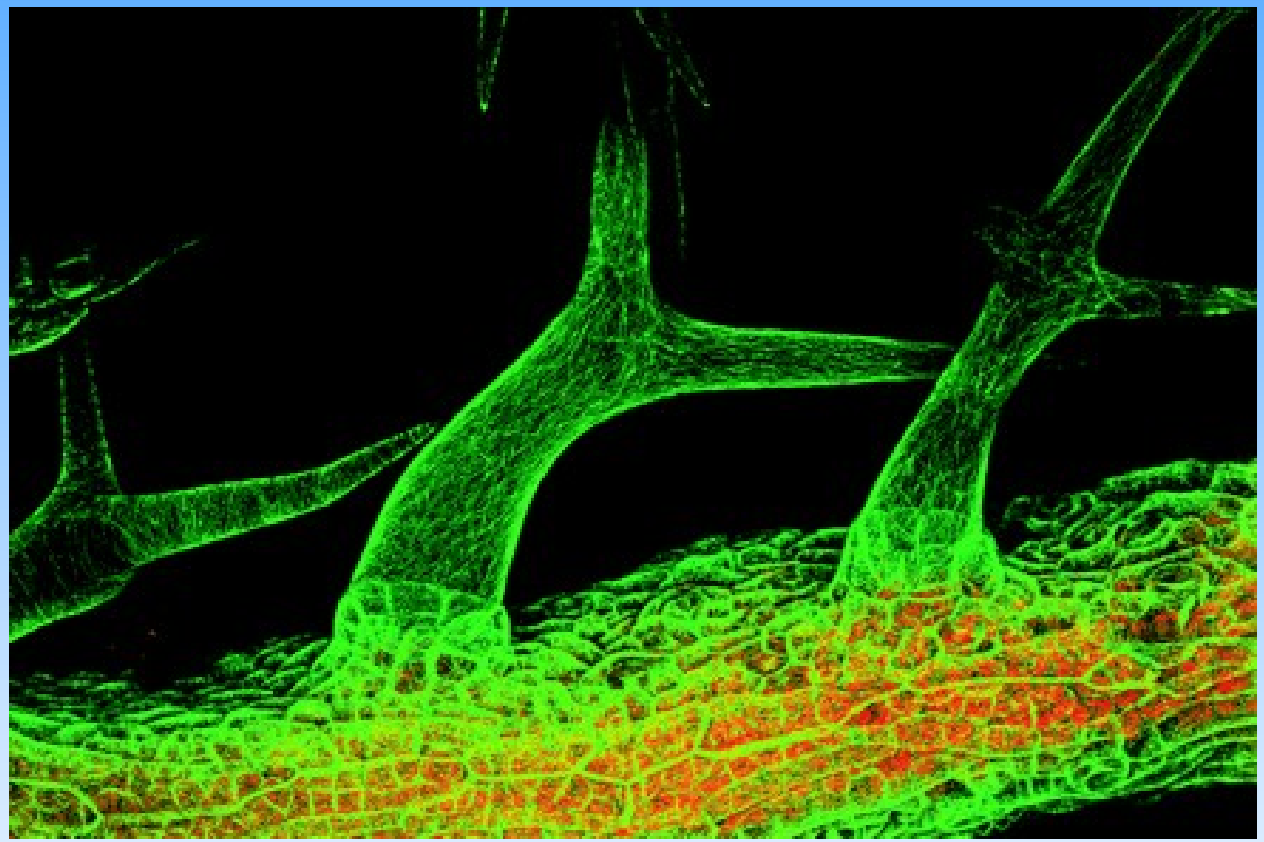
- teplota 0°C
- přítomnost Ca^{2+}
- rezistence mikrotubulů mnoha vyšších rostlin vůči chladové depolymeraci tubulinu

Vizualizace tubulinu



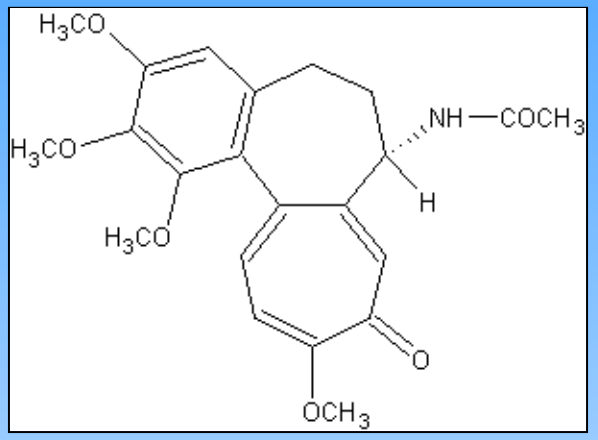
Fluorescenční mikroskopie: metoda FITC, barvení jader DAPI

