

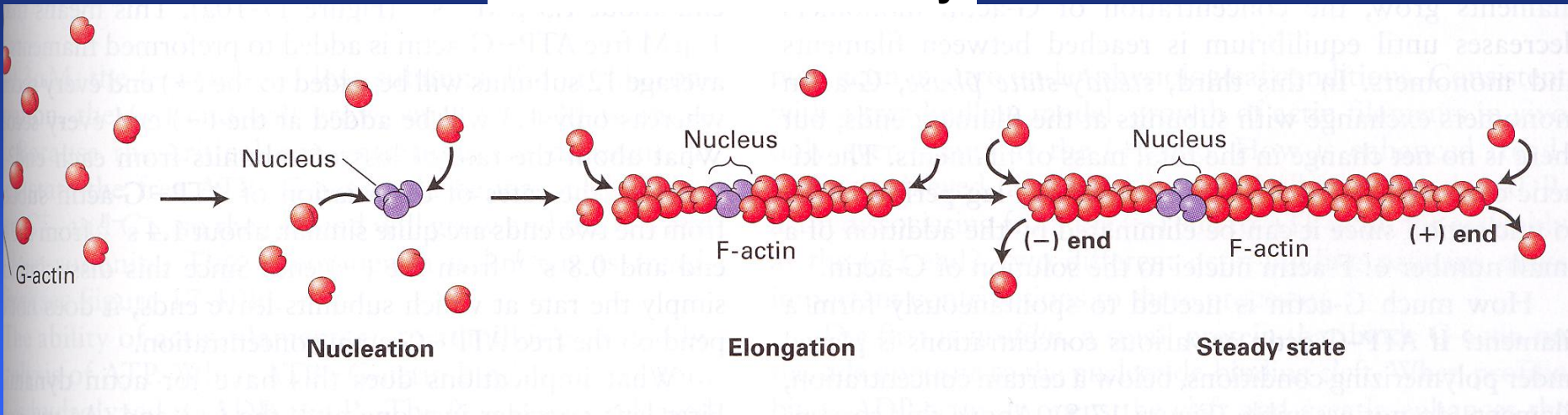
Cytológia

Milan Bobák, Jozef Šamaj

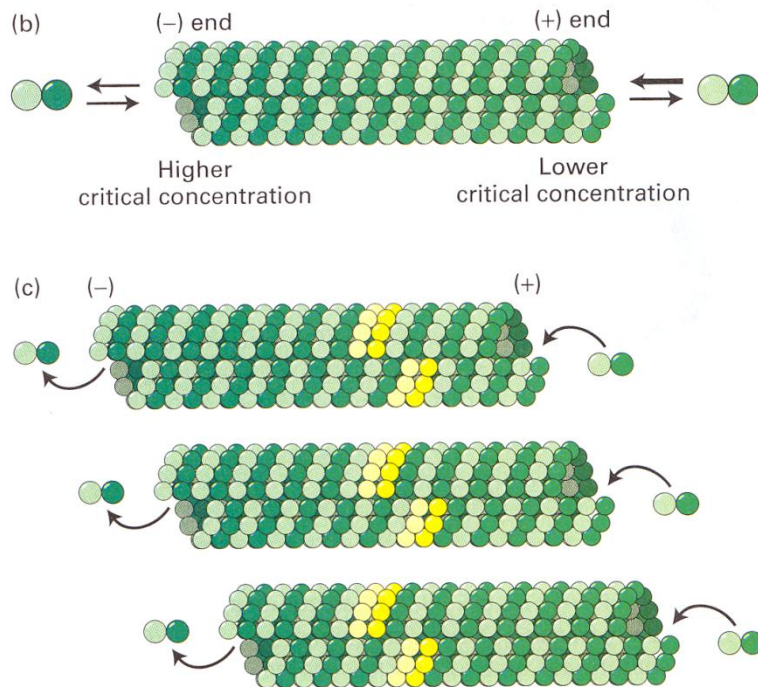
UK v BA

Cytoskelet a pohyb

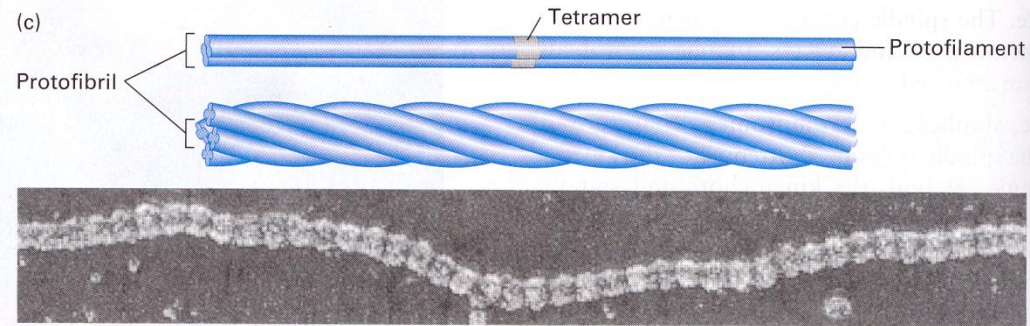
Aktínové mikrofilamenty



Tubulínové mikrotubuly



Intermediárne filamenty



Microfilaments

Actin

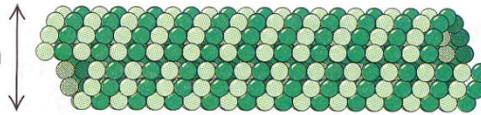
7 – 9 nm



Microtubules

$\alpha\beta$ -Tubulin dimer

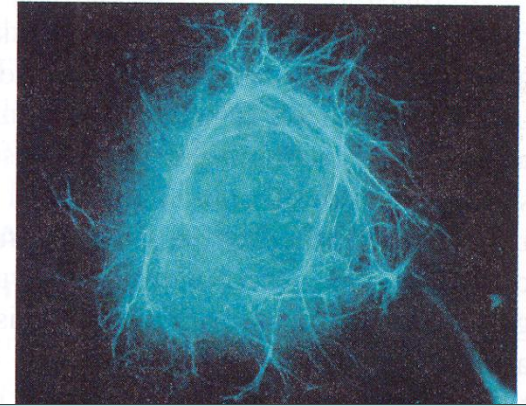
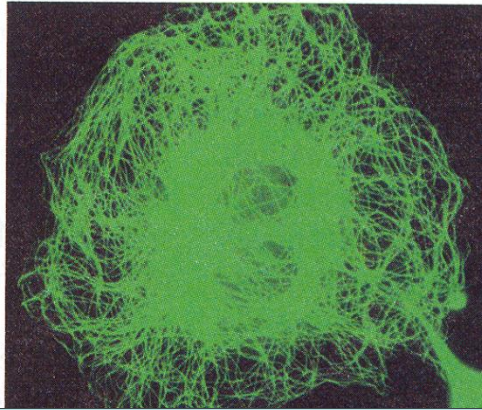
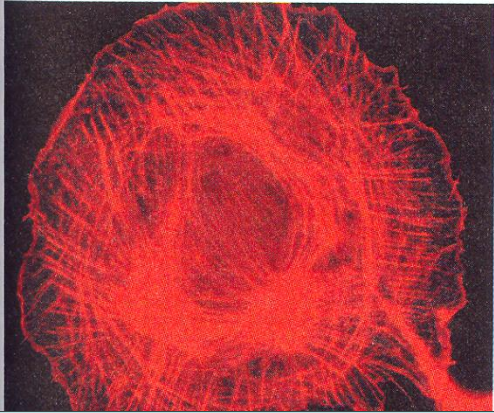
25 nm



Intermediate filaments

Various

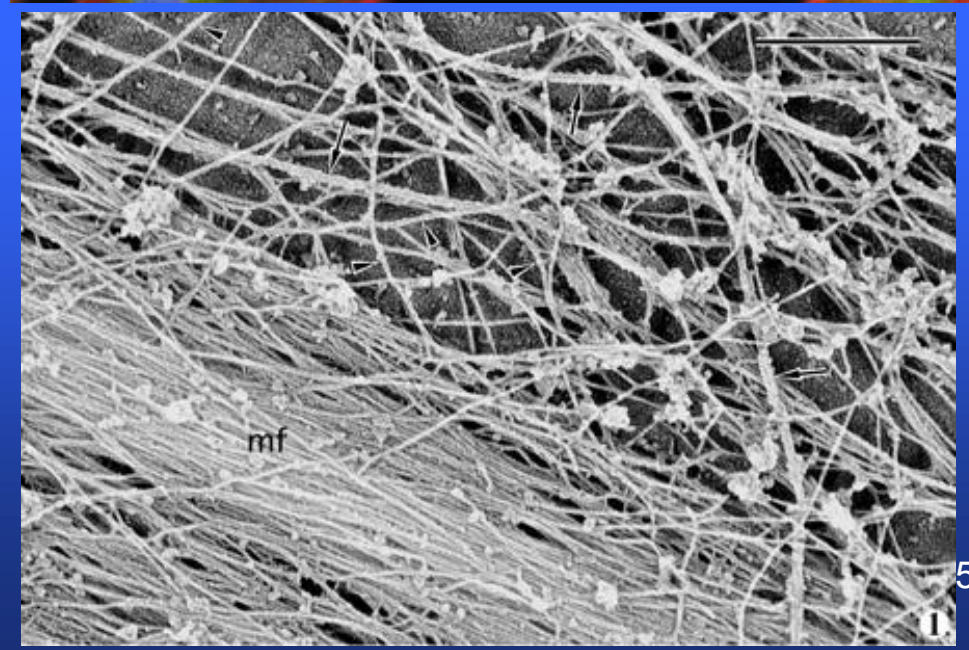
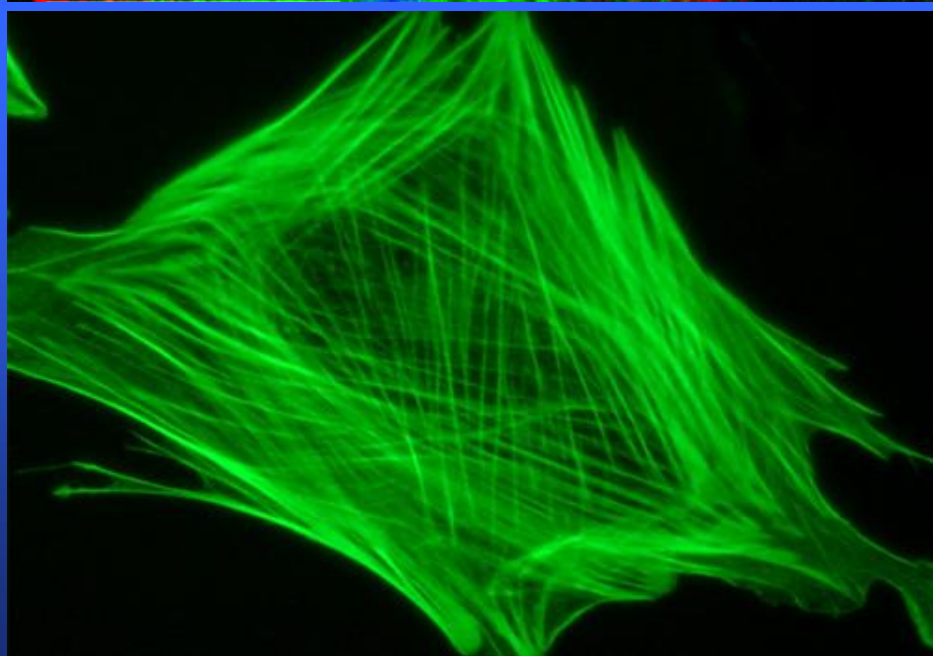
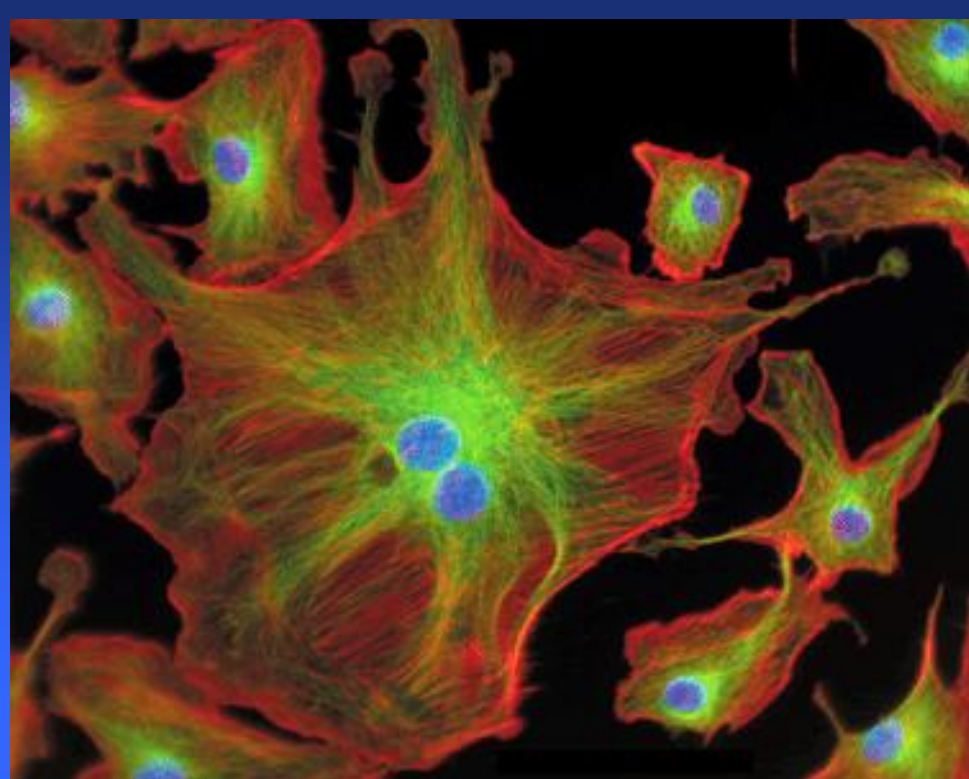
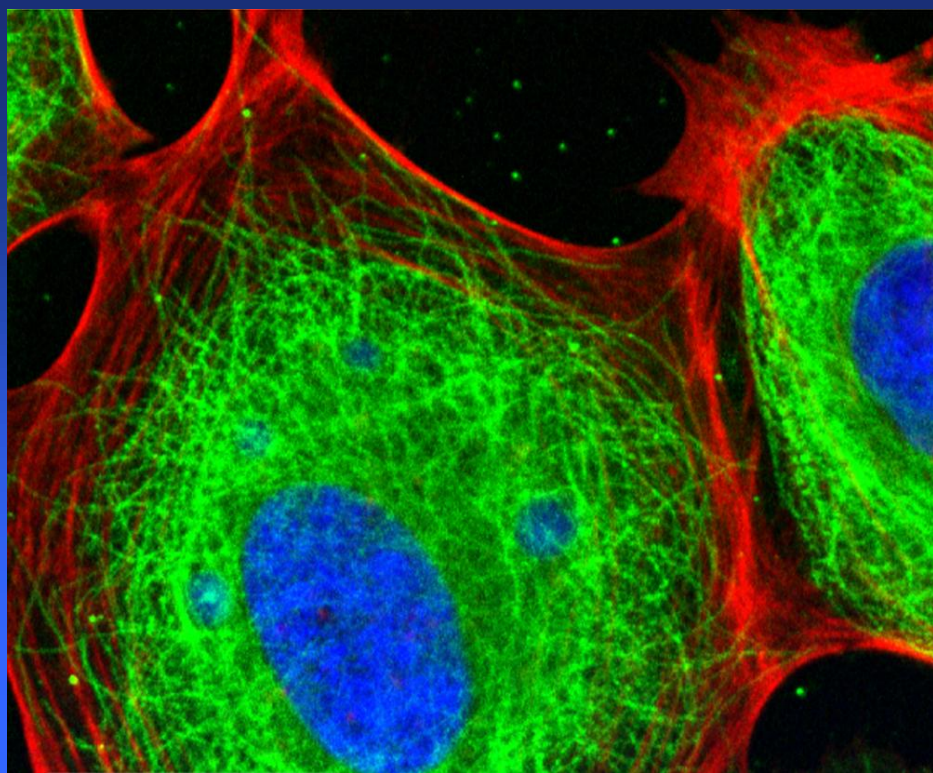
10 nm



