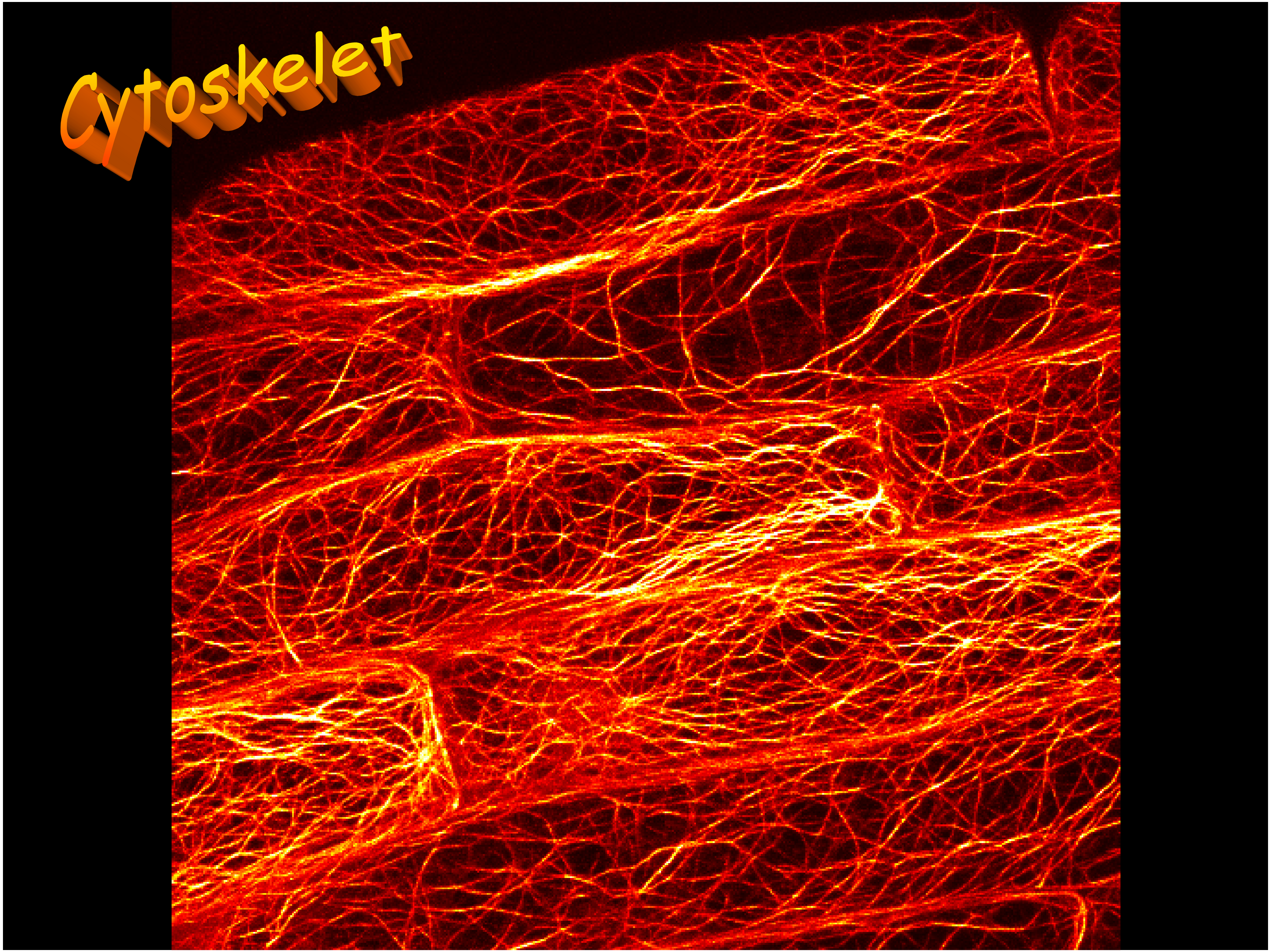
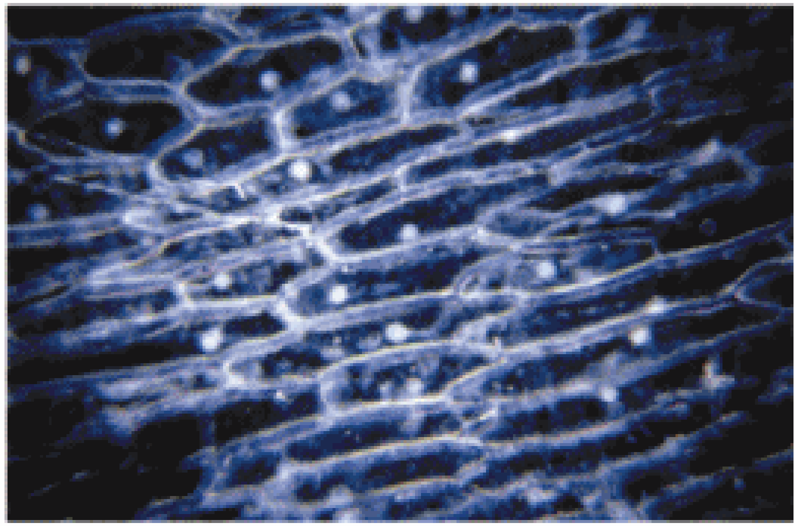

Cytoskelet

Cytoskelet rostlinné buňky

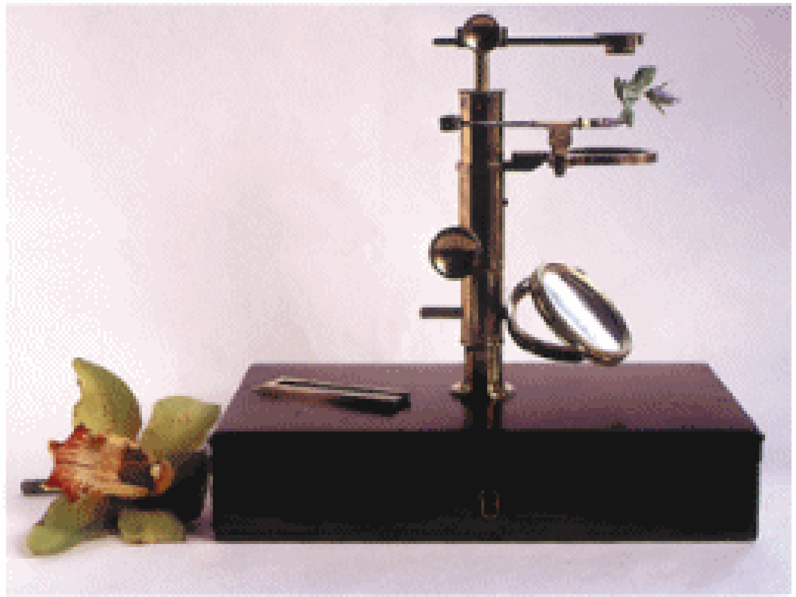
- Jak o něm víme (historie a metody)
- Obecné rysy a funkce cytoskeletálních systémů
- Aktin a asociované proteiny
- Tubulin, mikrotubuly a asociované proteiny
- Molekulární motory
- Koordinace cytoskeletálních systémů a …
  - buněčného cyklu
  - exocytosy, polarity, buněčné stěny
  - organel

(A)

(B)

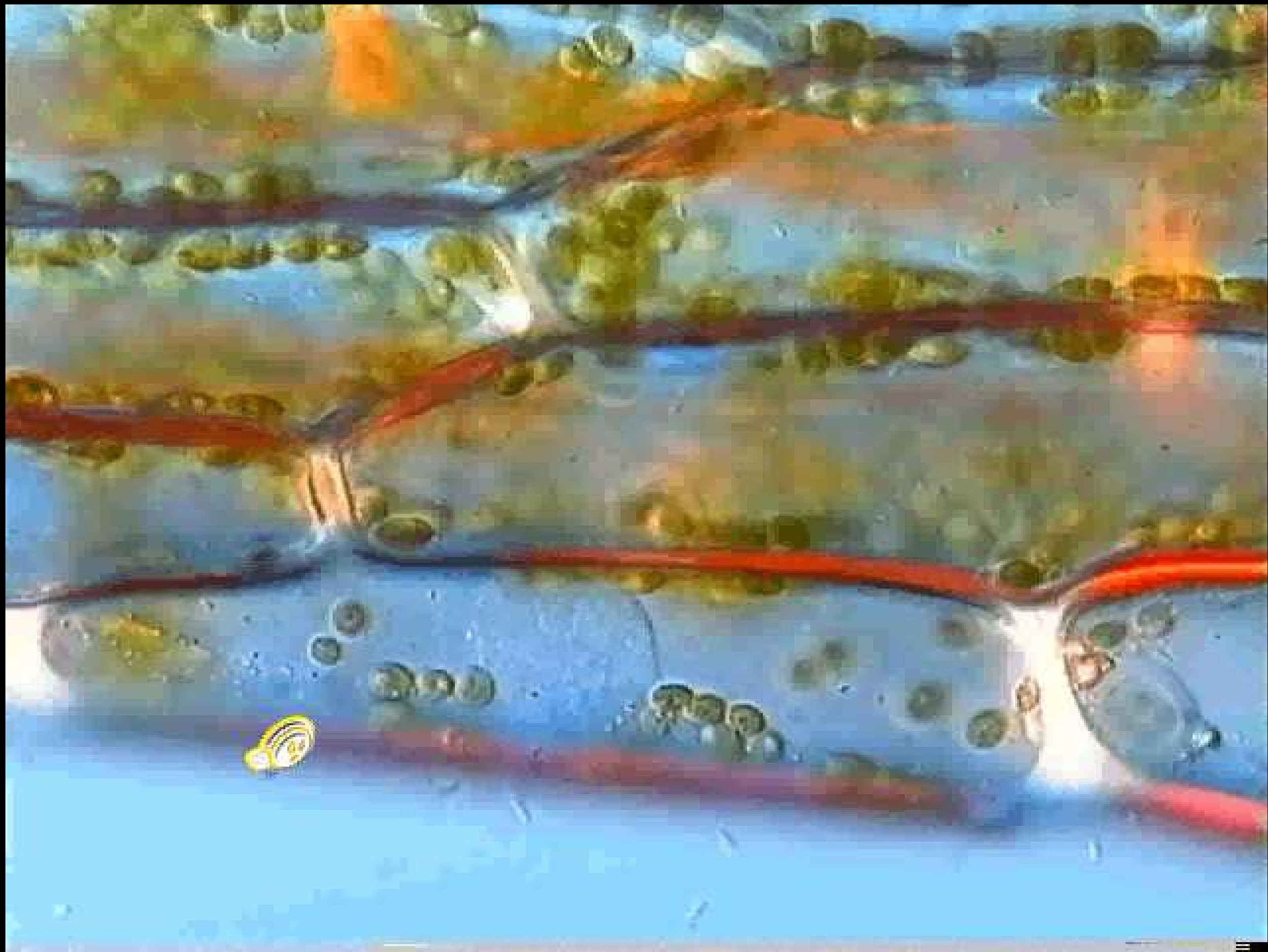


(C)



Robert Brown (1827):
ne všechny pohyby jsou dílem života

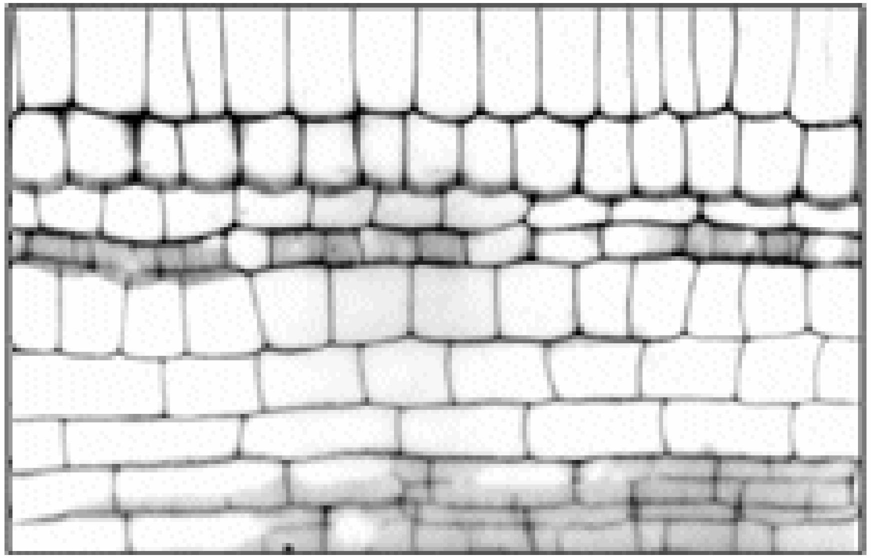
Proudění cytoplasmy



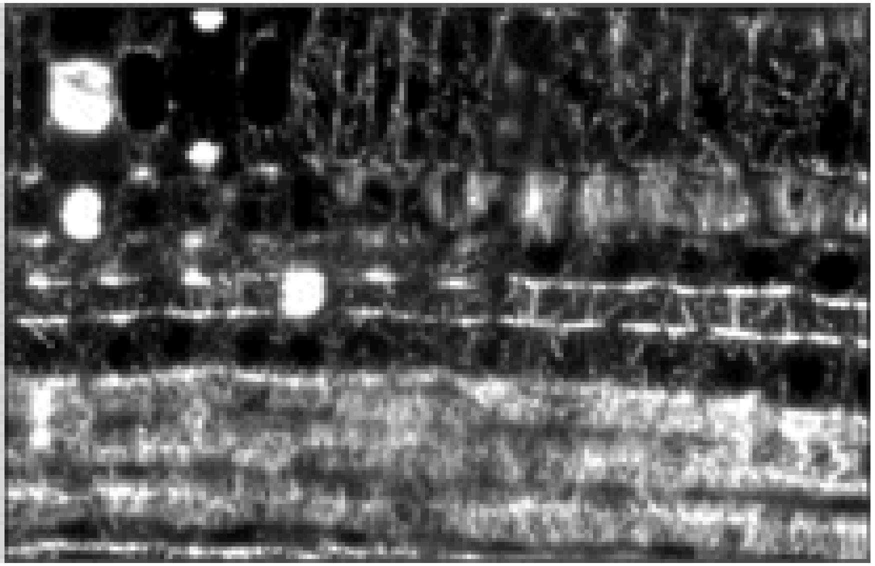
(Elodea sp.?)

Co je vidět v buňkách?

(řez kořenem Arabidopsis)

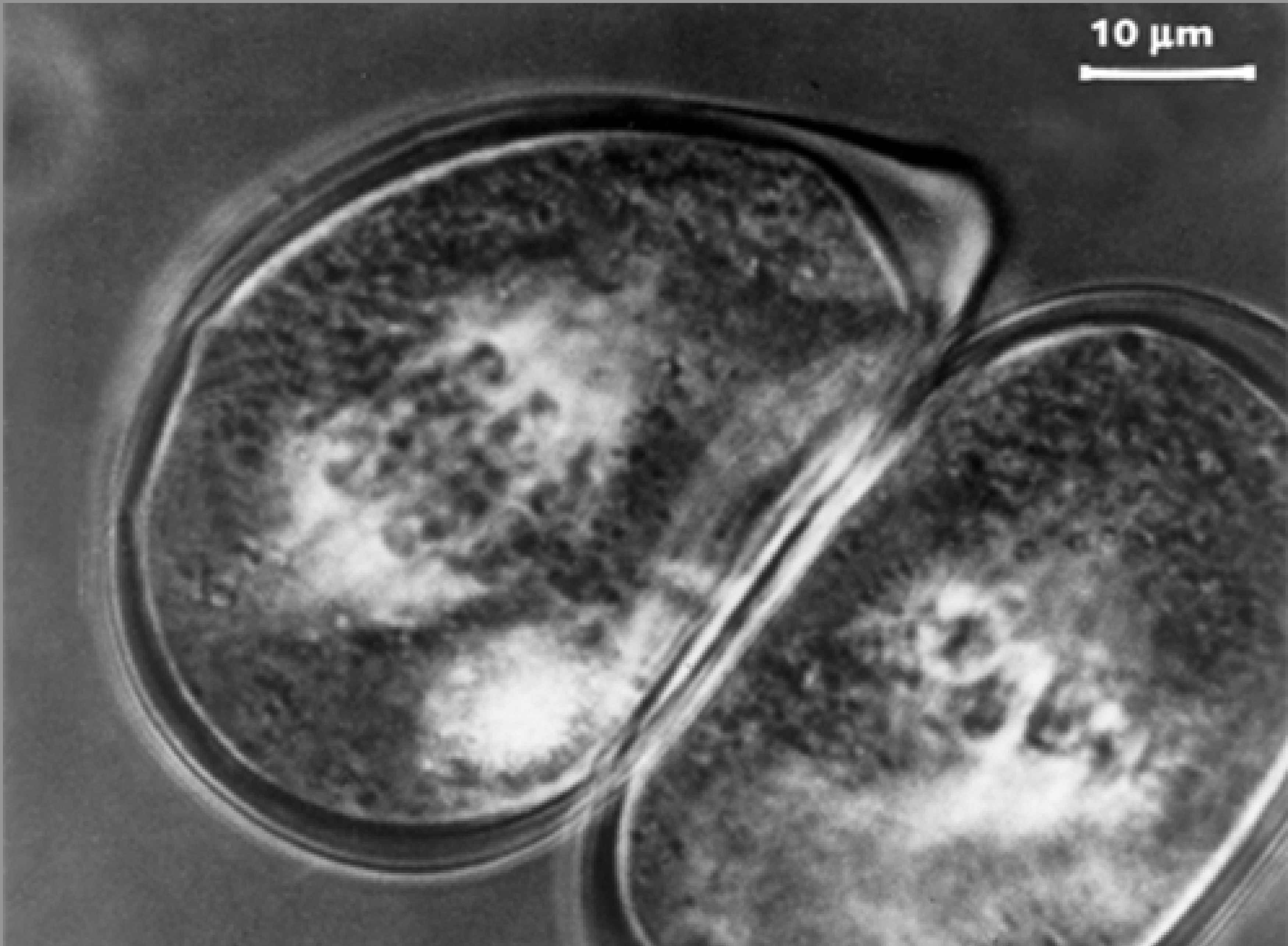


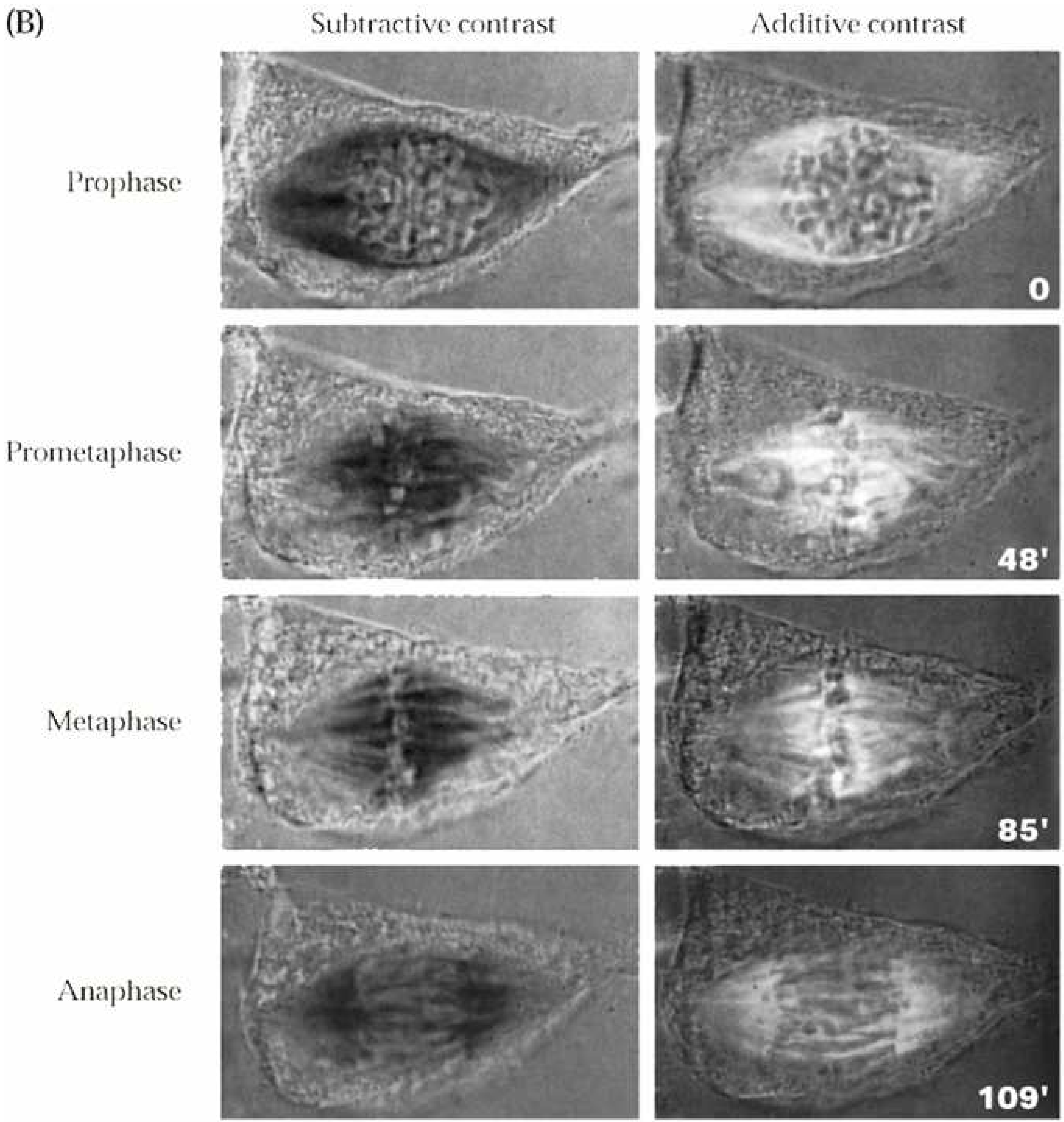
(C)



Ledbetter MC, Porter KR:
A „microtubule“ in plant cell fine structure.
J Cell Biol 1963:239-250

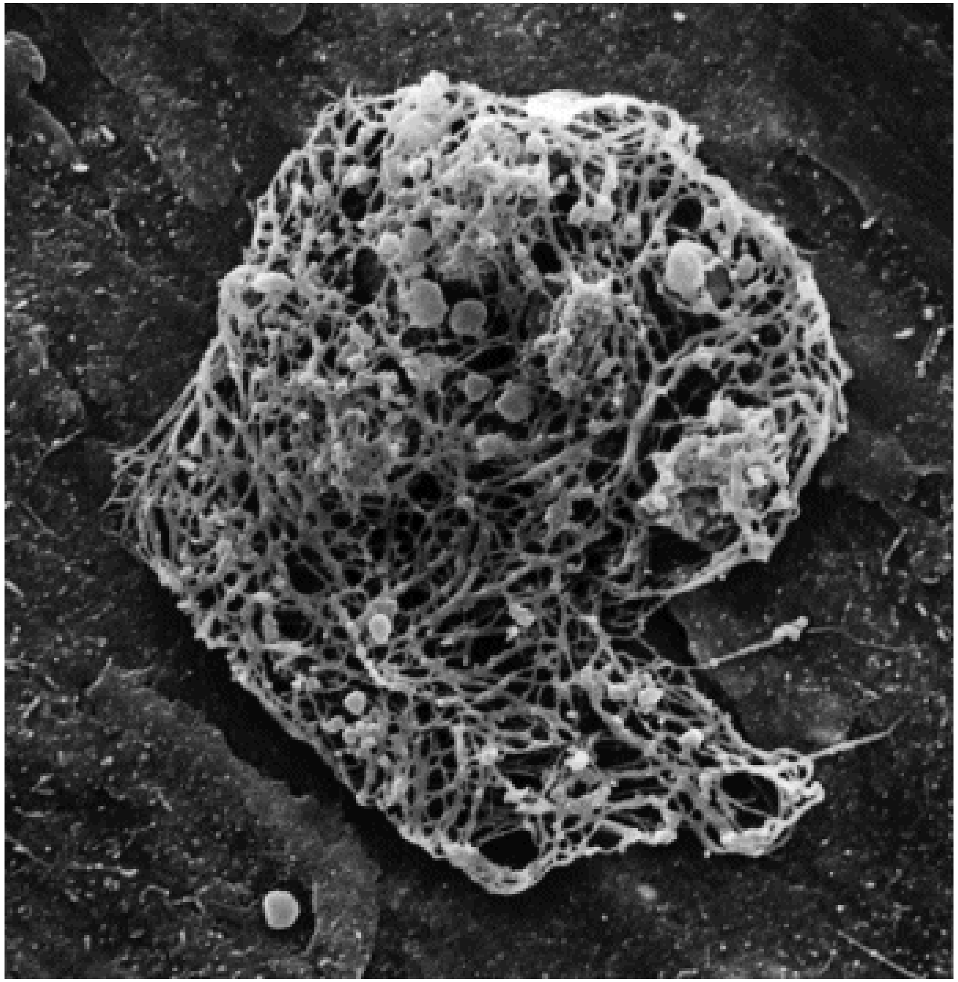
Inoue, 50. léta: „vláknité struktury“ (meiose mikrospor *Lilium longiflorum*)





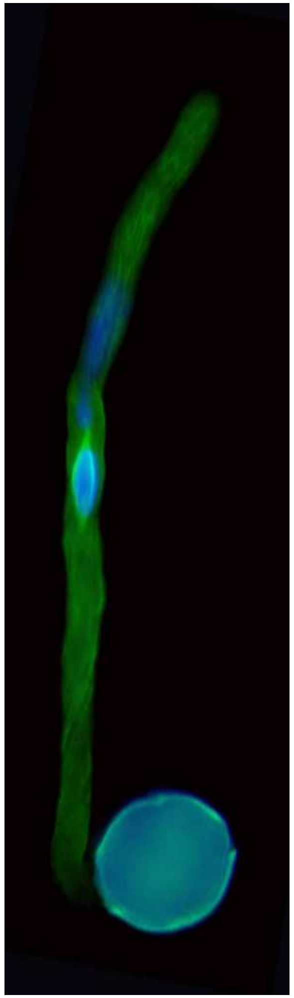
Endosperm,
Haemanthus sp.

Studium cytoskeletálních systémů: fixované buňky



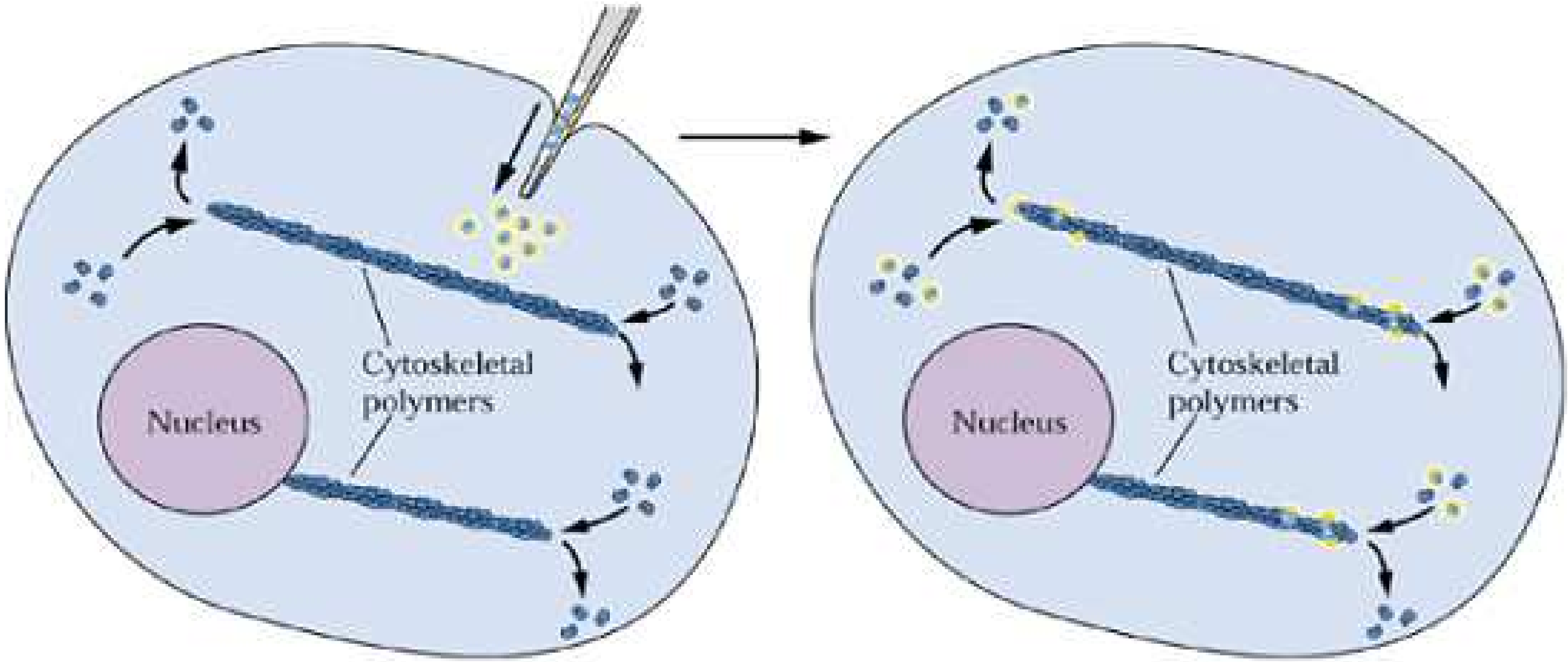
El. mikroskopie
(mrkev - protoplast TK)

pylové láčky tabáku (L. Synek)



Tubulin (Ab - nepřímo)
Aktin (faloidin – přímo)
DNA (DAPI)

Studium cytoskeletu *in vivo*: od mikroinjekce přes expresi GFP-značených proteinů po farmakologii

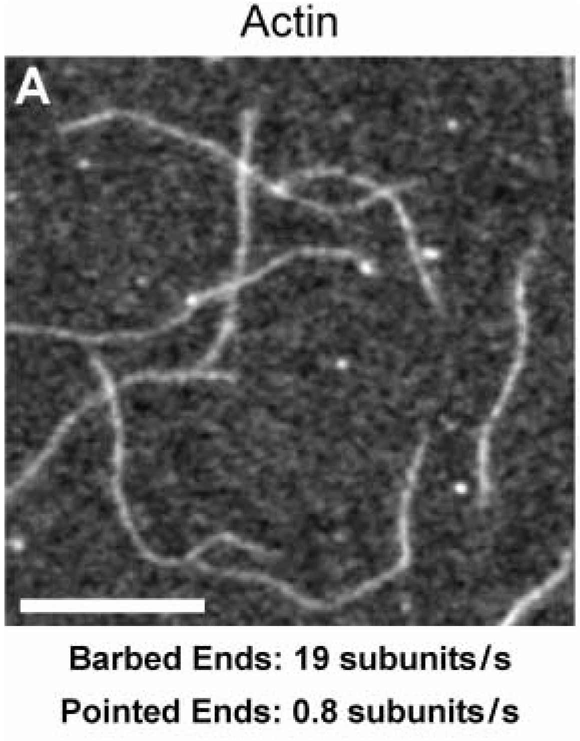


Infiltrace listů - tabák, N. benthamiana)

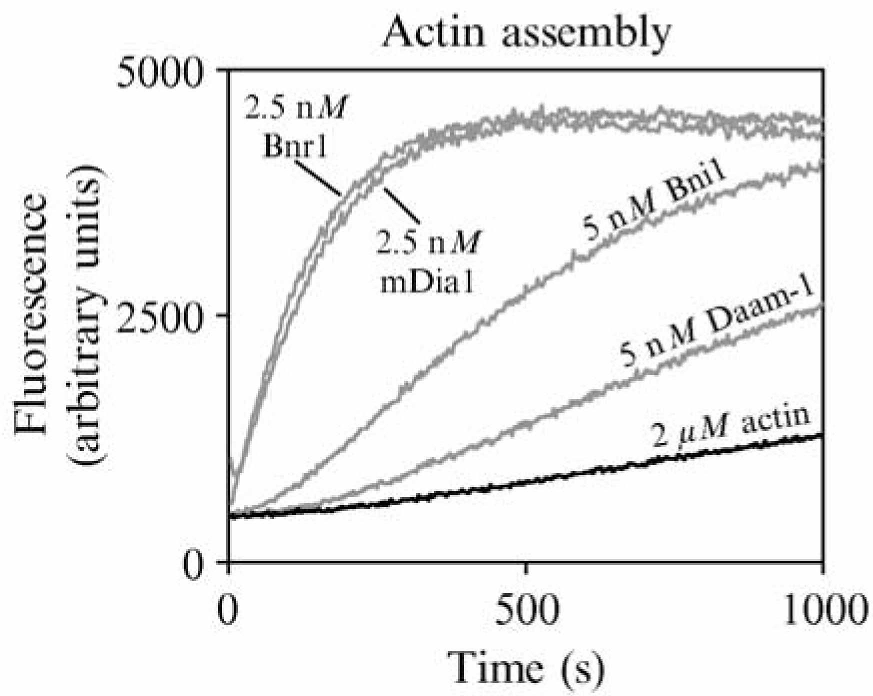
Farmakologické metody – cytoskeletální jedy

tubulin-destabilizing	tubulin-stabilizing	actin-destabilizing	actin-stabilizing
oryzalin	taxol	latrunculin B	phalloidin
nocodazole propyzamid		cytochalasin D	

Studium cytoskeletu *in vitro*



TIRF mikroskopie –
Kovar et al. 2003)