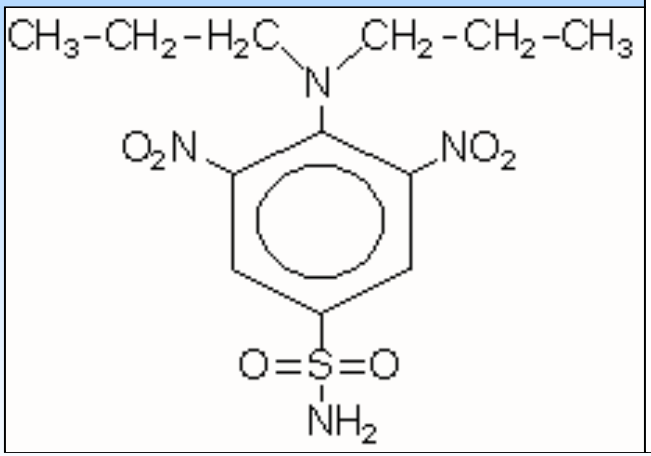
Arabidopsis - cytoskelet

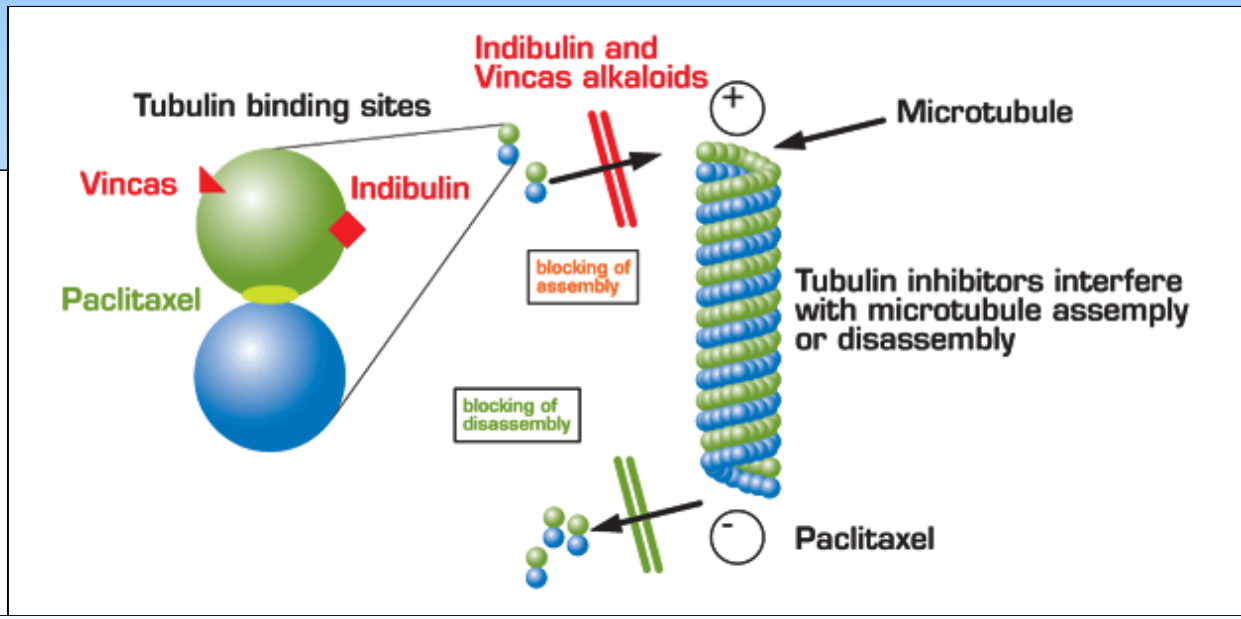


Disagregace tubulinu - rostlinné alkaloidy a herbicidy

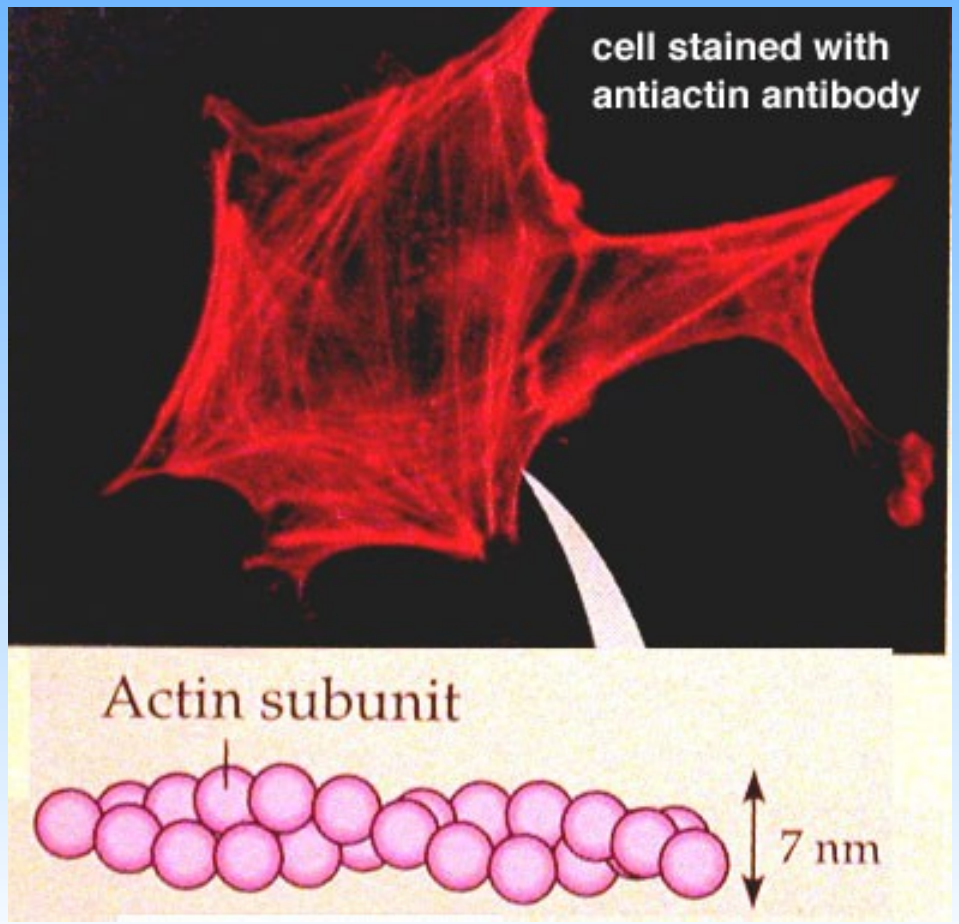
- kolchicin
- vincristin
- oryzalin







Mikrofilamenta - aktin



Formace cytoskeletu v průběhu buněčného cyklu

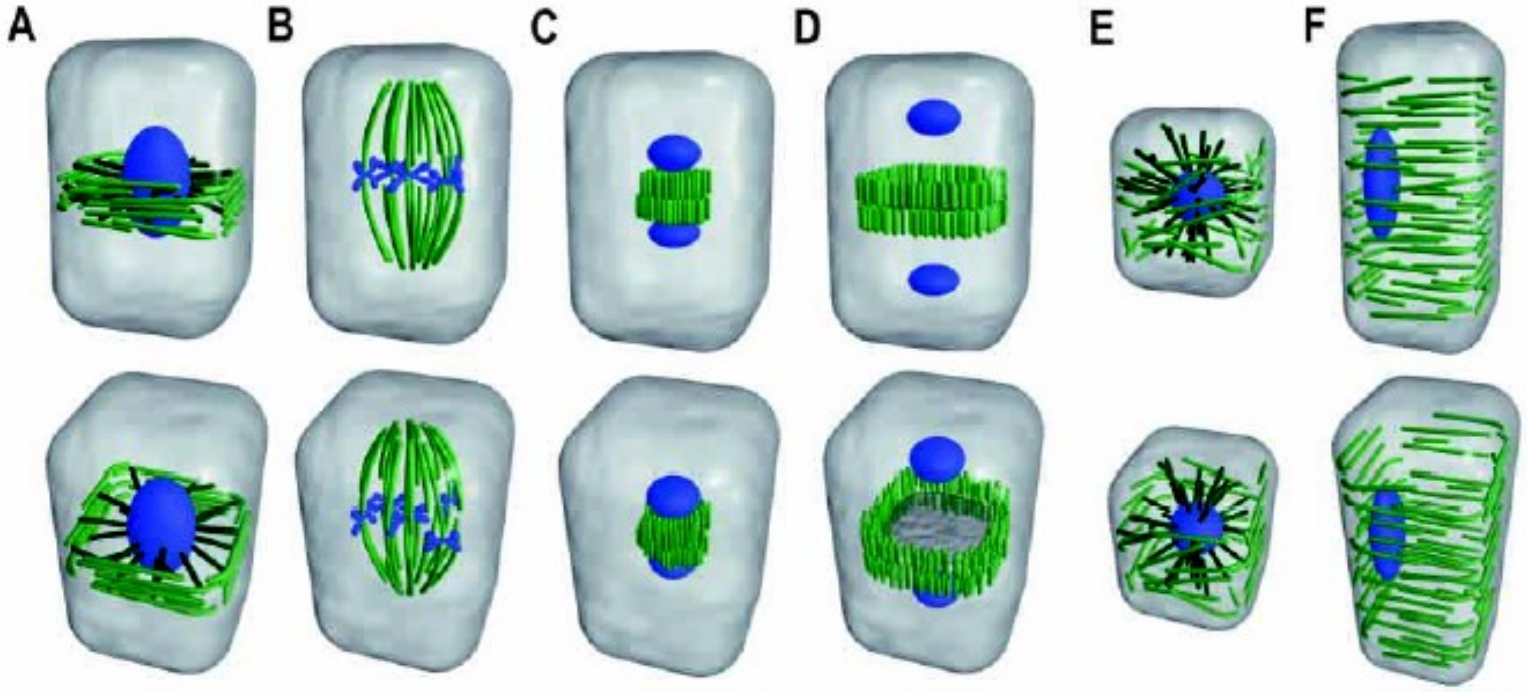


Fig. 1. These schematic illustrations, rendered in 3D at two aspects, show microtubule arrays through the plant cell cycle. (A) A preprophase band, linked to the nucleus by phragmosome microtubules, marks the future division site. (B) Metaphase spindle with a dispersed polar region. (C) In telophase, the phragmoplast forms as a concentrated cylinder of microtubules between daughter nuclei. (D) The cytokinetic phragmoplast expands centrifugally, leading the cell plate towards attachment sites previously established by the preprophase band. Microtubule plus ends meet at midplane. (E) Once cytokinesis is complete, microtubules extend from the nucleus toward the cell cortex and plasma membrane-associated microtubules appear. (F) Plant cells in interphase and those entering terminal differentiation often expand predominantly in one direction. During cell elongation, cortical microtubules are usually arranged in parallel arrays whose predominant orientation is at right angles to the axis of expansion.

Rho GTPázy řídí mj. „cytoskeletální" funkce

- asociace s organizací aktinu a jinými morfogenetickými ději (syntéza stěny u kvasinek); stresová odpověď
- podtřídy Rho, Rac,
  - Cdc42 u živočichů
  - Rho a Cdc42 u kvasinek
  - Rop u rostlin
- fyziologické funkce: pučení kvasinek, růst pylových láček, odpověď na ABA