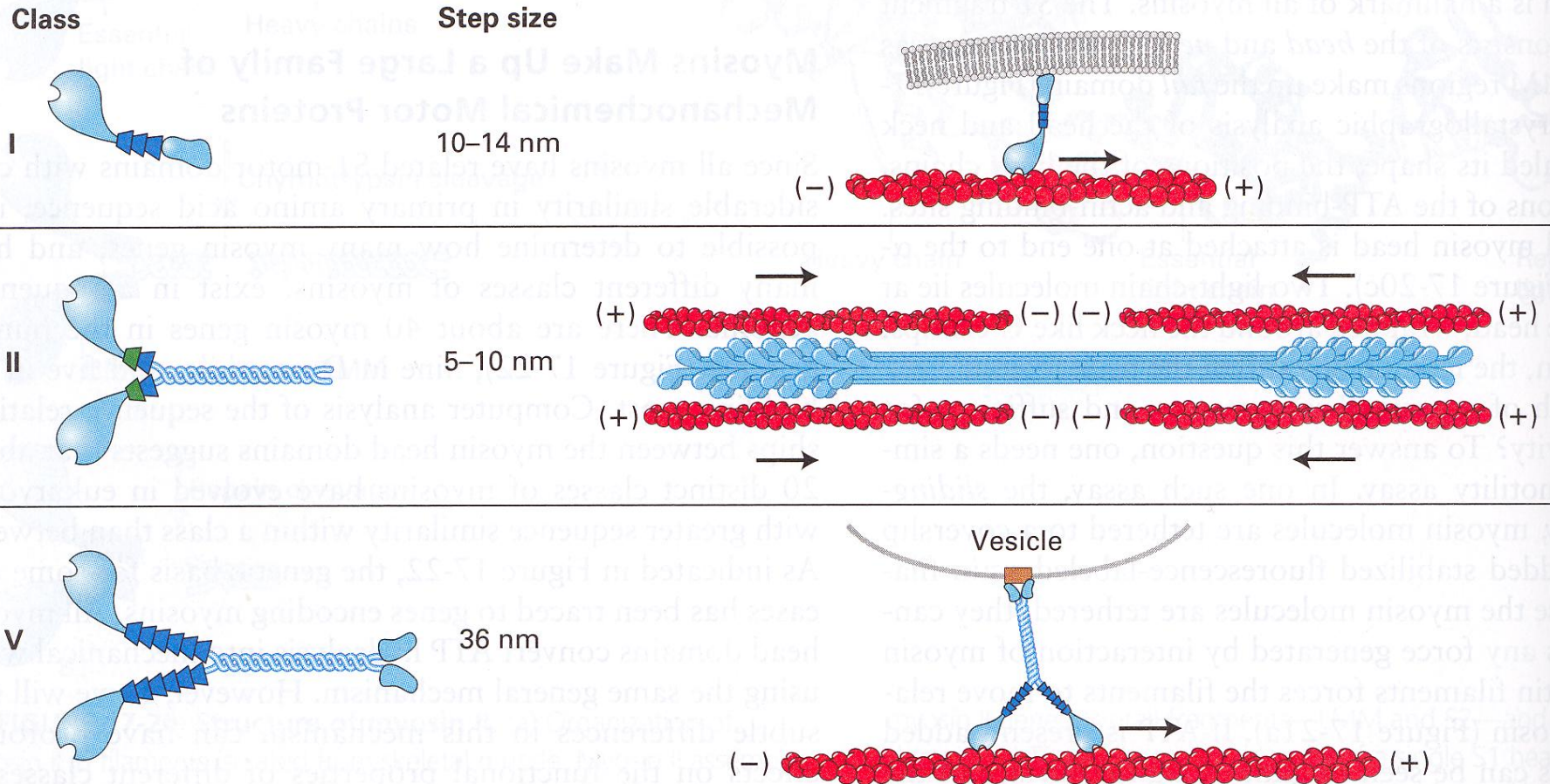
Mikrofilamenty – G-aktín (celé filamenty - F-aktín), polarizované (+ a - koniec), viažu ATP/ADP, dynamické, funkcia – kontrakcia, bunková sieť, trate pre myozíny.

Mikrotubuly – $\alpha\beta$ -tubulín (protofilamenty), polarizované (+ a - koniec), viažu GTP/GDP, dynamické, funkcia – organizácia, diaľkový transport organel, trate pre kinezíny a dyneíny. MT predprofázneho pásu, MT kortikálne, MT deliaceho aparátu.

Intermediárne filamenty – biochemicky heterogénne, veľká pevnosť v ťahu, nemajú polaritu, neviažu nukleotidy, stabilné, len živočíchy, funkcia – integrita buniek a tkanív, bez motorov.

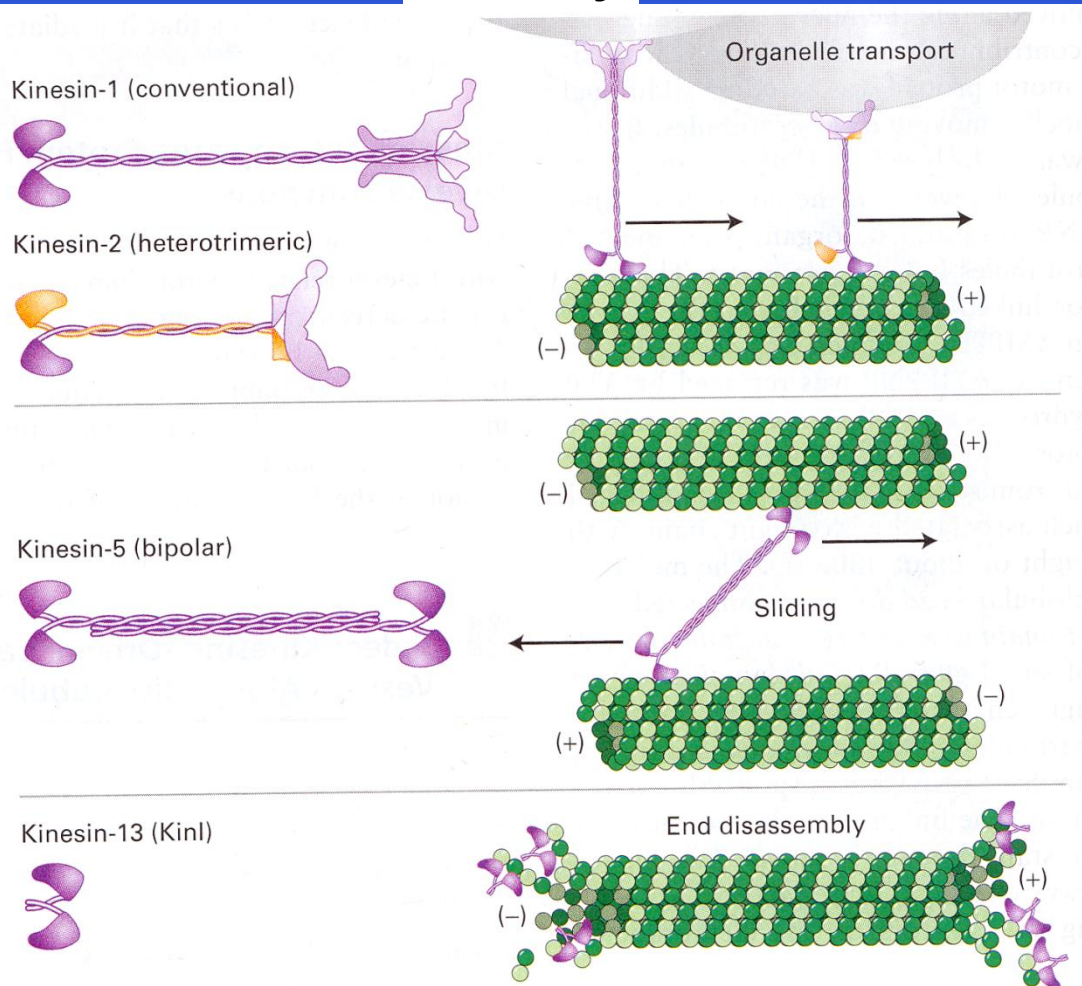


Myozíny - motory zabezpečujúce endocytózu, kontrakciu, transport organel (max. 7 $\mu\text{m/s}$) pozdĺž aktínových mikrofilamentov (spotrebovávajú ATP).

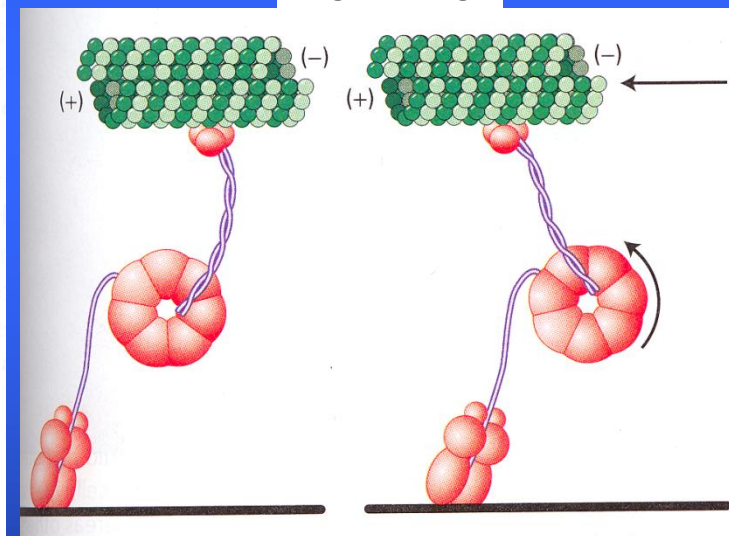


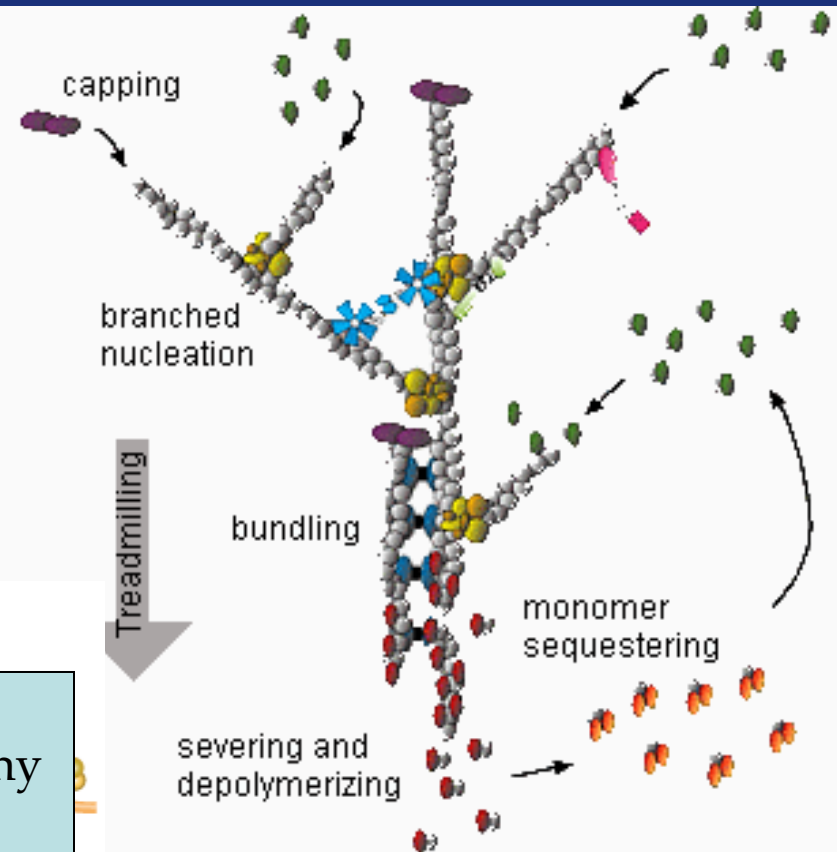
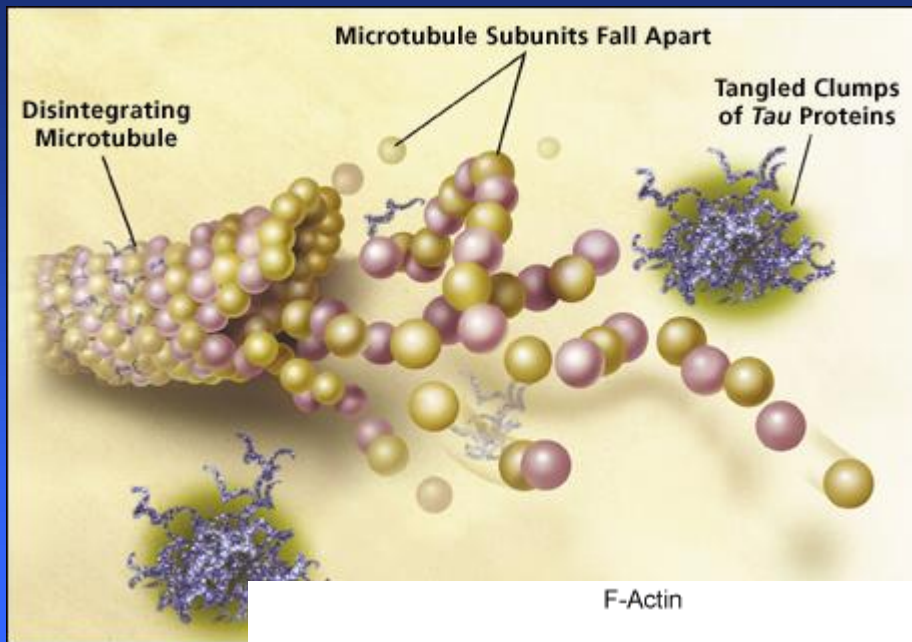
Kinezíny a dyneíny - motory zabezpečujúce transport (max. 3 $\mu\text{m/s}$) pozdĺž tubulínových mikrotubúl, vzájomný pohyb mikrotubúl, depolymerizáciu mikrotubúl (spotrebovávajú ATP).