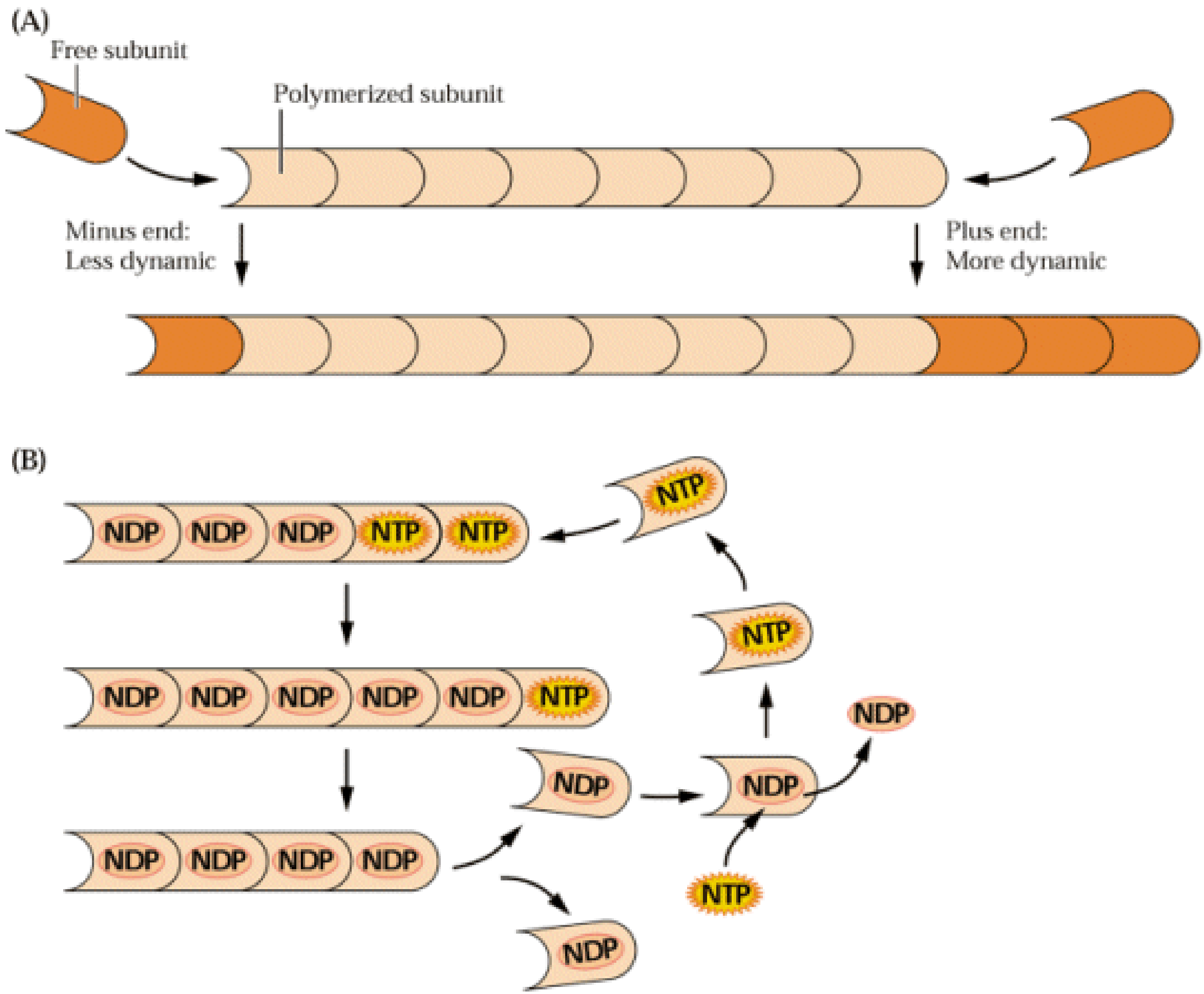


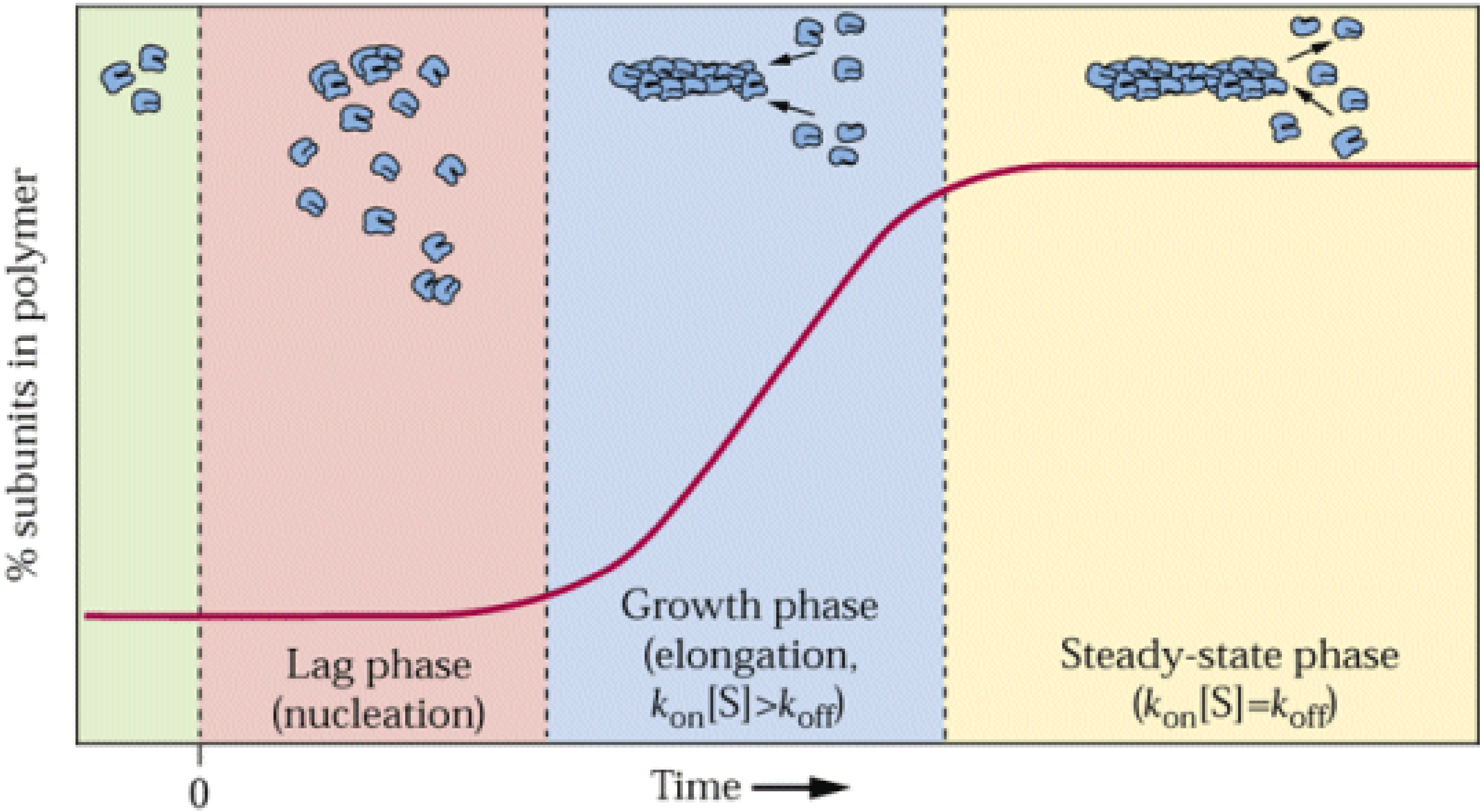
(fluorescenčně značený aktin v přítomnosti proteinů stimulujících tvorbu vláken – Moseley et al. 2006)

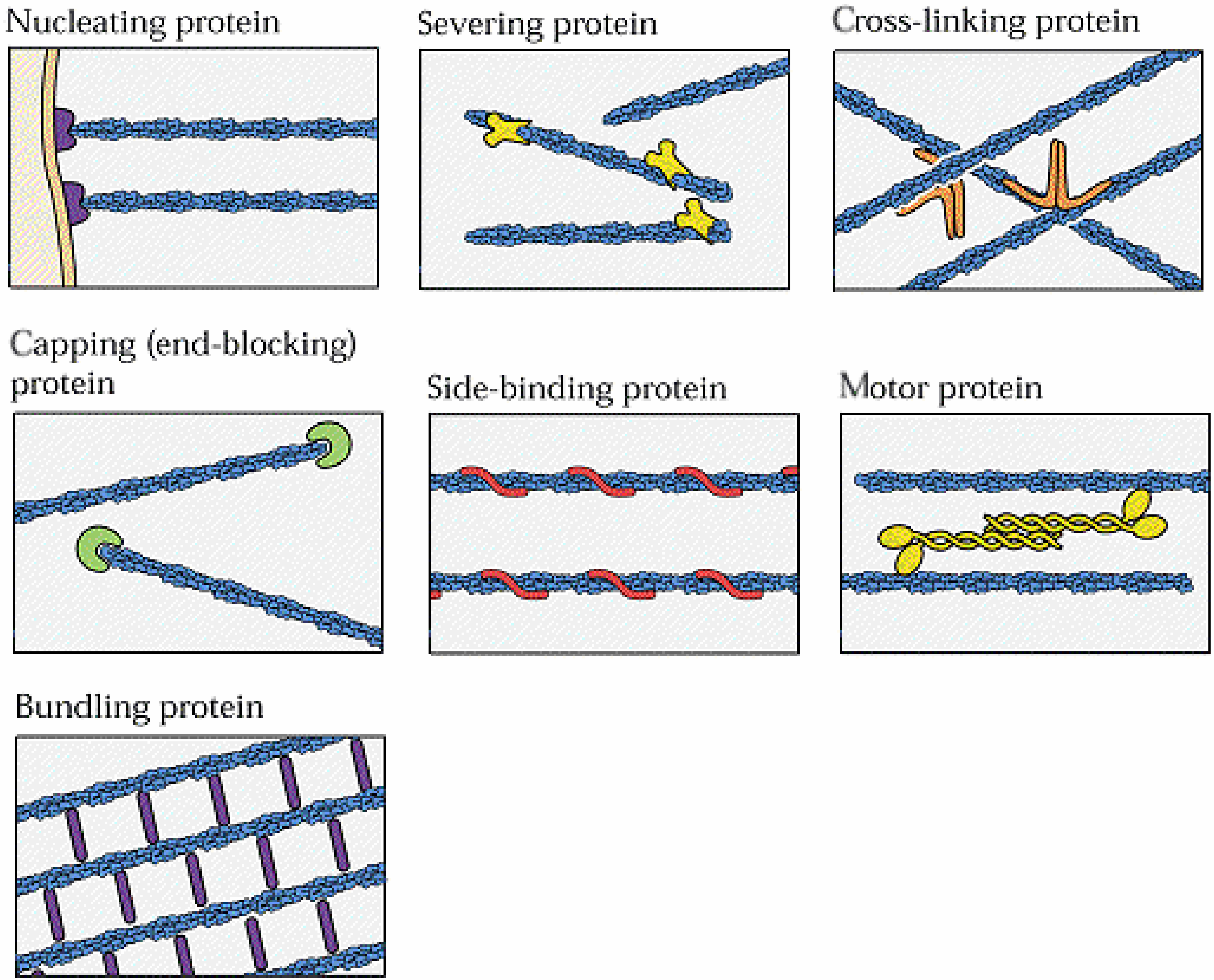
Obecné rysy cytoskeletálních systémů

- vlákna jsou polymery z 1 až několika typů podjednotek
- asociované proteiny kontrolují
  - strukturu/přestavby
  - diverzitu funkce
- cytoskelet jakožto „mechanický“ systém reaguje také na ryze fyzikální podněty
  - a to nejen pasivně

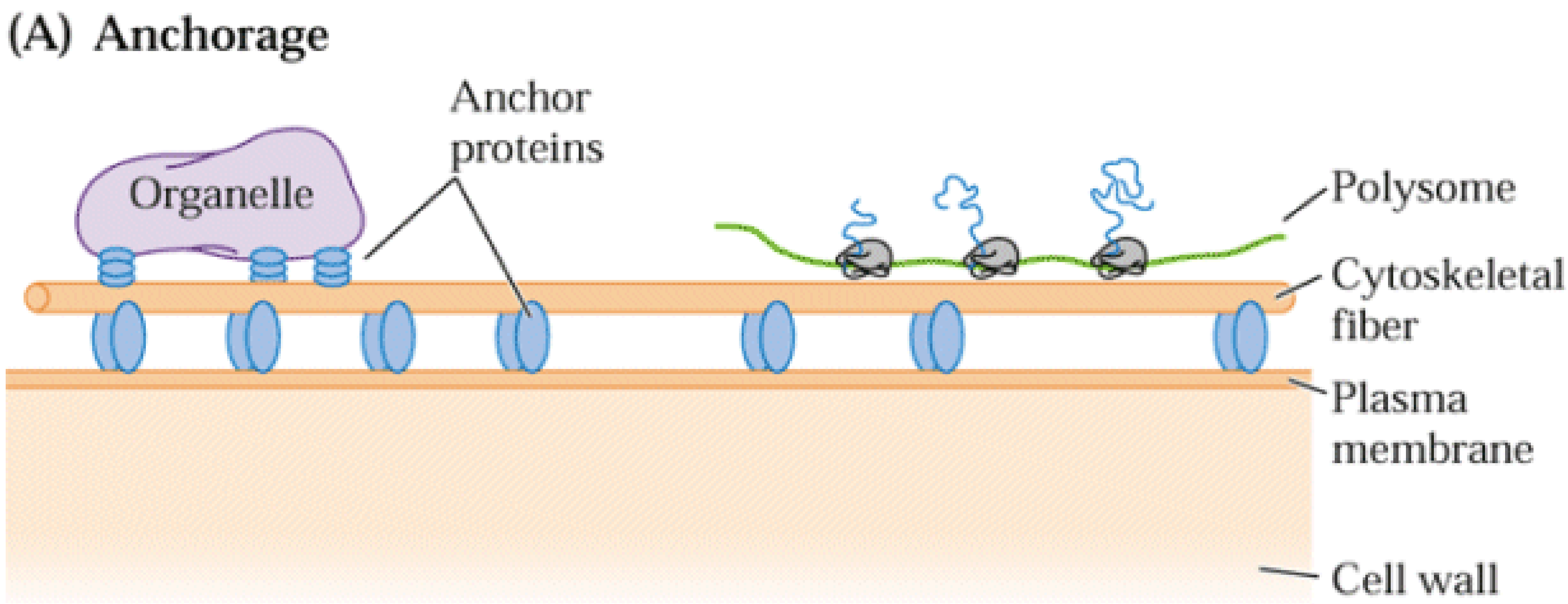
Vznik polárního vlákna z monomerů - obecný princip

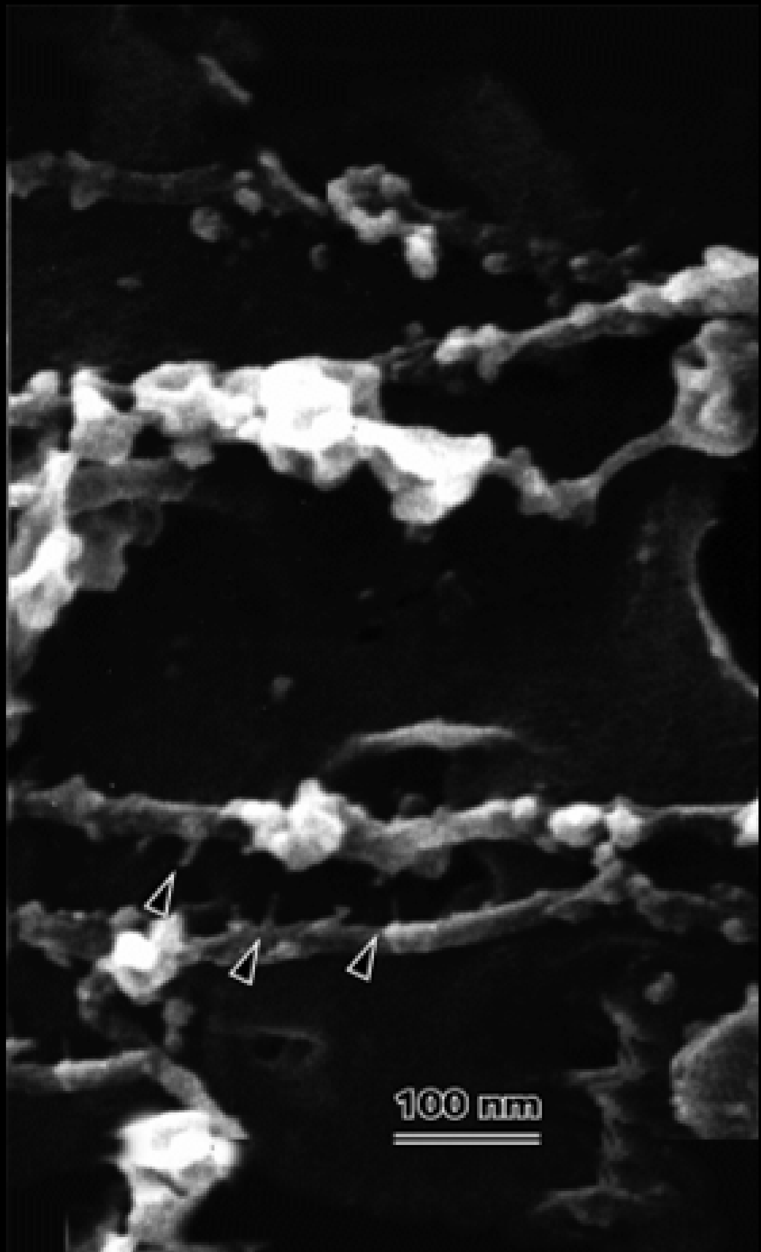


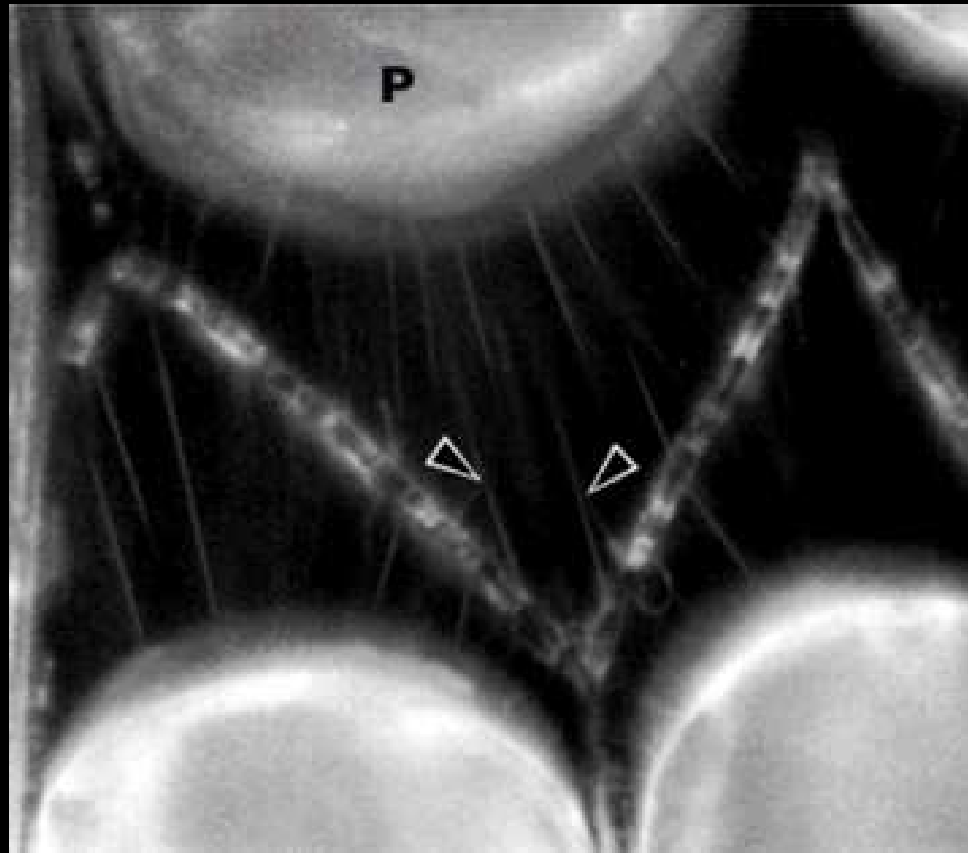

% subunits in polymer
Lag phase
(nucleation)
Growth phase
(elongation,
$k_{on}[S]>k_{off}$)
Steady-state phase
($k_{on}[S]=k_{off}$)
0
Time


Nucleating protein
Severing protein
Cross-linking protein
Capping (end-blocking) protein
Side-binding protein
Motor protein
Bundling protein

Obecné funkce cytoskeletu



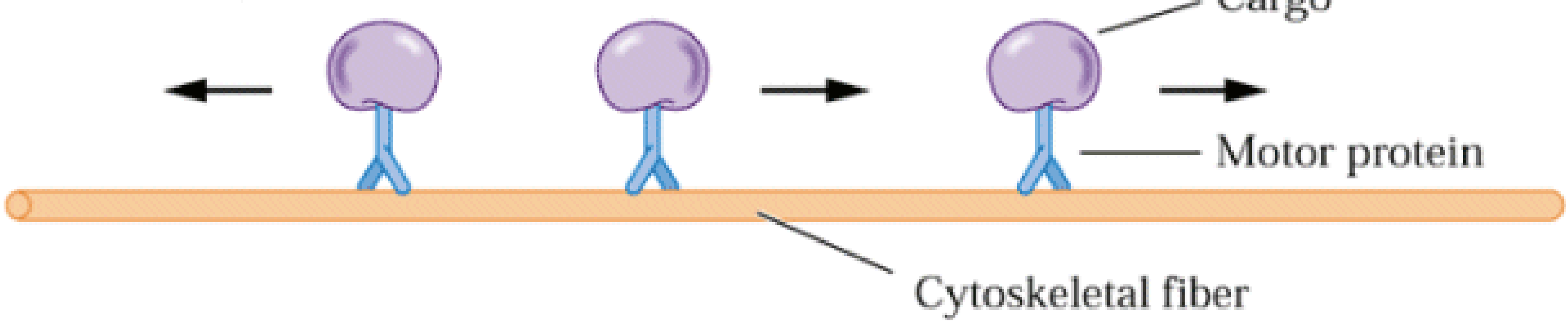


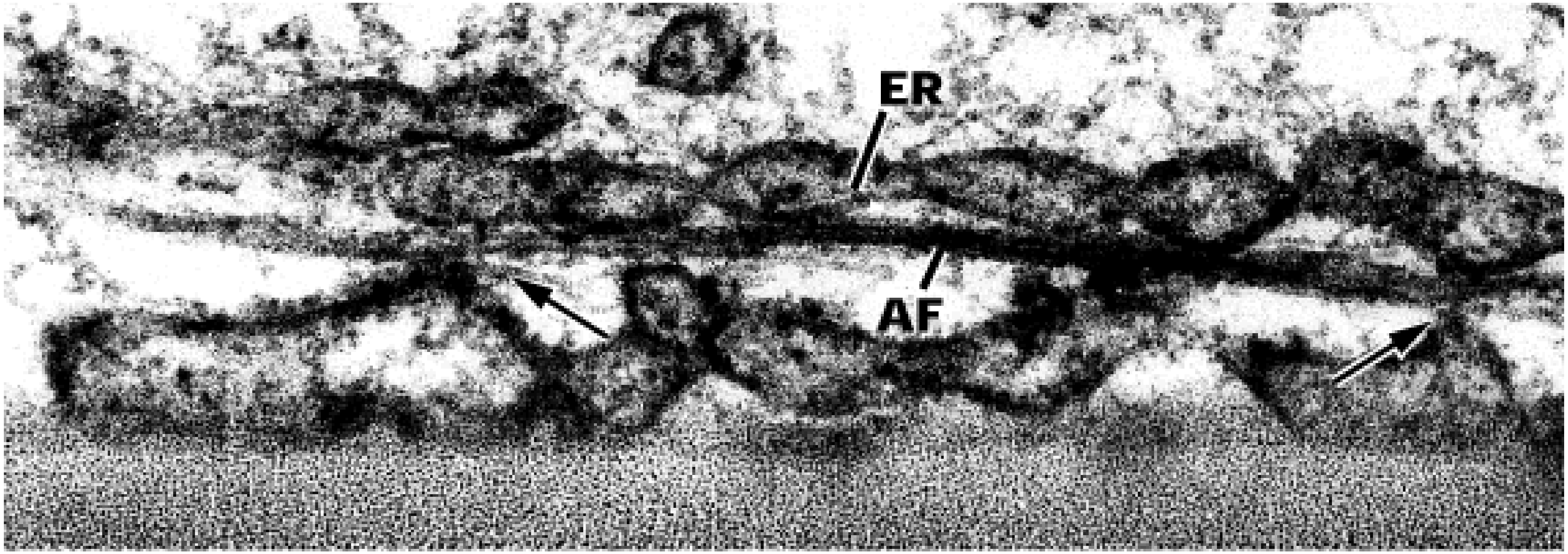


Hechtovy provazce

asociace kortikál. mt s membránou (cibule)

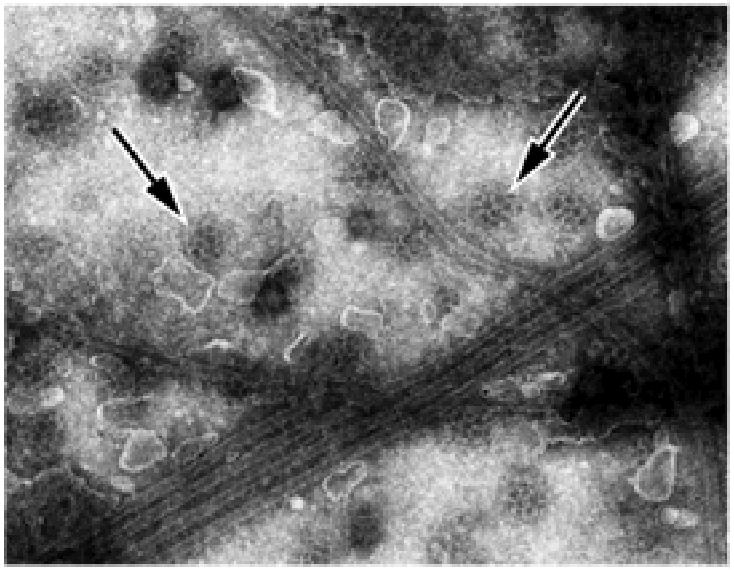
(B) Motility



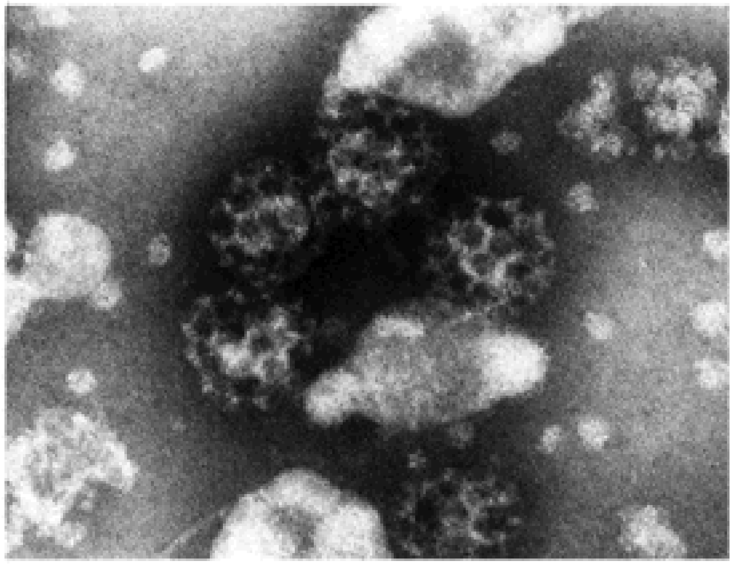


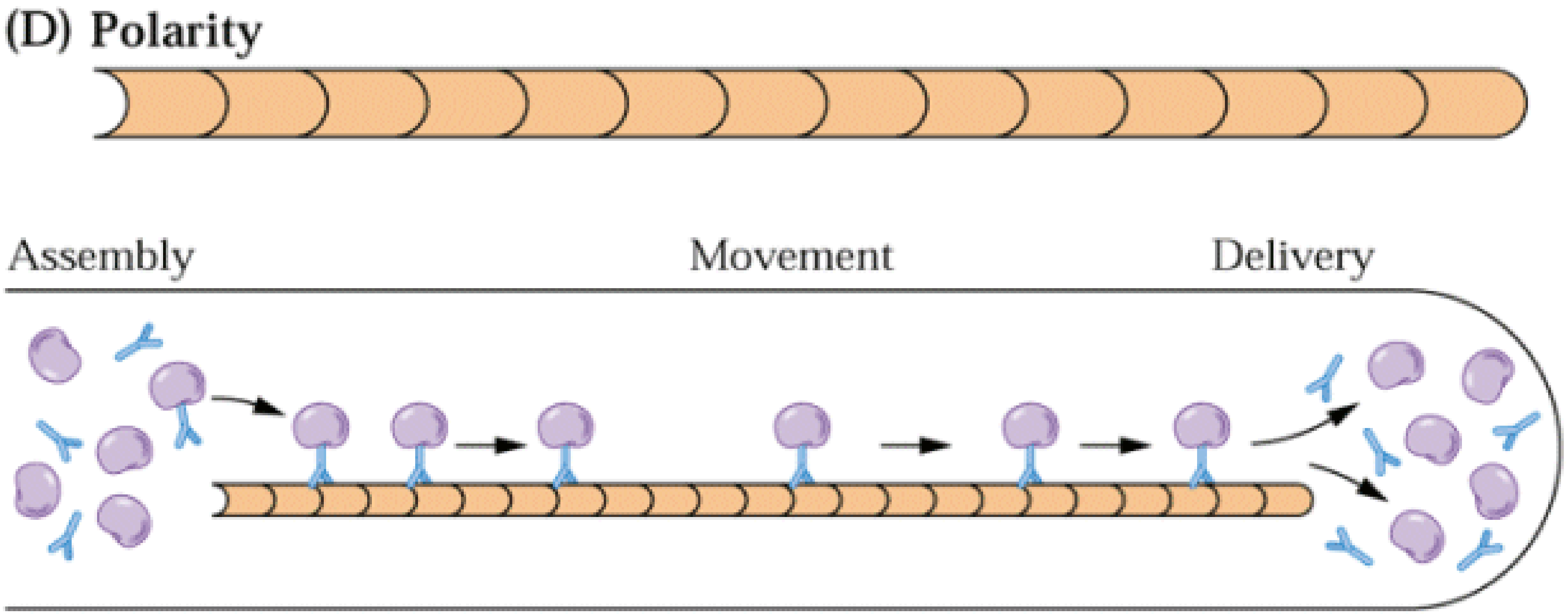
ER and clathrin-coated vesicles associated with actin and mt

(A)

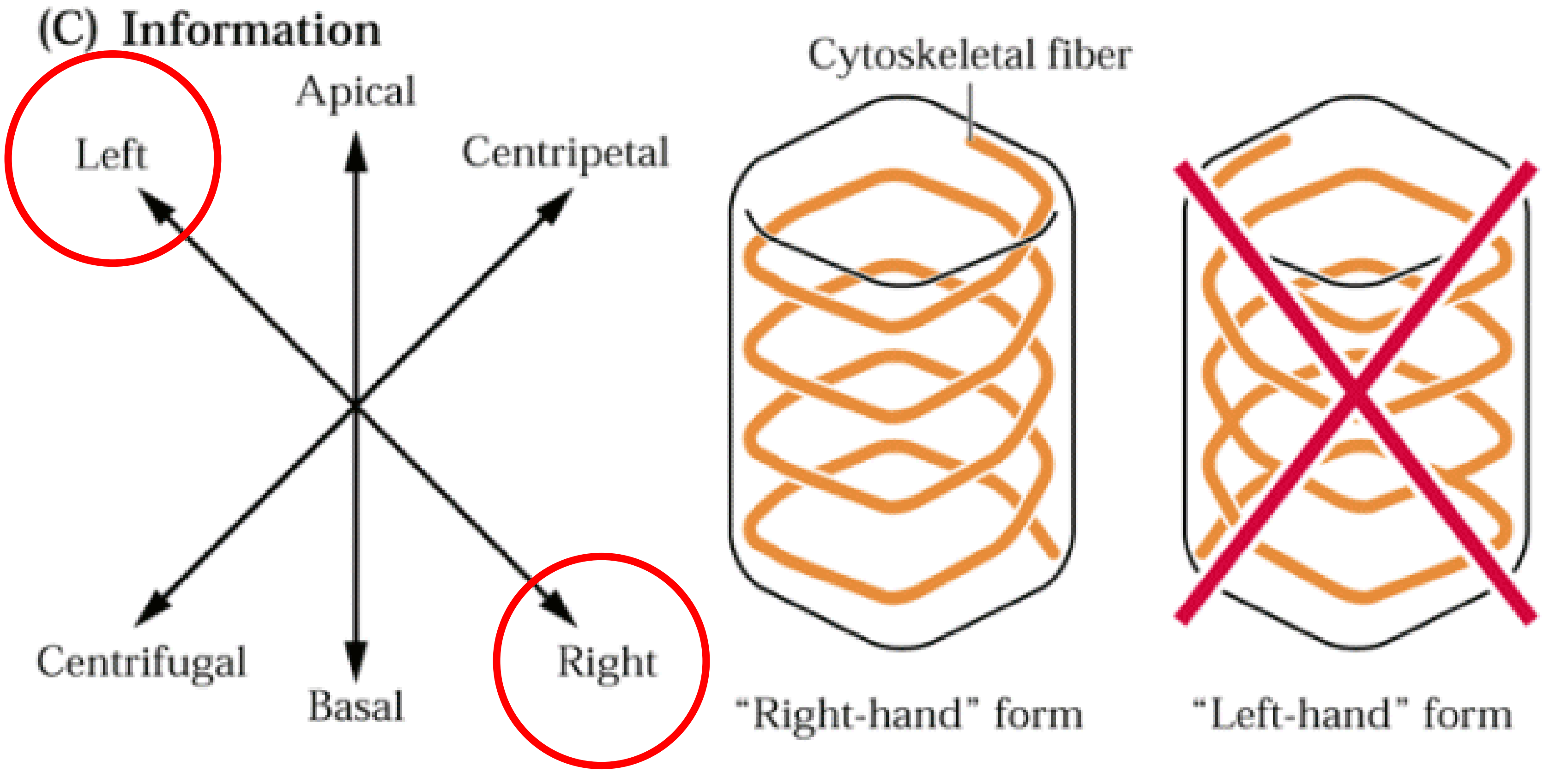


(B)