Osudy aktinu v BC kvasinky

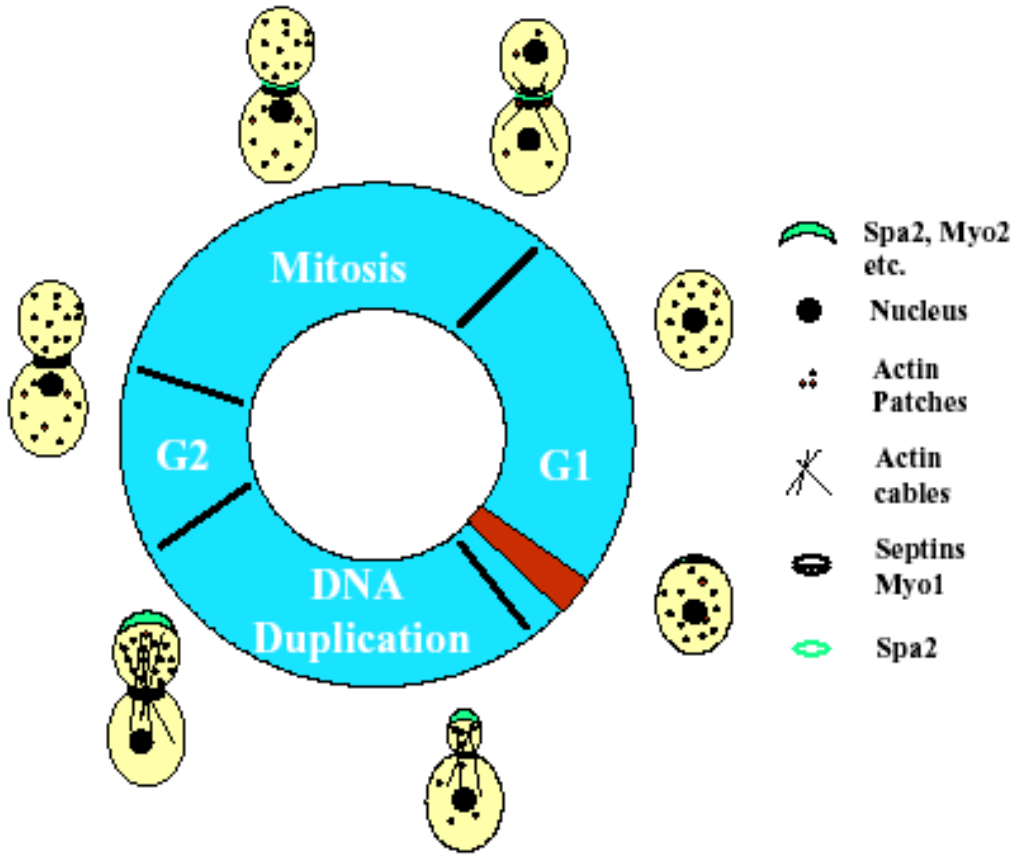
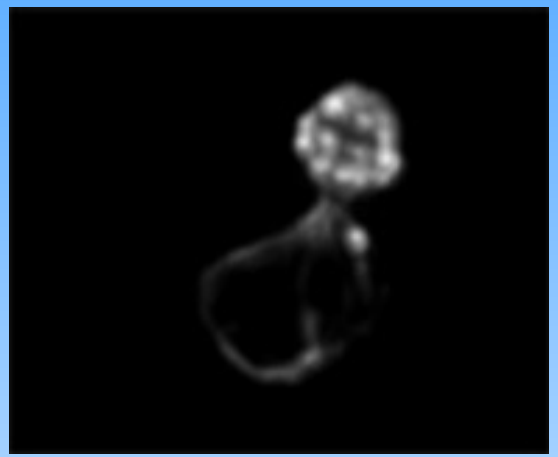


Fig. 3. Rearrangements of F-actin and bud assembly components through the cell cycle in *Saccharomyces cerevisiae*.

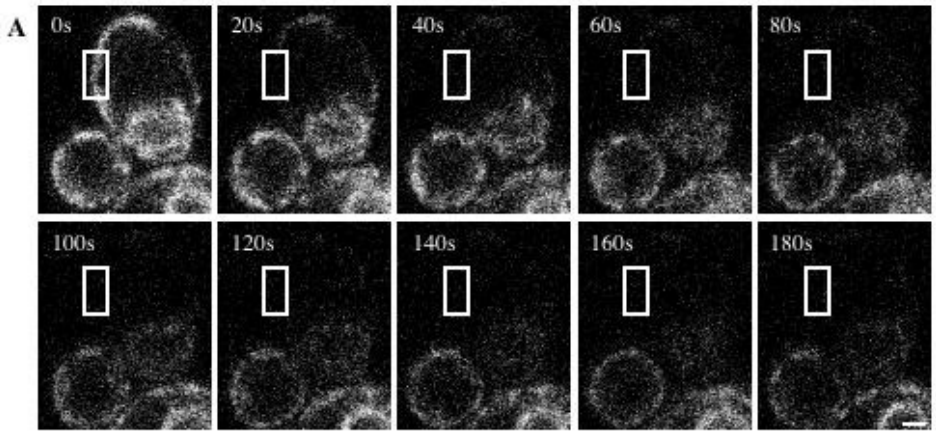


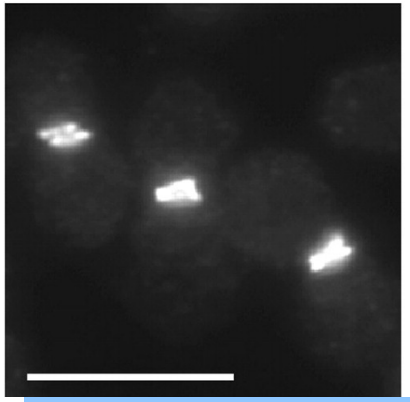
(L. Pon lab)

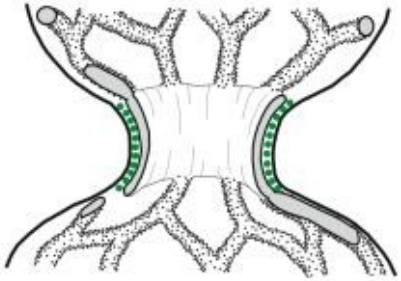
(Surana and Balasubramanian 2000)

Septiny – „neck filaments"

Figure 1



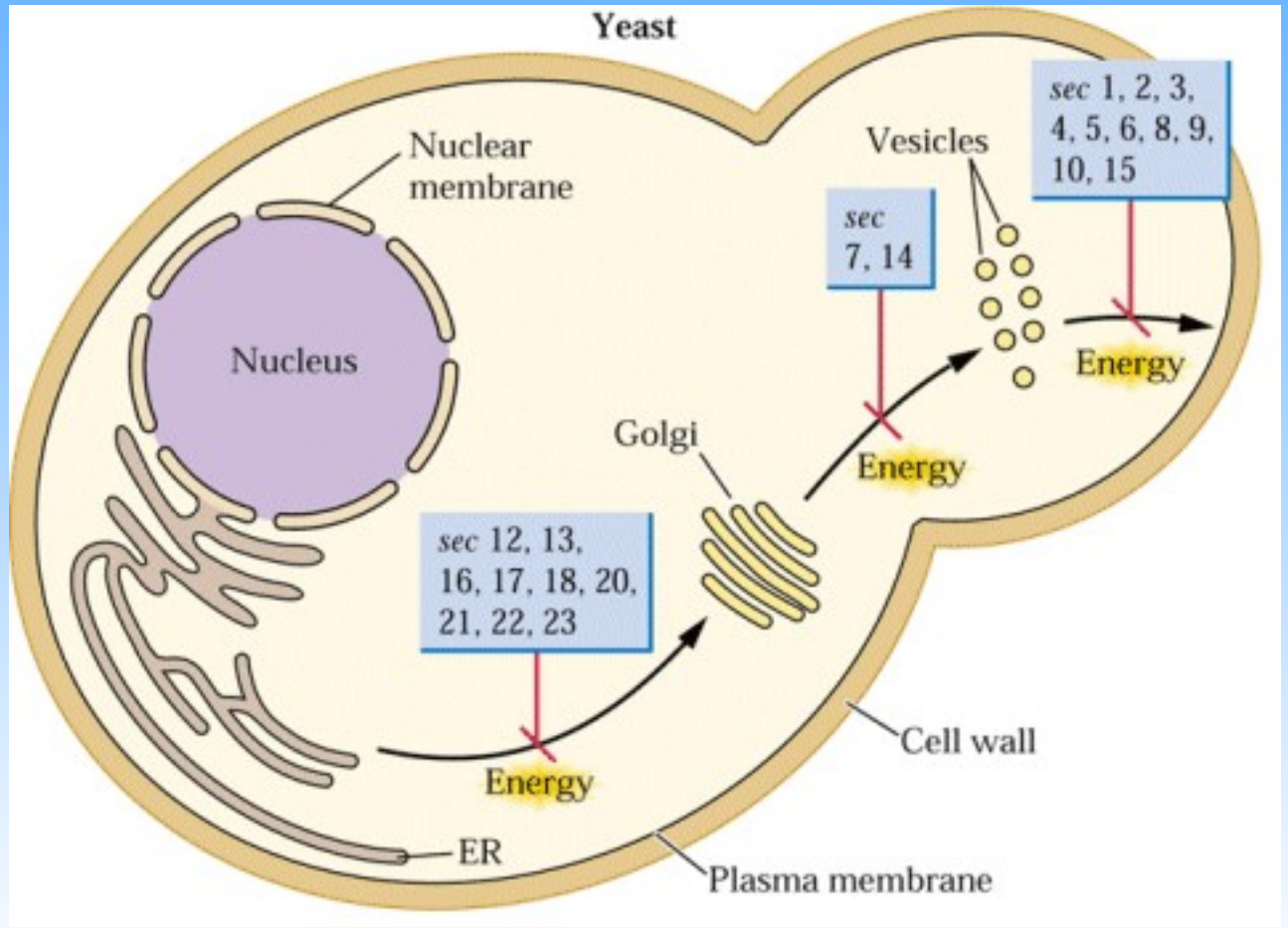




difusní bariéra, hranice pupene ...