Kinezíny



Dyneíny





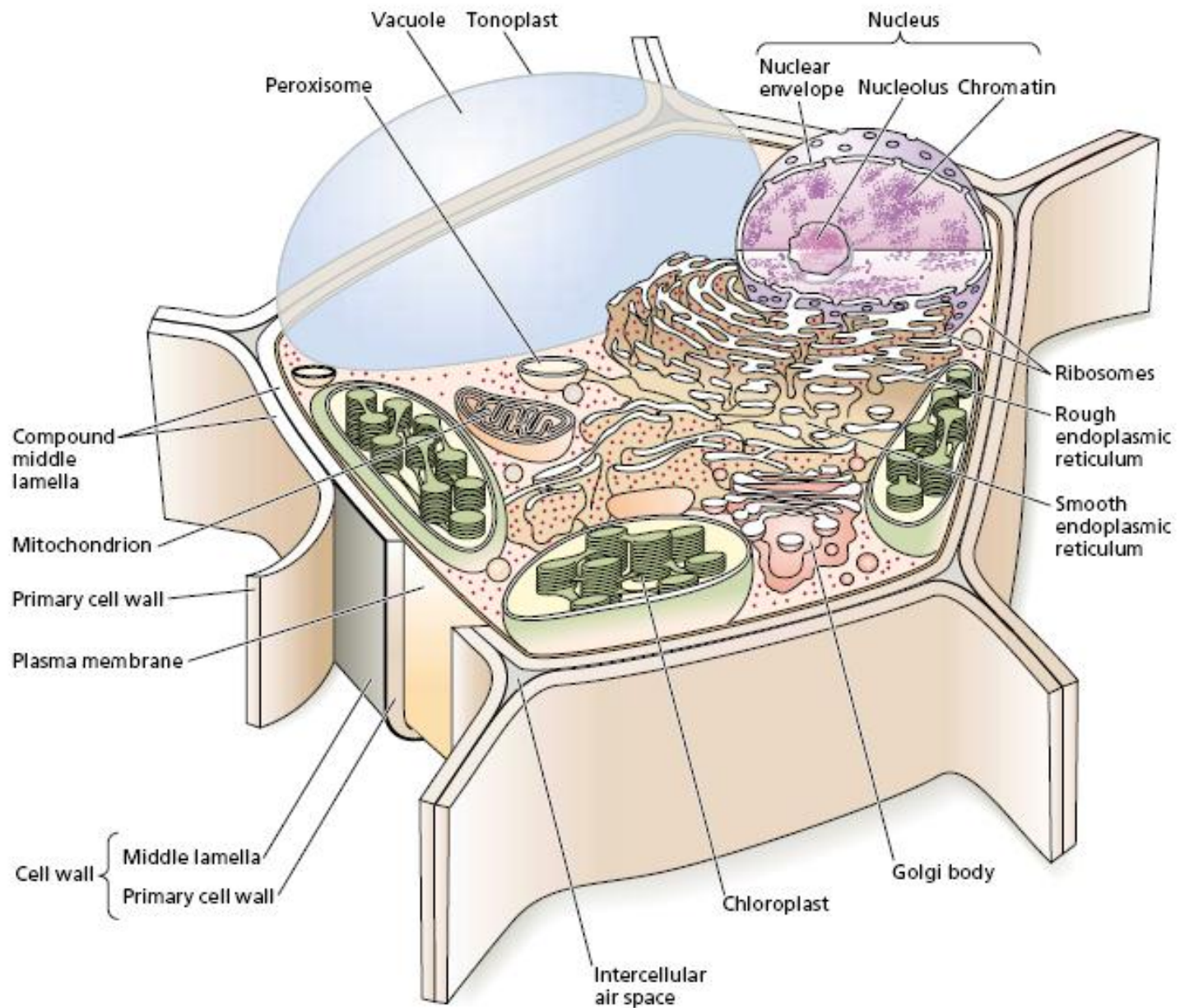
Dynamický cytoskelet zohráva kľúčové úlohy v bunkovej signalizácii, vezikulárnom transporte, pohybe a integrite organel, diferenciácii buniek a pohybe buniek.

Dynamika cytoskeletu je v bunkách významne regulovaná na mnohých úrovniach (transkripcia génov, posttranslačné modifikácie proteínov, atď.).

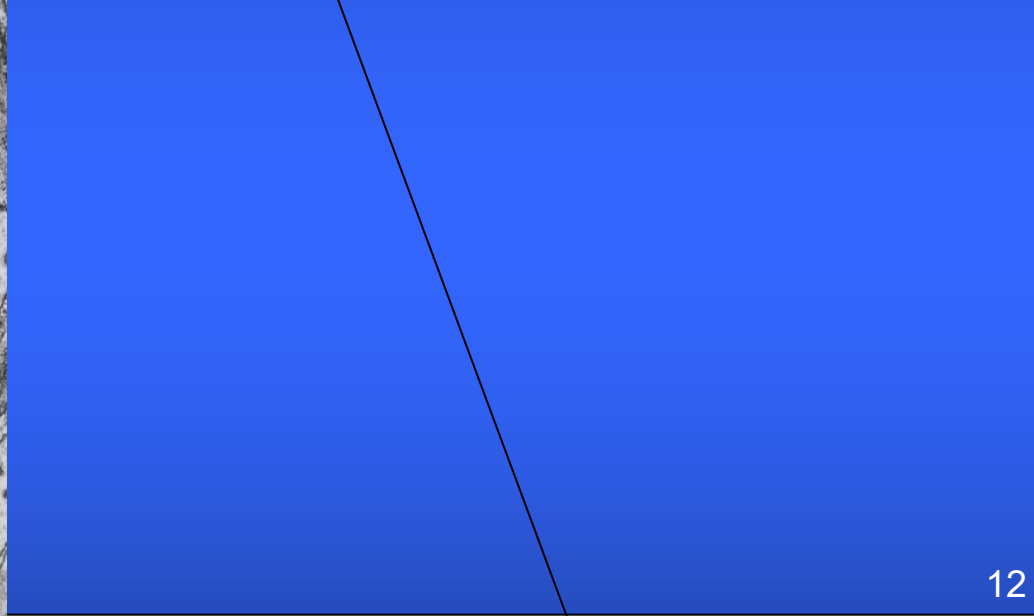
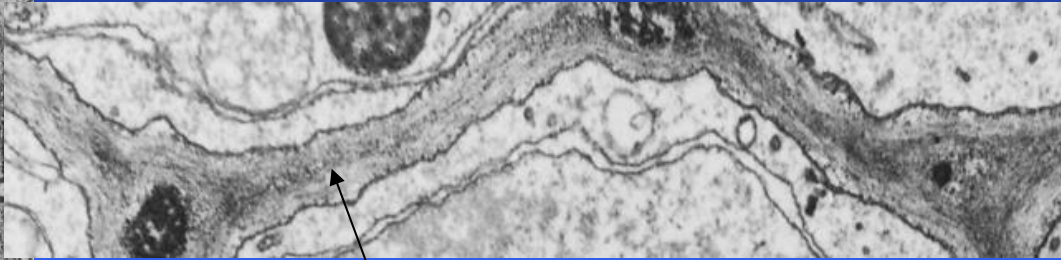
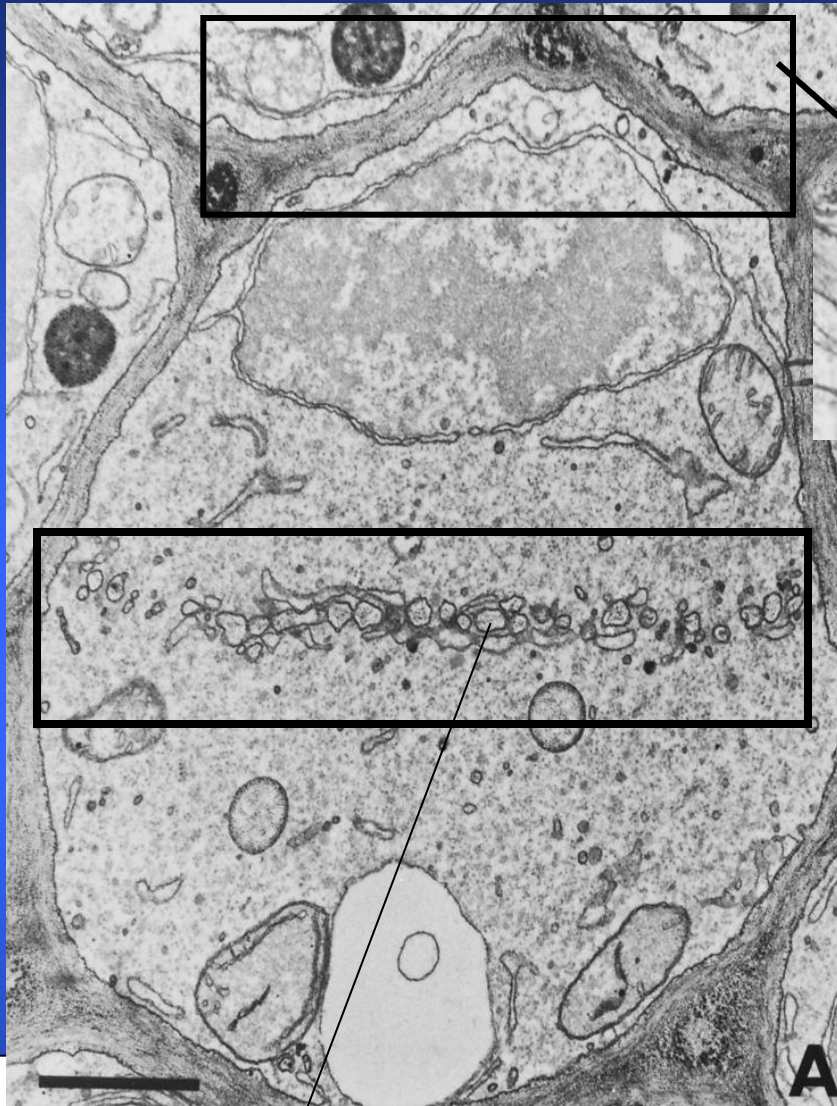
<https://www.youtube.com/watch?v=gDb8ENGOrvw>

Bunková stena

Bunková stena (*cell wall*)

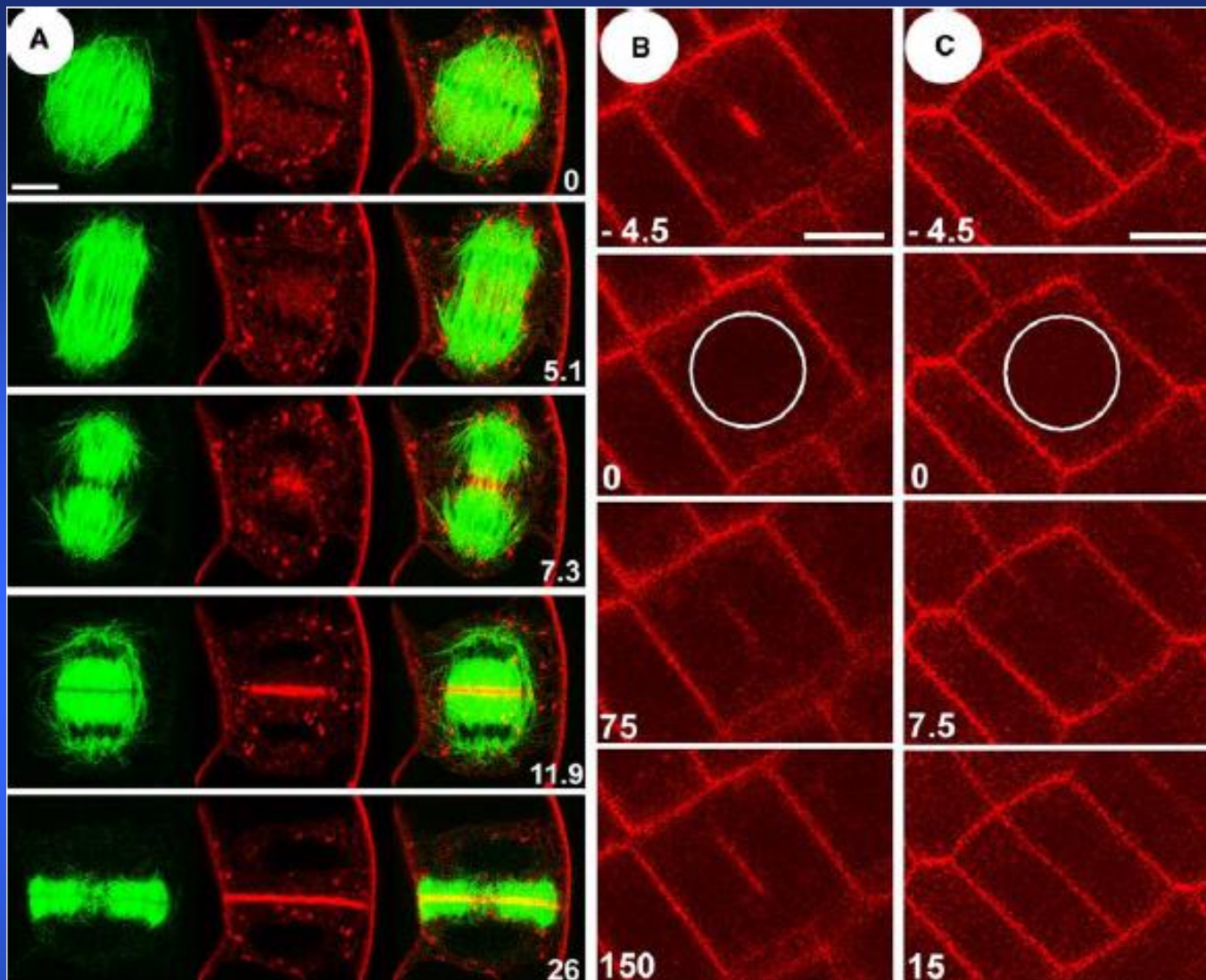


Bunková stena a jej biogenéza



12

Nová bunková stena sa začína tvoriť pri cytokinéze. Zoskupením vezikúl (GA, endocytóza) vzniká **fragmoplast** a ich postupnou fúziou **plazmatická platnička**. Tá tvorí základ pre formovanie **primárnej bunkovej steny**. Materiál plazmatickej platničky obsahuje pektíny medzi dvoma bunkami (ich primárnymi stenami) ostáva tzv. **stredná pektínová lamela**.