(dáno polaritou podjednotek)



(existuje preference směrů)

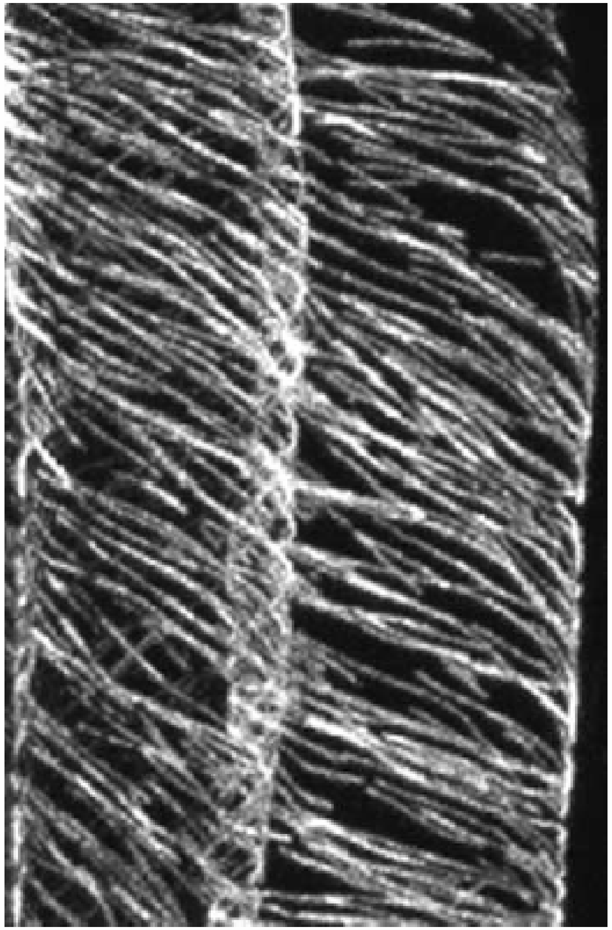
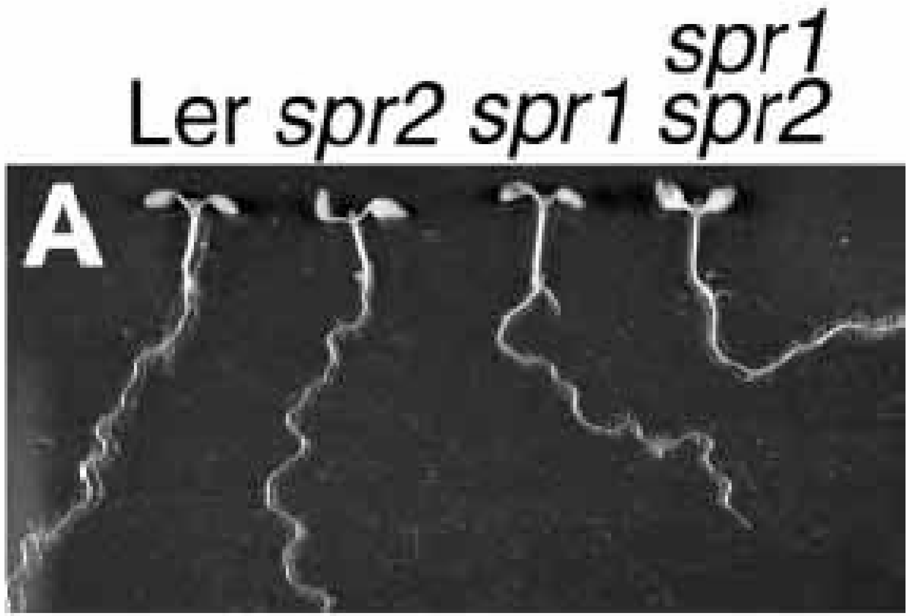
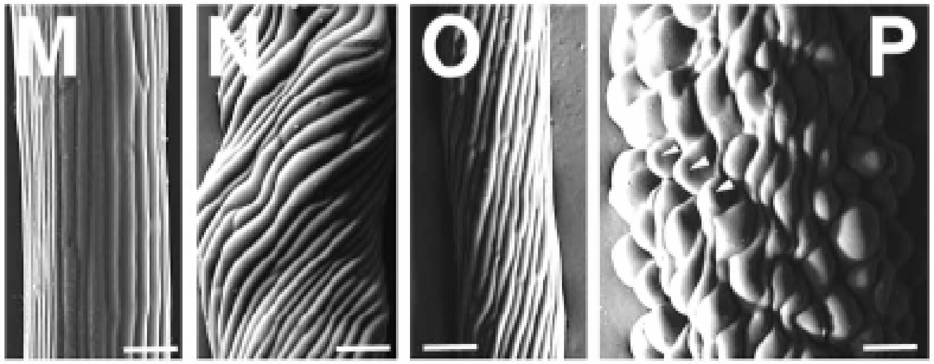


Figure 1. Microtubules in the *spiral* Mutant.

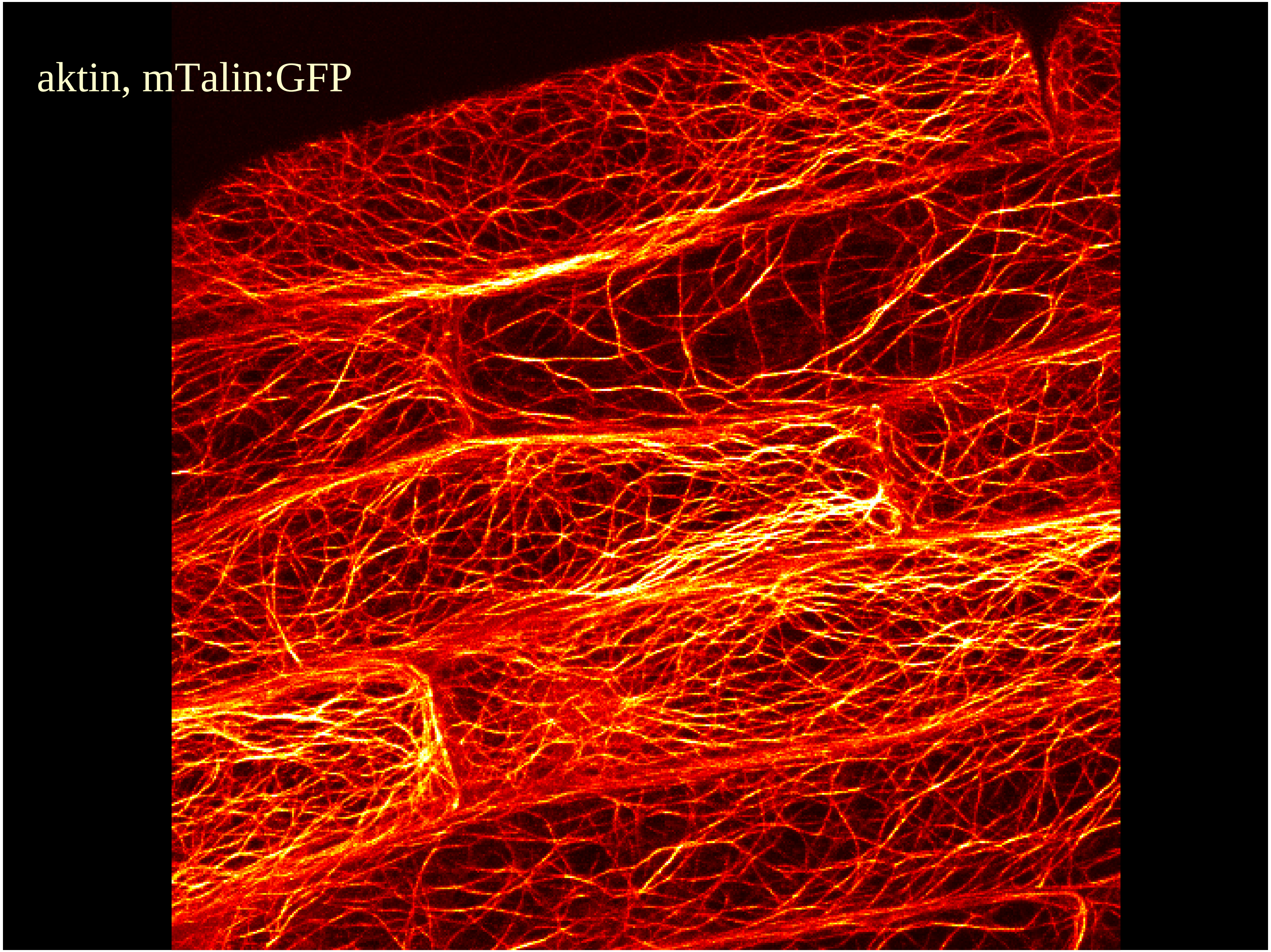
In the *spiral* mutant of Arabidopsis, the cortical microtubules of two epidermal cells wind around the cortex in left-handed *S*-shaped helices. Each single fluorescent strand is likely to represent a bundle of several microtubules. (*Figure courtesy of Keiko Sugimoto.*)



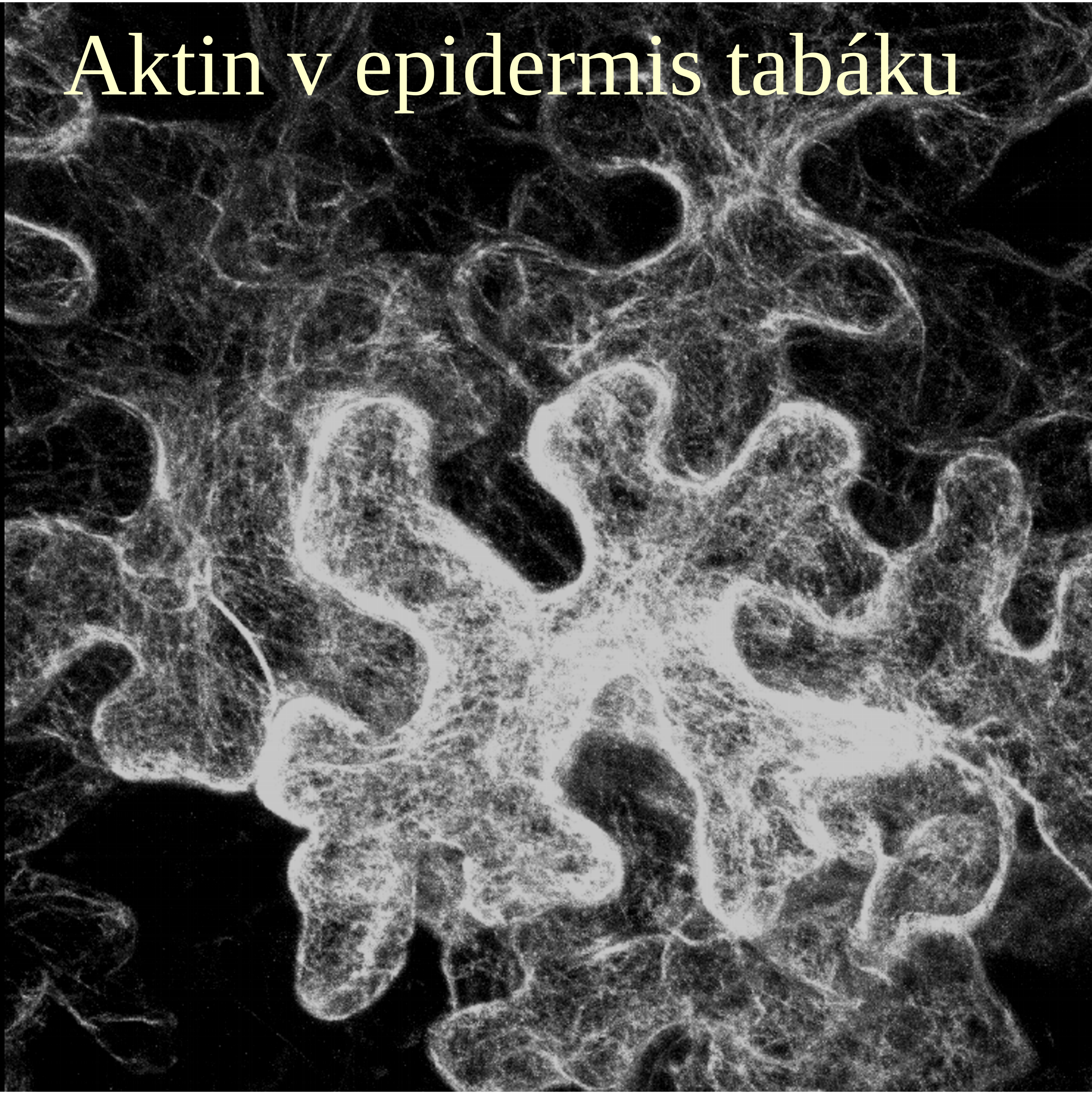


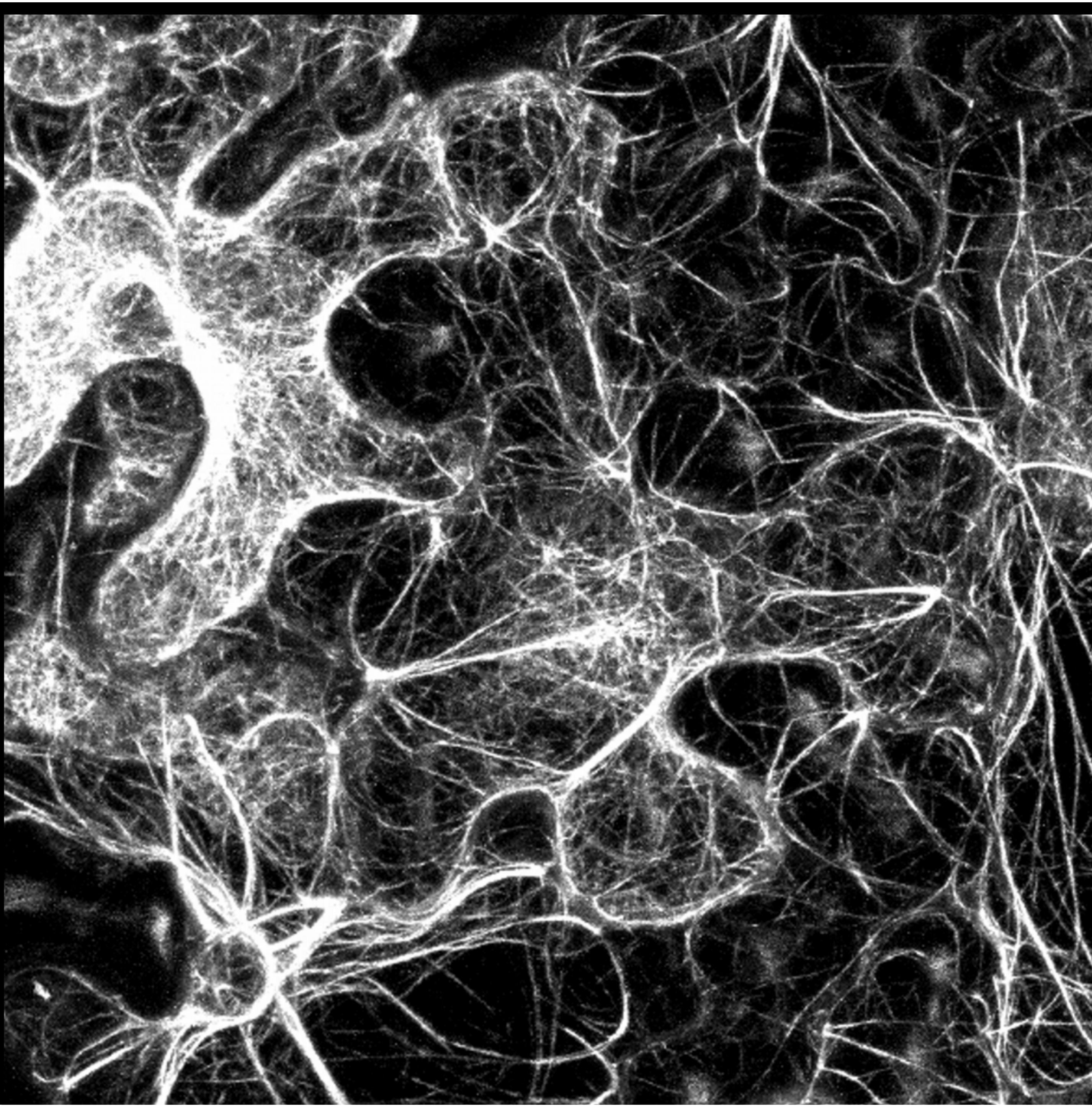
Základní cytoskeletální systémy eukaryot (?)

- Aktinový (mikrofilamenta)
  - všude (... prokaryotní MreB)
- Tubulinový (mikrotubuly)
  - všude (... prokaryotní FtsZ)
- Intermediální filamenta
  - „klasická“ zatím jen u Metazoí? (keratin, vimentin, neurofilamenta, laminy...)

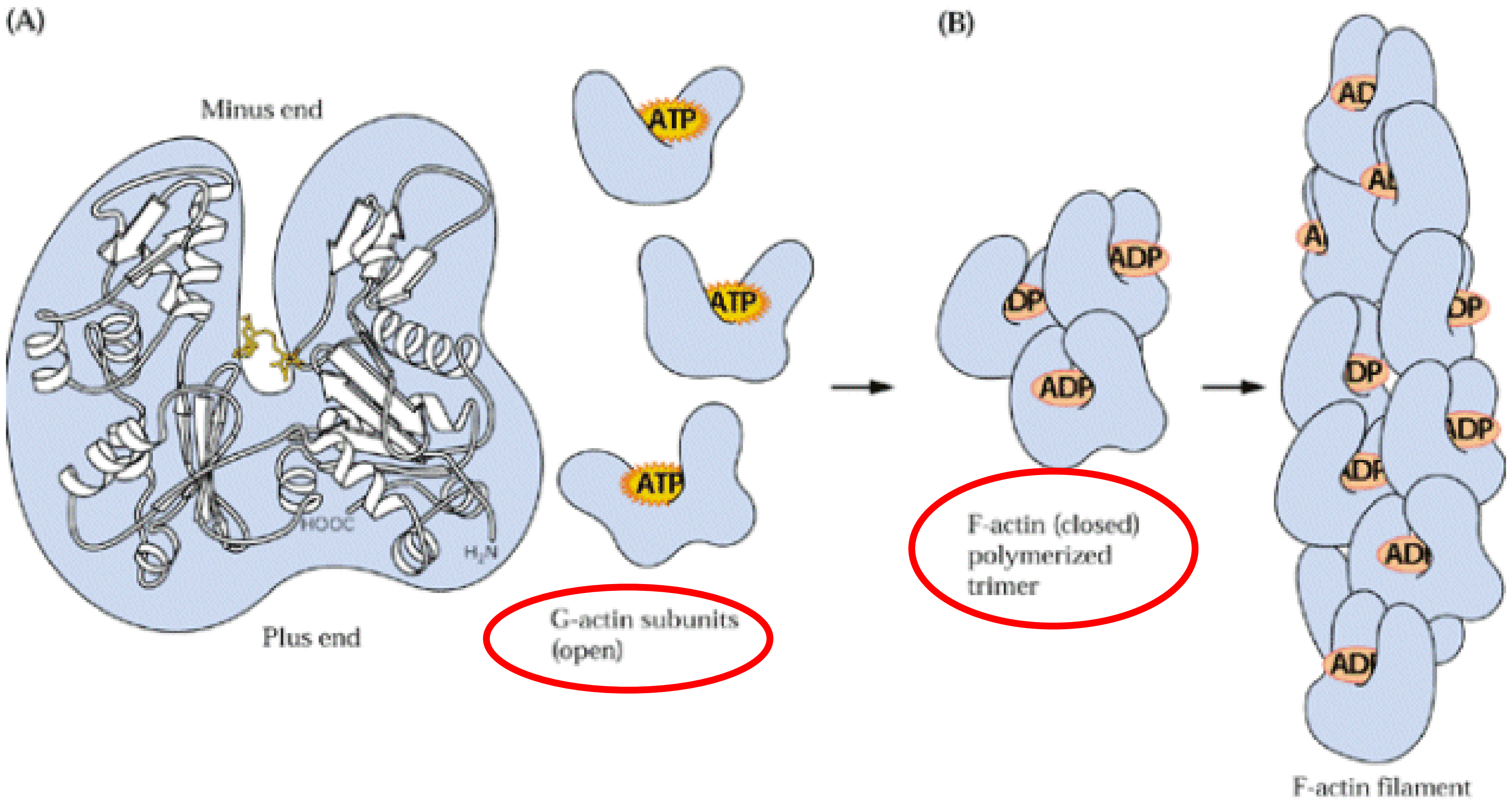

aktin, mTalin:GFP

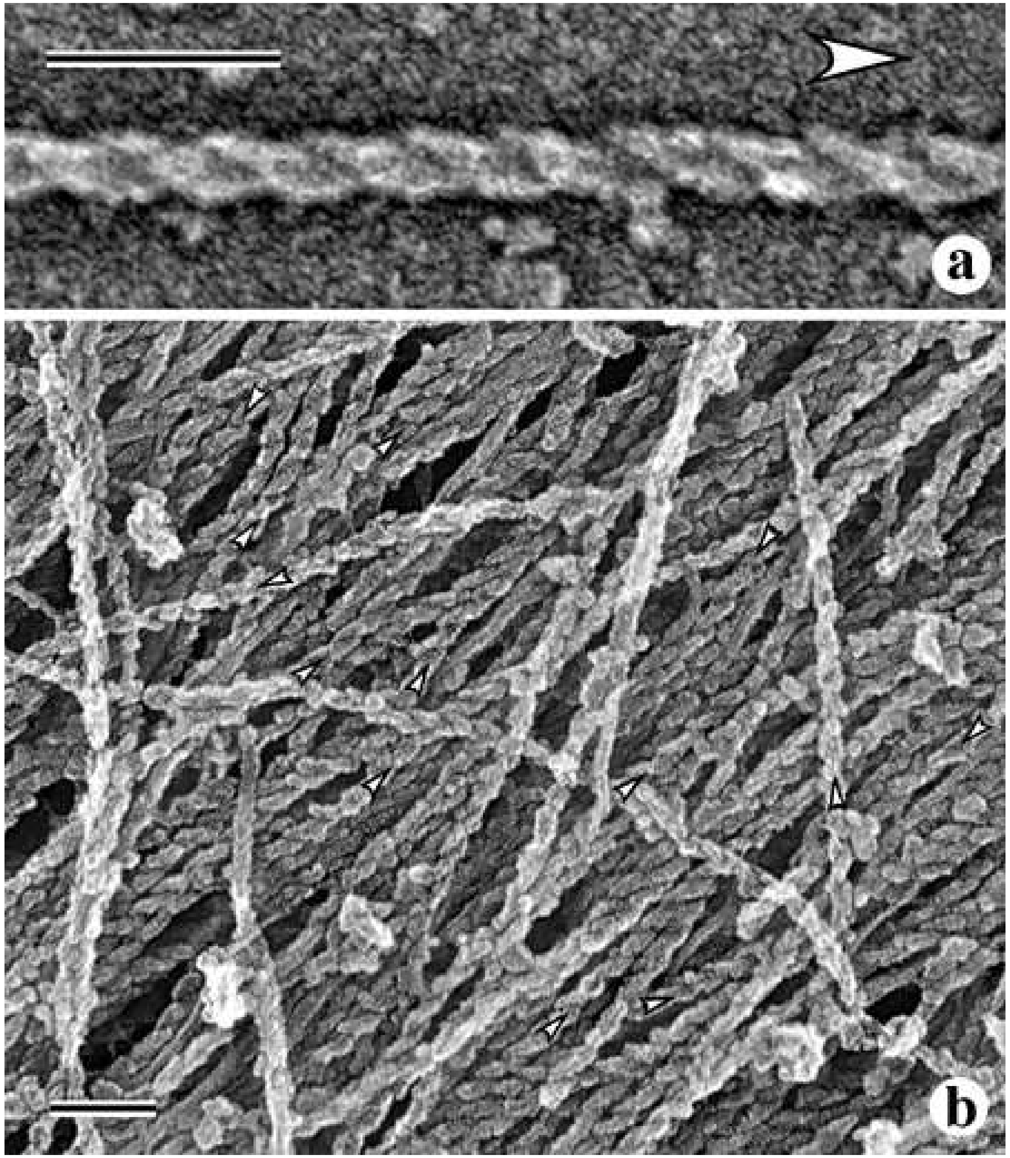
Aktin v epidermis tabáku



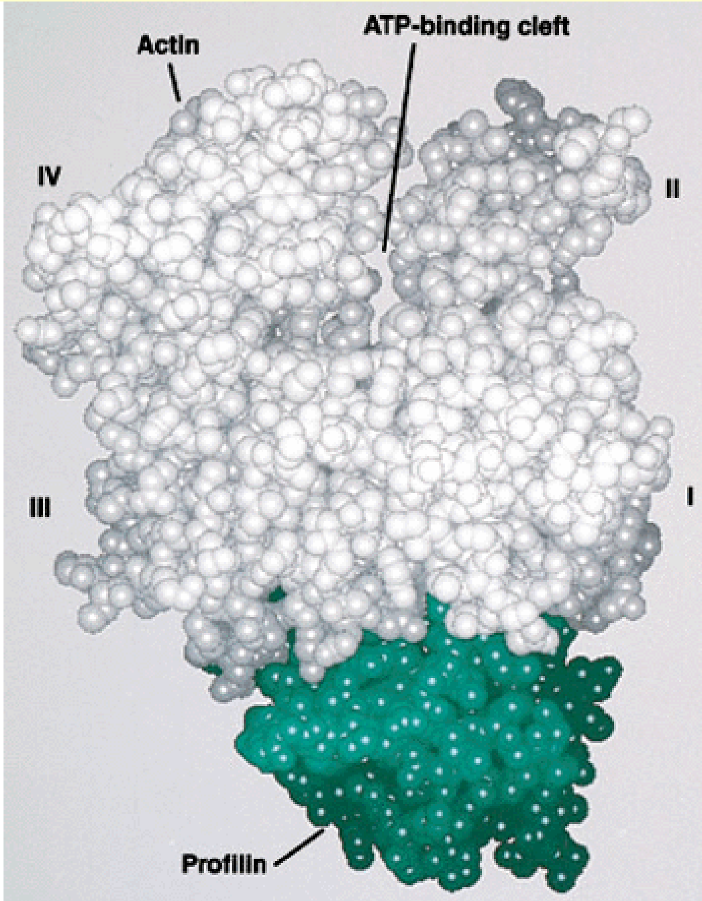


Aktinové vlákno a jeho monomer



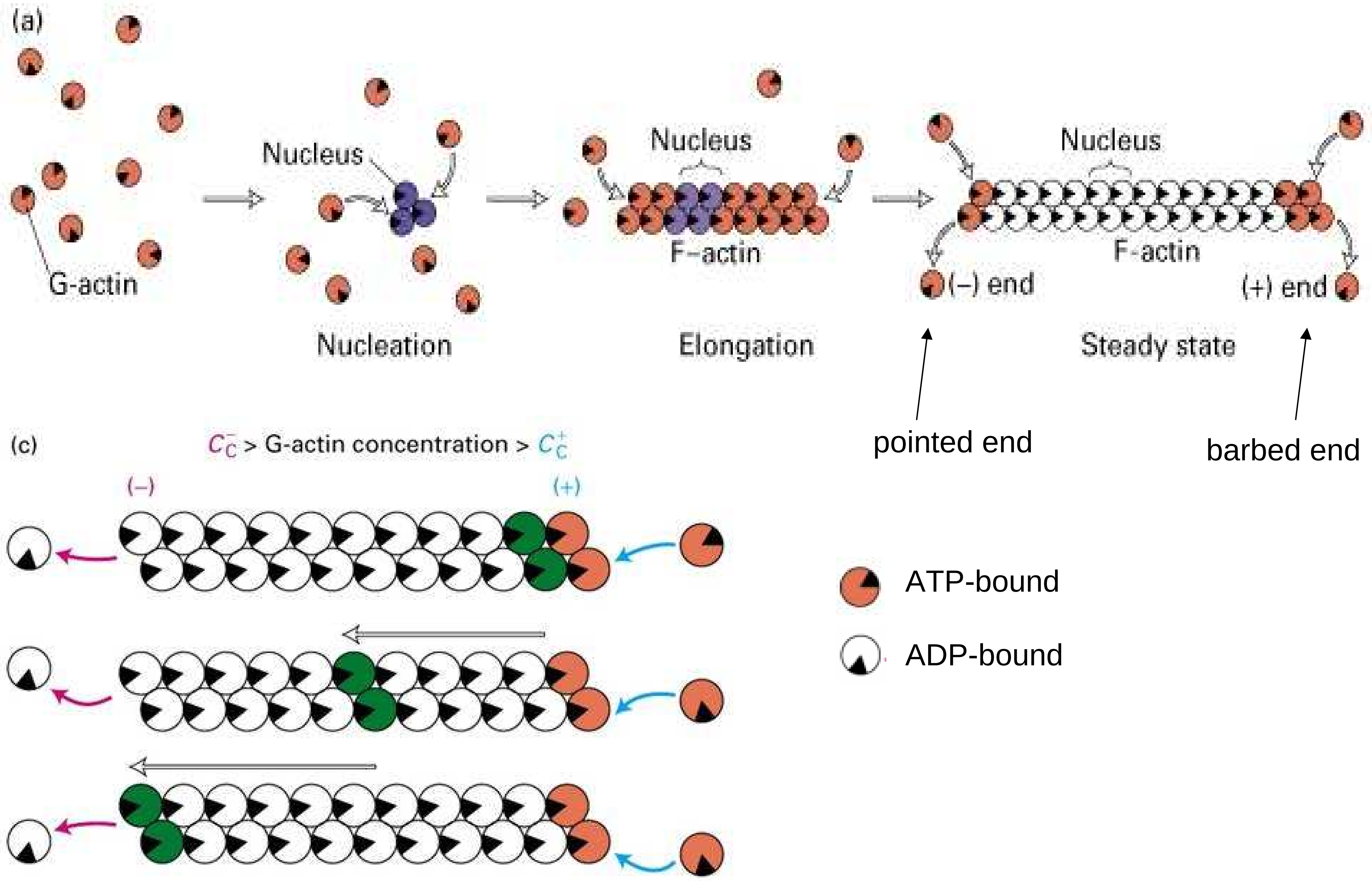


(a). S1 (myosin fragment)-decorated actin filament displays a helical rope-like appearance; the thicker part of a turn is directed to the pointed end of a filament (direction of the arrowhead). (b), part of an actin filament bundle from a REF-52 fibroblast after S1 decoration. Bars, 0.1 um

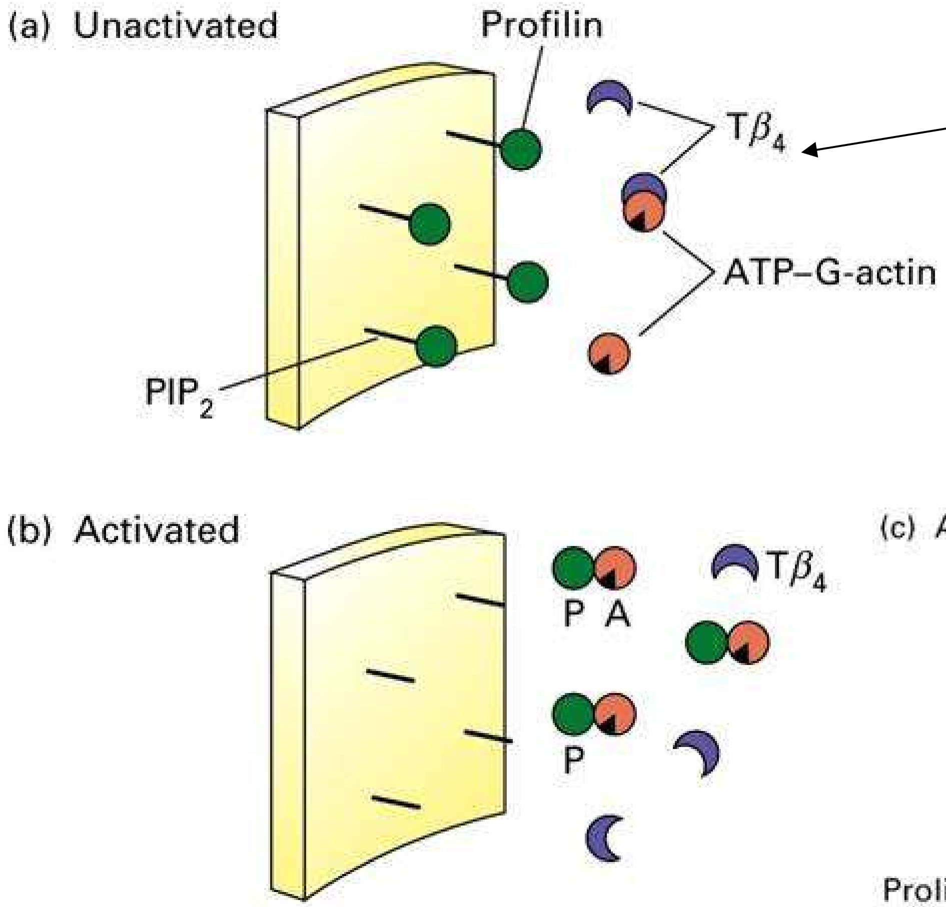


T. M. Svitkina and G.G. Borisy, "Methods in Enzymology", volume "Molecular Motors and the Cytoskeleton: Part B", 298: 570-92, 1998.