A. Dynamics of the translocon subunit Sec61 throughout the yeast ER during metaphase. Diffusion from the mother cortex to the bud cortex is slow. FLIP (fluorescence loss in photobleaching) was performed on a metaphase cell expressing Sec61-GFP. Pictures were taken every 20s.. The bleaching region is depicted by an empty rectangle. **B.** Model of rough and smooth ER in the bud-neck of yeast cells. Sections through the septin filaments are shown in green.

Sekreční dráha a exocytosa – provoz váčků



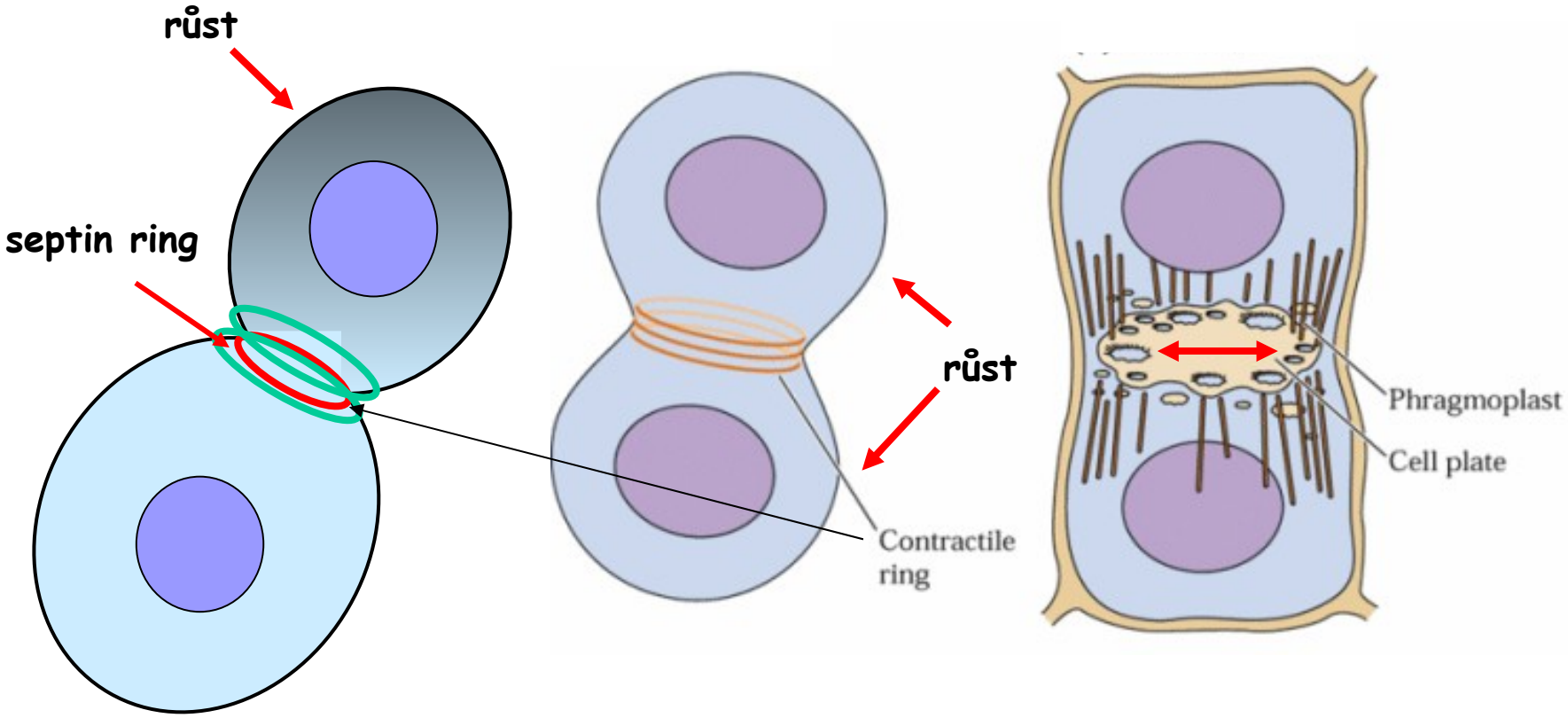
Sec mutace (P. Novick)

Cytokineze

kvasinka

živočišná buňka

rostlinná buňka



Je rozdíl mezi rostlinami a živočichy opravdu tak zásadní?

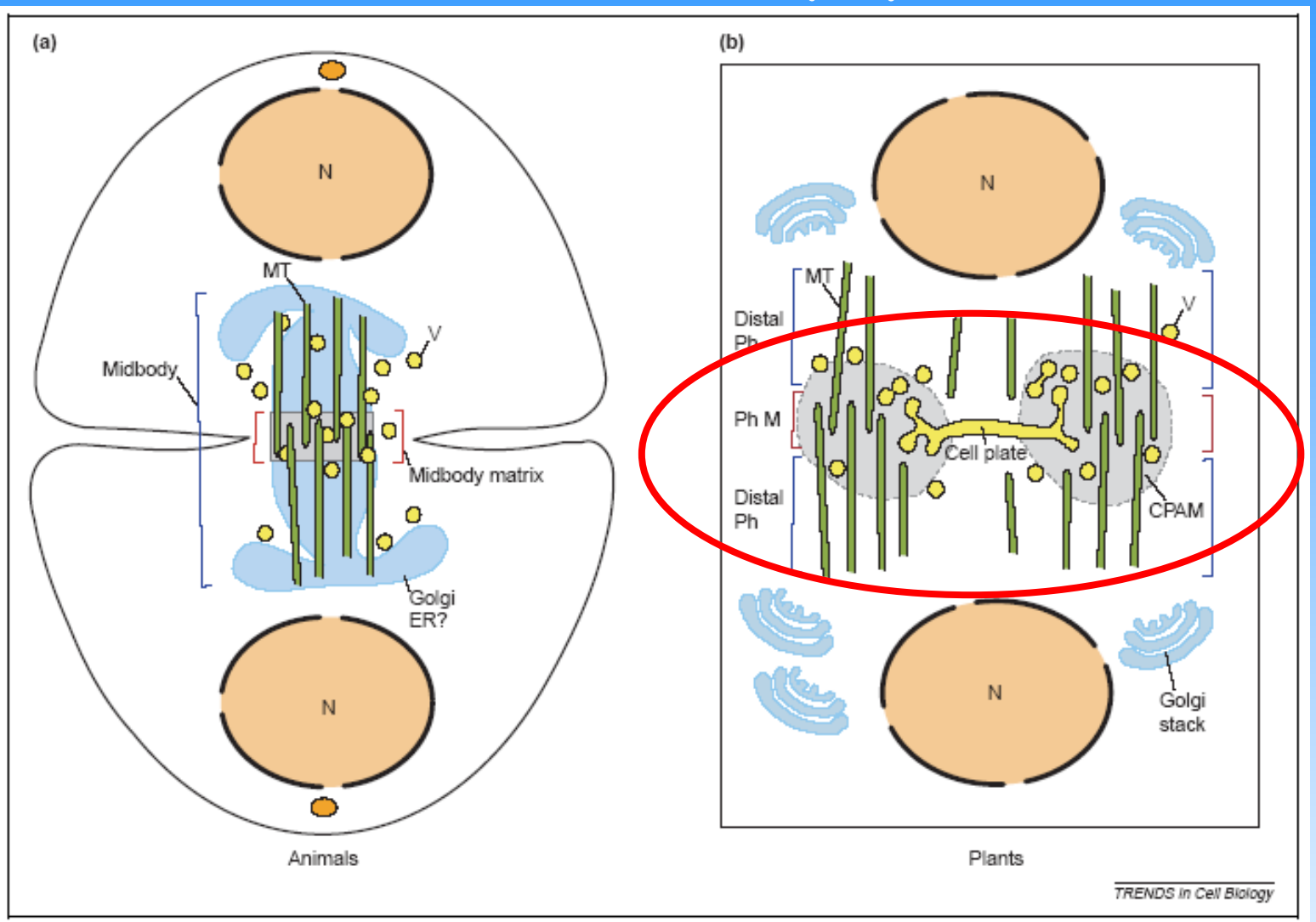
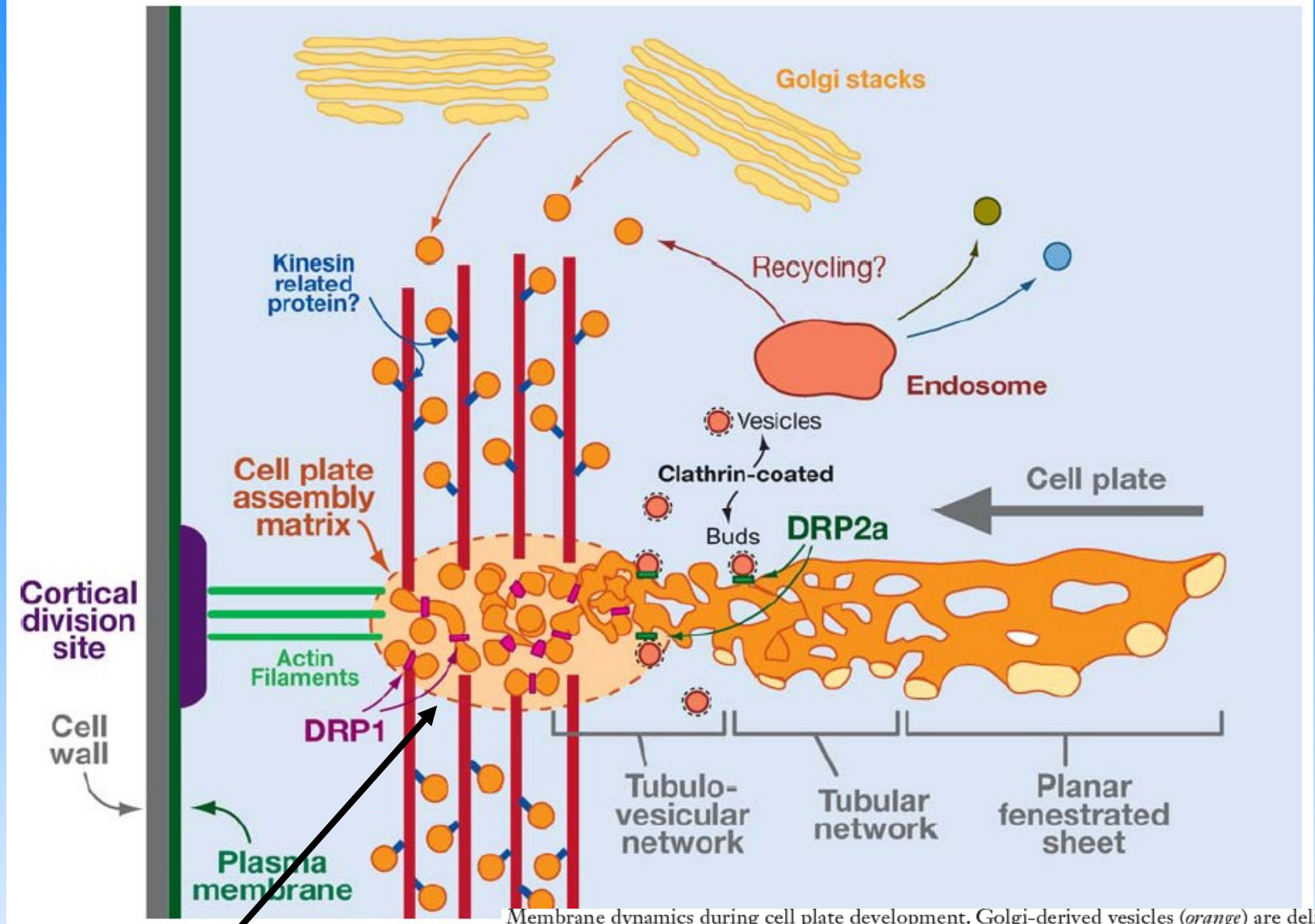