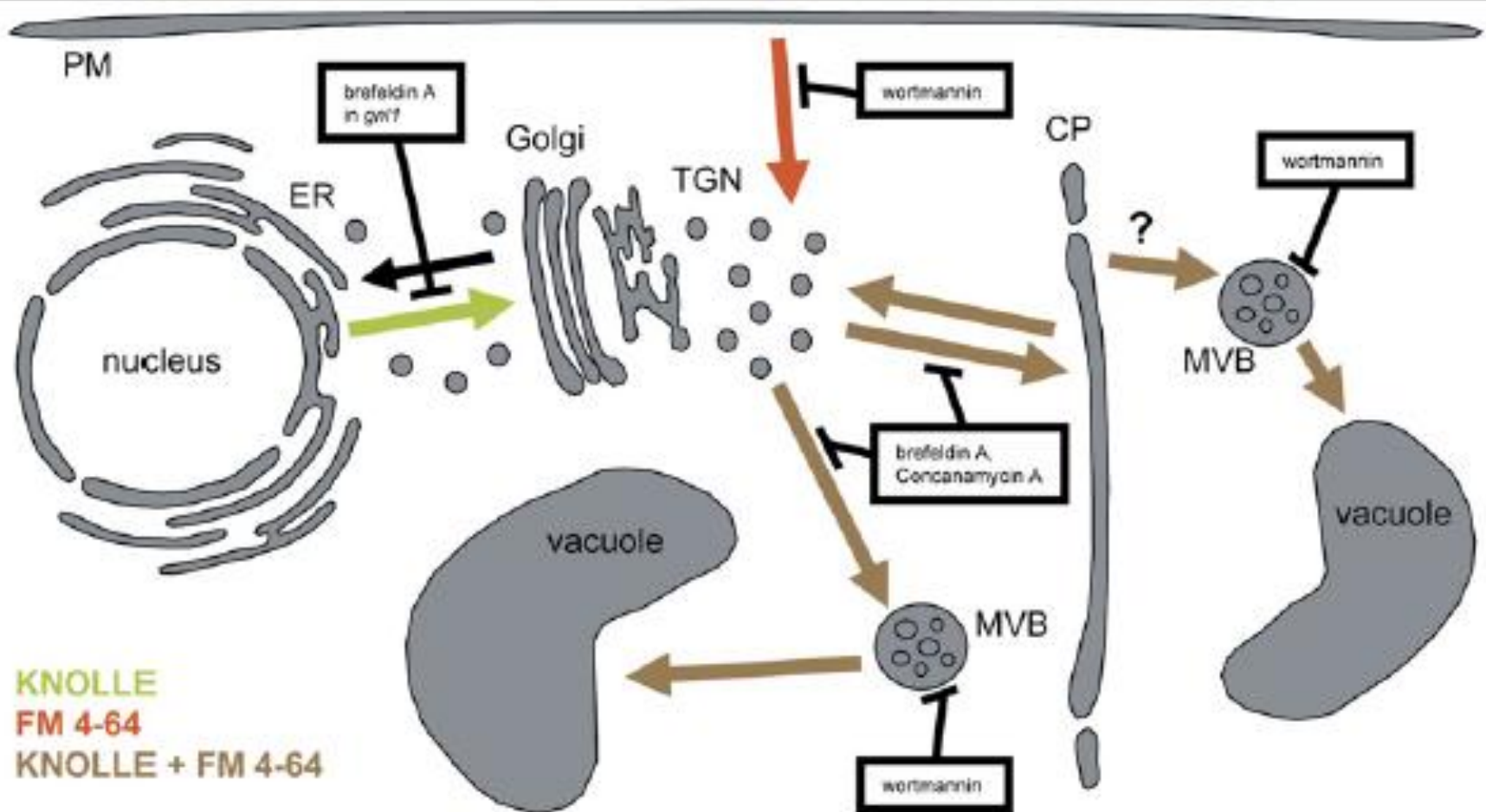


A: marker endocytózy **FM4-64** a jeho postupná lokalizácia do plazmatickej platničky (rastlinné bunky endocytujú molekuly/materiál z apoplastu, a teda i bunkovej steny)

B: FRAP (**fluorescence recovery after photobleaching**) FM4-64 – formujúca sa plazm. plat.

C: FRAP FM4-64 plazmatických platničiek pripojených k cytoplazmatickej membráne

F



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Jiří Friml,^{2,*} and Theodorus W.J. Gadella, Jr.^{1,*}

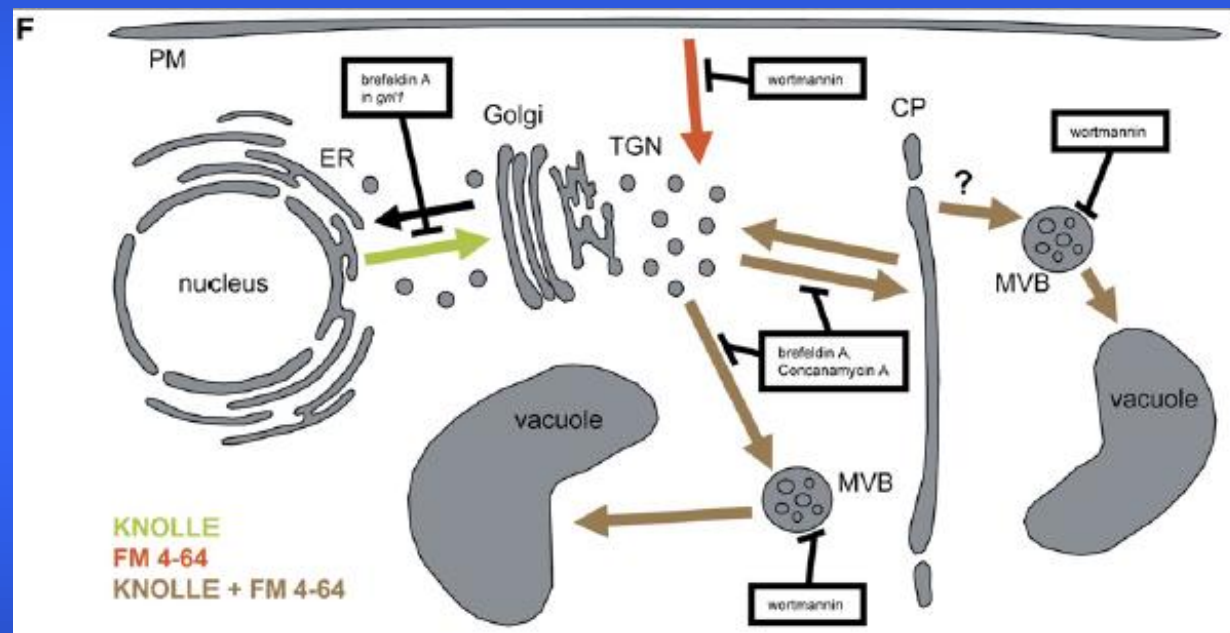
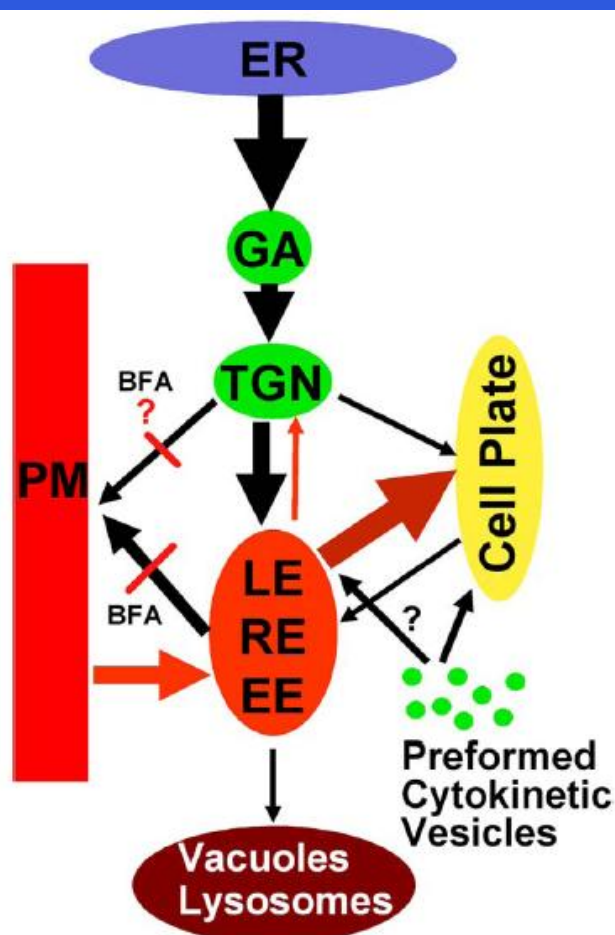
Introduction

Cytokinesis in immobile plants striking

Plant Cytokinesis Requires De Novo Secretory Trafficking but Not Endocytosis

Ilka Reichardt,¹ York-Dieter Stierhof,² Ulrike Mayer,¹
Sandra Richter,¹ Heinz Schwarz,³ Karin Schumacher,⁴

Results and Discussion



Zloženie bunkových stien

Látky spoločné pre väčšinu bunkových stien:

- voda
- ióny
- proteíny (štruktúrne proteíny a mnohé enzýmy)

Zloženie bunkových stien

Baktérie – peptidoglykany a teicholové kyseliny

Archea – glykoproteíny, proteínové S vrstvy, pseudopeptidoglykany, polysacharidy

Huby – chitín a chitozan, manoproteíny = manány

Riasy – glykoproteíny a polysacharidy (manány, xylány, algínové kyseliny, sulfonované polysacharidy – agaróza, karagenan, porfyran, furccleran, furonan)

Vyššie rastliny – celulóza, hemicelulózy, lignín, suberín

Ontogenéza

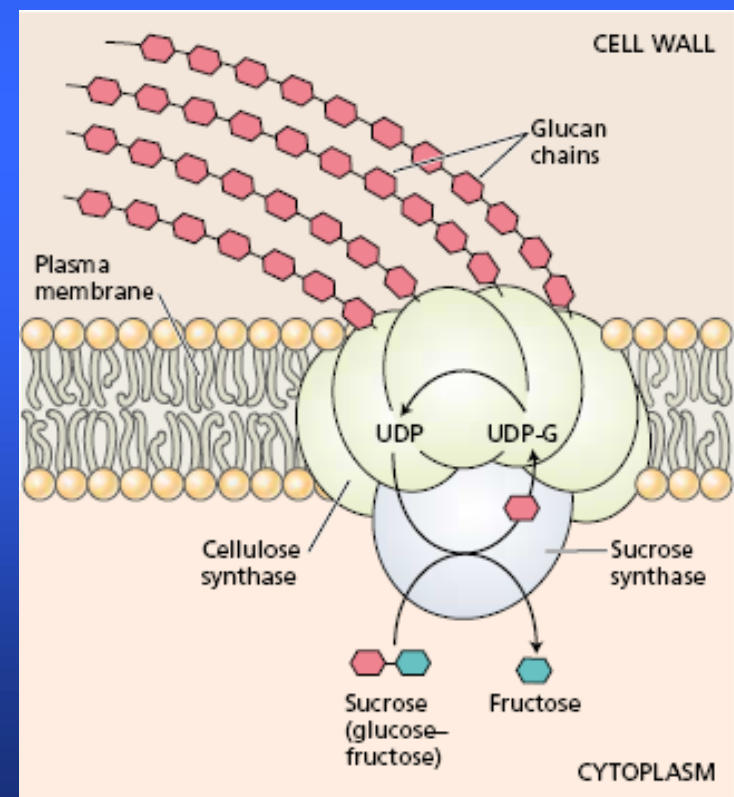
- Delenie buniek
- Stredná lamela
- Primárna bunková stena
- Sekundárna bunková stena
- Terciárna bunková stena