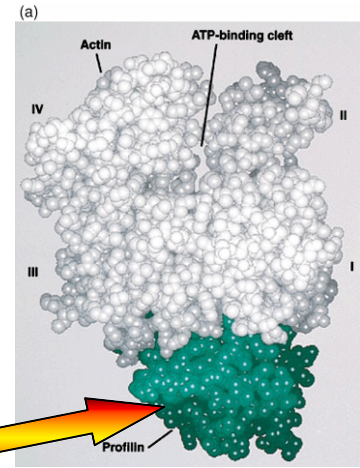
Dynamika aktinových vláken: polymerace a treadmilling



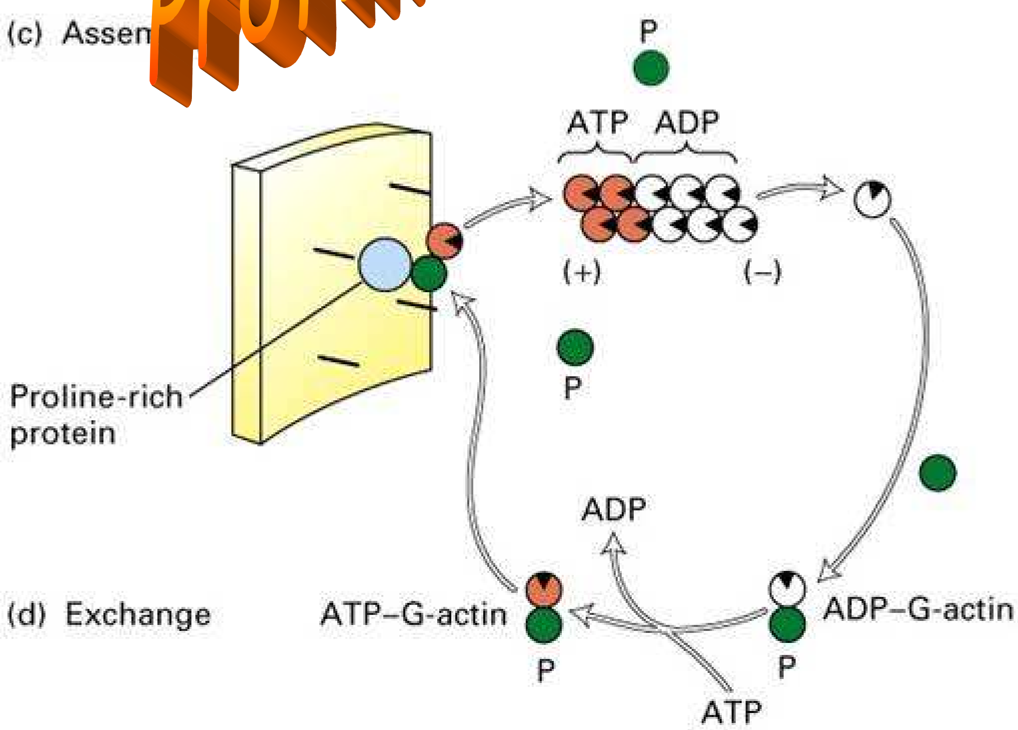
G-aktin-vazebné proteiny: regulace dostupnosti podjednotek



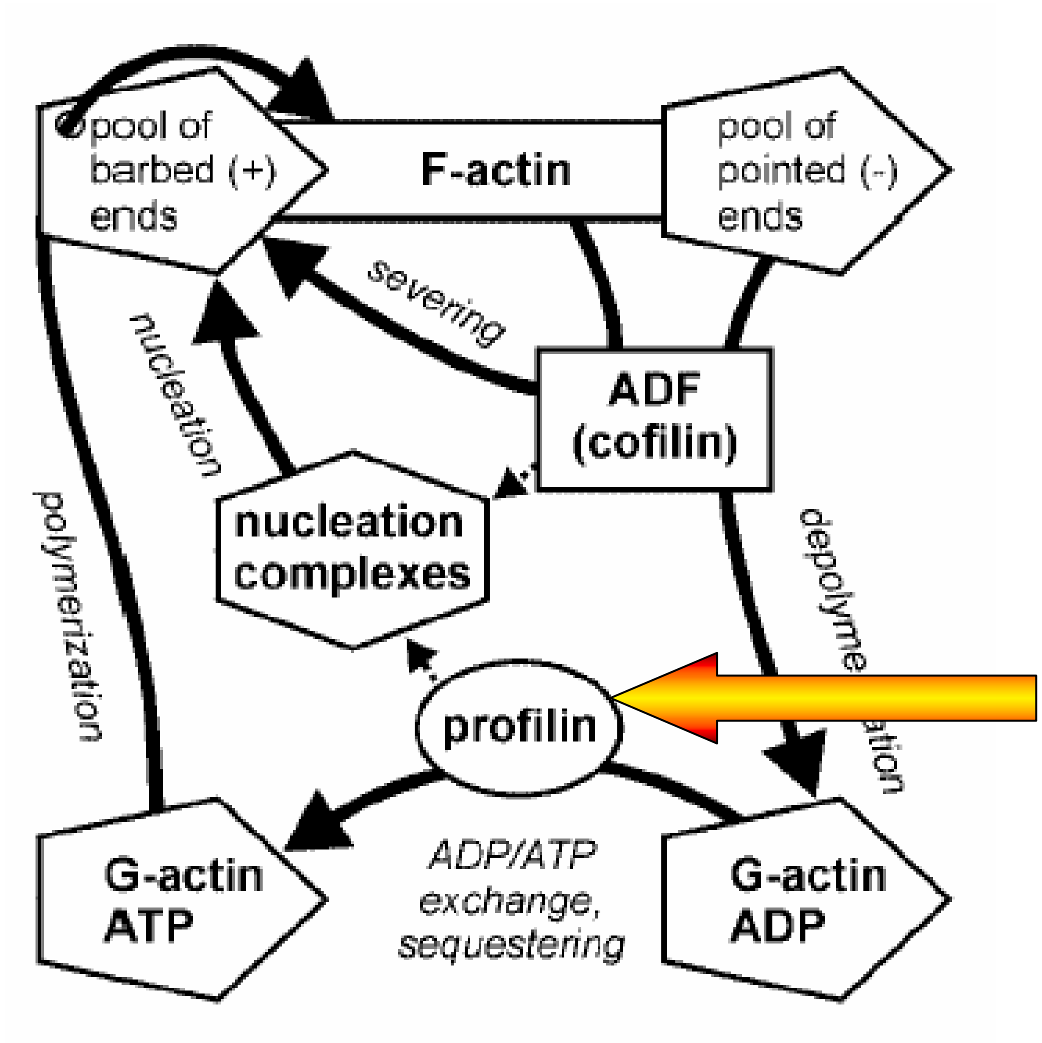
sequestered G-actin here:
thymosine beta4 may be vertebrate-specific?



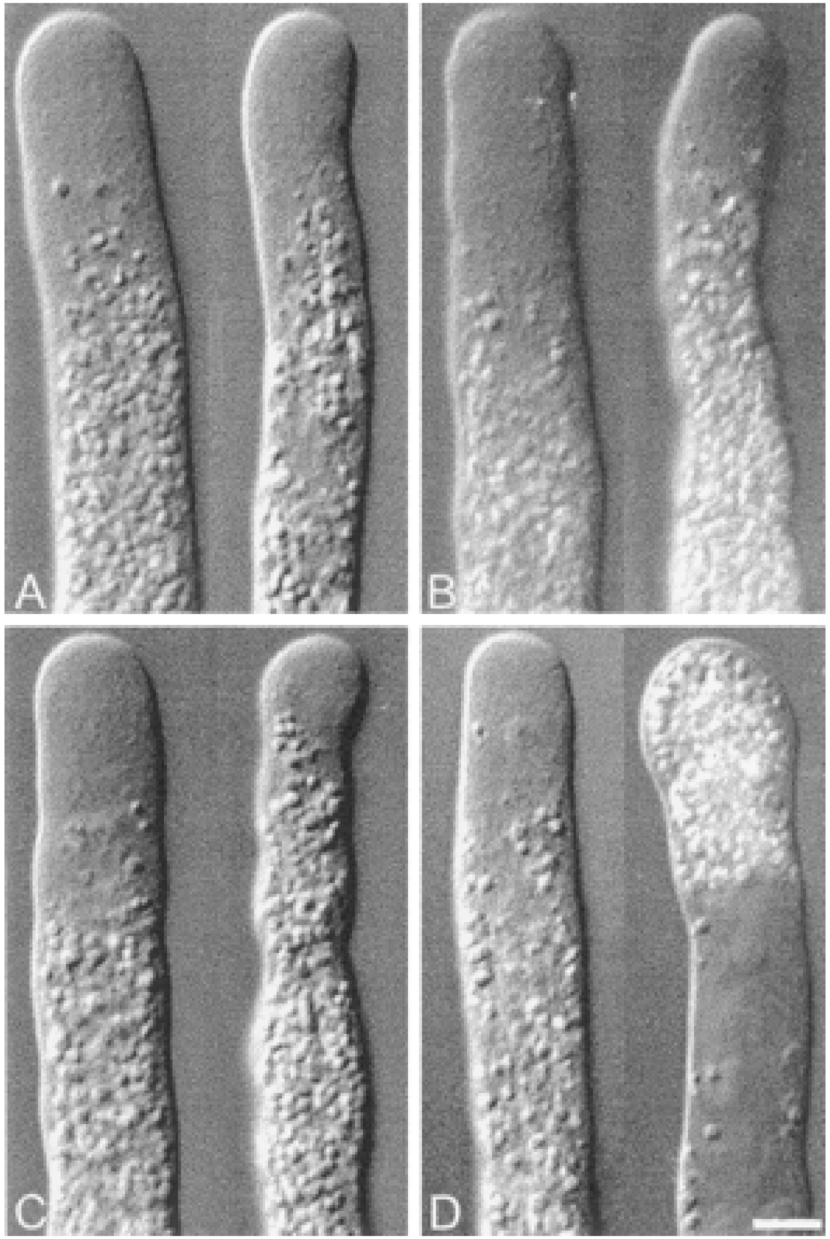
Profilin



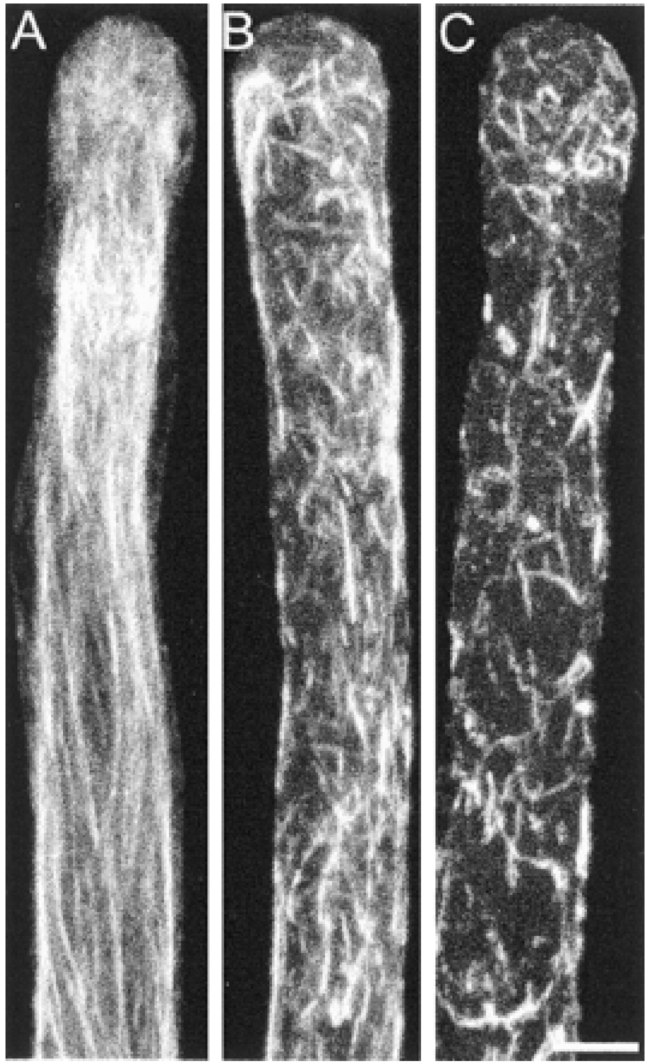
Profilin a jiné proteiny ovlivňující dynamiku aktinu



Injekce profilinu inhibuje růst pylových láček a narušuje strukturu aktinu



Pollen tubes injected with increasing doses of native pollen profilin. Leftmost cell - before injection, rightmost 20 min after injection. (A) 10 µM, (B) 16 µM, (C) 22 µM, and (D) 62 µM. Bar, 10 µm.

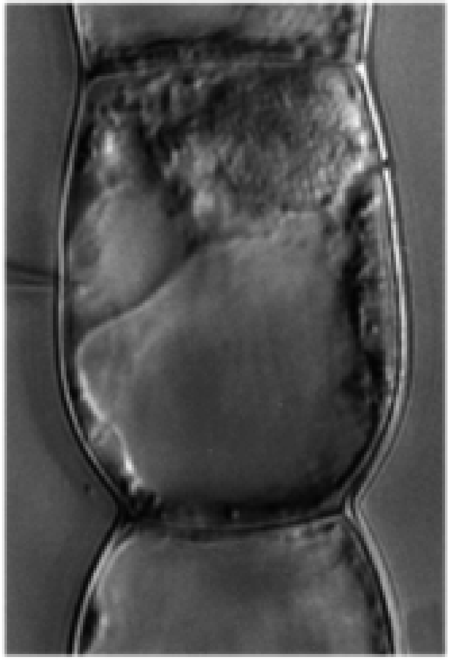


F-actin distribution of profilin-injected cells after chemical fixation and Alexa-phalloidin staining. Twenty minutes after injection cells were chemically fixed and stained with 0.3 µM Alexa-phalloidin. (A) Control cell. (B) Cell injected with a low concentration of profilin (~30 µM). (C) Cell injected with a higher concentration of profilin (>60 µM).

Vidali et al. 2001

(A)

Cytoplasmic streaming along transvacuolar strands is evident in an uninjected cell.



Ten minutes after profilin is microinjected, streaming has stopped and most of the transvacuolar strands have broken down.

Streaming continues unabated 10 minutes after bovine serum albumin (BSA) is microinjected into a control cell.

Tradescantia stamen hairs

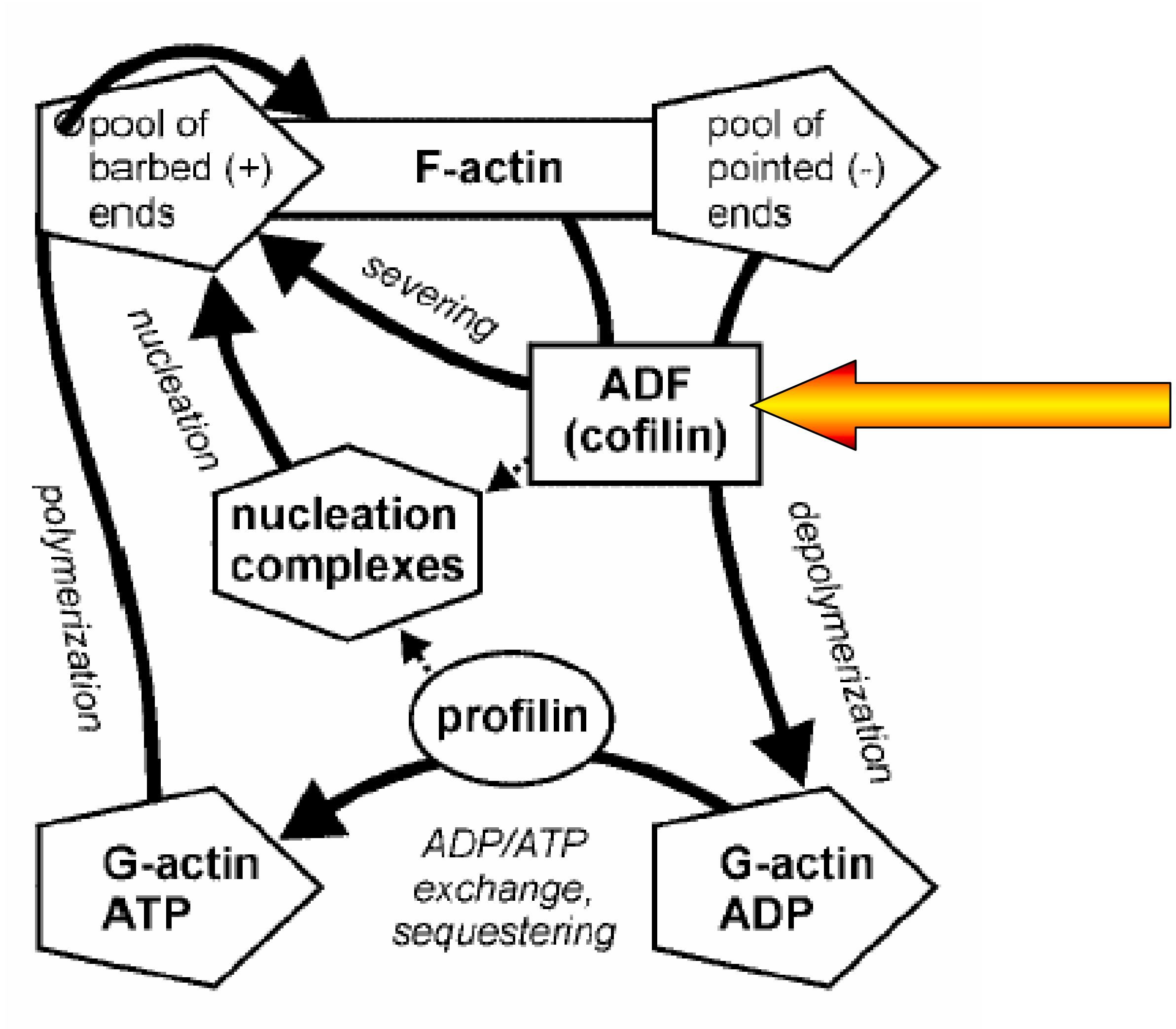
(B)

Anaphase cell undergoing chromosomal separation in an uninjected cell.

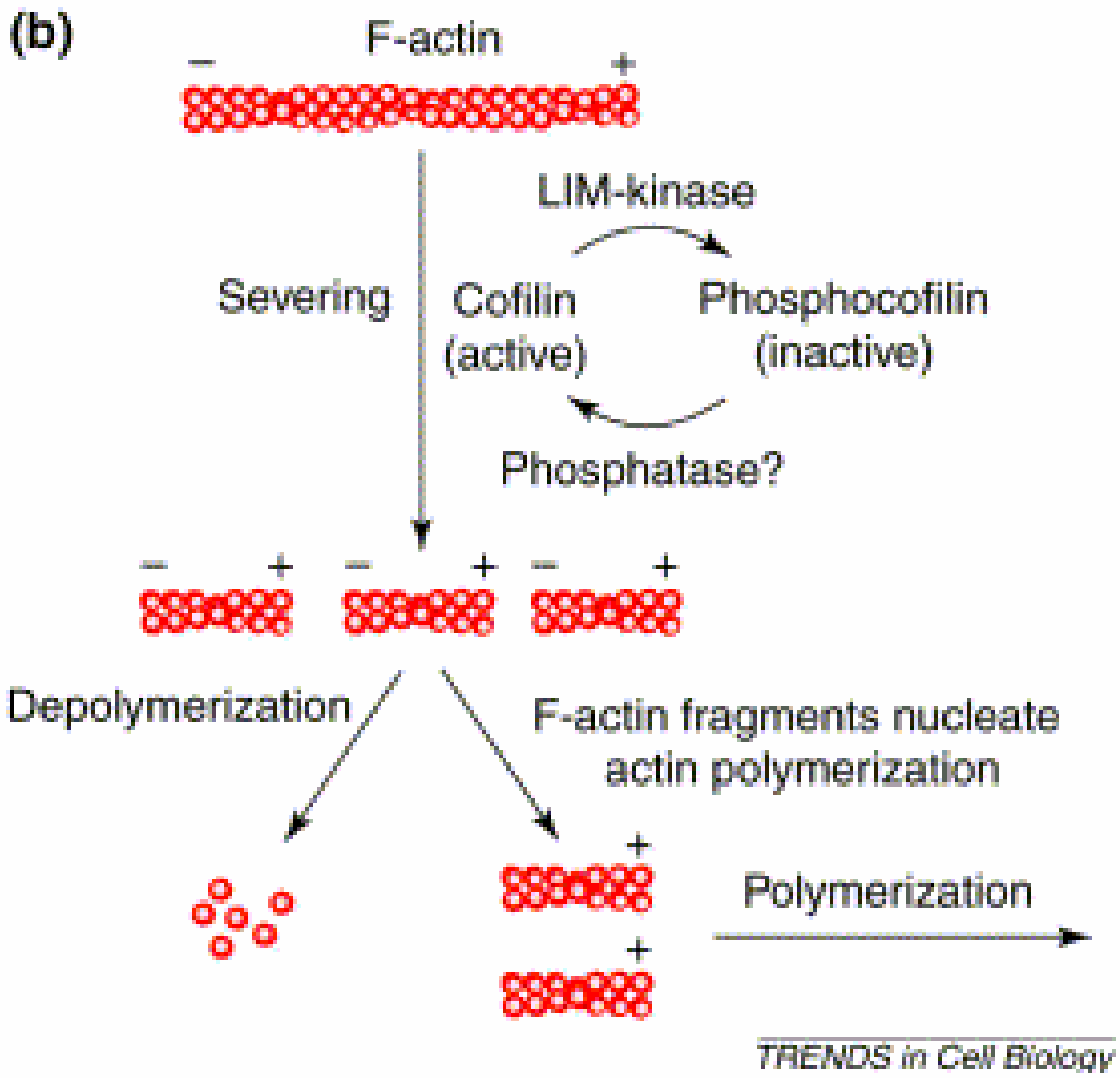
Fifty minutes after profilin is microinjected, chromosomal separation is complete, but a cell plate has not formed.

Twenty-five minutes after BSA is microinjected, a cell plate separates the control cell into two daughter cells.

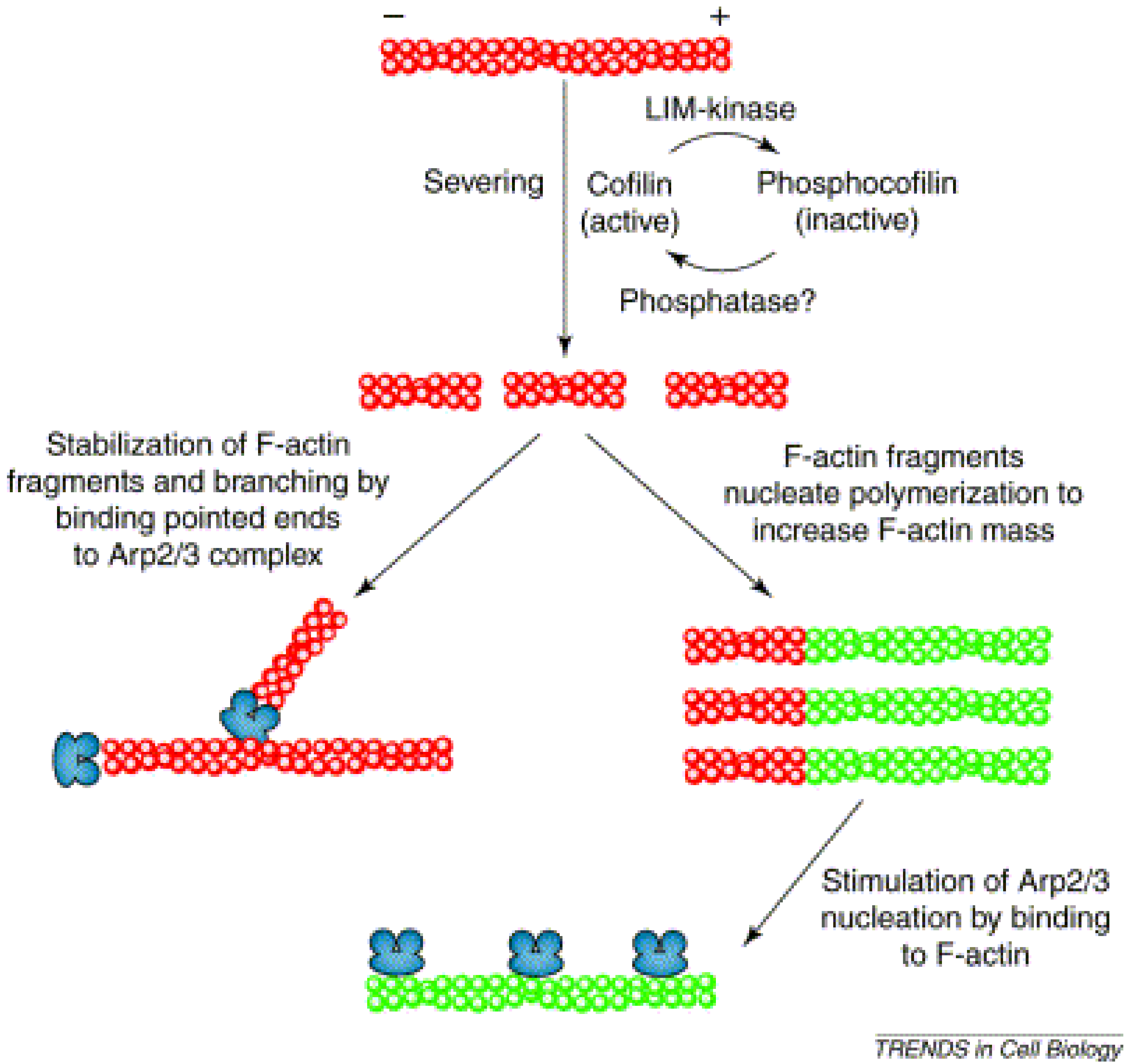
ADF (Actin depolymerizing factor)/cofilin



„Stříhání“ filamentů: ADF/cofilin

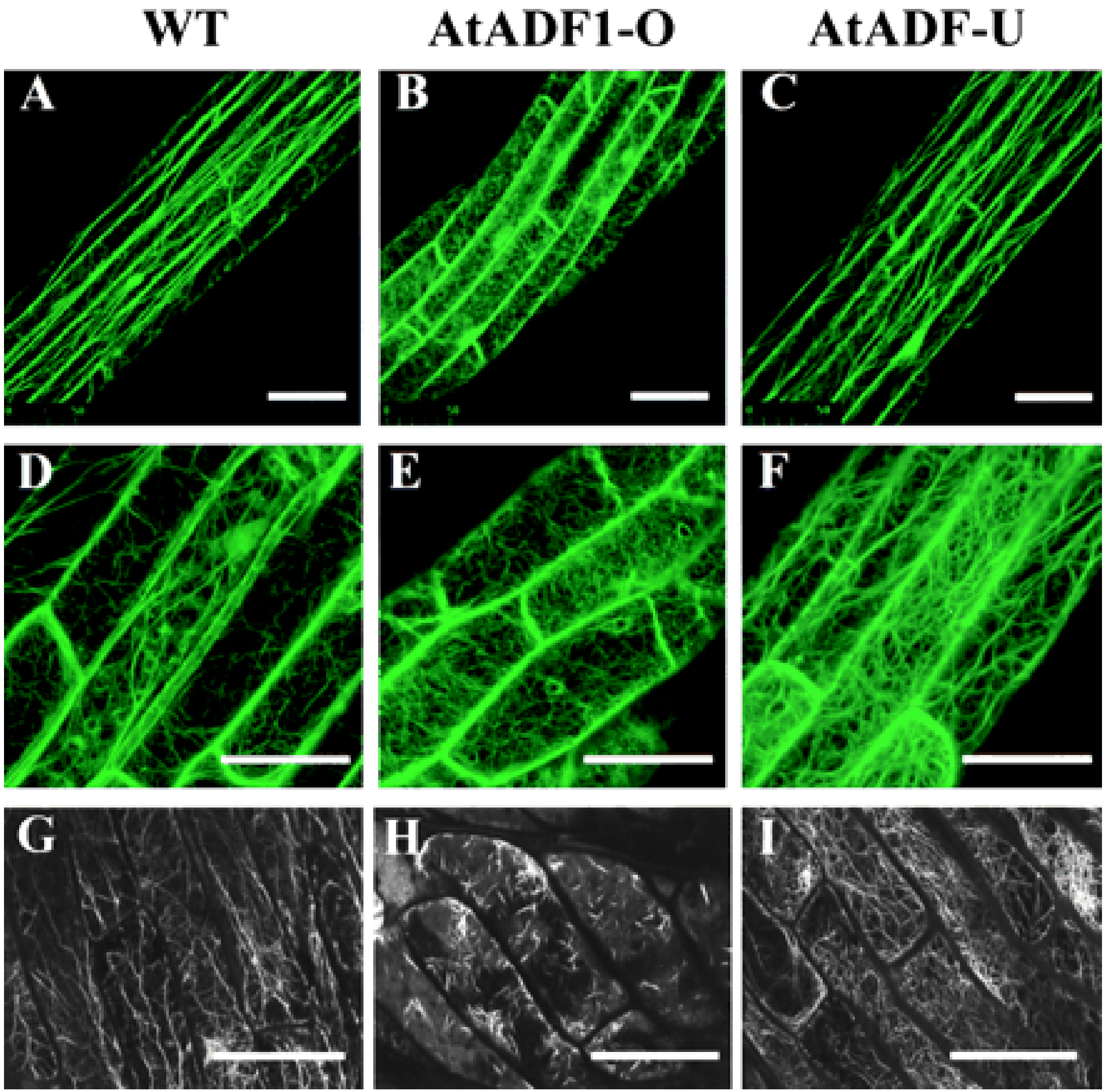


Condeelis 2001

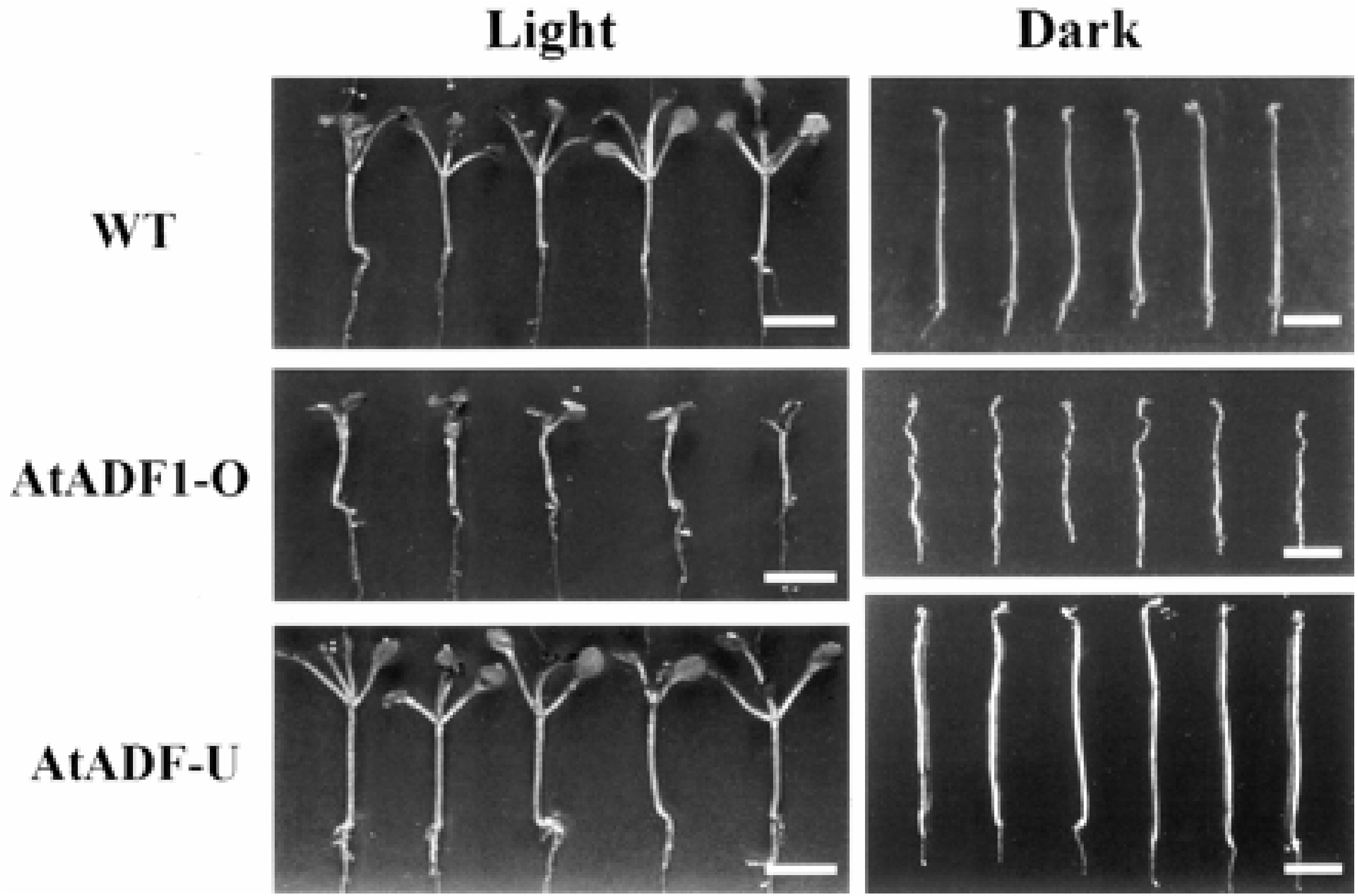


Condeelis 2001

Změny hladiny ADF1 u Arabidopsis ...