Figure 1. Overview of animal and plant cytokinesis. (a) Cytokinesis in animal cells. The spindle midzone/midbody forms when microtubules (MTs) from opposite poles overlap. It consists of the overlapping microtubules as well as associated proteins that bundle these MTs and other proteins that together form a dense protein matrix. This matrix excludes antibodies against MTs, giving a stereotypical region devoid of staining. As the furrow ingresses, the midzone is swept into one larger structure called the midbody. The Golgi and endoplasmic reticulum (ER) membranes are also found in the midbody during telophase to cytokinesis. It is proposed that vesicles (V) traffic along the midbody microtubules toward the ingressing furrow. (b) Cytokinesis in somatic plant cells. The forming cell plate is assisted by the phragmoplast at the future site of the new cell wall. Two topographic regions can be distinguished in the phragmoplast: the phragmoplast midline (Ph M), where the opposing set of microtubules interdigitate, and the distal phragmoplast (distal Ph), at both sides of the phragmoplast midline. A filamentous cell-plate assembly matrix (CPAM) accumulates at the phragmoplast midline. Key: MT, microtubule (green); N, nucleus (tan); V, vesicle (yellow); Golgi (pale blue); midbody matrix (gray box); CPAM (gray circles).



Membrane dynamics during cell plate development. Golgi-derived vesicles (*orange*) are delivered along phragmoplast microtubules (*red*), by a putative kinesin-related protein (*blue*), to the cell plate assembly matrix. Vesicle fusion generates fusion tubes and tubulo-vesicular networks as a result of the constricting activity of class I dynamin-related proteins (DRP1) (*magenta*). The tubulo-vesicular network is successively transformed into a tubular network and a planar fenestrated sheet. Lateral expansion of the cell plate (*large arrow*) toward the cortical division site is guided by actin filaments. Endocytosis from the tubulo-vesicular network and tubular network removes excess membrane, which is delivered to endosomes via clathrin-coated buds and vesicles. Dynamin-related protein 2a (DRP2a; *green*) is involved in the formation of clathrin-coated vesicles. The endosome sorts proteins for trafficking to various destinations (*blue*, *green*, *orange*), possibly including recycling to the margin of the cell plate.

fúze váčků

Vývoj fragmoplastu a CP

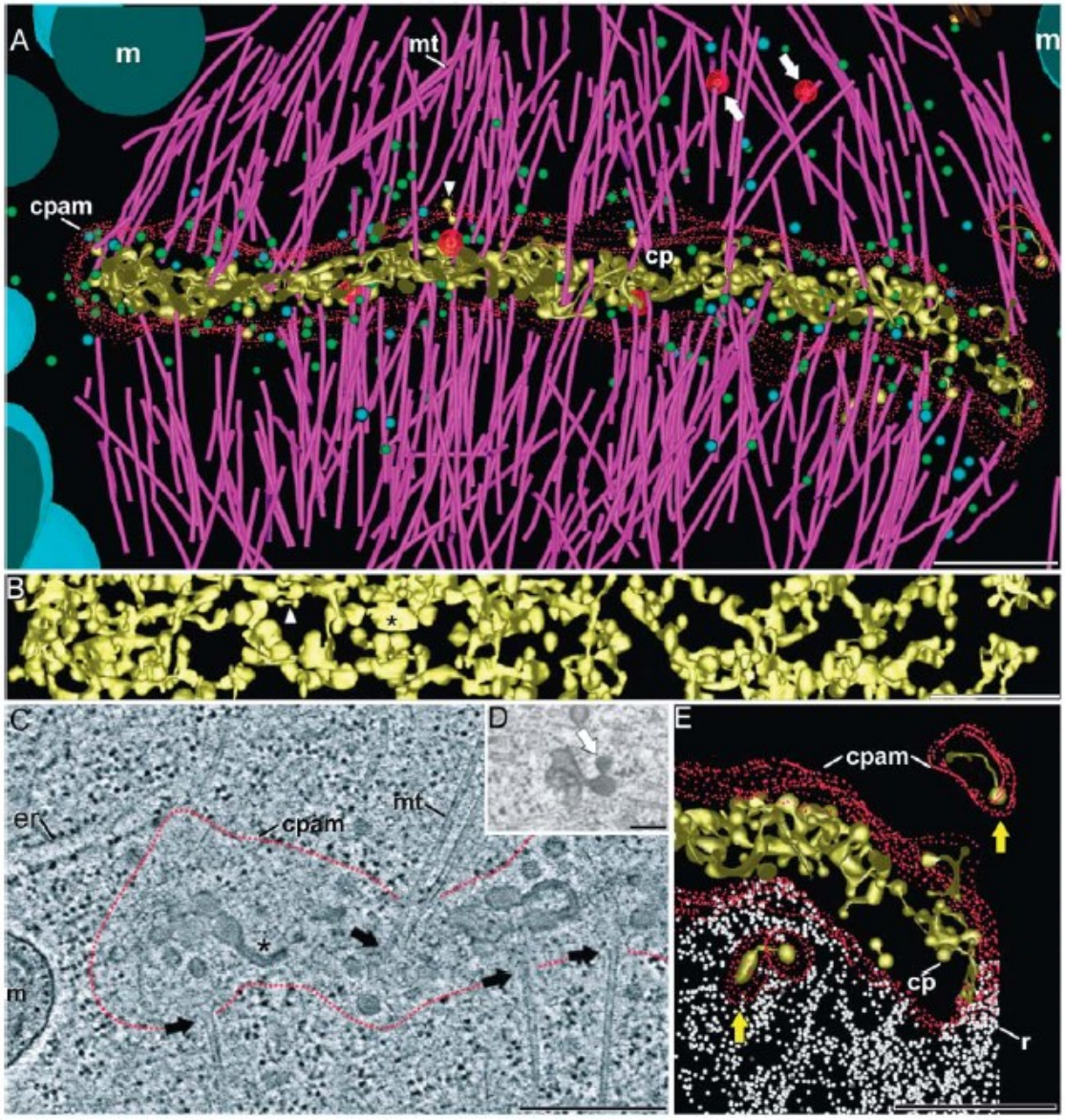


Figure 7. Solid Phragmoplast with CPAM and TVN Stage Cell Plate.