Štruktúra a biogenéza primárnej bunkovej steny

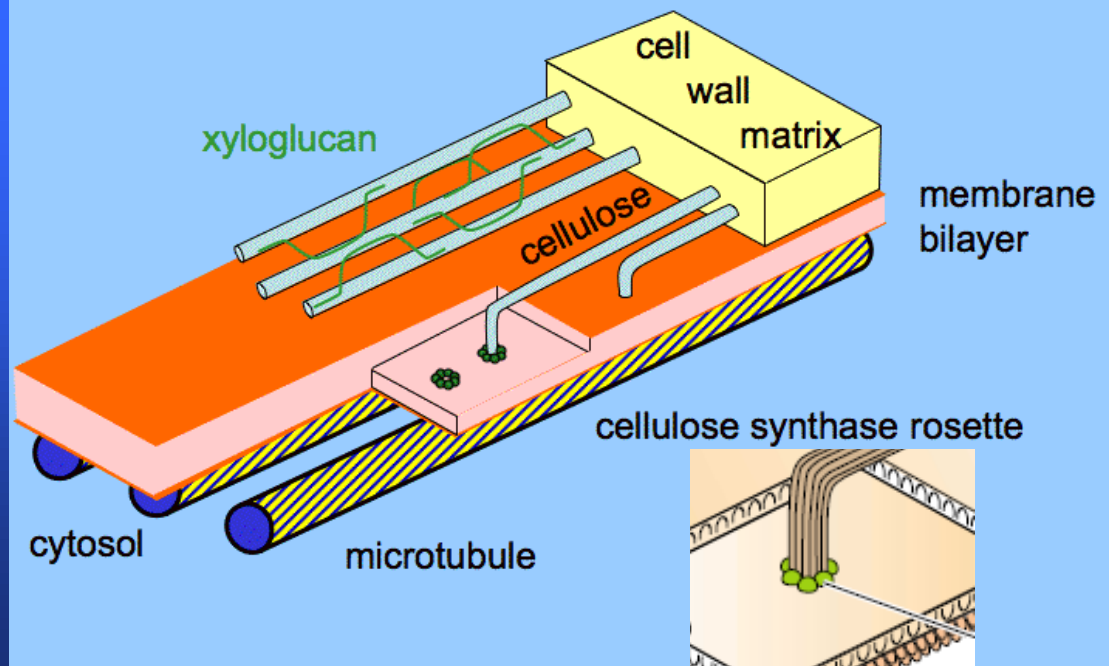


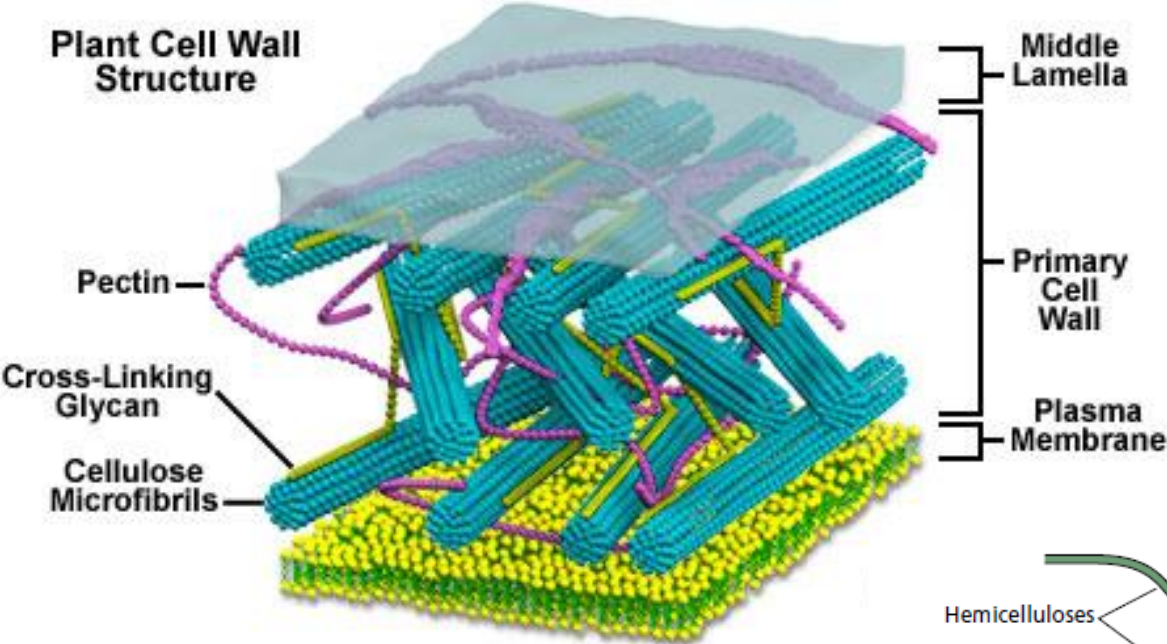
Primárna bunková stena pozostáva z celulózových mikrofibríl a matrix (pektíny, hemicelulózy...).

Celulózové polyméry sú syntetizované enzýmovým komplexom – **celulóza-syntáza** (*cellulose synthase*), ktorá je vnorená v cytoplazmatickej membráne. Jednotlivé komplexy CS sa zoskupujú a vytvárajú **rozety**, ktoré na vonkajšej strane CM uvoľňujú materiál pre formovanie celulózových mikrofibríl.



Cellulose synthesis is aligned with microtubules





Zložky bunkovej steny:

Celulóza (8 – 45 %)

Hemicelulózy

Pektíny

Lignín (exoderma, endoderma, xylém, sklerenchým)

Bielkoviny (<12 %)

Lipidy

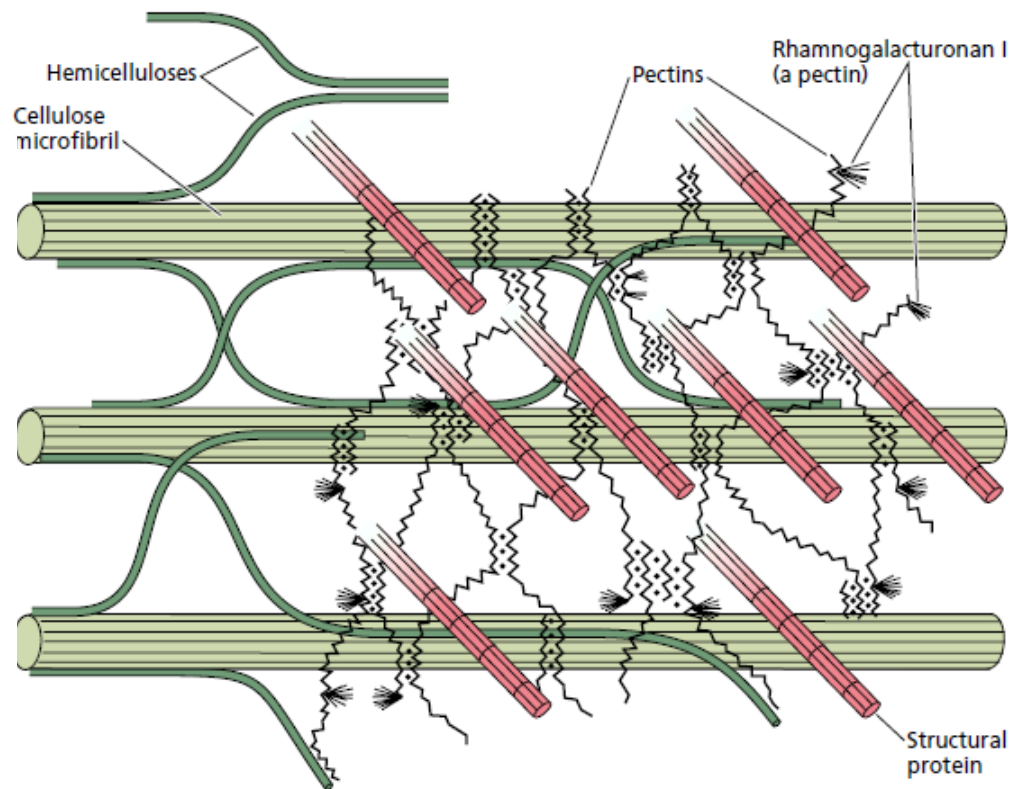
Suberín (exoderma, endoderma, korok)

Kutín (epiderma)

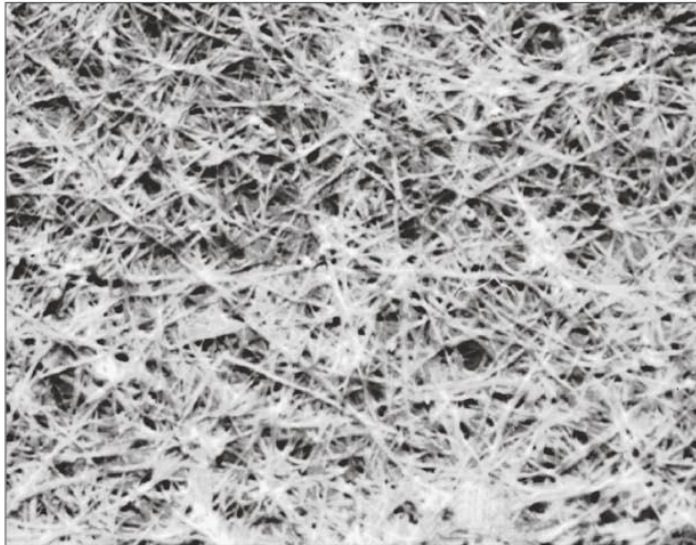
Chitín (bunky nižších rastlín, hmyzu a húb)

Voda

Ióny



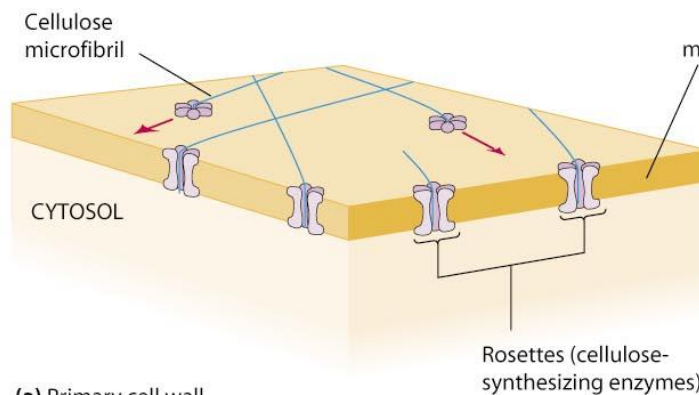
Usporiadanie a dynamika roziet CS určujú usporiadanie celulóзовých mikrofibríl v bunkovej stene (úloha cytoskeletu)



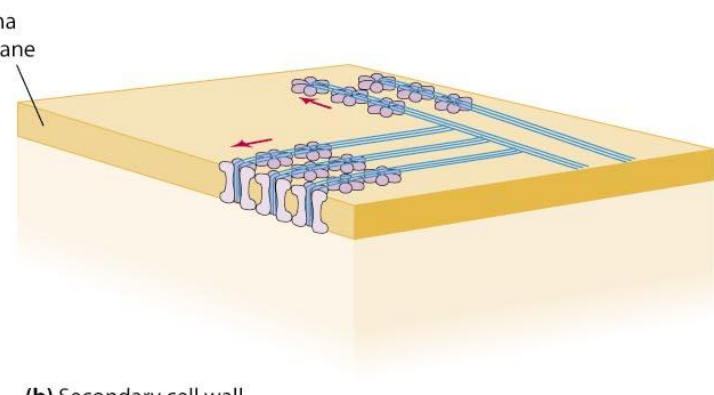
Anizotropná orientácia CM 1 μm



Izotropná orientácia CM 1 μm

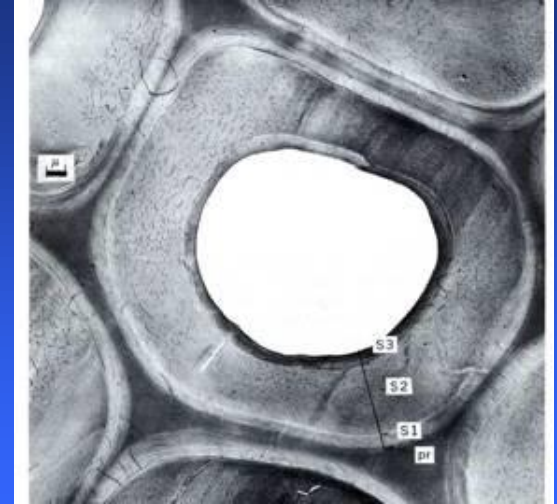
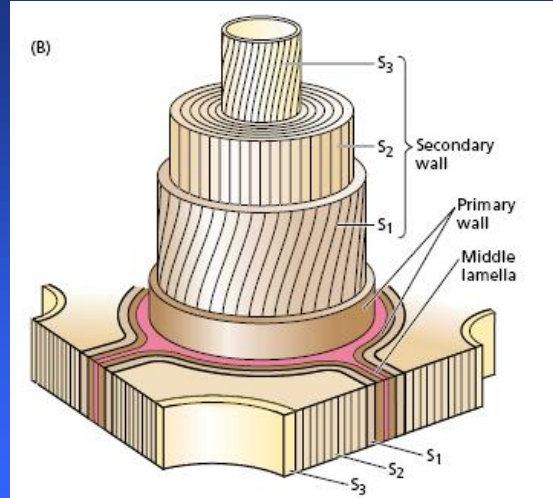
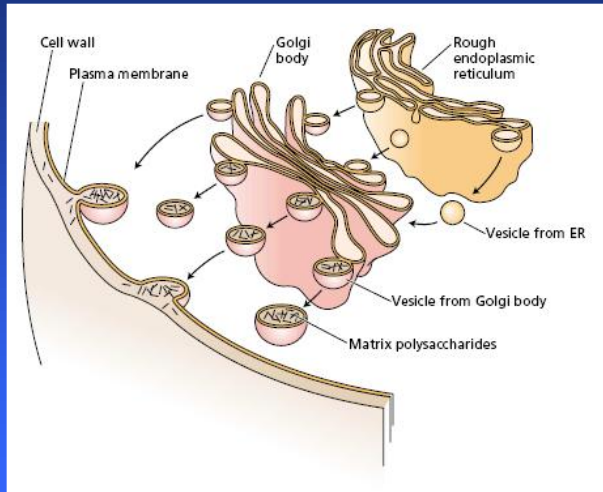