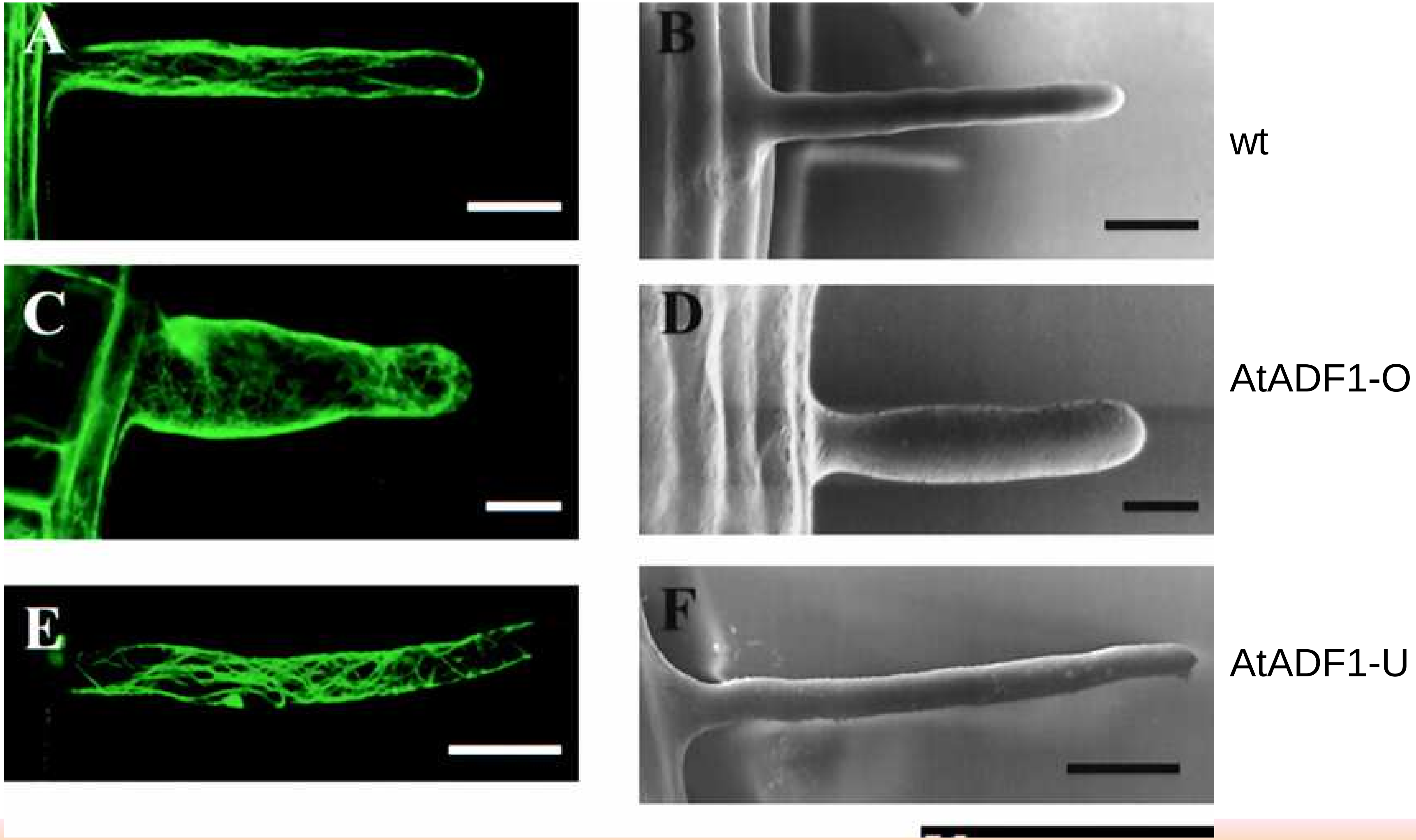
Dong et al. 2001



Overexprese: redukce dlouživého růstu
Inhibice: stimulace růstu

Dong et al. 2001

... vliv na růst kořenových vlásků a indukci kvetení

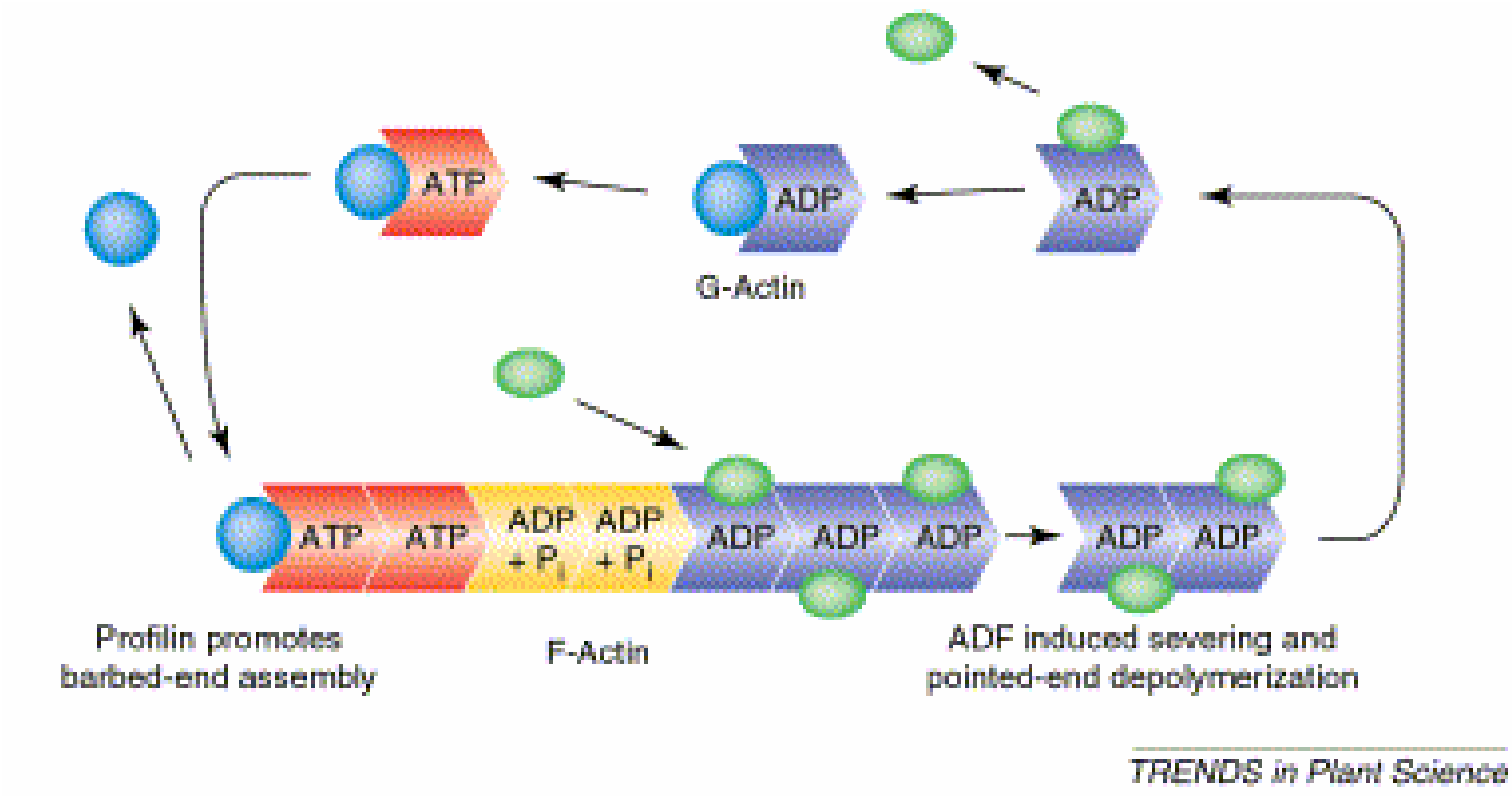


Rosette leaves before flowering:

wt 16.57±0.95 AtADF1-O 16.46±1.10 AtADF1-U 25.61±1.90

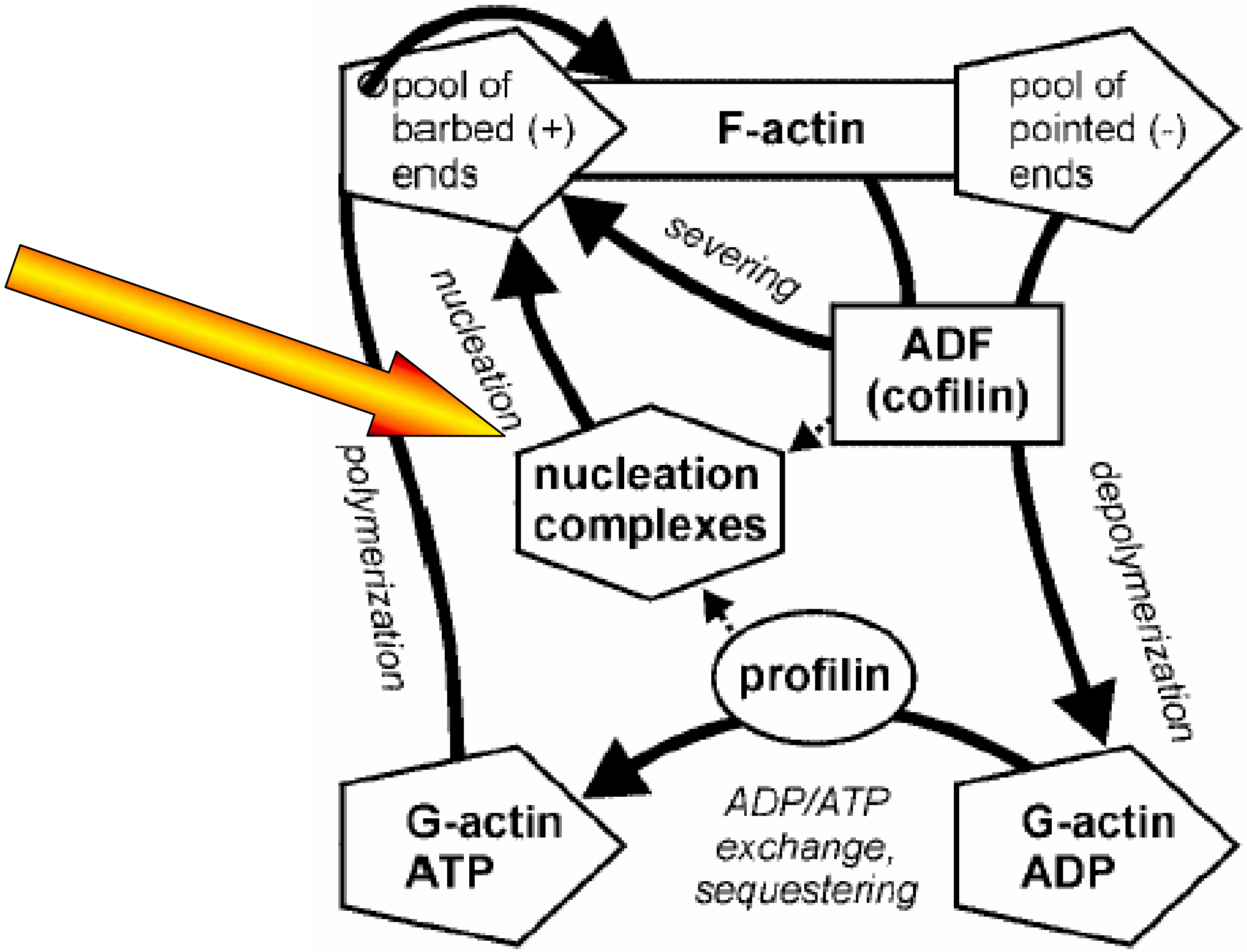
Dong et al. 2001

Cofilin versus profilin

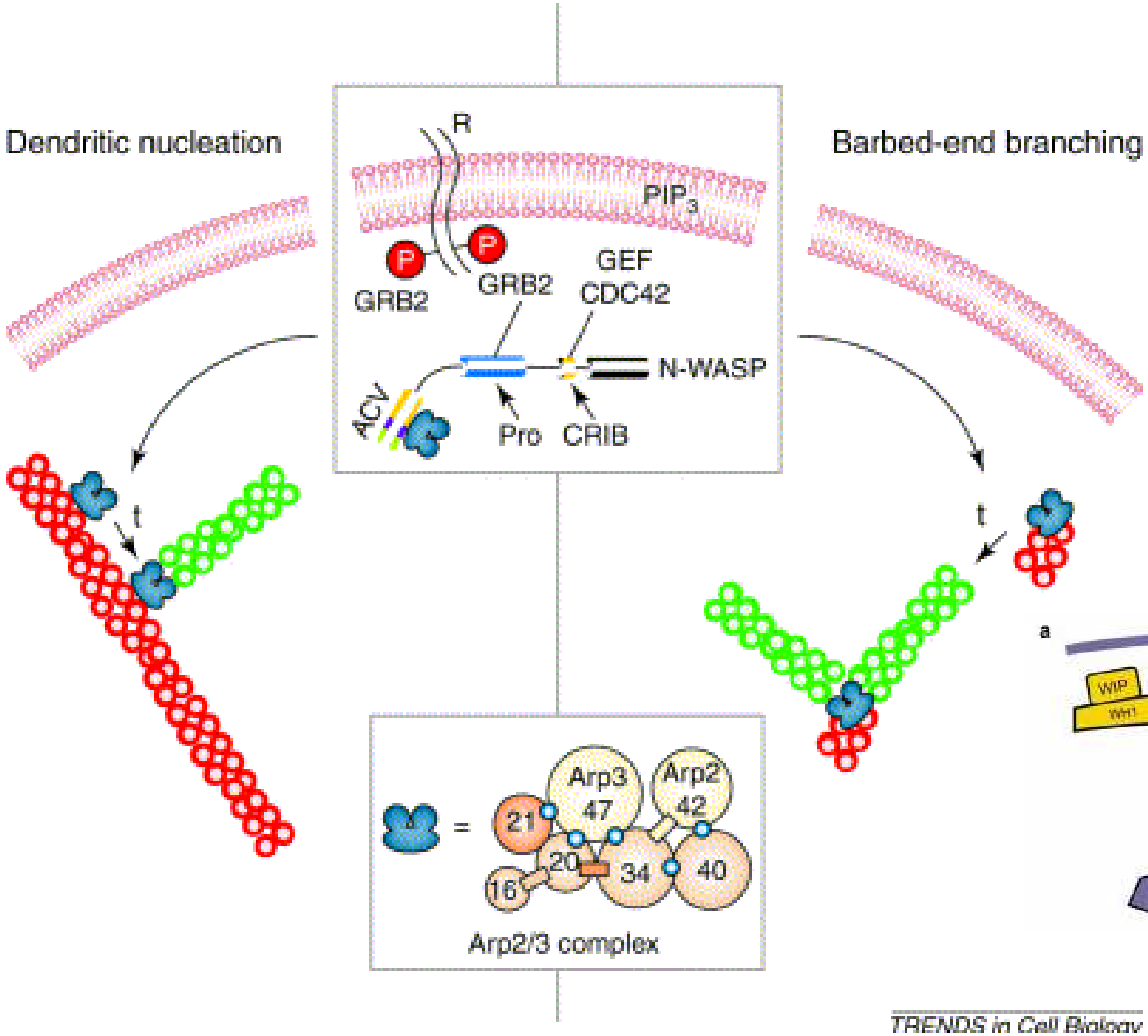


Deeks et al. 2002

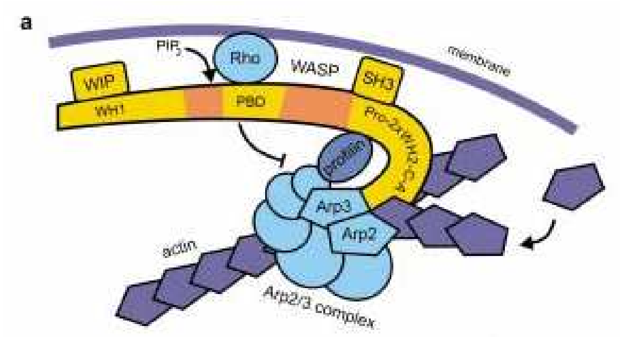
Nukleace aktinových filament *de novo*



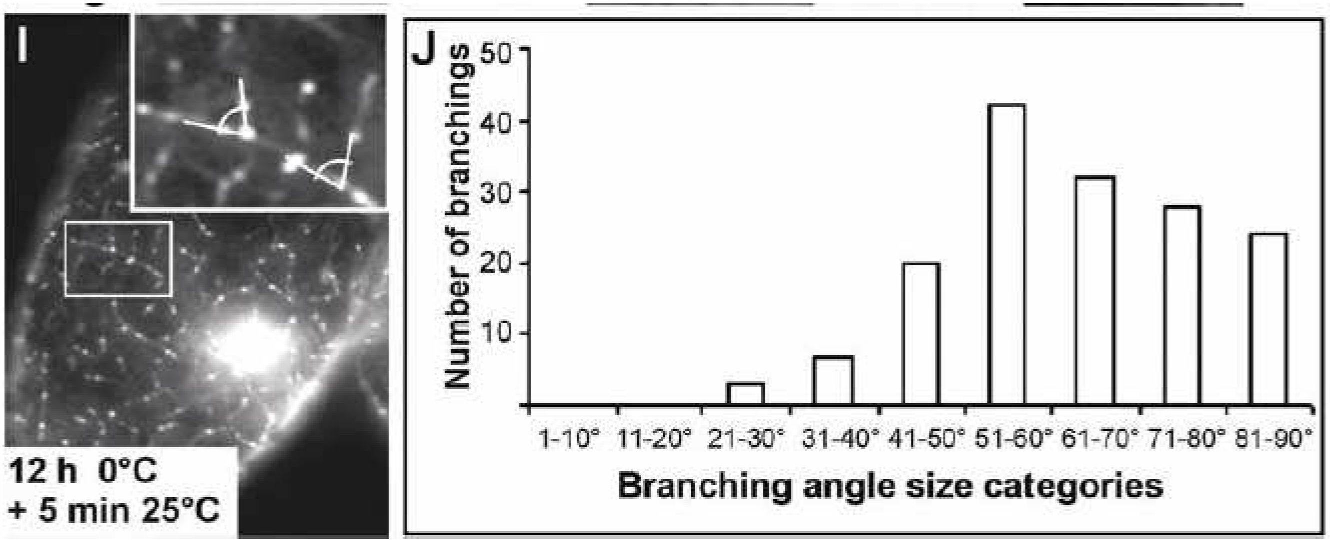
Nukleace aktinových filament de novo: Arp2/3 komplex



regulátory:
WASP
SCAR/WAWE



Condeelis, 2001



I and **J** Angles formed by two branches of newly formed AFs. **I** Branching of thin AFs with a dotted signal formed in the cortical cytoplasm. Rhodamine-phalloidin staining. **J** Frequency of branching angles of defined size categories. The angle of branching was measured for 156 cases in a total of 50 optical fields. Data are from one representative experiment. Bar: 10 μm

ARP2 and ARP3 are localized to sites of actin filament nucleation in tobacco BY-2 cells

J. Fišerová[1,*], **K. Schwarzerová**[1], **J. Petrášek**[1,2], and **Z. Opatrný**[1]

Protoplasma (2006) 227: 119–128
DOI 10.1007/s00709-006-0146-6

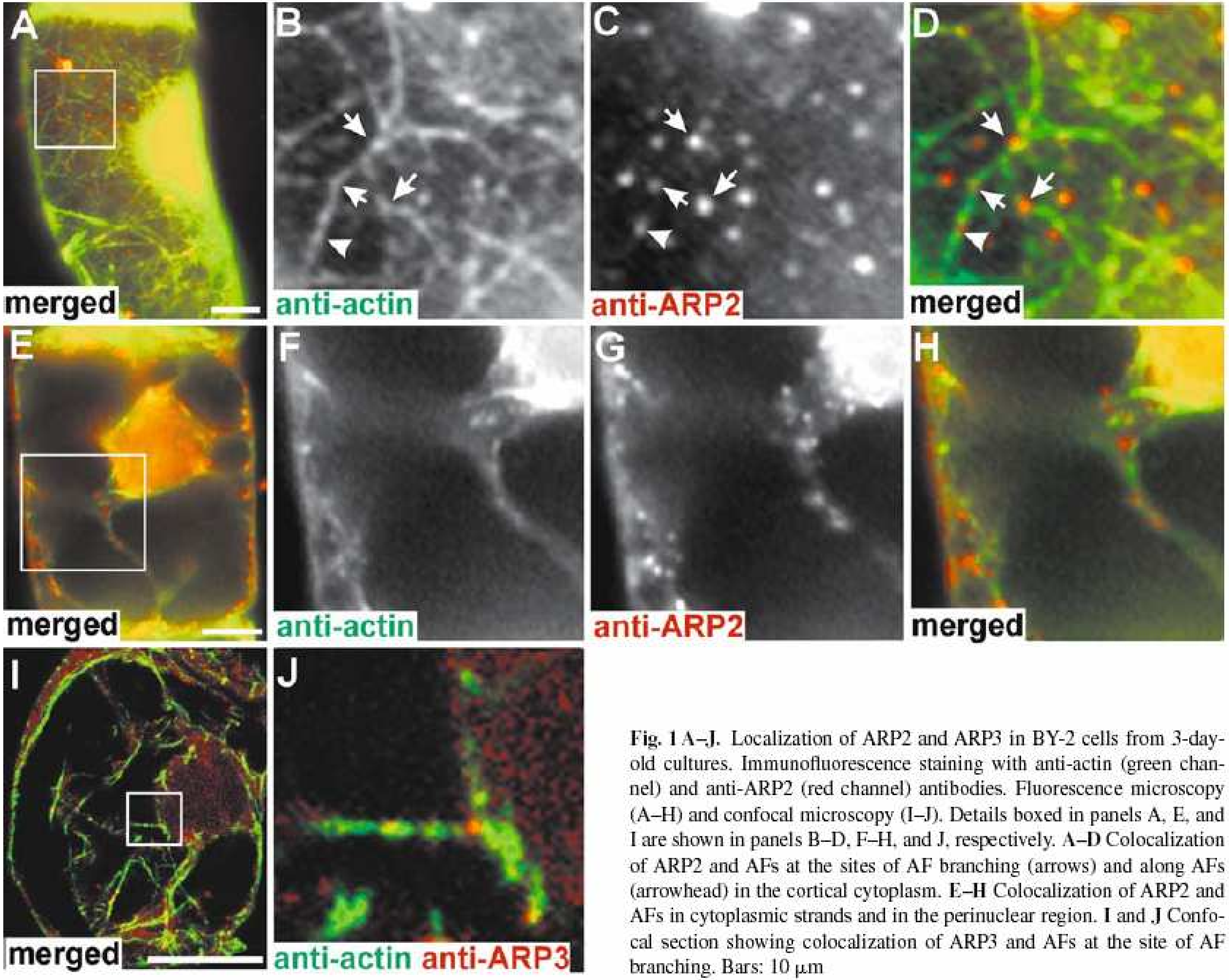
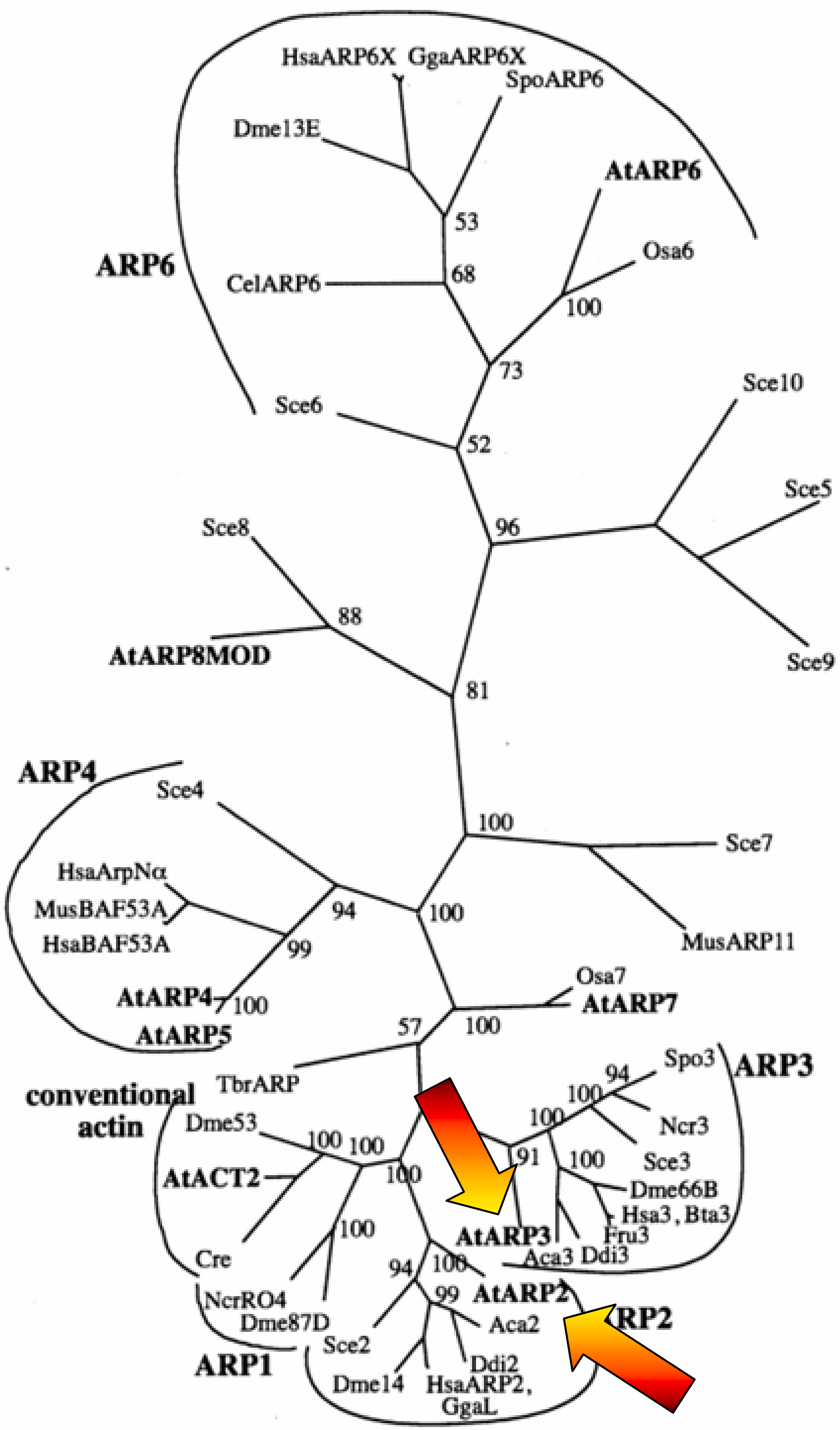


Fig. 1 A–J. Localization of ARP2 and ARP3 in BY-2 cells from 3-day-old cultures. Immunofluorescence staining with anti-actin (green channel) and anti-ARP2 (red channel) antibodies. Fluorescence microscopy (A–H) and confocal microscopy (I–J). Details boxed in panels A, E, and I are shown in panels B–D, F–H, and J, respectively. **A–D** Colocalization of ARP2 and AFs at the sites of AF branching (arrows) and along AFs (arrowhead) in the cortical cytoplasm. **E–H** Colocalization of ARP2 and AFs in cytoplasmic strands and in the perinuclear region. **I** and **J** Confocal section showing colocalization of ARP3 and AFs at the site of AF branching. Bars: 10 μm



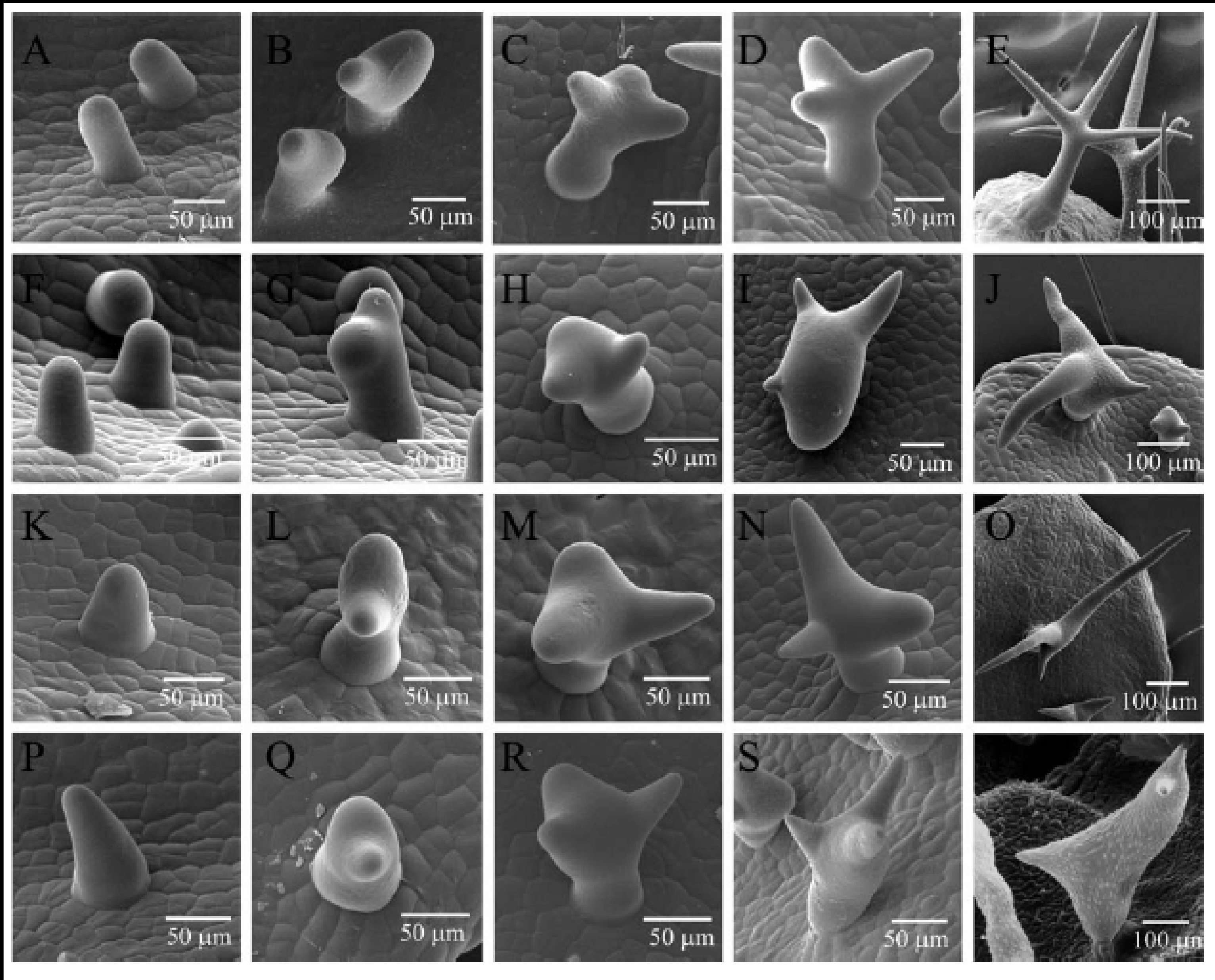
Podjednotky Arp2/3 komplexu jsou evolučně staré

Arabidopsis:

- ARPC1/Arc40p/p40 (2)
- ARPC2/Arc35p/p35 (2)
- ARPC3/Arc18p/p21 (1)
- ARPC4/Arc19p/p20 (1)
- ARPC5/Arc15p/p15 (1)