electronmicroscopic tomography
Hepler laboratory

Homotypická fúze váčků: SNARE et al., Exocyst?

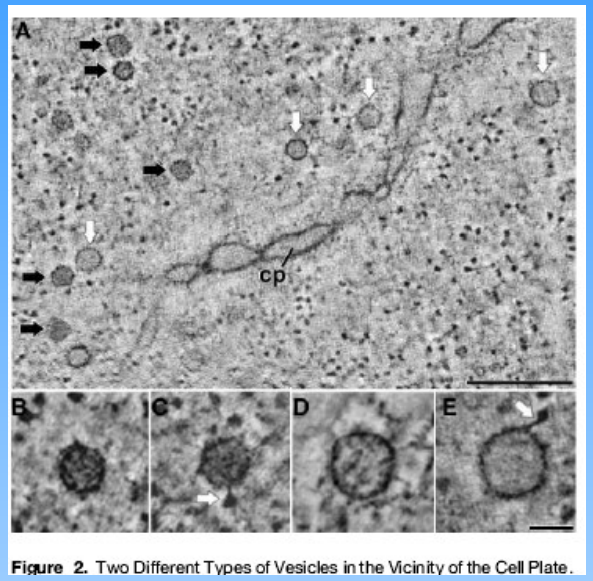
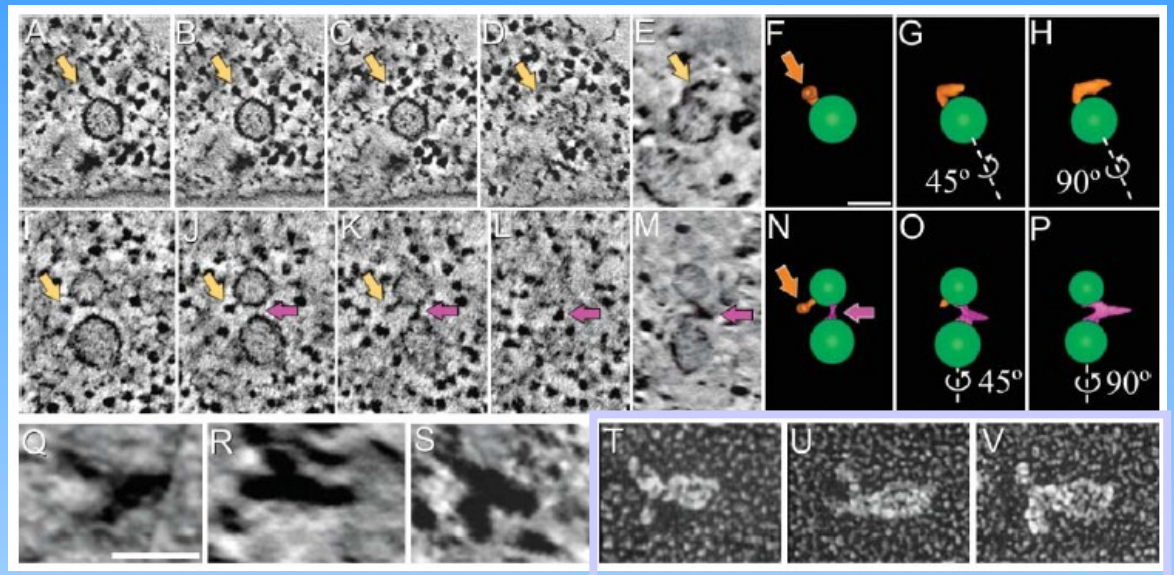


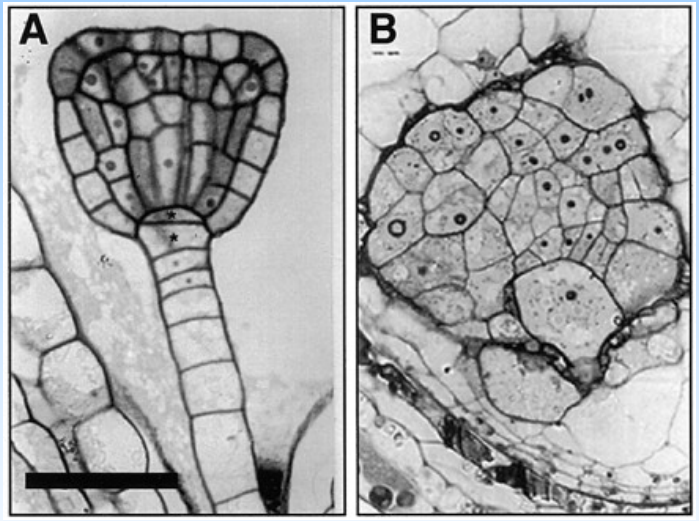
Figure 2. Two Different Types of Vesicles in the Vicinity of the Cell Plate.

(Segui-Simarro et al. 2004)



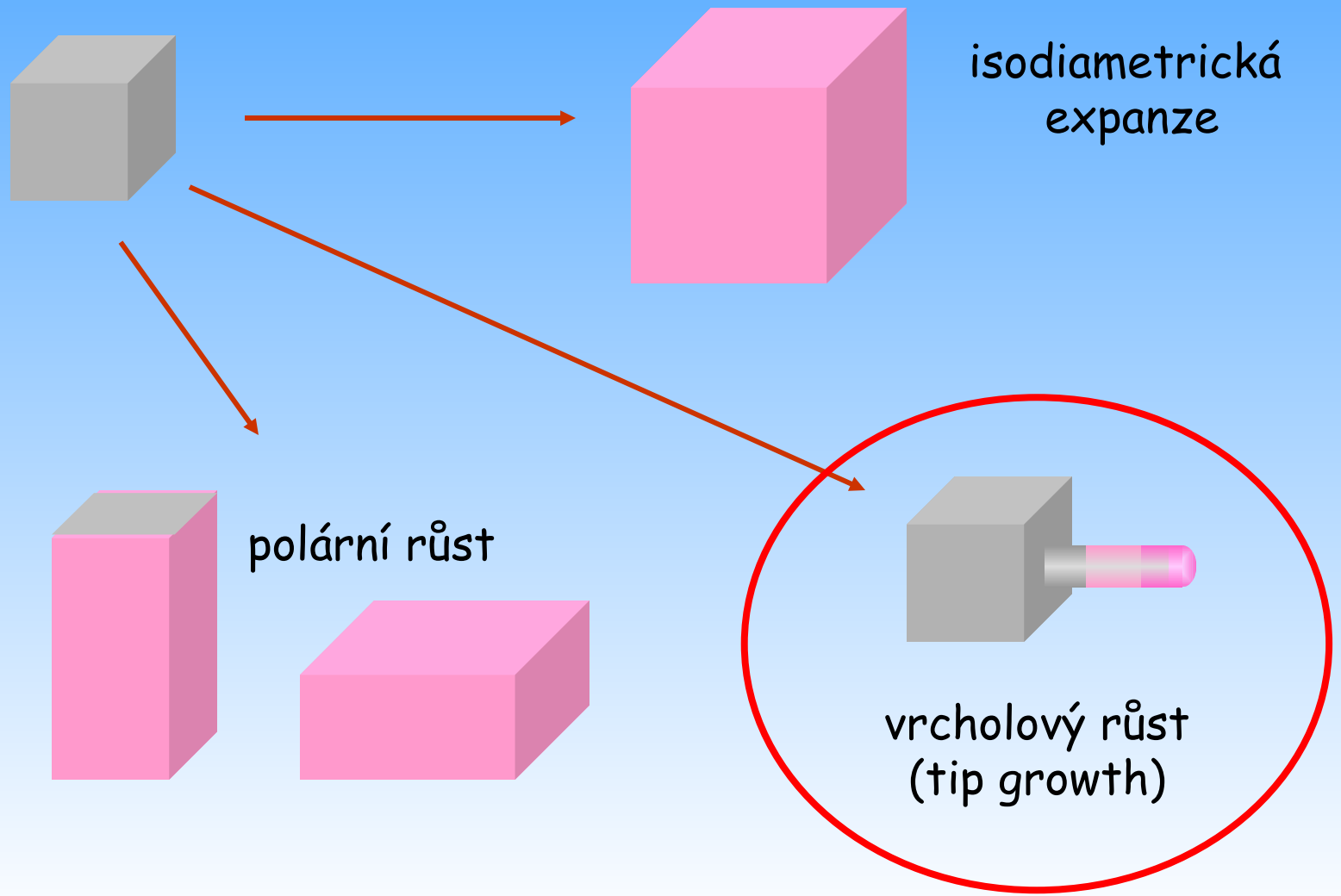
(exocyst)