(a) Primary cell wall



(b) Secondary cell wall

Hrubnutie bunkovej steny – tvorba sekundárnej bunkovej steny



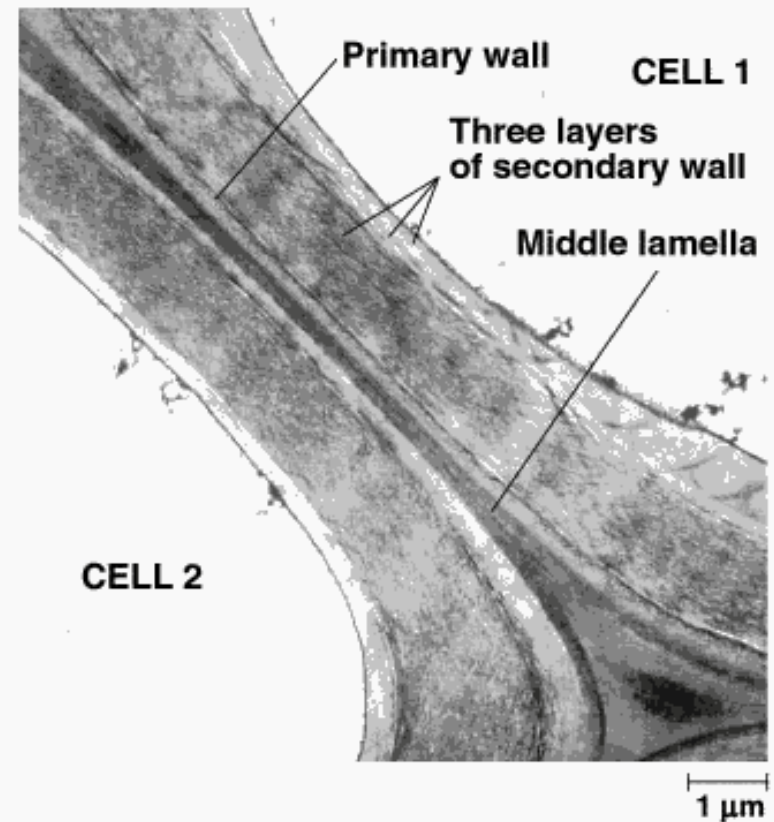
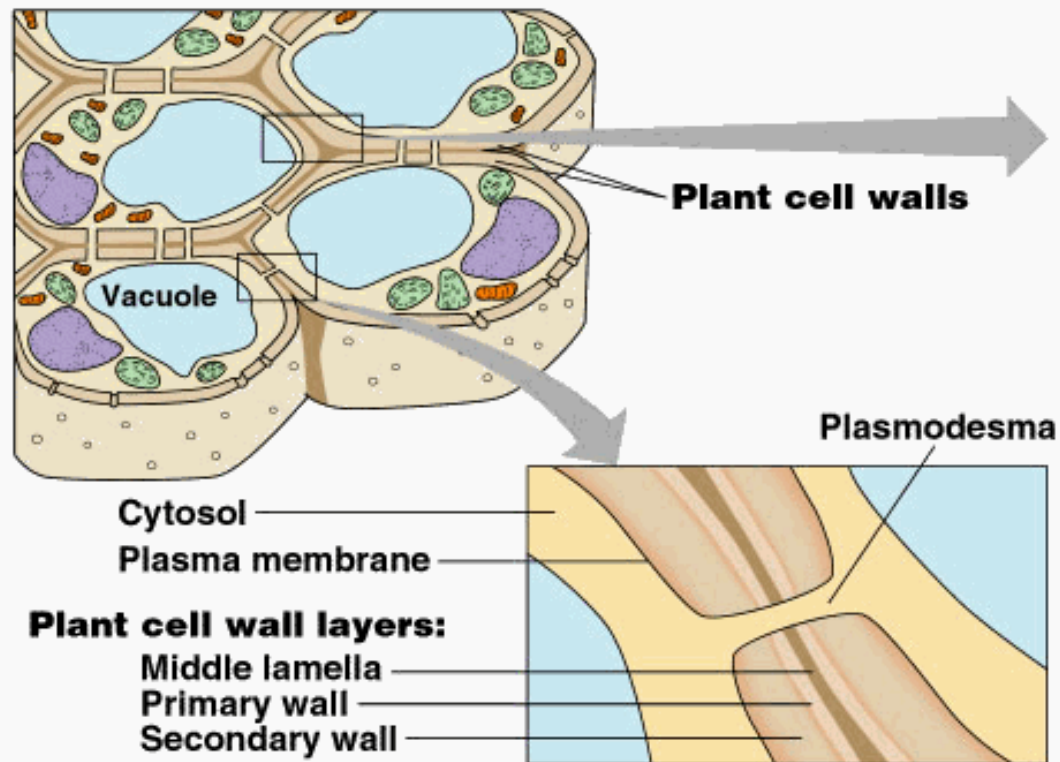
Primárna bunková stena rastie – expanduje. K novo-syntetizovaným celulóзовým mikrofibrilám sa pripájajú molekuly matrix (tento materiál môže byť k bunkovej stene dopravovaný prostredníctvom vezikúl).

Rast bunky úzko súvisí s rastom bunkovej steny. Môže byť vrcholový (*tip growth*), alebo difúzny/rovnomerný procesom nazývaným **intususcepcia**.

Ak bunka ukončí expanzívny rast, jej stena môže hrubnúť (**apozícia**) – vytvára sa **sekundárna bunková stena**, často lignifikovaná. Nerovnomerné hrubnutie bunkovej steny je typické pre bunky cievnych elementov.

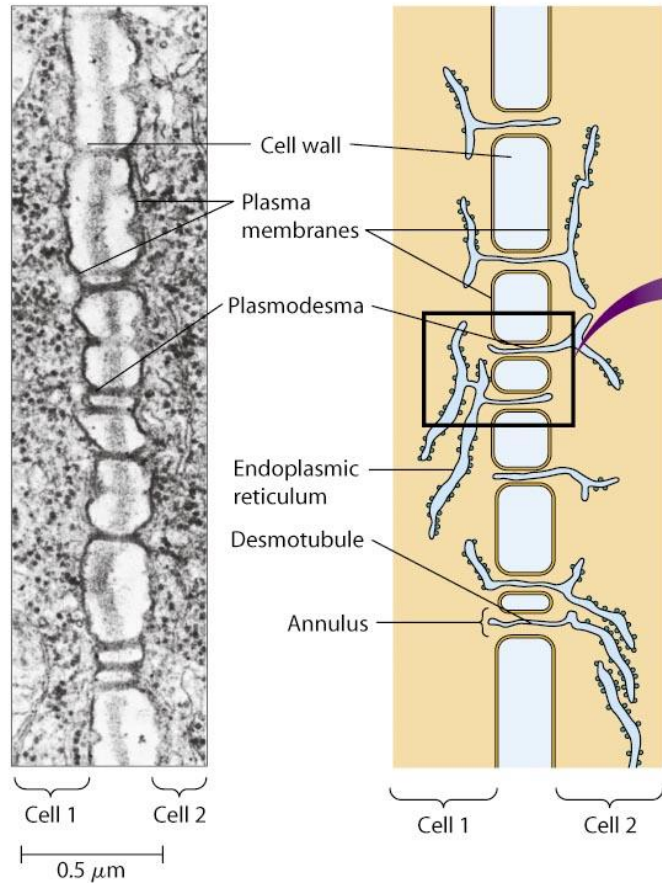
Funkcie bunkovej steny

- Kompartmentalizácia
- Spájanie
- Prenos látok
- Mechanika (pevnosť, sila)
- Obrana
- Ochrana
- Izolácia

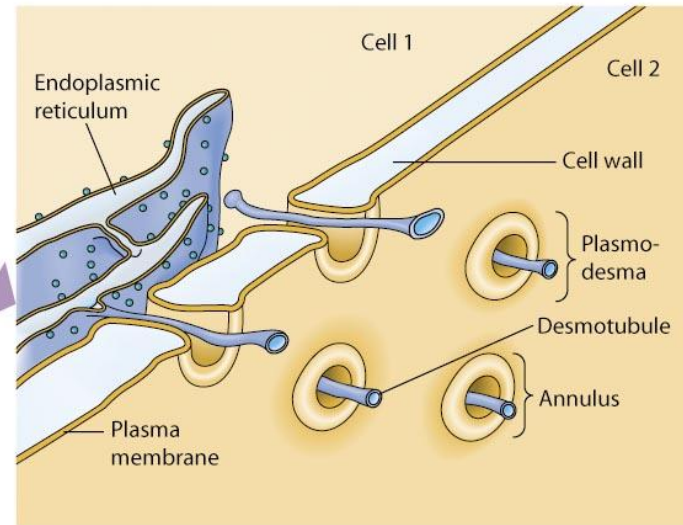


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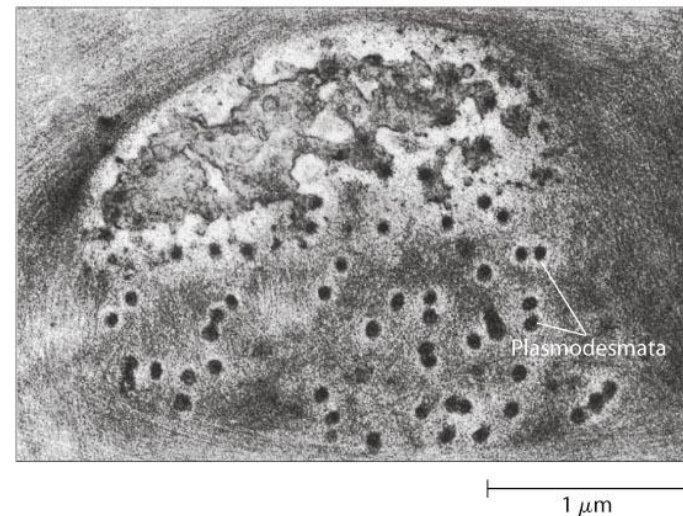
Plasmodesmy



(a) Electron micrograph and diagram of plasmodesmata in longitudinal section

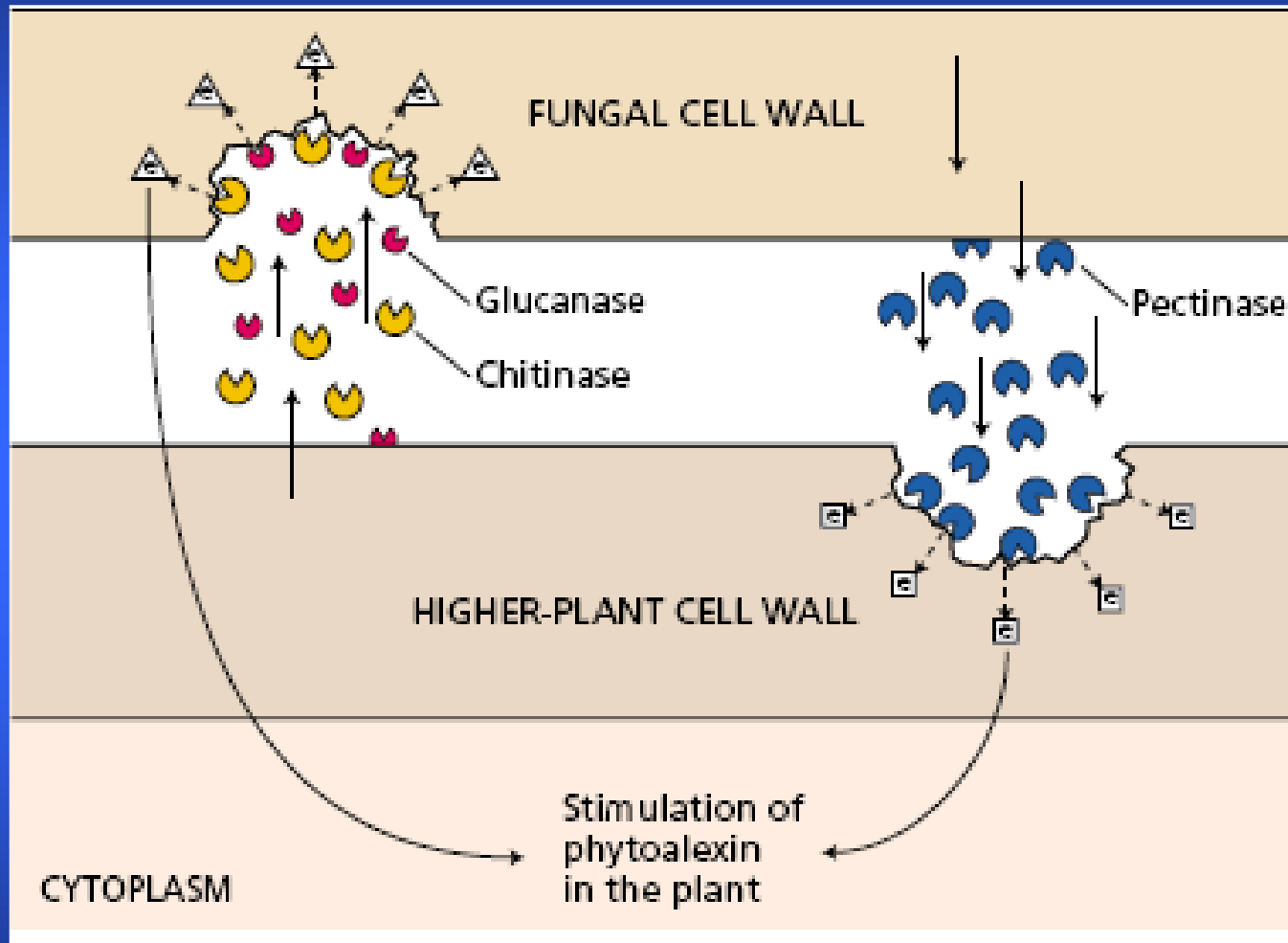


(b) Diagram of plasmodesmata



(c) Electron micrograph of plasmodesmata in cross section

Pektinázy a celulázy hydrolyzují primární bunkovou stenu



Podkladové údaje pre pochopenie formovania
plazmatickej platničky (nasledovných 6 strán – nebudú súčasťou skúšky)

Mechanismus formovania plazmatickej platničky je stále nejasný

Developmental Cell 10, 137–150, January, 2006 ©2006 Elsevier Inc. DOI 10.1016/j.devcel.2005.11.015