McKinney et al. 2002; Mathur , 2005

Arabidopsis: mutanti třídy *distorted*

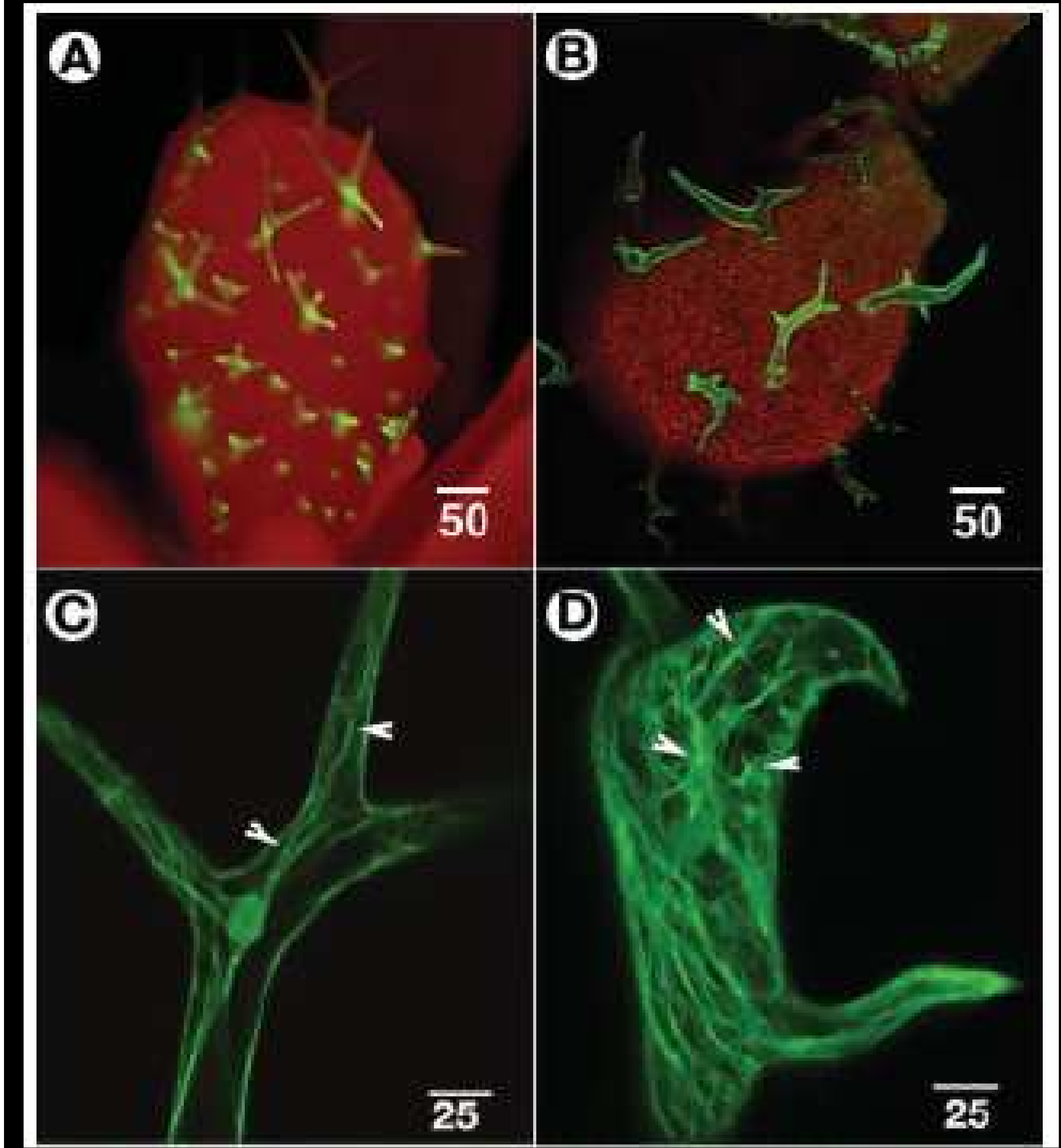


wt

crk ←

spi

wrm ←

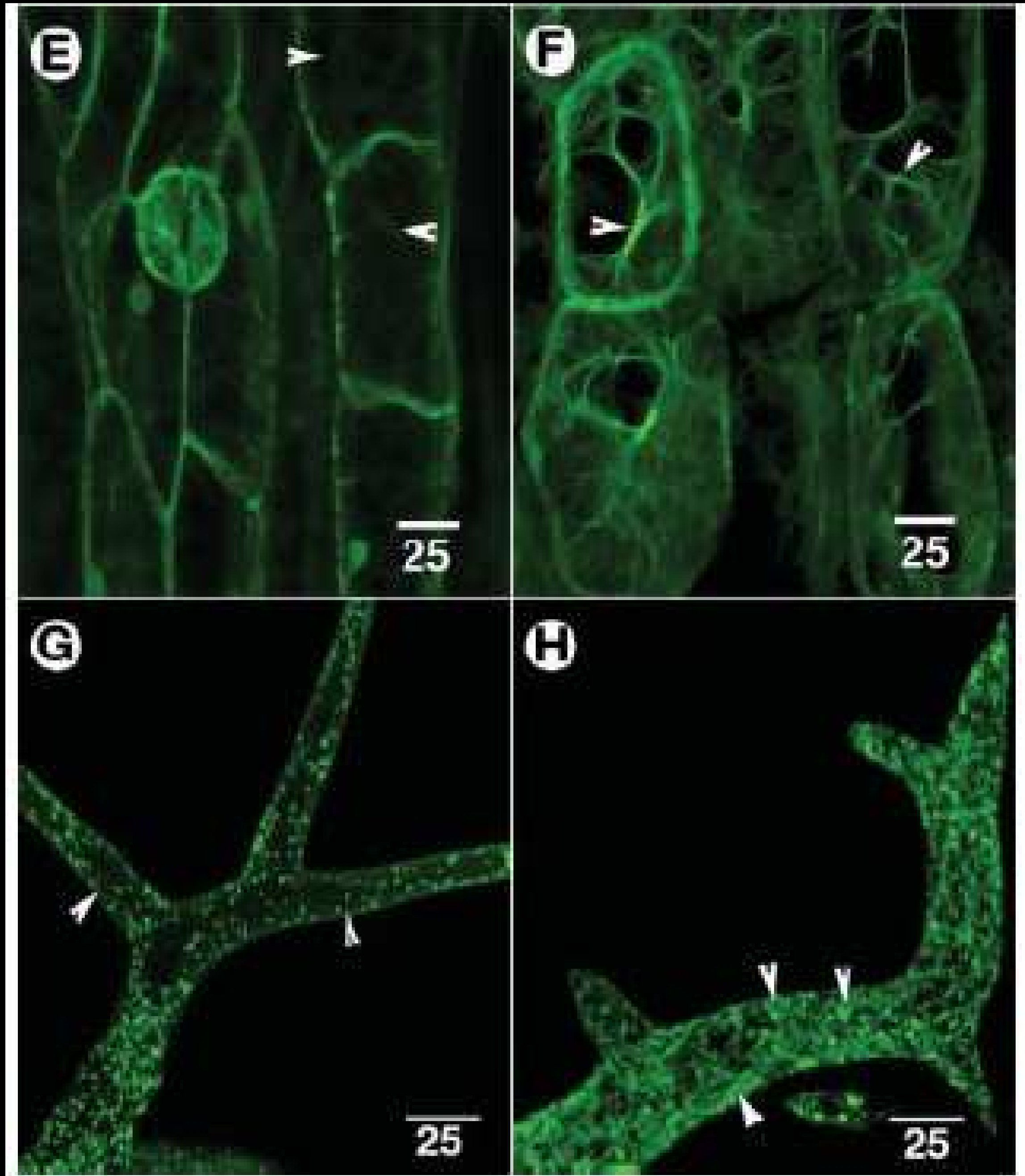


autofluorescence

GFP-mTalin

wt *wurm*

hypokotyl,
GFP-mTalin



Golgi

agregáty!

wt

crooked

Table 3. The *DISTORTED* class of *Arabidopsis* genes

Gene	Chr	AtDB Ac. No	Homolog for
ALIEN	4	unknown	unknown
→ *CROOKED*	4	At4g01710	ARPC5
→ *DISTORTED1*	1	At1g13180	ARP3
→ *DISTORTED2*	1	At1g30825	ARPC2
→ *GNARLED*	2	At2g35110	NAP135
→ *KLUNKER**	5	At5g18410	PIR121
SPIRRIG	1	Unknown	Unknown (WD40 domain)
→ *WURM*	3	At3g27000	ARP2

Podjednotky a regulátory Arp2/3 komplexu!

Proč má ztráta Arp2/3 komplexu jen relativně mírný fenotyp?
Physcomitrella: NENÍ letální!

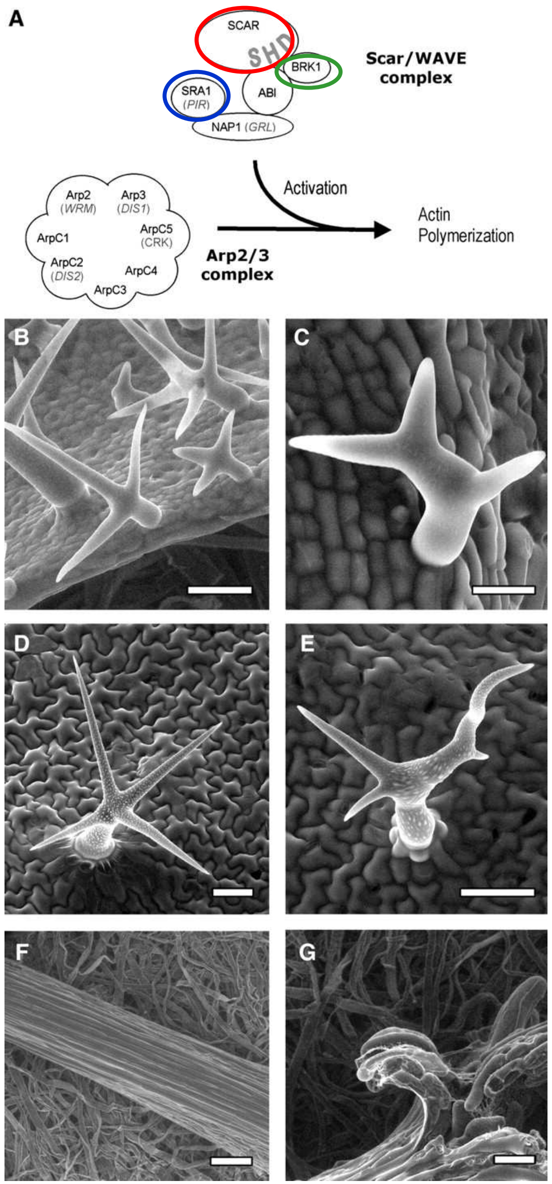
(Mathur, 2005)

SCAR/WAWE: regulátory Arp2/3

Arabidopsis BRICK1/HSPC300 Is an Essential WAVE-Complex Subunit that Selectively Stabilizes the Arp2/3 Activator SCAR2.
Current Biology, Volume 16, Issue 9, Pages 895-901
J. Le, E. Mallery, C. Zhang, S. Brankle, D. Szymanski

The evolutionarily conserved Rac-WAVE-Arp2/3 pathway links actin filament nucleation to cell morphogenesis. WAVE translates Rac-GTP signals into Arp2/3 activation by regulating the stability and/or localization of the activator subunit Scar/WAVE. The WAVE complex includes:

1) Sra1/PIR121/CYFIP1, KLUNKER
2) Nap1/NAP125, GNARLED
3) Abi-1/Abi-2,
4) Brick1(Brk1)/HSPC300,
5) Scar/WAVE



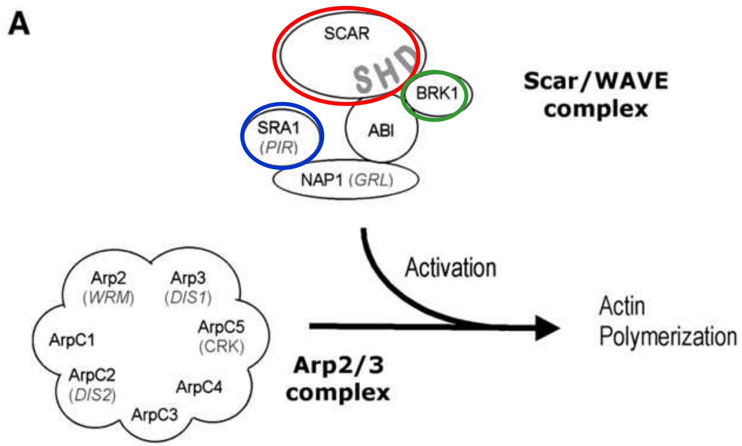
wt *itb1/scar2*

Zhang, X., et al. Plant Cell 2005;17:2314-2326

Development 133, 1091-1100 (2006) doi:10.1242/dev.02280

BRICK1/HSPC300 functions with SCAR and the ARP2/3 complex to regulate epidermal cell shape in *Arabidopsis*

Stevan Djakovic*, Julia Dyachok, Michael Burke†, Mary J. Frank‡ and Laurie G. Smith§



wt

arp2

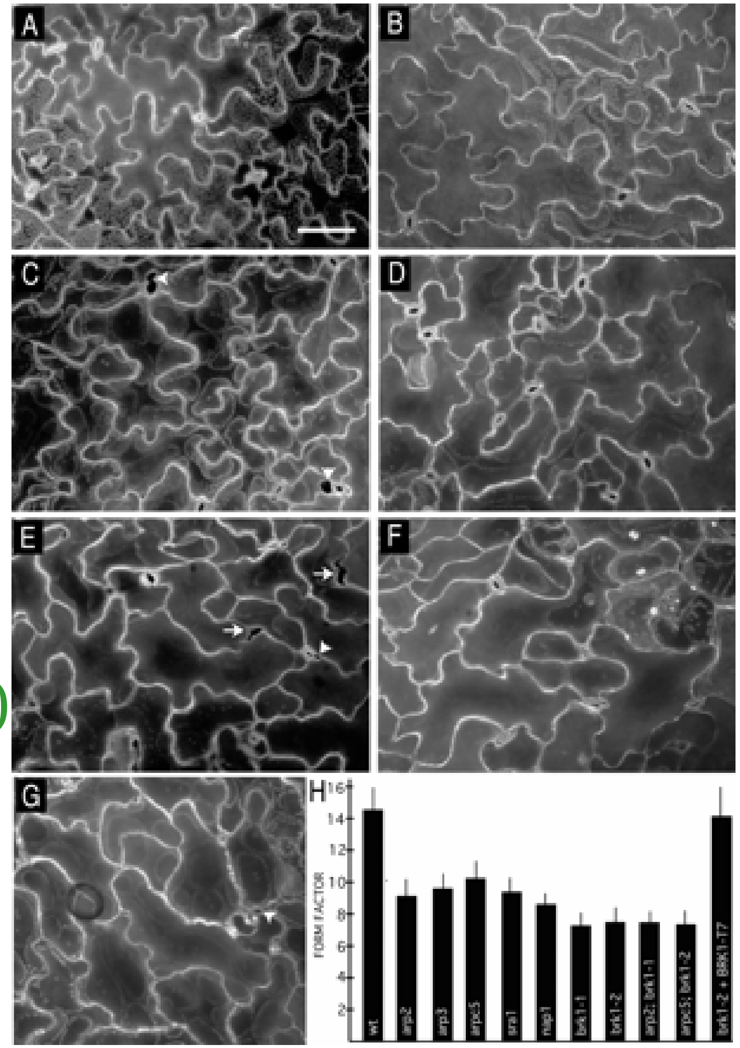
arpc5

sra1

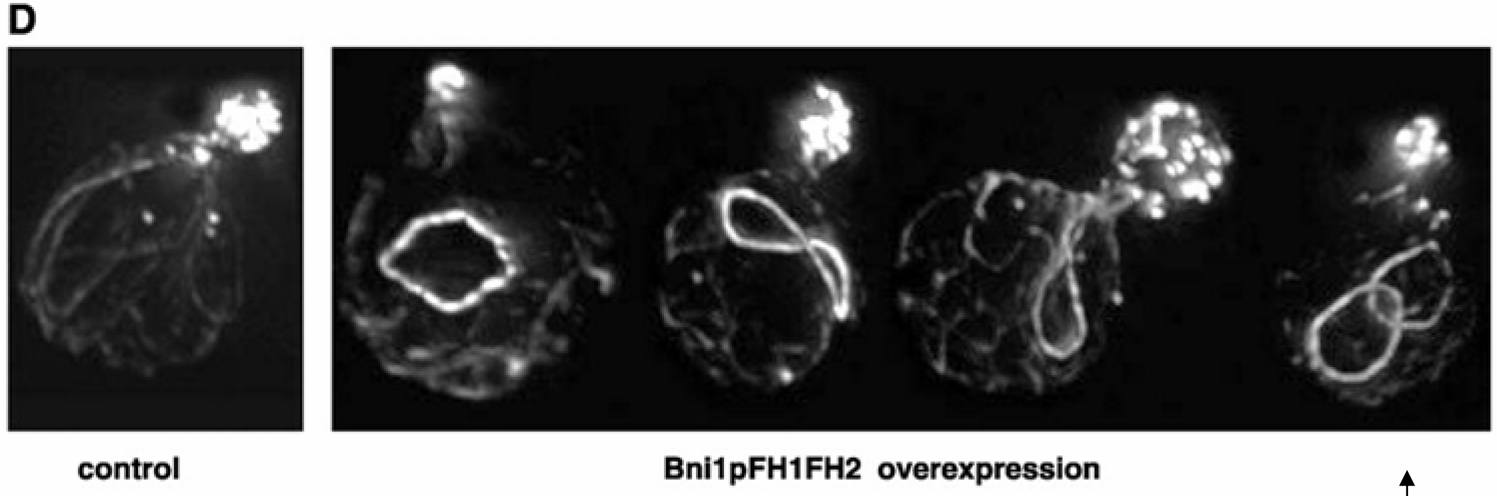
brk1 (BRICK)

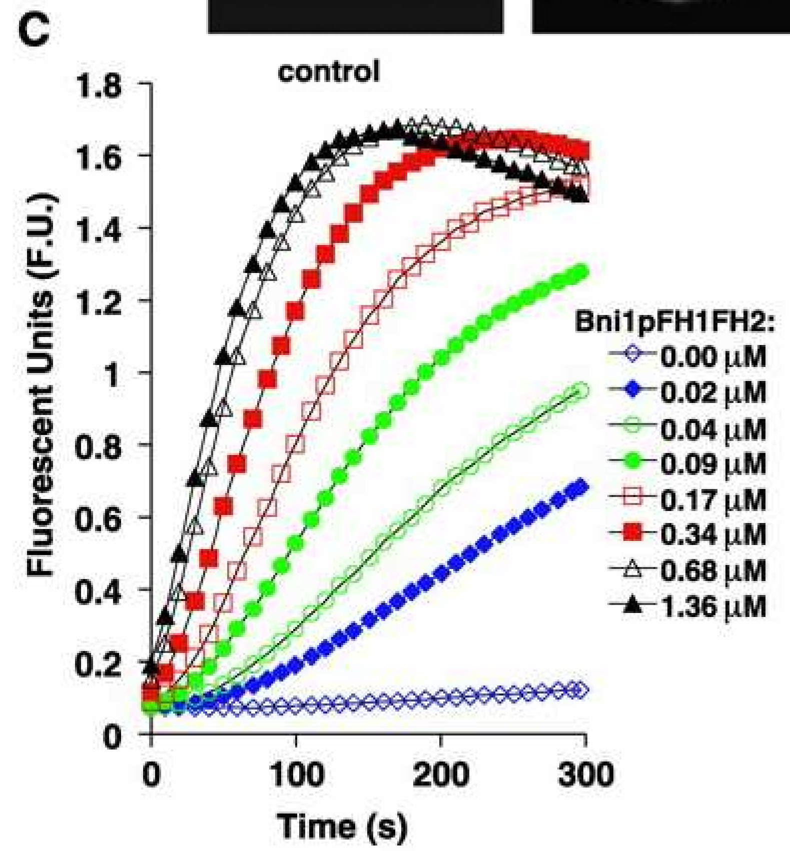
arp2 brk1

arpc5 brk1



Další způsoby nukleace: například forminy (FH2 proteiny)





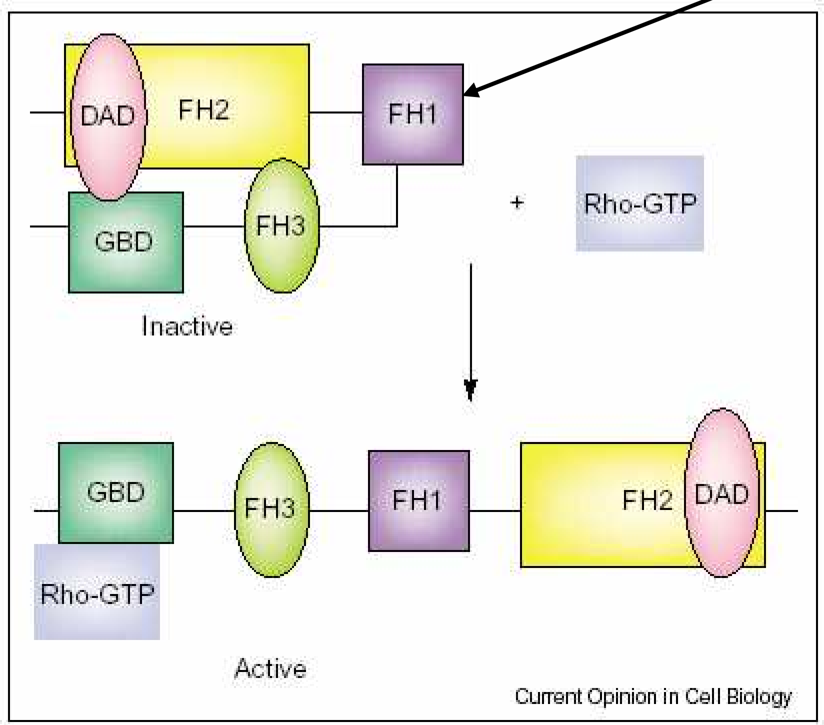
overexpression of a partial yeast formin causes formation of ectopic actin structures

and the protein stimulates filament formation in vitro

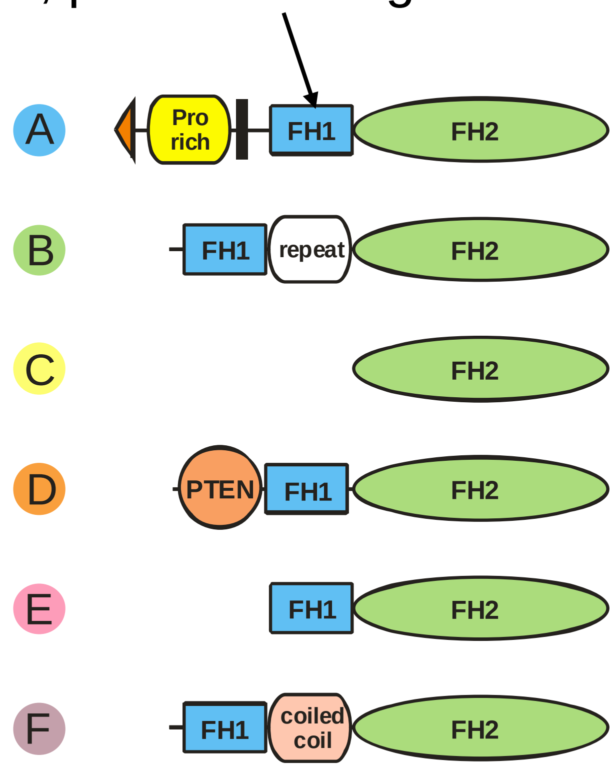
oligomerizace FH2,
vazba na „barbed end“,
nukleace nevětvených vláken,
„leaky cap“ model

Pruyne et al. 2002

Doménová struktura forminů



Activation of Diaphanous-related formin by Rho-GTPase. Diagram of domains present in Diaphanous-related proteins, illustrating the conformation change that occurs upon binding Rho-GTP.



Arabidopsis: 21 genů!

Zigmond, Curr. Opin. Cell Biol. 2003; Cvrčková et al., BMC Genomics 2004

Membránová lokalizace forminů třídy I: rostlinná specialita?

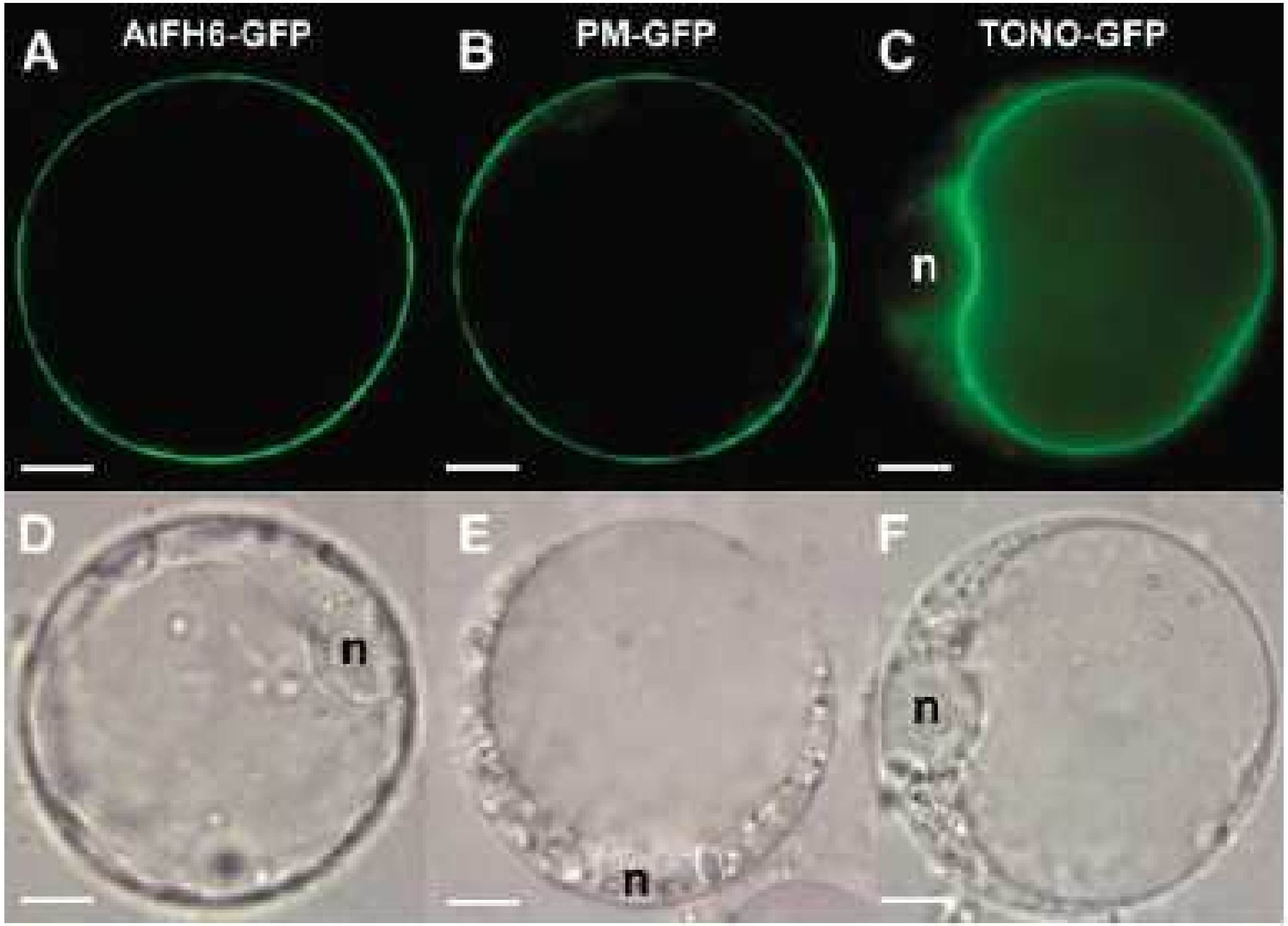
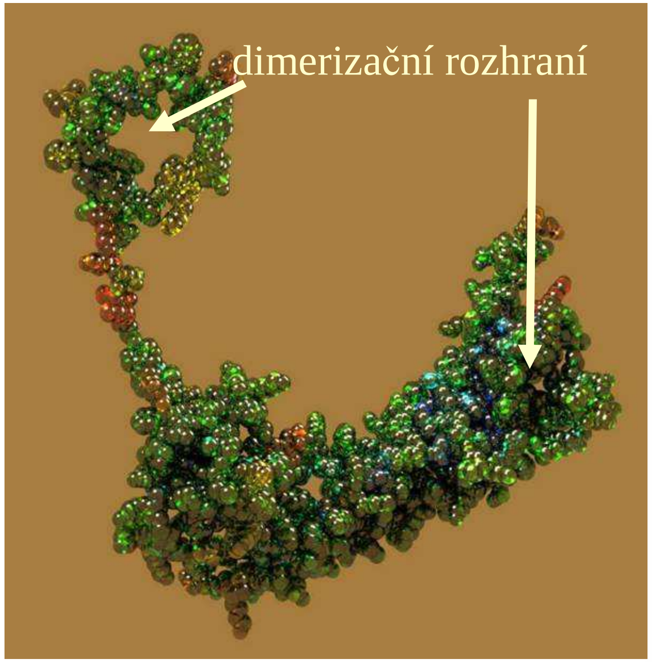
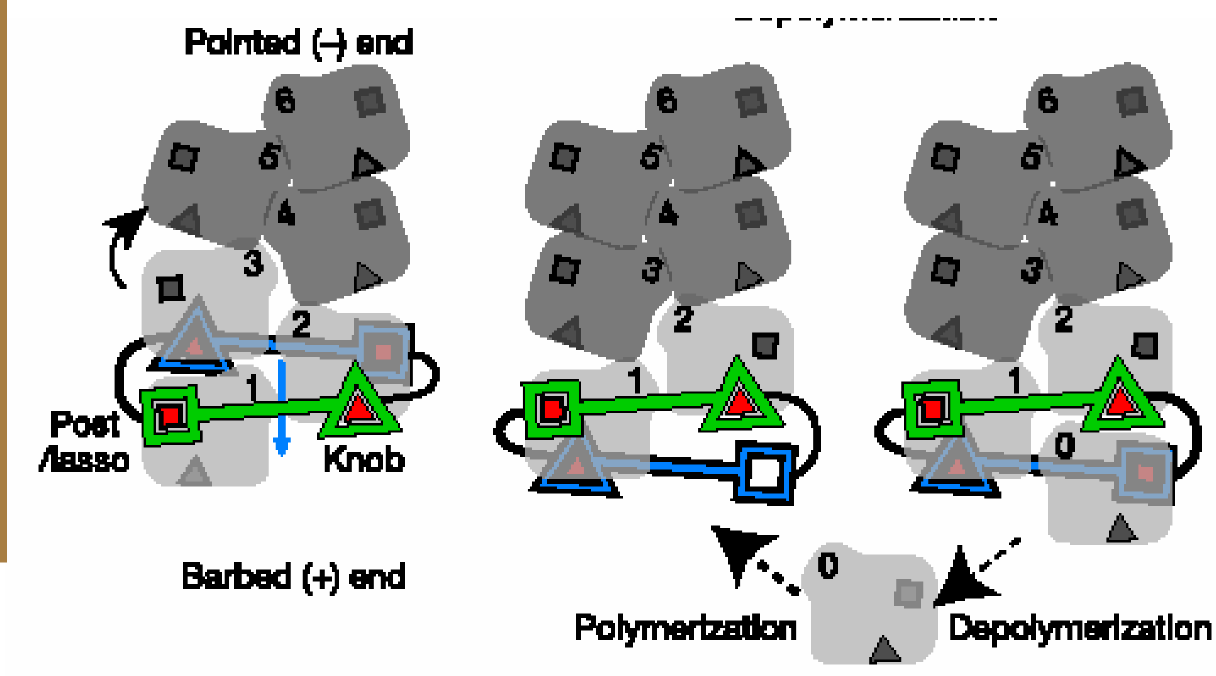


Figure 4. AtFH6 Is Targeted to the Plasma Membrane.

(Favery et a. 2005)

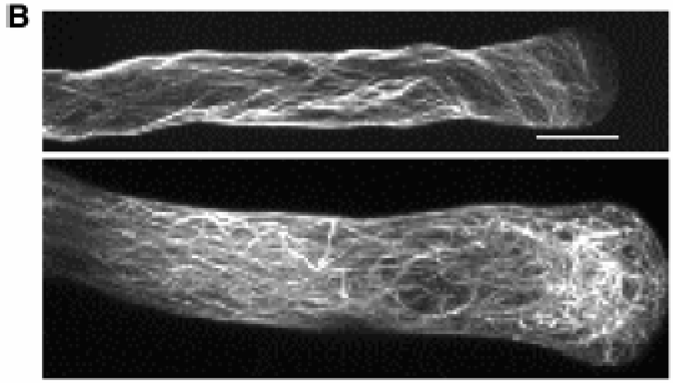
„Kráčející nukleační komplex“





Otomo et al. Nature 433:488-494, 2005

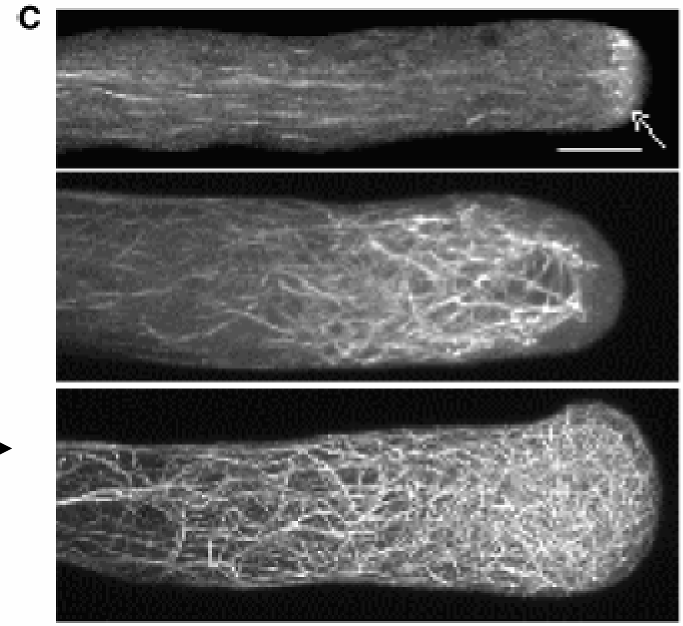
Overexprese AtFH1 v pylových láčkách

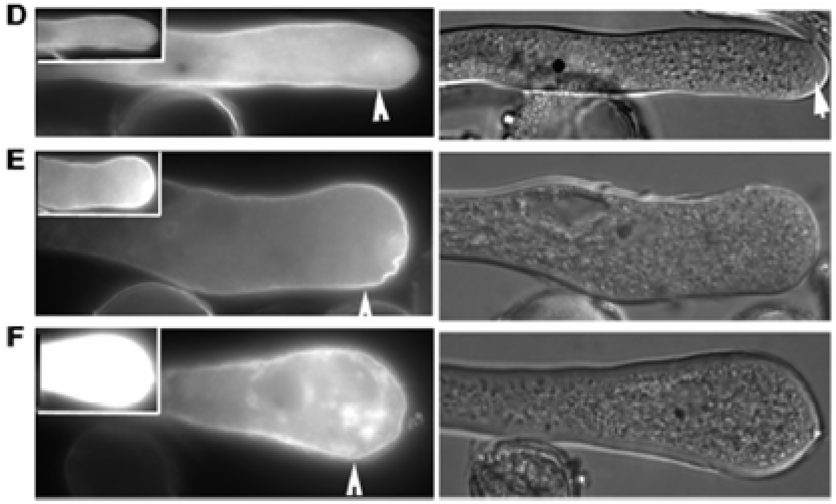


kontrola

AtFH1 (FH1/2)

talin





AtFH1:GFP

(Cheung a Wu, 2004)

Specifické funkce isoforem forminů

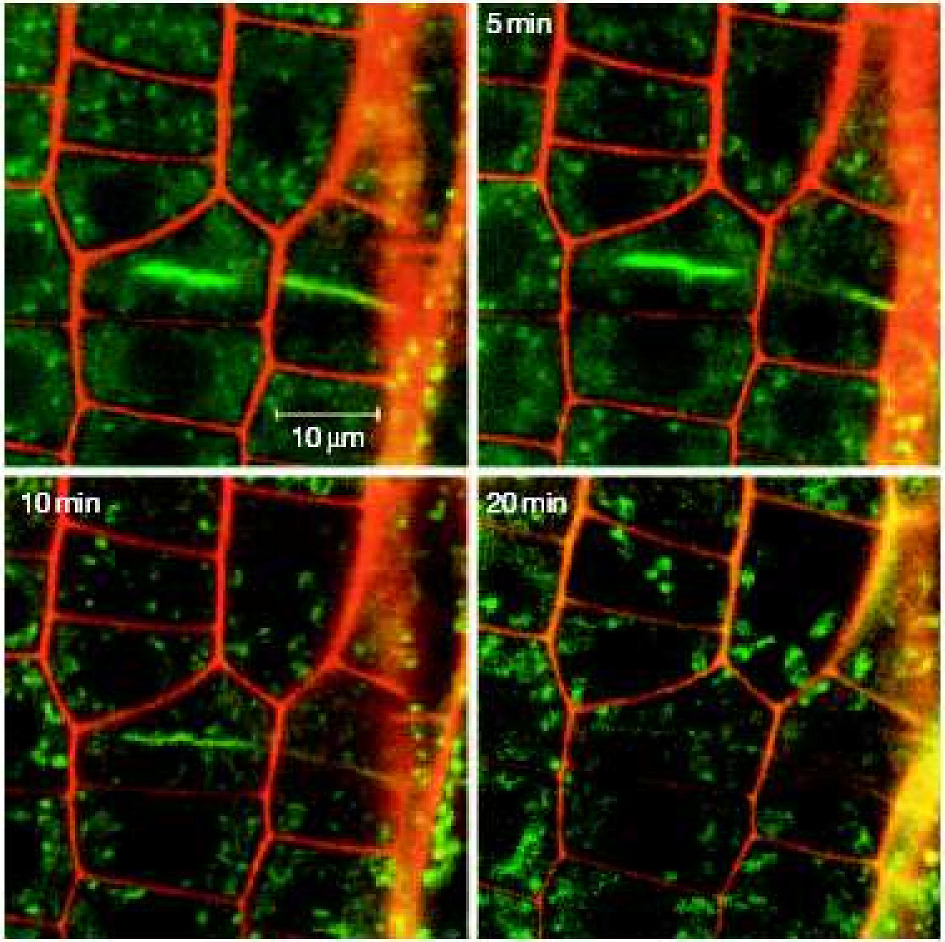


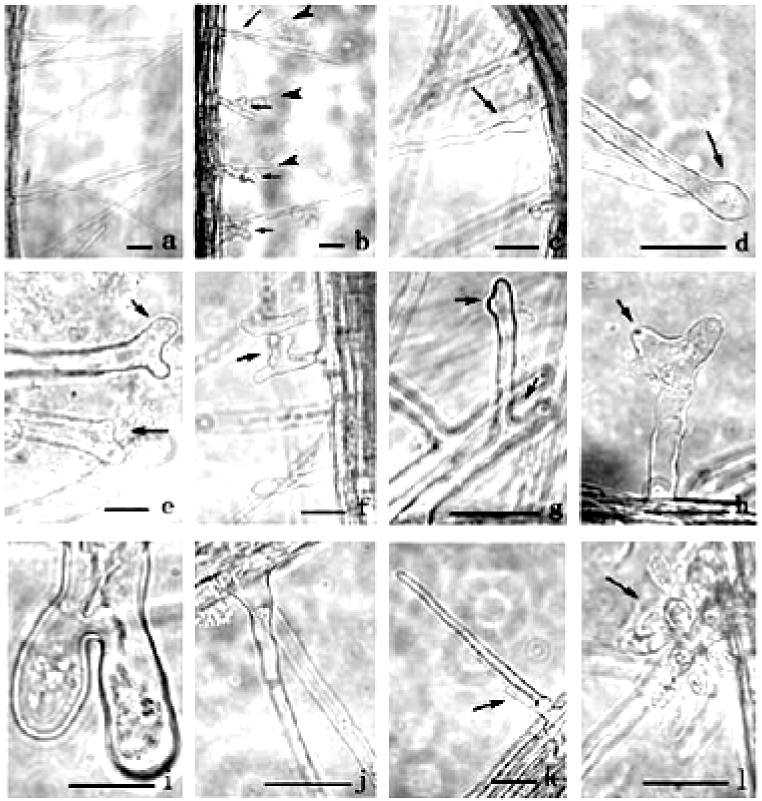
Figure 3 Formin AtFH5-GFP is targeted to the developing cell plate.