- KNOLLE : syntaxin (v-SNARE)
- přísluš. t-SNARE asi redundantní (SNAP33, SNAP29, SNAP30)
- KEULE : Sec1-related, interakce s KNOLLE
- KNOLLE a syntaxin SYP31: interakce s CDC48

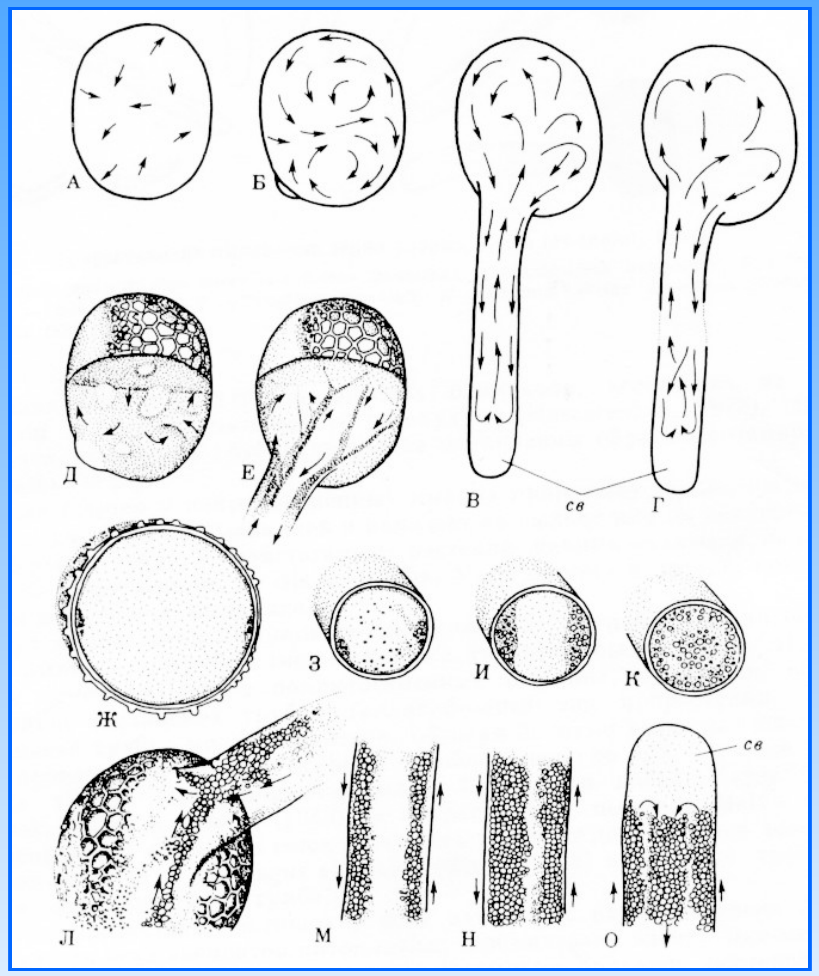


wt keule

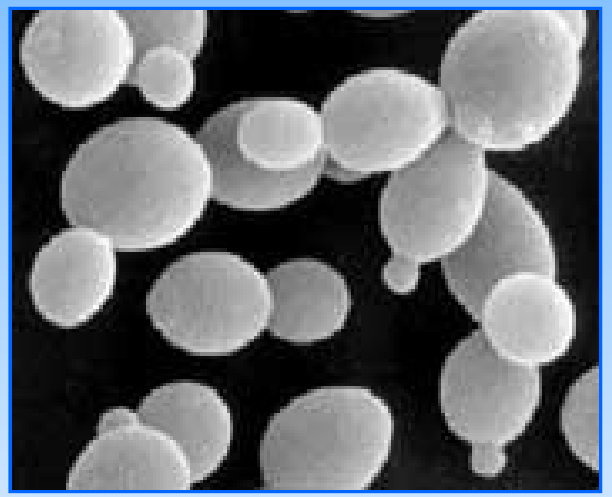
Typy buněčného růstu



Příklady vrcholového růstu:

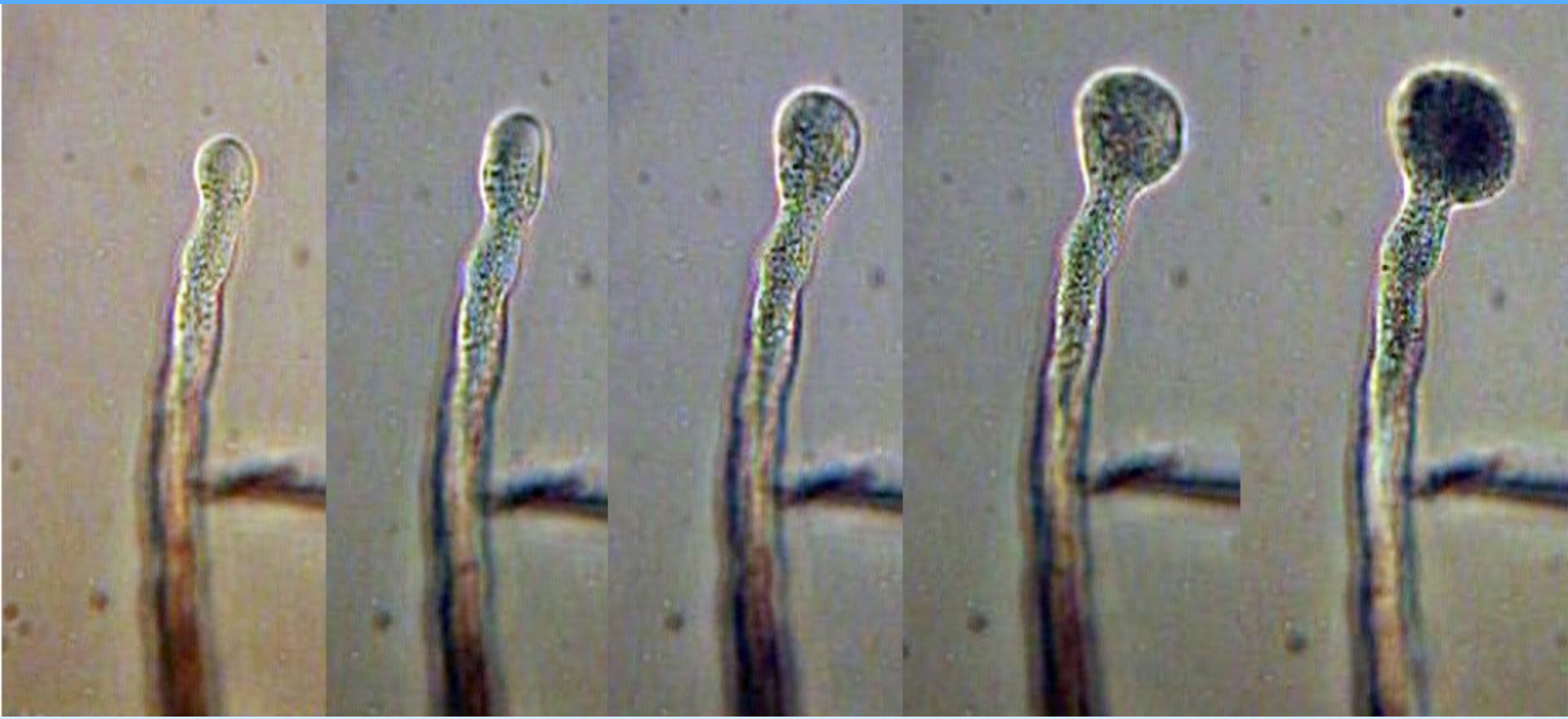


pylová láčka



(časný) pupen kvasinky

... a jsou důležité



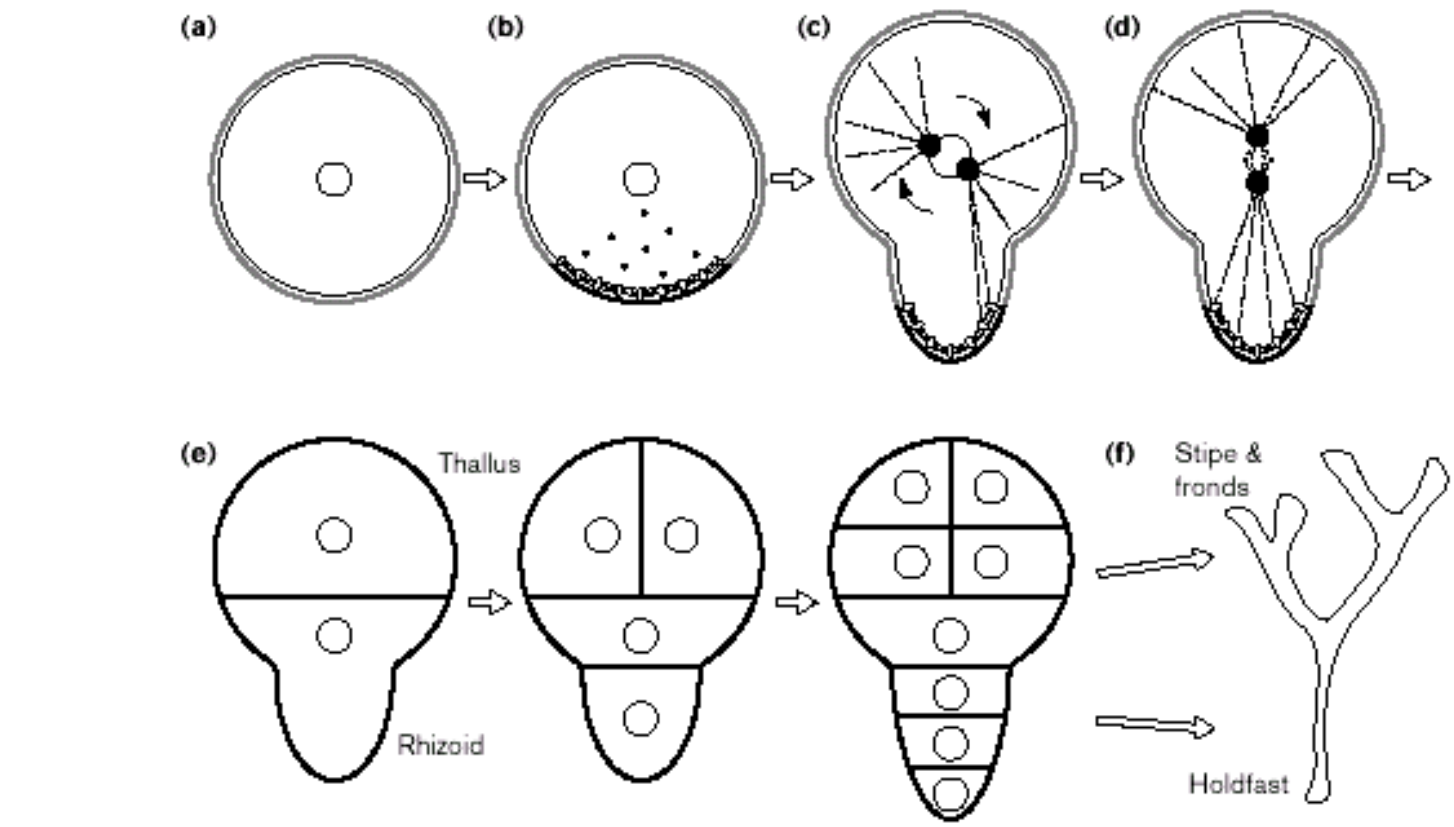
pylová láčka lilie po injekci inhibitoru GDP-b-S

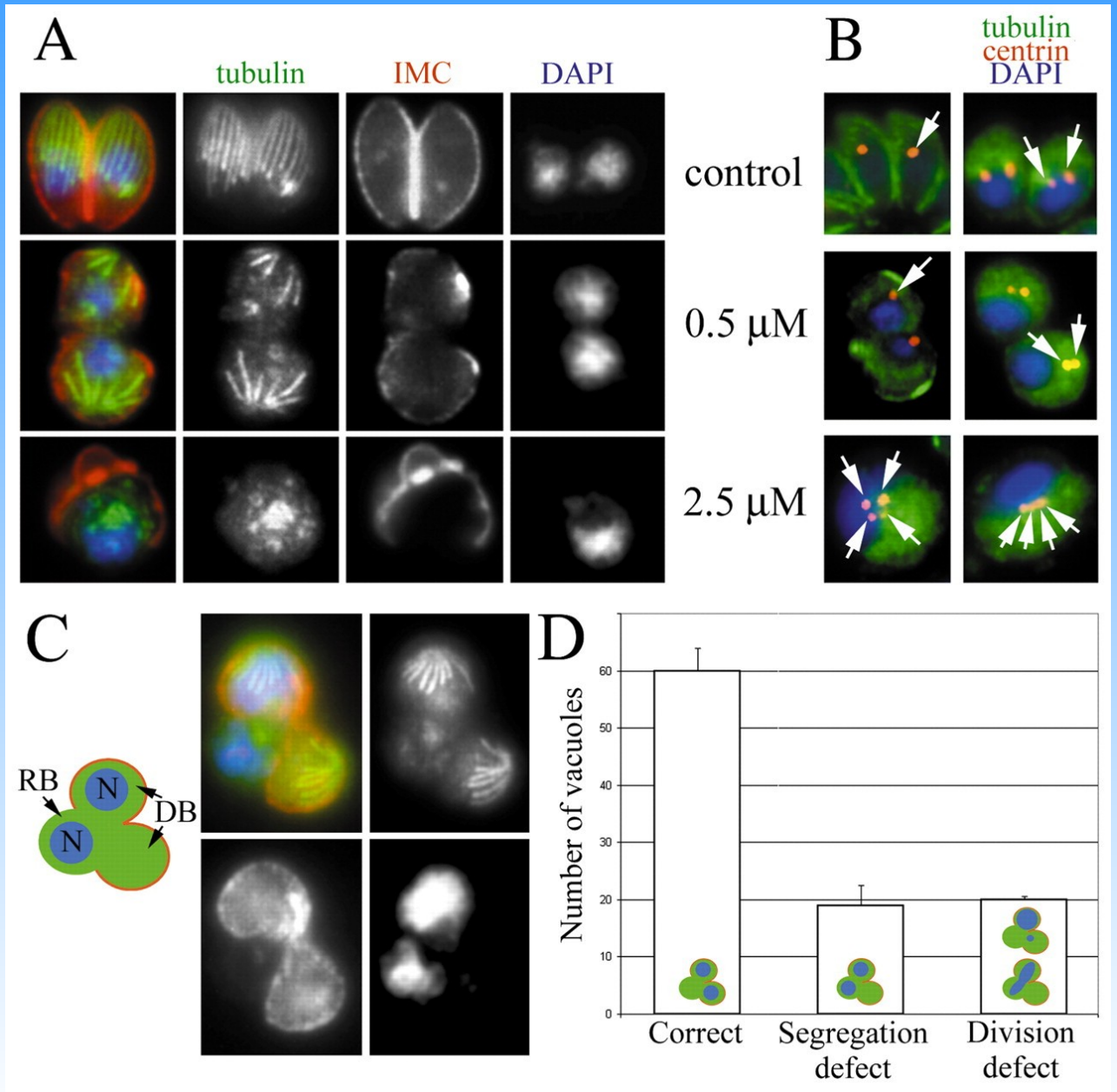
(M. Eliáš)

Mechanismy orientovaného růstu buněk

- depozice nového materiálu buněčných povrchů (membrána, stěna) - exocytóza, pohyb váčků, sekrece, …
- orientovaná povaha těchto procesů: cytoskelet, molekulární motory, …
- obojí těsně spřaženo: malé GTPázy

Polarizace - dělení zygoty (*Fucus*)




A
tubulin
IMC
DAPI
control
0.5 µM
2.5 µM
B
tubulin
centrin
DAPI
C
RB
N
N
DB
D
Number of vacuoles
0
10
20
30
40
50
60
Correct
Segregation defect
Division defect