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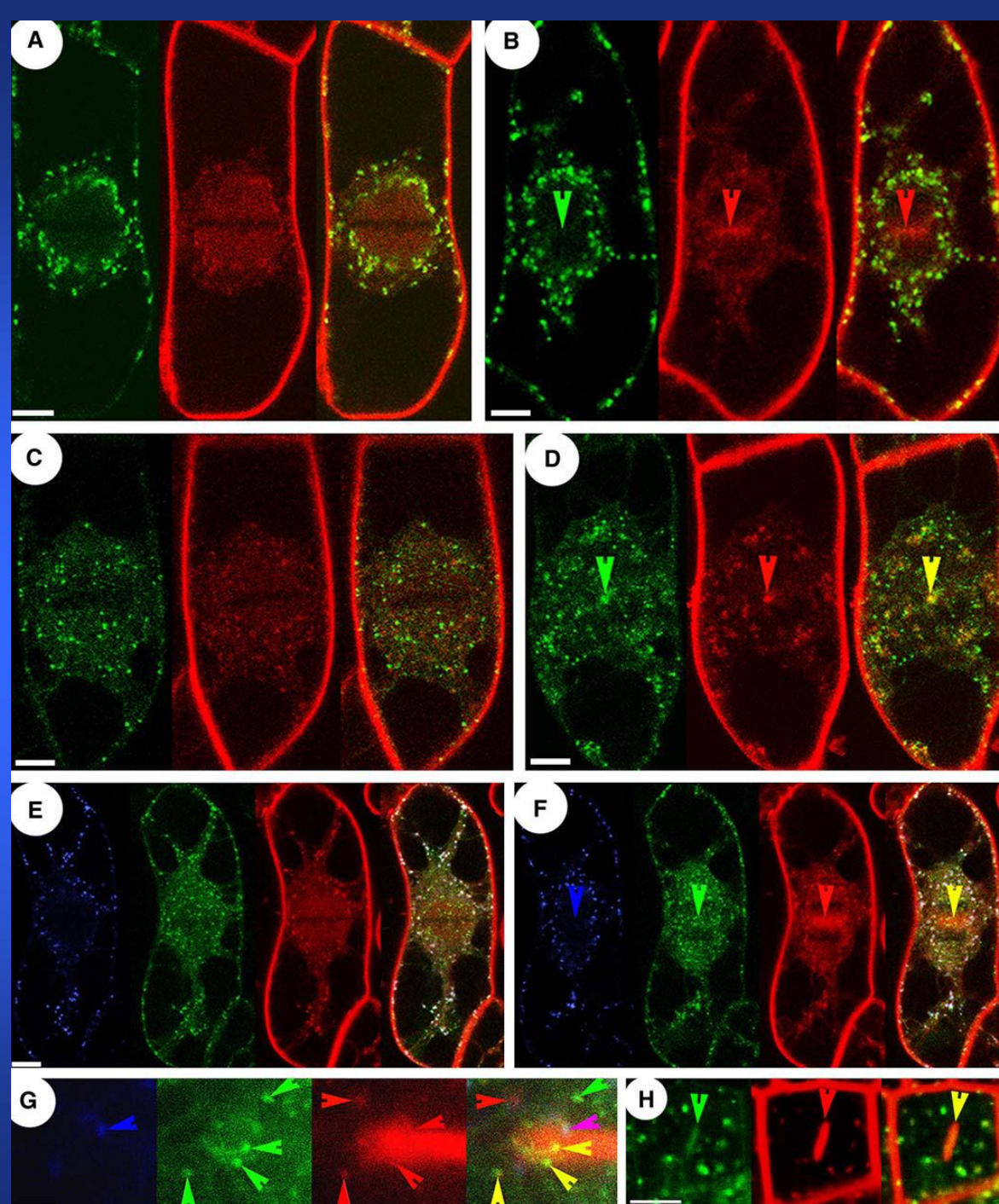
Cytokinesis in immobile plants striking

Current Biology 17, 2047–2053, December 4, 2007 ©2007 Elsevier Ltd All rights reserved DOI 10.1016/j.cub.2007.10.040

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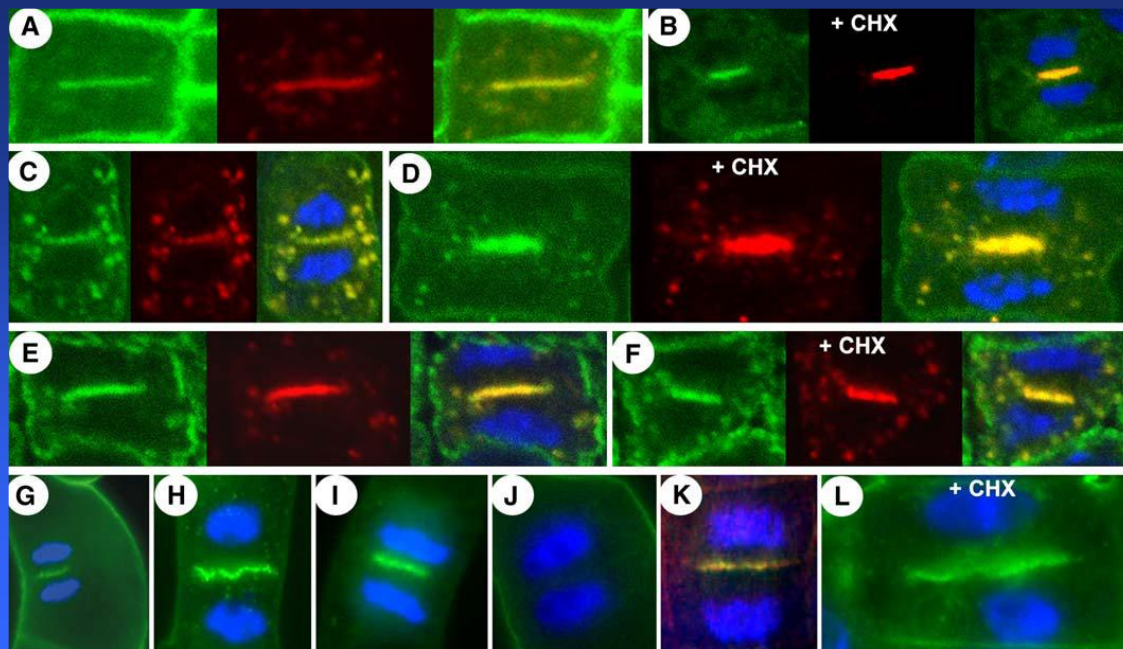
Ilka Reichardt,¹ York-Dieter Stierhof,² Ulrike Mayer,¹
Sandra Richter,¹ Heinz Schwarz,³ Karin Schumacher,⁴

Results and Discussion



A, C, E: pred iniciáciou plazma-
tickej planičky (PP);
B, D, F: iniciácia PP

ST-YFP - marker *trans*-Golgi (A, B)
FM4-64 - marker endozómov
GFP-Ara7 - marker endozómov (C, D)
ST-YFP - marker *trans*-Golgi (E, F)
GFP-Ara7



Kolokalizácia markera plazmatickej platničky (KNOLLE) s proteínmy PM:

A: **PIN2** a **KNOLLE** (+ cykloheximid > B)

C: **PIP2** a **KNOLLE**

E: **LT16b** a **KNOLLE**

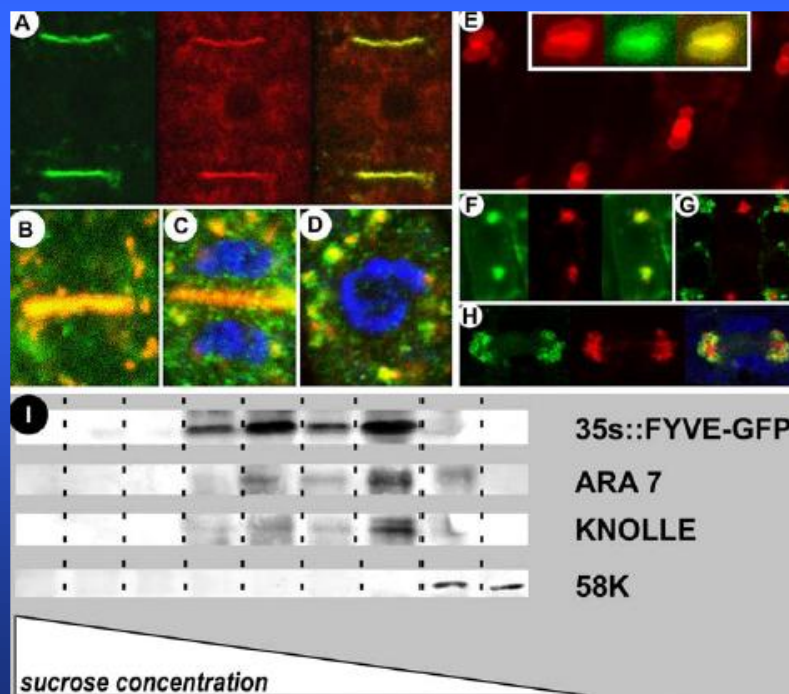
Lokalizácia pektínov:

G,H: pectin marker **RGII** (CW pectins)

I: **JIM5** pectins (CW partially esterified pectins)

J: **JIM7** pectins (esterified pectins in GA-vesicle)

K: **JIM5** a **KNOLLE**



Kolokalizácia markerov endozómov a plazmatickej platničky

A: **KNOLLE** a **GNOM-Myc**

B: **Ara7** a **KNOLLE**

C: **Ara6** a **KNOLLE**

F: **PIN1** a **KNOLLE** po BFA

H: **γCOP** a **KNOLLE** po BFA

I: Kofrakcionácia proteínov a následný Western blot

DAPI

Endocytosis of Cell Surface Material Mediates Cell Plate Formation during Plant Cytokinesis

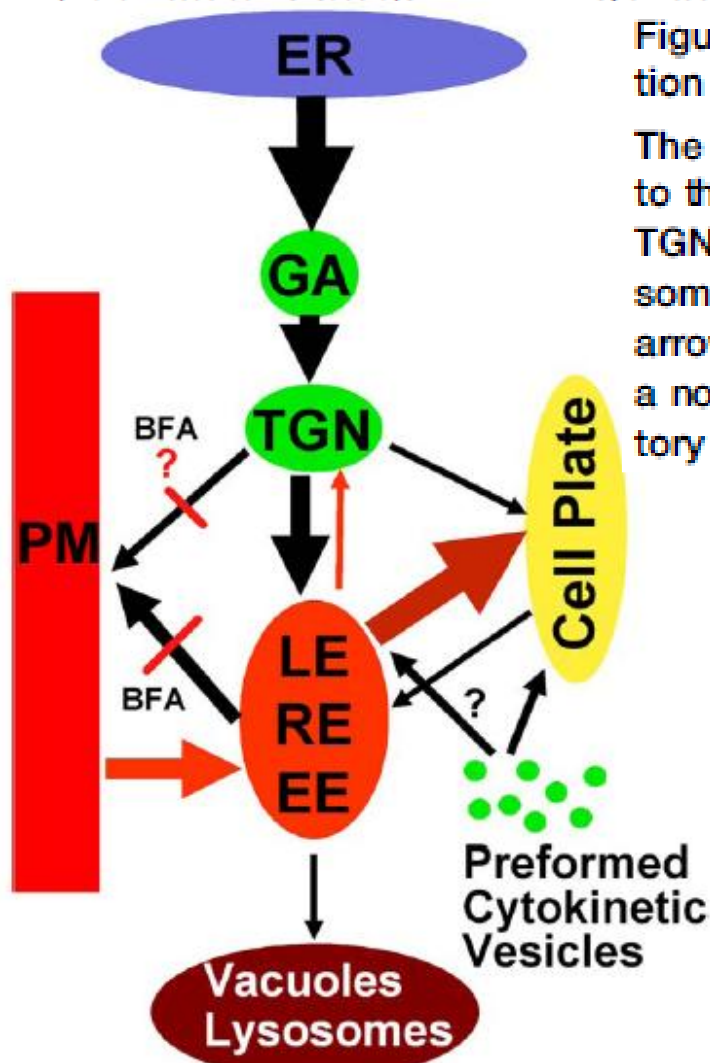
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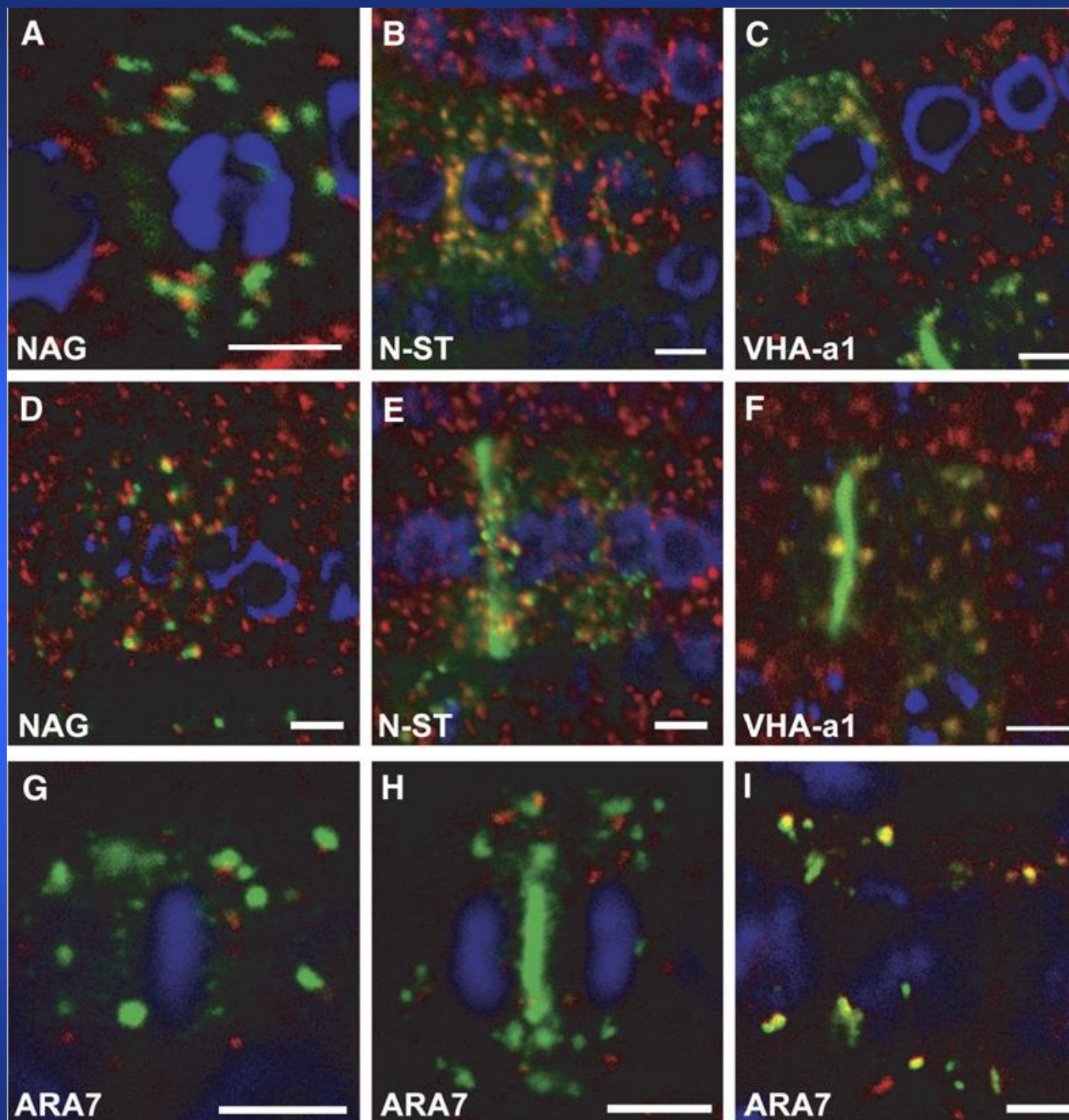
Cytokinesis in immobile plants striking

Figure 7. Membrane Trafficking Pathways during Cell Plate Formation

The model depicts the mass flow of endocytic material from the PM to the cell plate. ER, Endoplasmic Reticulum; GA, Golgi apparatus; TGN, Trans Golgi Network; PM, plasma membrane; EE, early endosome; RE, recycling endosome; LE, late endosome. The sizes of the arrows show the extent of membrane transport. Red arrows indicate a novel pathway revealed by our results. Red lines indicate inhibitory effects of BFA.