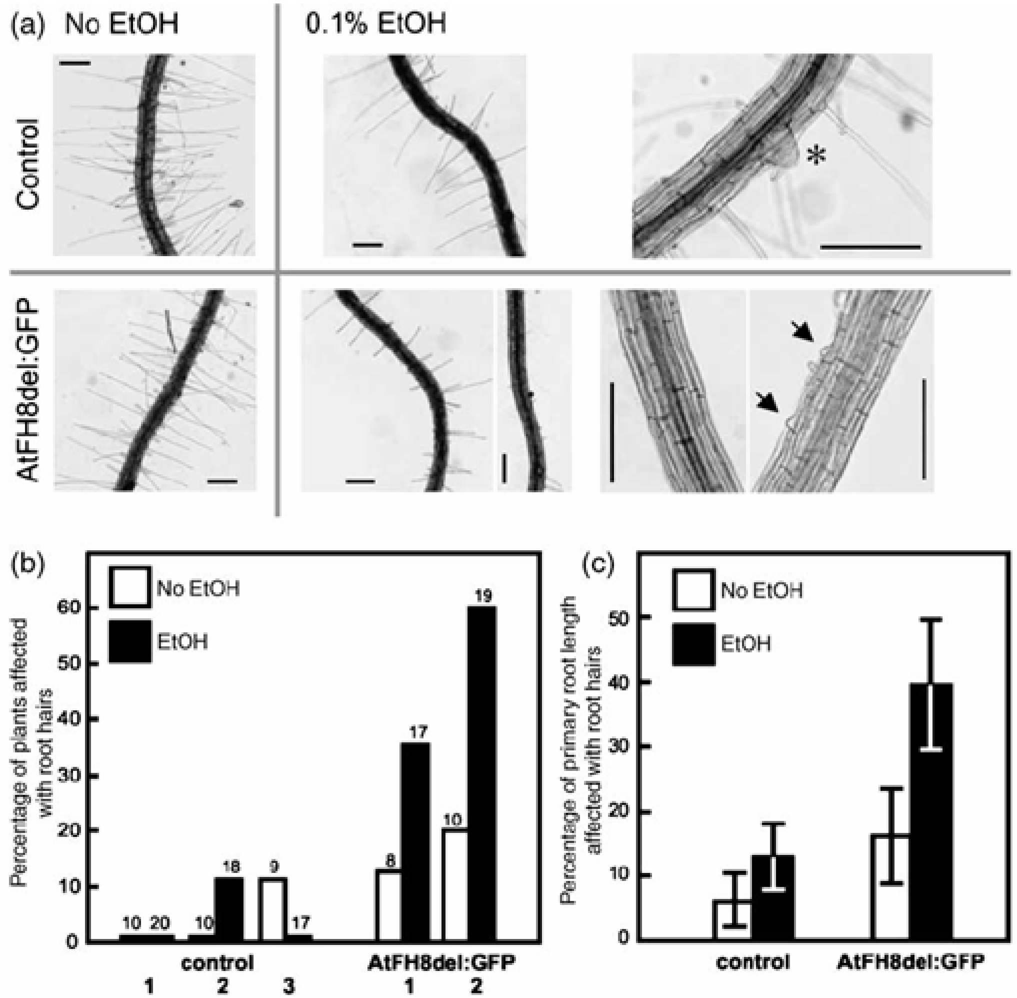
Mutant: zpožděná celularizace endospermu (jinde redundantní)

(Ingouff et al. 2005)

- AtFH1: bundling!
- AtFH5: cytokineze endospermu
- AtFH6: zvýšená exprese v hálkách indukovaných hlísticemi
- AtFH4/AtFH8: kořenově specifické, koř. vlásky?
- AtFH3: pyl?? – ALE redundance

Overexprese wt a mutantního AtFH8 - koř. vlásky





wt

mutant

(Yi et al. 2005)

(Deeks et al. 2005)

Proč mají rostliny právě tyto geny v tolika kopiích?

- Krom funkčního rozrůznění i „jemné ladění“ pro proměnlivé podmínky
  - v čase (rostlina je přisedlá)
  - v (mikro)prostoru buňky obklopené stěnou?
- Možnosti se nevylučují

(viz též Nasmyth, Dirick, Surana, Amon, Cvrčková, 1991)

Nukleátorů aktinu je víc…

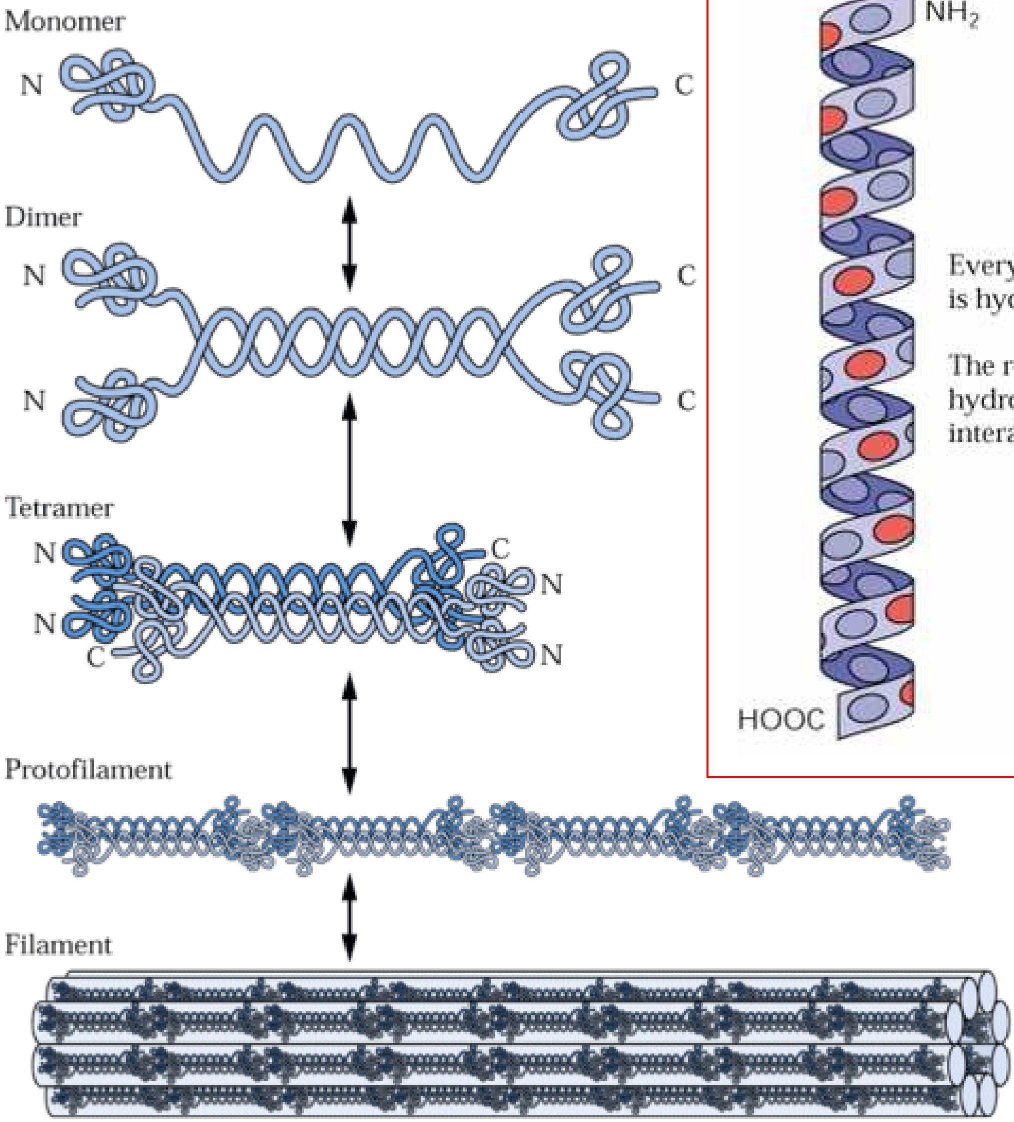
- Spire (Spir) – Drosophila, 2005
- Cordon Bleu (Cobl) – neurony, 2007
- Leiomodin
- …

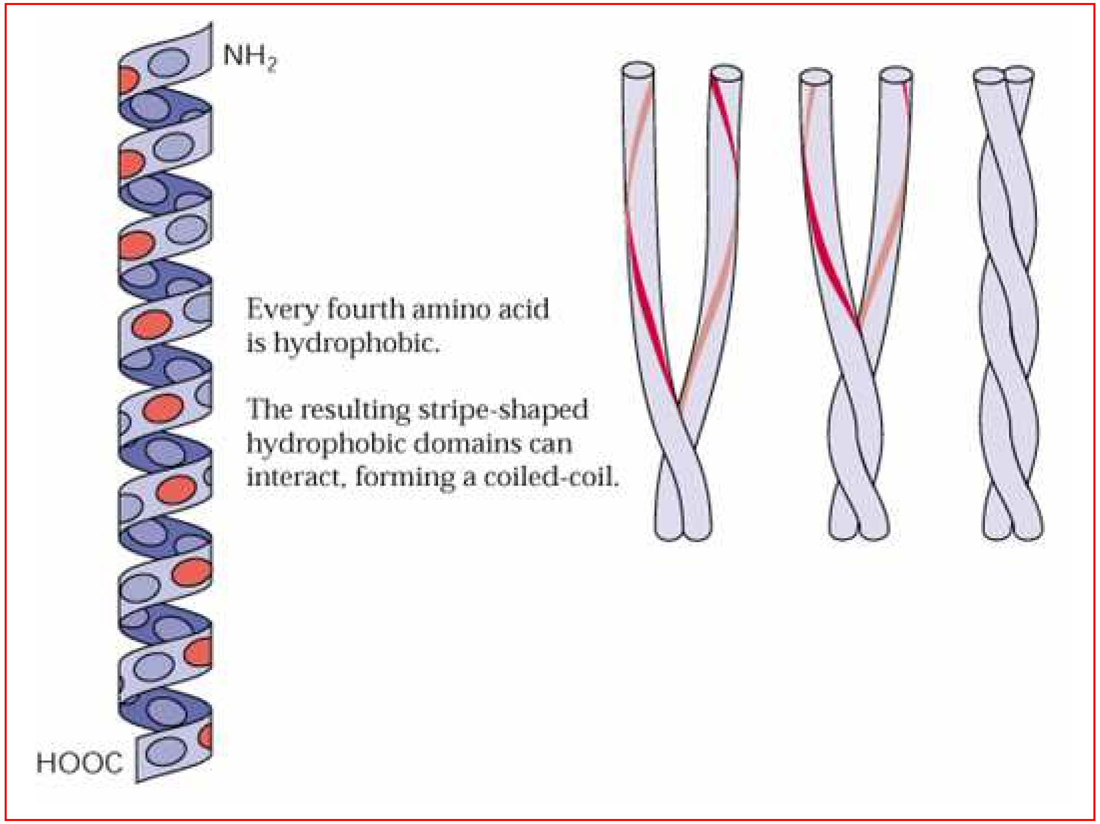
… ale zatím specifické pro Metazoa

Základní cytoskeletální systémy eukaryot (?)

- Aktinový (mikrofilamenta)
  - všude (... prokaryotní MreB)
- Intermediální filamenta
  - „klasická“ zatím jen u Metazoí? (keratin, vimentin, neurofilamenta, laminy...)
  - IF asociované proteiny ... spektrin
  - septiny u kvasinek?
  - jsou epitopy u rostlin??
- Tubulinový (mikrotubuly)
  - všude (... prokaryotní FtsZ)

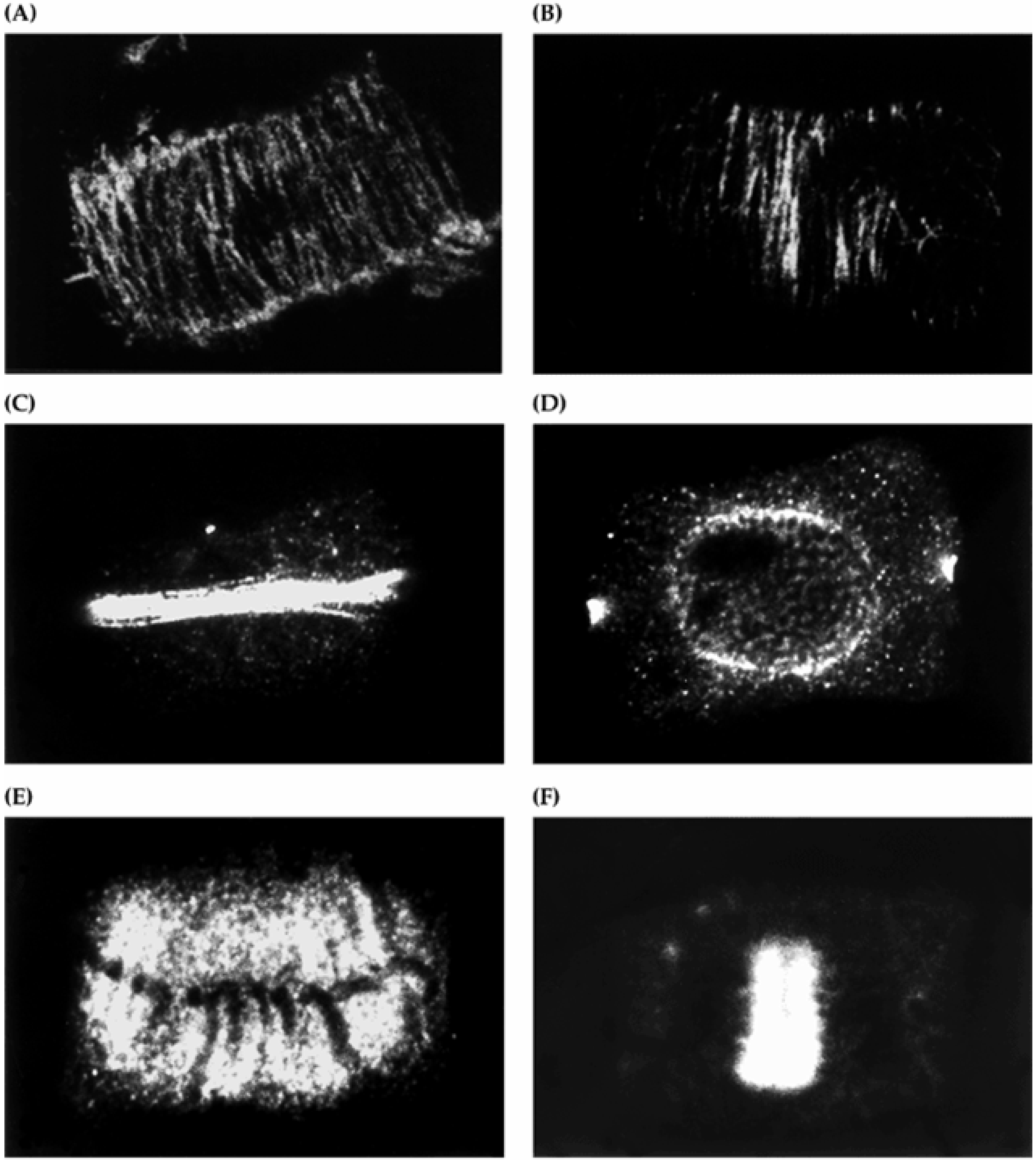
Intermediální filamenta: konvergence?





regulace např. fosforylací (CDK ... jader. lamina)

Výjimka z pravidla: rovnocenné konce!



Epitopy příbuzné IF v rostlinách?

interfáze

(kolokalizace s **mt**?)

profáze

mitóza a cytokineze

wheat root tip,
anti-keratin

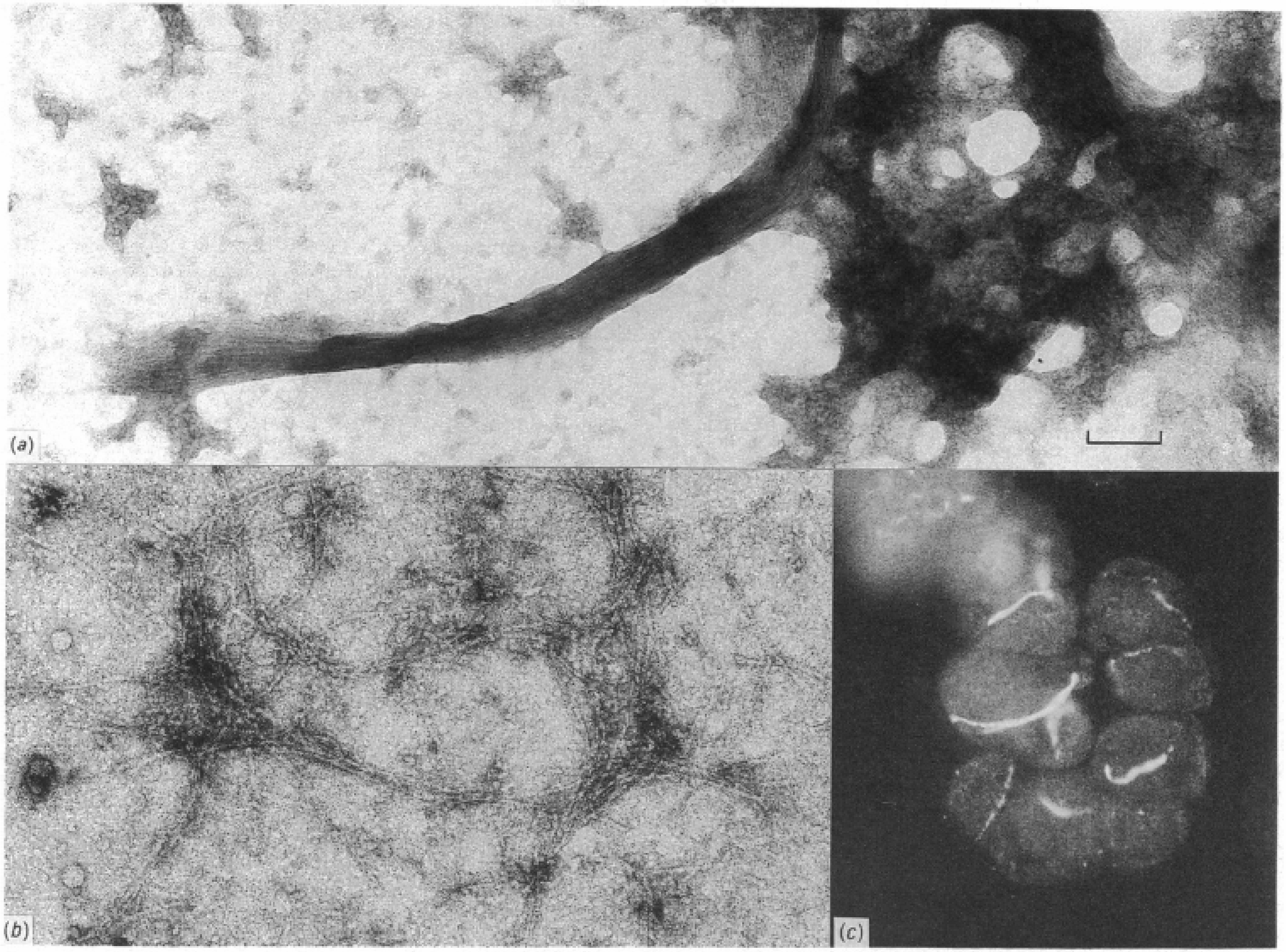


Fig. 1. Ultrastructural characterization of reconstituted 10 nm filaments and filament bundles

Shown are electron micrographs of a reconstituted filament bundle (*a*) and reconstituted 10 nm filaments (*b*), together with indirect immunofluorescence of carrot cells showing native fibrillar bundles stained with AFB followed by a fluorescein-conjugated goat anti-rat IgM (*c*). The bar (shown only in *a*) represents 100 nm in (*a*) and (*b*) and 10 μm in (*c*).

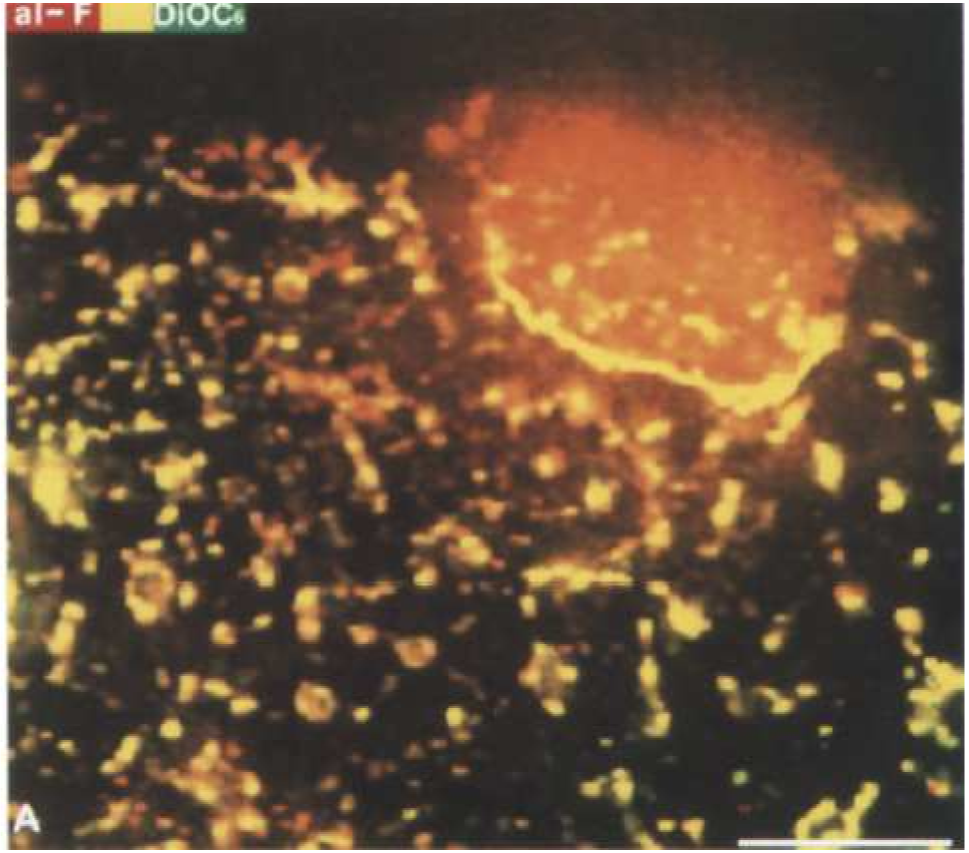
Biochem J. 1989 July 15; 261(2): 679–682.

Mikroinjekce značených protilátek

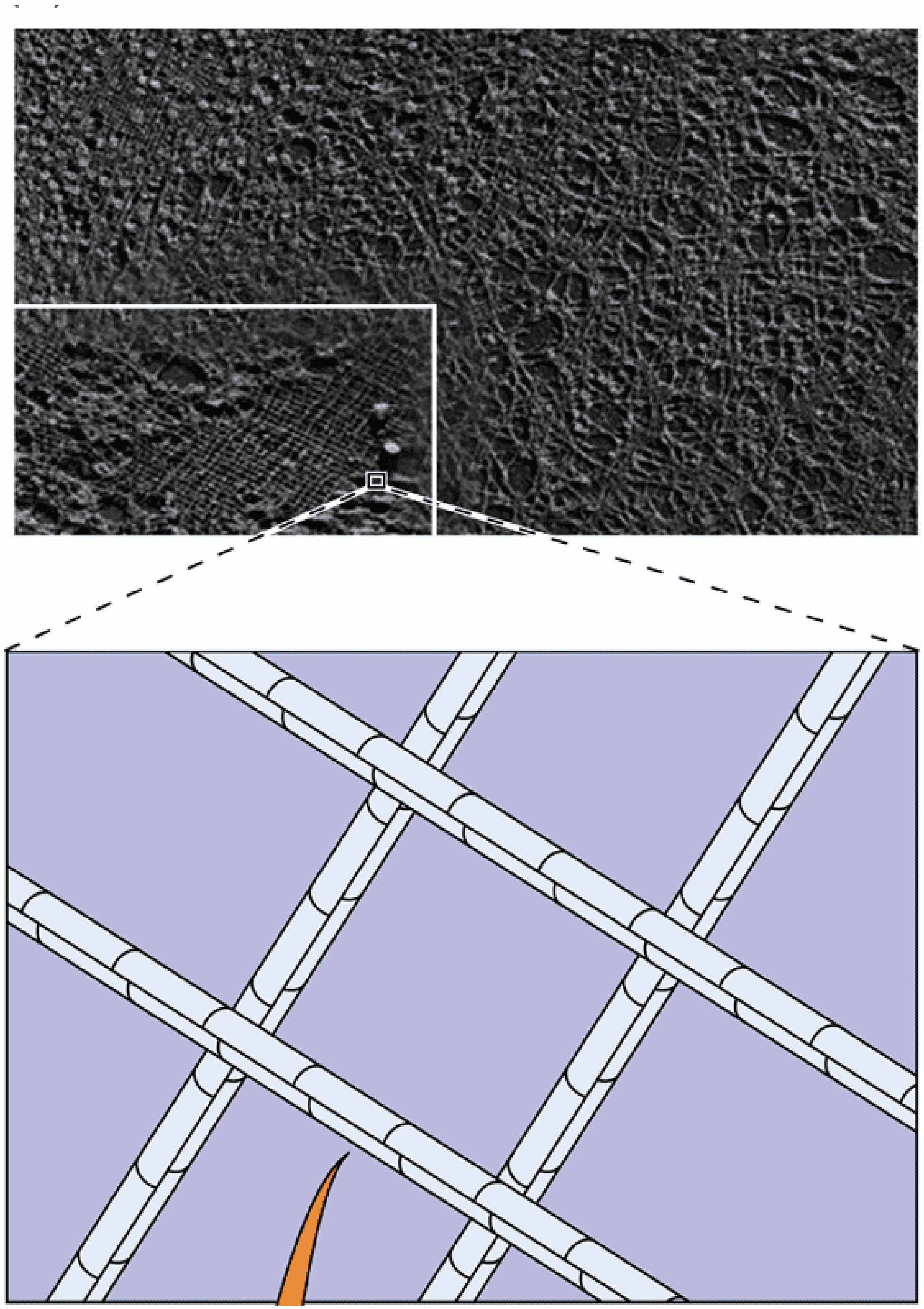
Covisualization in living onion cells of putative integrin, putative spectrin, actin, putative intermediate filaments, and other proteins at the cell membrane and in an endomembrane sheath

Protoplasma (1997) 199: 173–197

Christophe Reuzeau**, **Keith W. Doolittle**, **James G. McNally**, and **Barbara G. Pickard***

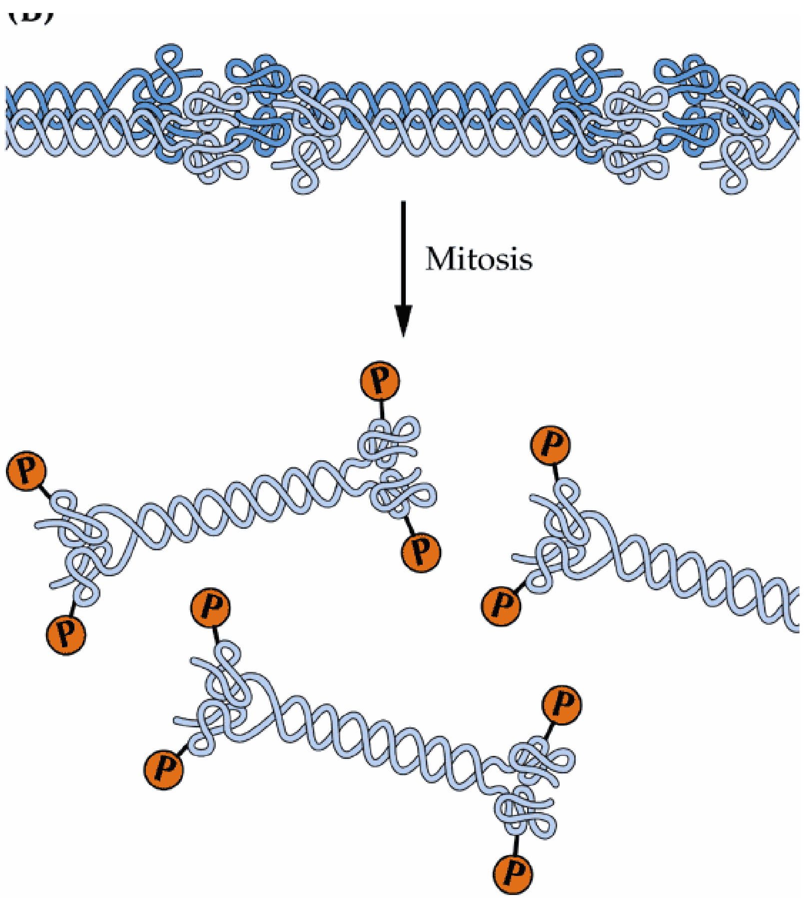


anti-IF
DIOC6 - membrány



Jaderná IF - laminy

Asi jen u živočichů?



Jak u rostlin?