Griffiths, 2002). In this view, the cell plate can be regarded as some sort of specialized endosomal compartment that receives material from the Golgi apparatus directly as well as from the PM through sorting and recycling endosomes (see our model in Figure 7). Our view of cell plate formation involving multiple membrane fusion events and cargo received from multiple pathways would also provide more stability and back up for this process. One can imagine that inhibition of one pathway may temporarily upregulate the other to rescue the defects. This would explain the robustness of the cytokinetic machinery, which is indicated by multiple observations of CW stubs and normal, complete CWs in various reported cytokinetic mutants.



Pred formovaním plazmatickej platničky: A - C
Počas formovania PP: D - F
G: metafáza, H: telofáza,
I: koniec cytokinézy

DAPI - DNA marker

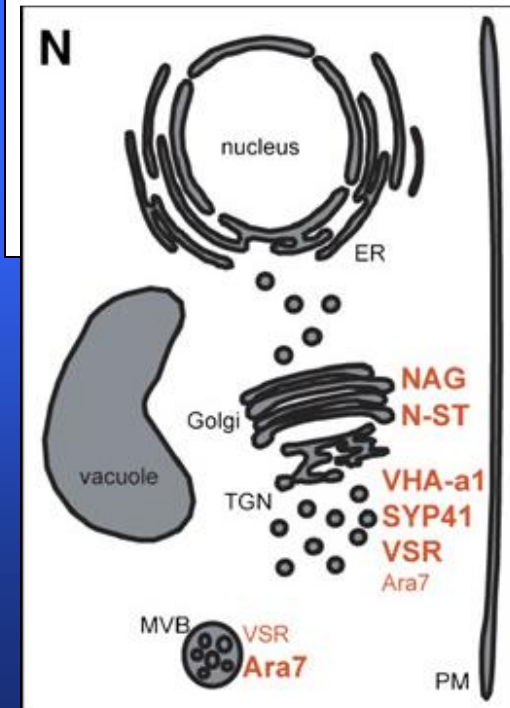
KNOLLE - marker PP

NAG - medial-Golgi

N-ST - *trans*-Golgi

VHA-a1 - TGN (*trans*-Golgi network)

ARA7 - MVB marker



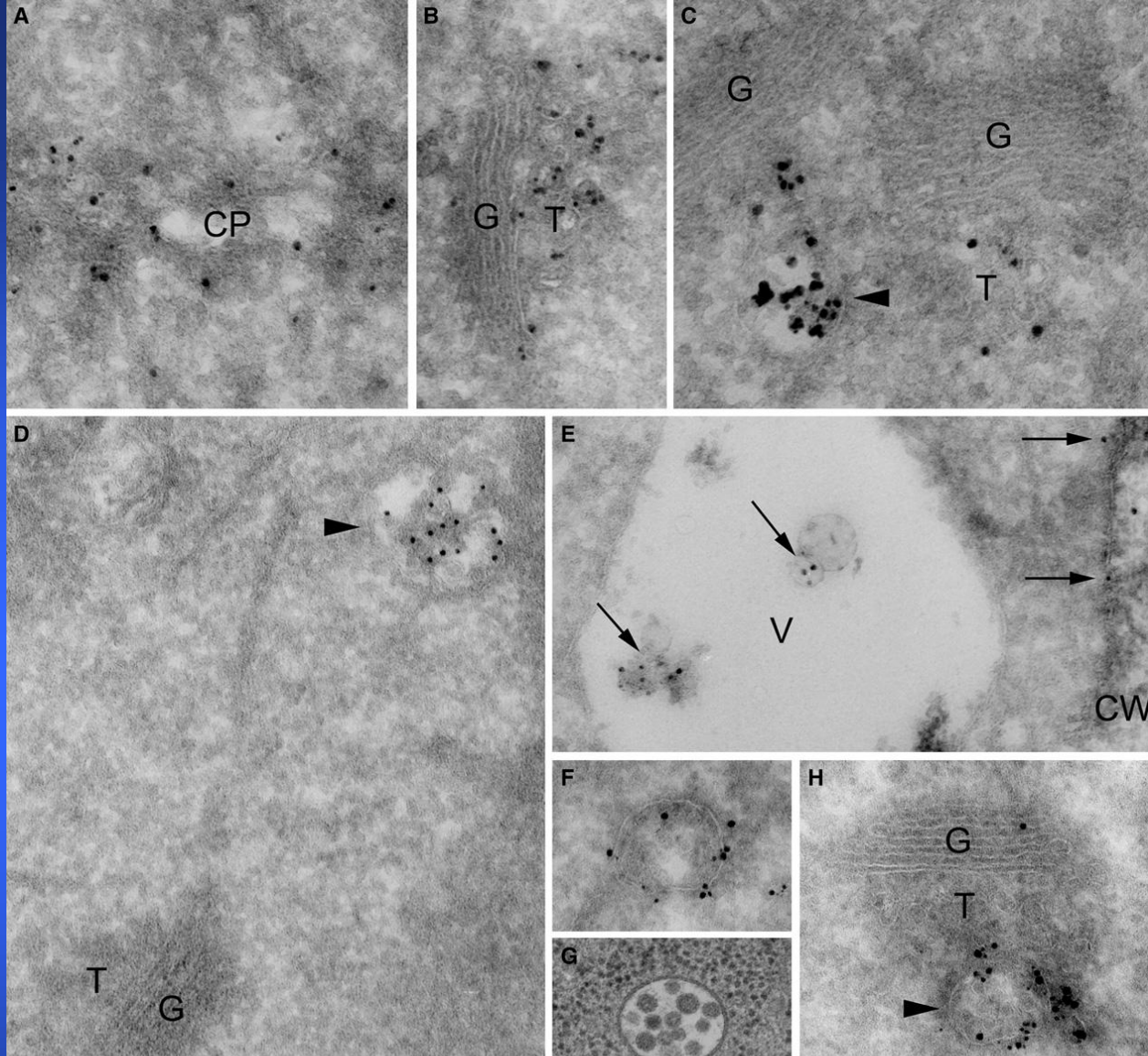


Figure 2. KNOLLE-Trafficking Pathway in Dividing Cells

(A and B) KNOLLE on cell-plate ([A], "CP") and neighboring vesicles, (B) TGN (early telophase; overview: [Figure S2D](#)).

(C) KNOLLE label reduced on TGN, increased on internal vesicles of MVB (arrowhead) (early interphase; overview: [Figure S2E](#)).

(A–F and H) Immunogold labeling of cryosections from cryofixed root tips.

(D and E) GFP-KNOLLE labeling of vesicles inside MVB ([D], arrowhead) and intra-vacuolar vesicles ([E], arrows) and residual labeling of the plasma membrane ([E], arrows).

(F–H) GFP-ARA7 on the membrane of MVBs ([F]; [H], arrowhead); (G) MVB. CP, cell plate; CW, cell wall; G, Golgi stack; MVB, multivesicular body;

T, *trans*-Golgi network (TGN); V, vacuole. The scale bar represents 250 nm.

(G) Resin section of cryofixed root-tip cells.

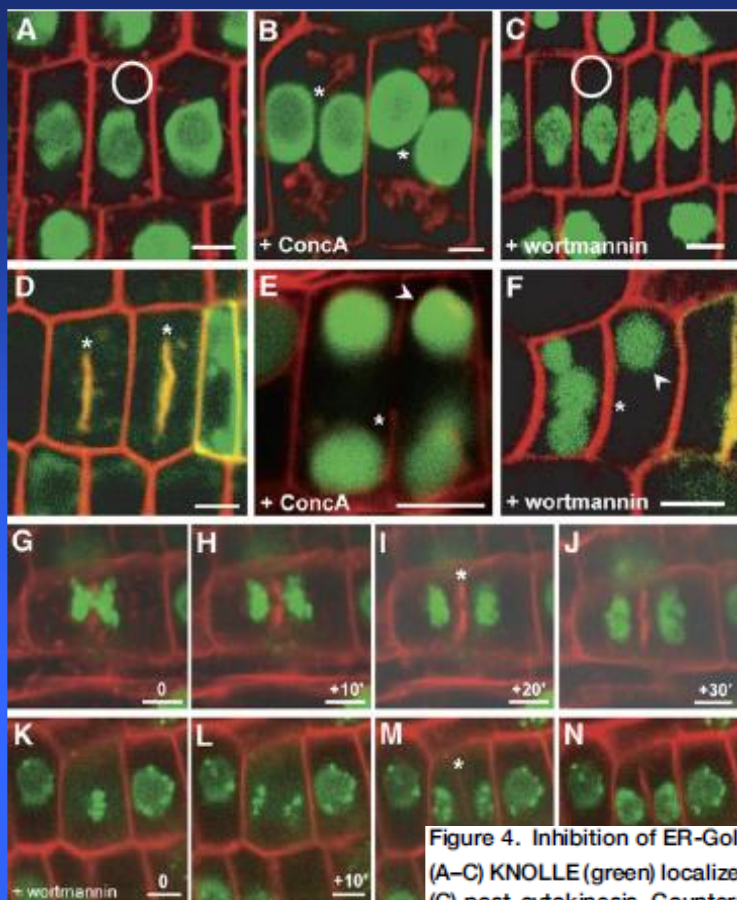


Figure 3. Concanamycin A but not Wortmannin Inhibits Cell-Plate Formation

(A–N) Fluorescence imaging of root cells. The scale bars represent 5 μ m (D) and 500 nm (E).
 (A–C) Nuclear GFP (green) and FM4-64: (A) control; (B) ConcA-treated (2 hr); (C) wortmannin-treated (2 hr). Note the absence of labeled intracellular vesicles in (C), circle) compared to (A).
 (D–F) GFP-KNOLLE (green) and FM4-64: (D) control; (E) ConcA-treated (2 hr); (F) wortmannin-treated (2 hr). Note the absence of FM4-64 label in vacuolar membrane (arrowheads) in (F) compared to (E).
 (G–N) Time-course of FM4-64 accumulation in cell plate.
 (G–J) H2B-YFP (green) and FM4-64.
 (K–N) H2B-CFP (green) and FM4-64; wortmannin-treated (2 hr). Arrowheads: (E and F) vacuolar membrane. Asterisks: (D, F, I, and M) cell plate, (B) binucleate cell, (E) cell-wall stubs. Circles: (A and C) FM4-64 internalization. The scale bar represents 5 μ m.

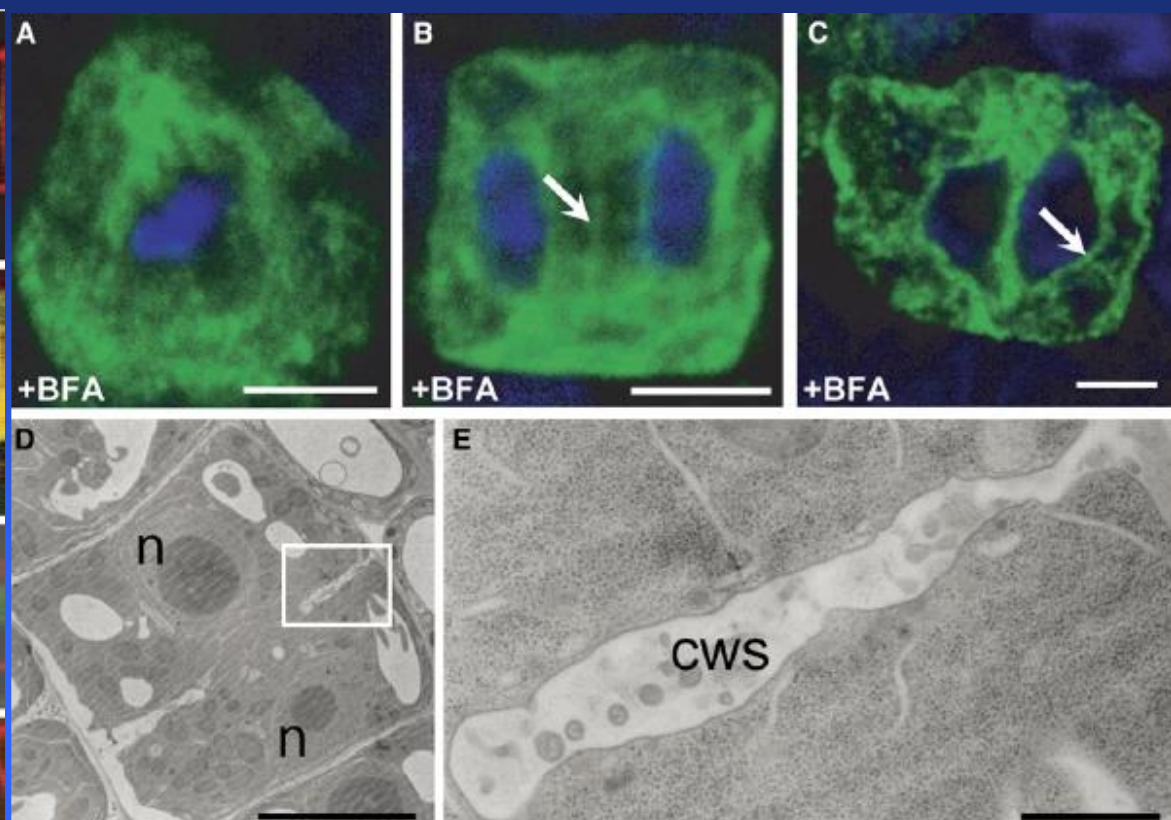


Figure 4. Inhibition of ER-Golgi Trafficking Strongly Affects Cell-Plate Formation

(A–C) KNO1 (green) localizes to ER (arrows) in BFA-treated (25 μ M, 2 hr) BFA-sensitive GNL1 seedling root cells: (A) metaphase, (B) telophase, (C) post-cytokinesis. Counterstained with DAPI (blue). The scale bar represents 5 μ m.

(D and E) Electron micrographs of BFA-treated *gnl1* homozygous seedling root-tip cells after cryofixation and resin embedding: (D) Binucleate cell with cell-wall stubs (boxed area); n, nucleus. The scale bar represents 5 μ m. (E) Higher magnification of cell-wall stub (cws) from (D), boxed area.