- Jsou „laminové“ epitopy, ale není ortolog (A.th. genom)
- ALE rekonstituce jader Xenopus v bezbuň. extraktech z tabáku (Lu and Zhai 2001)
- ALE savčí LBR se lokalizuje do NE v tabáku (Irons et al. 2003)

Cell Biology International
Volume 27, Issue 3, 2003, Pages 233-235 - C. de la Torre

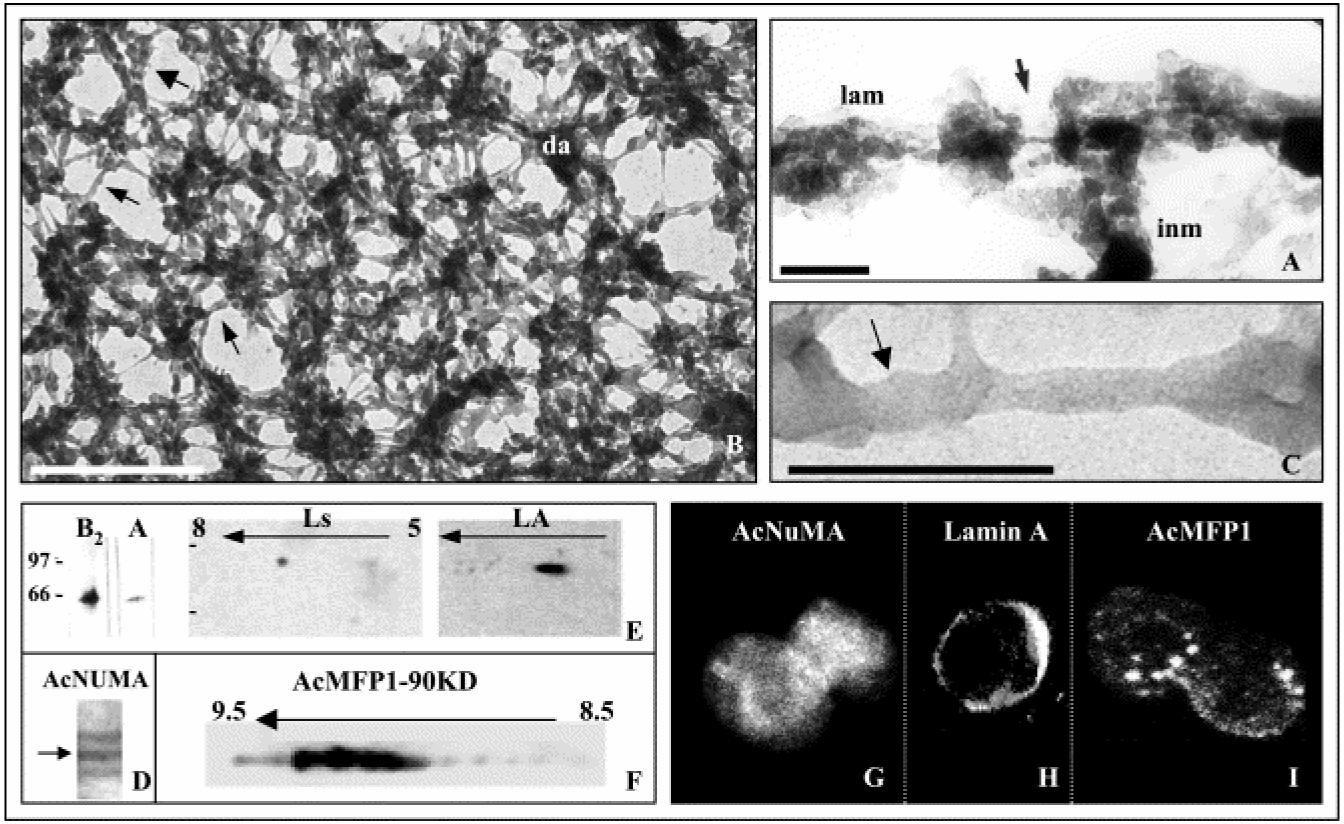


Fig. 1. Ultrastructural organisation of the plant NM in resinless sections. (A) Portion of the peripheral lamina with a complex structure (lam), connected with the internal matrix (inm). Individual filaments are observed (arrow). (B) A delicate anastomosed network of filaments forms the internal matrix: 15–25 nm knobbed filaments (arrows) and thicker ones, covered by globular structures (big arrow;dense aggregates (da). (C) Higher magnification of a branched filament of the internal matrix, showing the junction of two filaments and a typical 25 nm knob. Bar in (A), 0.1 µm; in (B), 0.5 µm; and in (C), 0.1 µm. Identification of the IF proteins by Western blot in the onion NM. (D) The anti-NuMA serum, S2, reveals three onion isoforms, the major one at 220 kD (arrow). (E) Antibodies against chicken B2and A lamins, and a serum against the chicken lamina (Ls) recognise in all cases protein spots at 65 kD with pI varying from 5.65 to 6.8. (F) 2-D blot with serum 288 against LeMFP1. The 90 kD AcMFP1 shows up to 12 basic spots. In situ localisation of the IF proteins in the NM. (G) NuMA is associated with the internal matrix network and accumulates in small foci with a punctate pattern. (H) Lamin-like proteins accumulate not only at the peripheral lamina, but also in internal foci. (I) AcMFP1 accumulation in large replication foci from a late-S phase NM.

Rostliny sice nemají laminy, ale ...

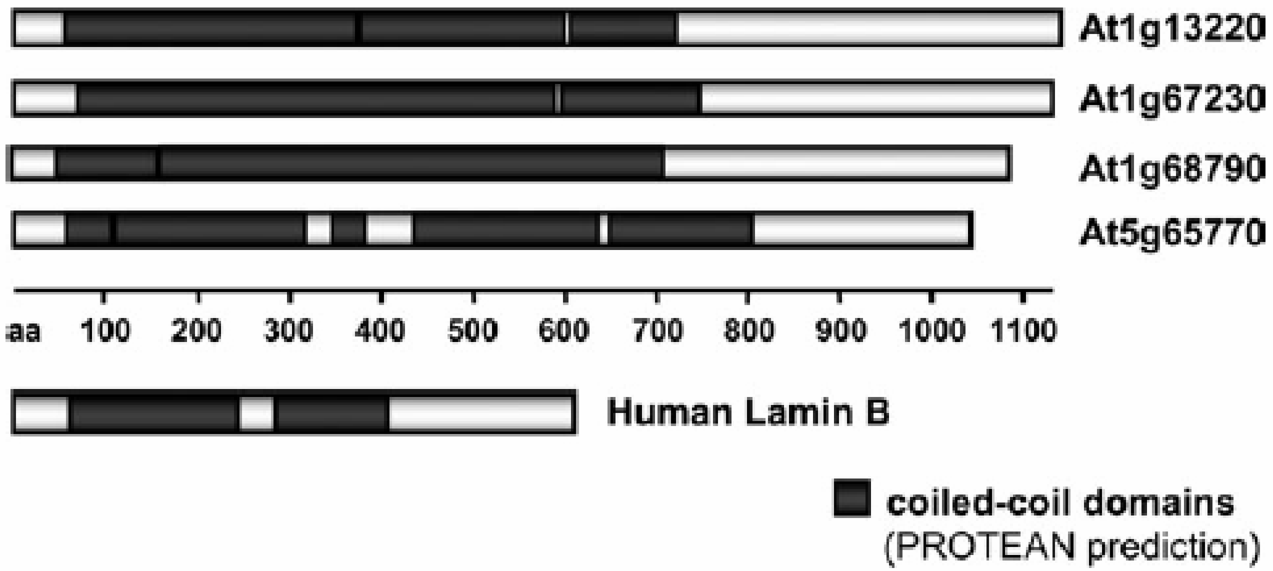


Fig. 1. Comparison of localization and structural organization of human lamin B and *Arabidopsis* nuclear matrix constituent proteins (NMCPs). While almost twice the size of lamins, NMCPs have a comparable domain organization of short head, coiled-coil centre, and longer tail domain. The gene identifiers of the four *Arabidopsis* homologues of DcNMCP1 are shown.

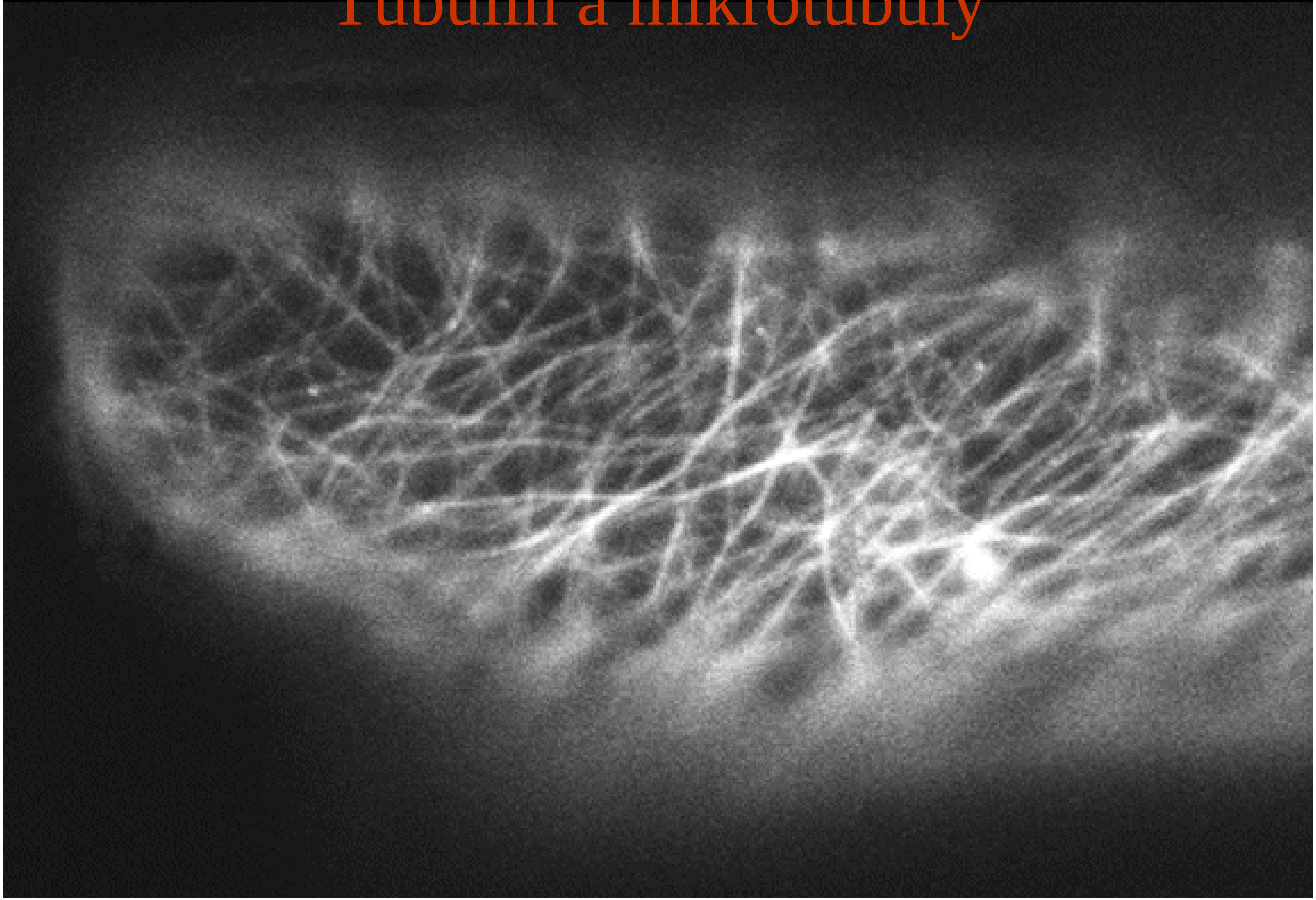
Základní cytoskeletální systémy eukaryot (?)

- Aktinový (mikrofilamenta)
  - všude (... prokaryotní MreB)
- Intermediální filamenta
  - „klasická“ zatím jen u Metazoí? (keratin, vimentin, neurofilamenta, laminy...)
- Tubulinový (mikrotubuly)
  - všude (... prokaryotní FtsZ)

Cytoskelet rostlinné buňky

- Jak o něm víme (historie a metody)
- Obecné rysy a funkce cytoskeletálních systémů
- Aktin a asociované proteiny
- Tubulin, mikrotubuly a asociované proteiny
- Molekulární motory
- Koordinace cytoskeletálních systémů a …
  - buněčného cyklu
  - exocytosy, polarity, buněčné stěny
  - organel

Tubulin a mikrotubuly



Mikrotubulární systémy rostlinné buňky

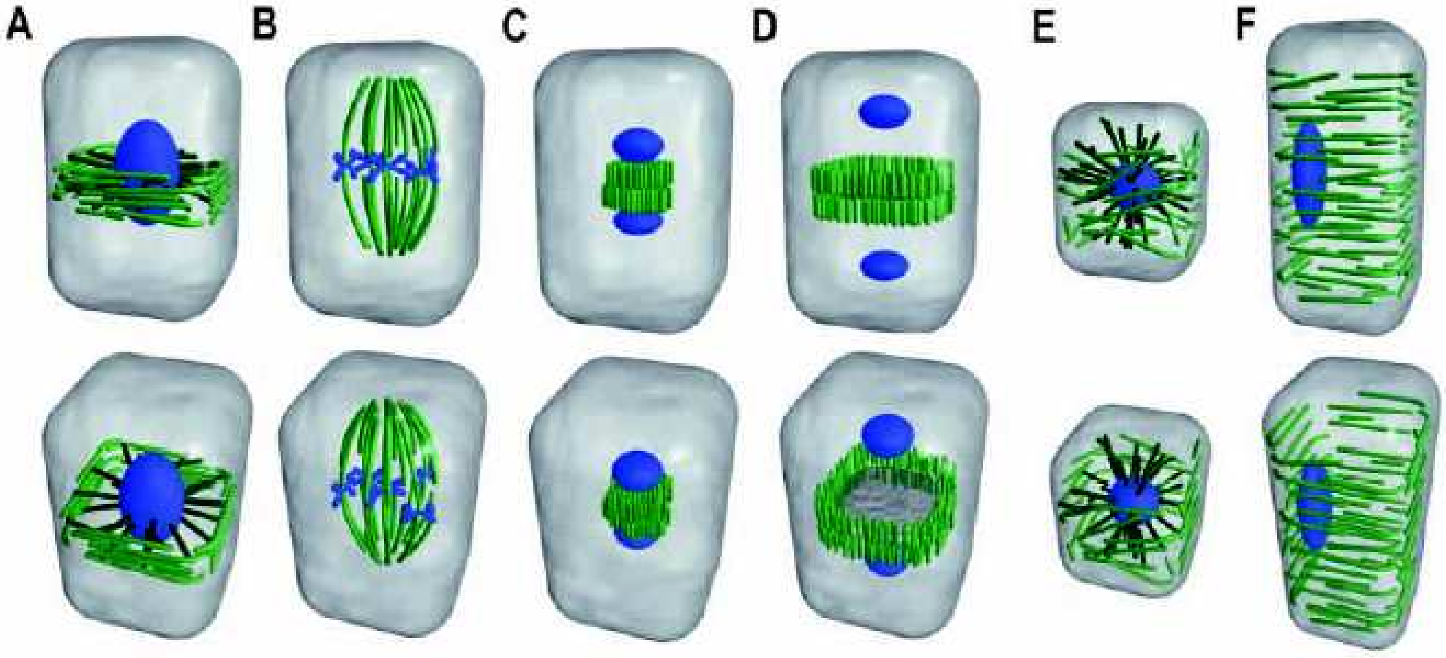
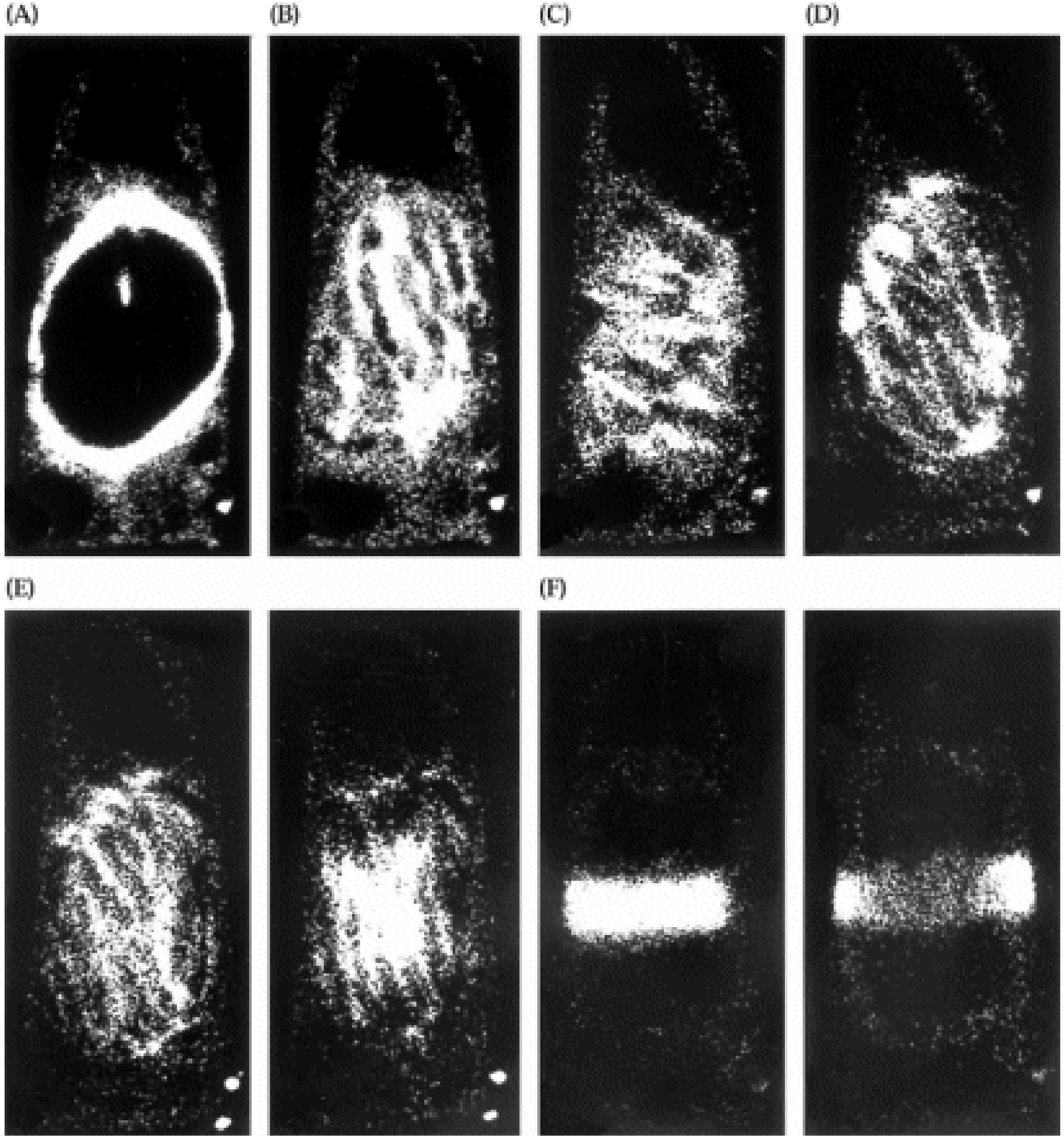


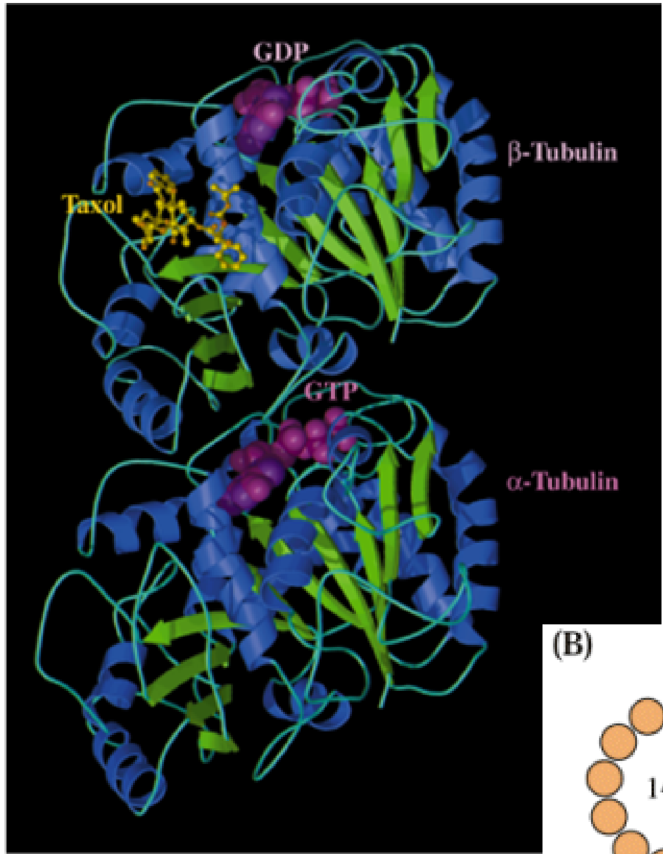
Fig. 1. These schematic illustrations, rendered in 3D at two aspects, show microtubule arrays through the plant cell cycle. (A) A preprophase band, linked to the nucleus by phragmosome microtubules, marks the future division site. (B) Metaphase spindle with a dispersed polar region. (C) In telophase, 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.

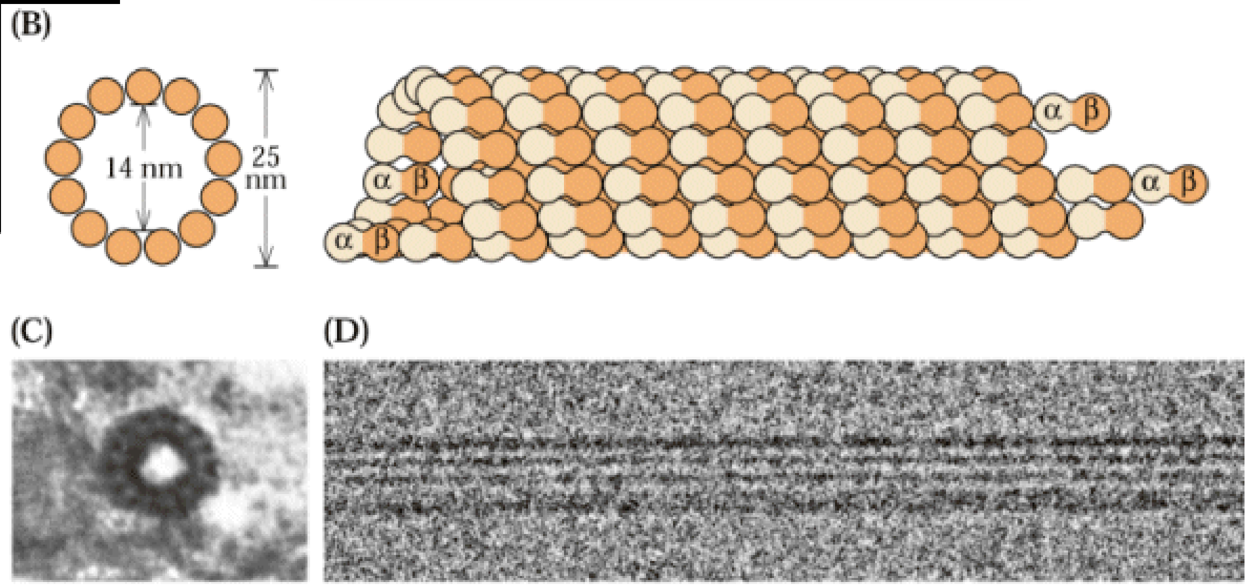


Tradescantia,
injected
bovine tubulin

Mikrotubuly: podjednotky a stavba

Co je uvnitř? ... např. K40 acetylace!





Video:
Dr. E. Nogales

The Royal Society

Quantum computation in brain microtubules? The Penrose–Hameroff 'Orch OR' model of consciousness

By Stuart Hameroff

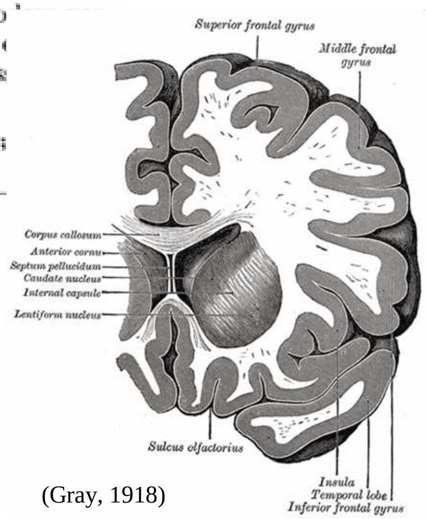
Departments of Anesthesiology and Psychology, The University of Arizona, Tucson, AZ 85724, USA

Potential features of quantum computation could explain enigmatic aspects of consciousness. The Penrose–Hameroff model (orchestrated objective reduction: 'Orch OR') suggests that quantum superposition and a form of quantum computation occur in microtubules—cylindrical protein lattices of the cell cytoskeleton within the brain's neurons. Microtubules couple to and regulate neural-level synaptic functions, and they may be ideal quantum computers because of dynamical lattice structure, quantum-level subunit states and intermittent isolation from environmental interactions. In addition to its biological setting, the Orch OR proposal differs in an essential way from technologically envisioned quantum computers in which collapse, or reduction to classical output states, is caused by environmental decoherence (hence introducing randomness). In the Orch OR proposal, reduction of microtubule quantum superposition to classical output states occurs by an objective factor—Roger Penrose's quantum gravity threshold stemming from instability in Planck-scale separations (superpositions) in spacetime geometry. Output states following Penrose's objective reduction are neither totally deterministic nor random, but influenced by a non-computable factor ingrained in fundamental spacetime. Taking a mo psychist view in which protoconscious experience and Platonic values are in Planck-scale spin networks, the Orch OR model portrays consciousnes activities linked to fundamental ripples in spacetime geometry.

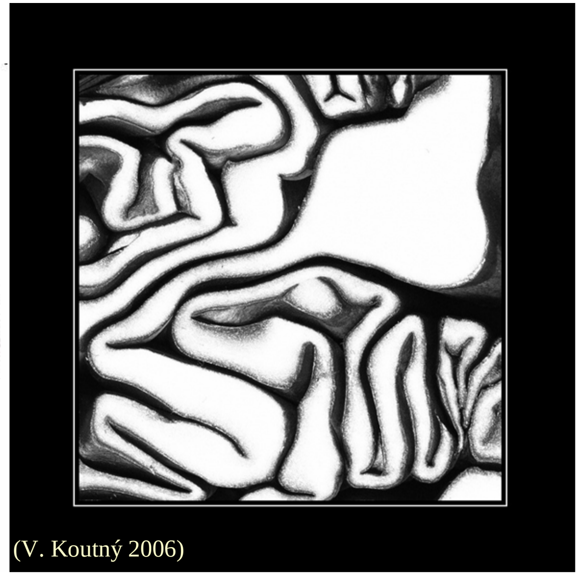
Keywords: consciousness; quantum computation; objective reduction; orchestrated objective reduction (Orch OR); microtubules; brain

...ale co za biologicky relevantních podmínek?

- Současné modely: spíš ne
- Obecné ponaučení: nehledejme (lidské) vědomí v něčem, co sdílí vše živé, navzdory povrchní podobnosti

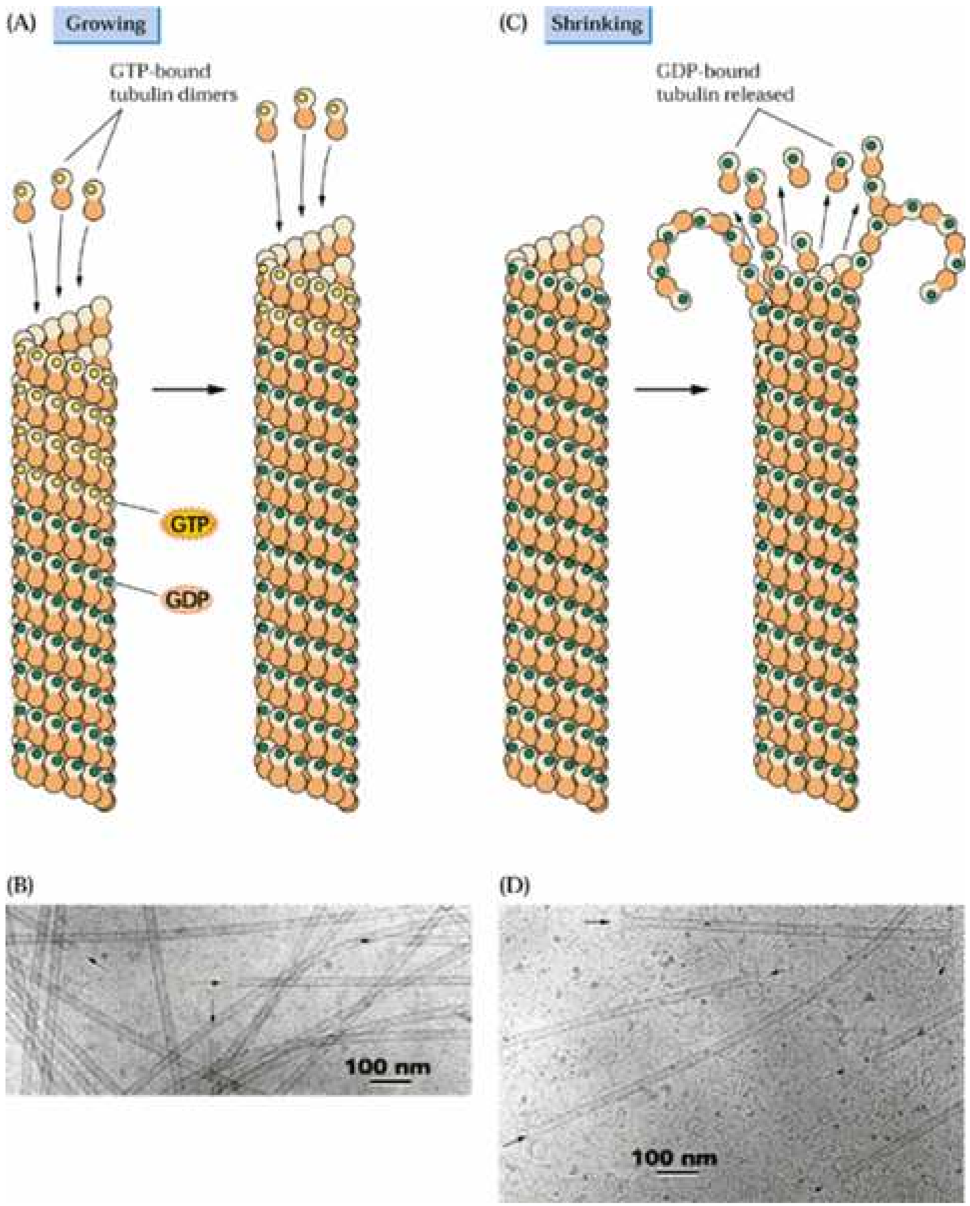


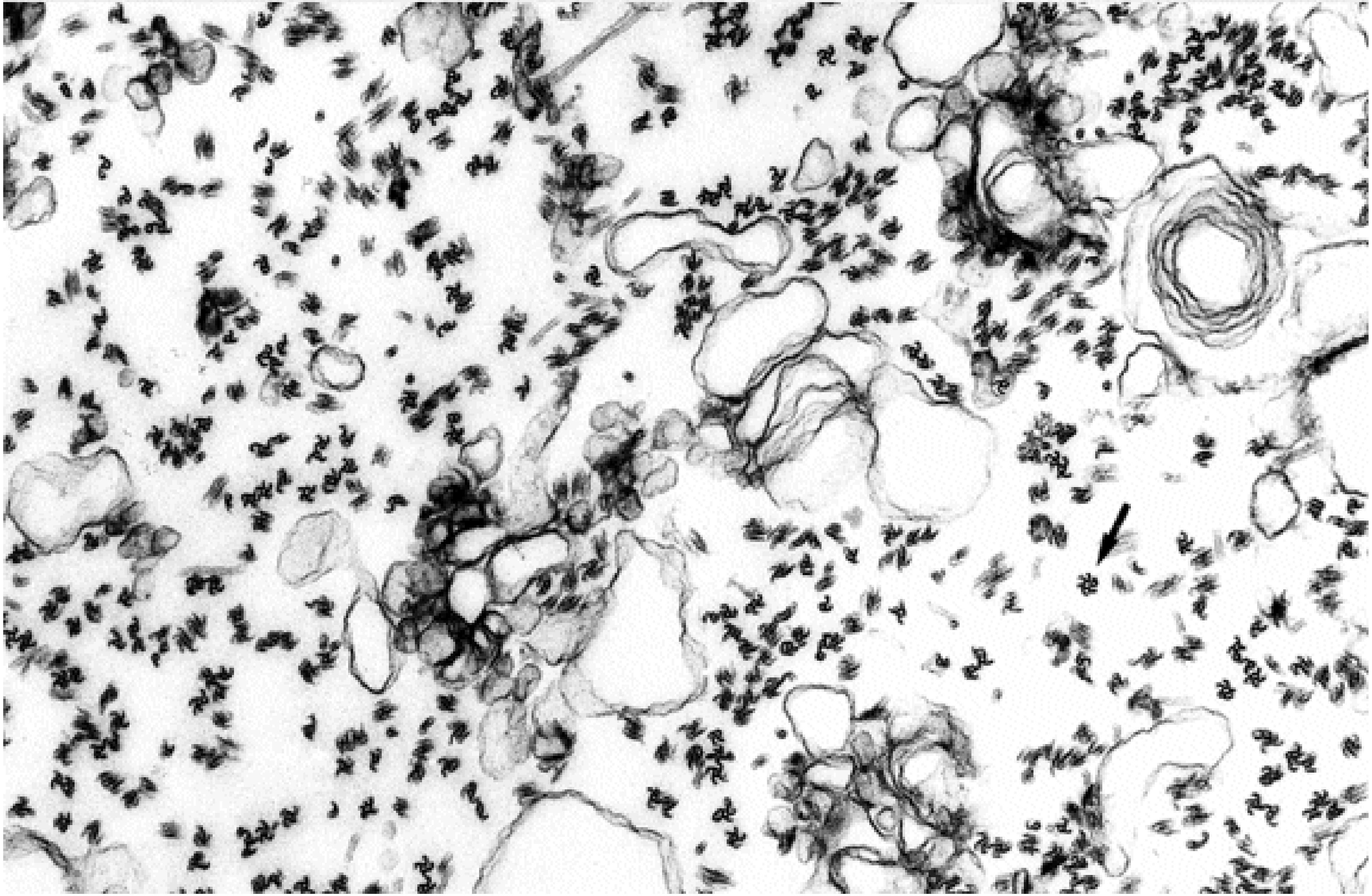
(Gray, 1918)



(V. Koutný 2006)

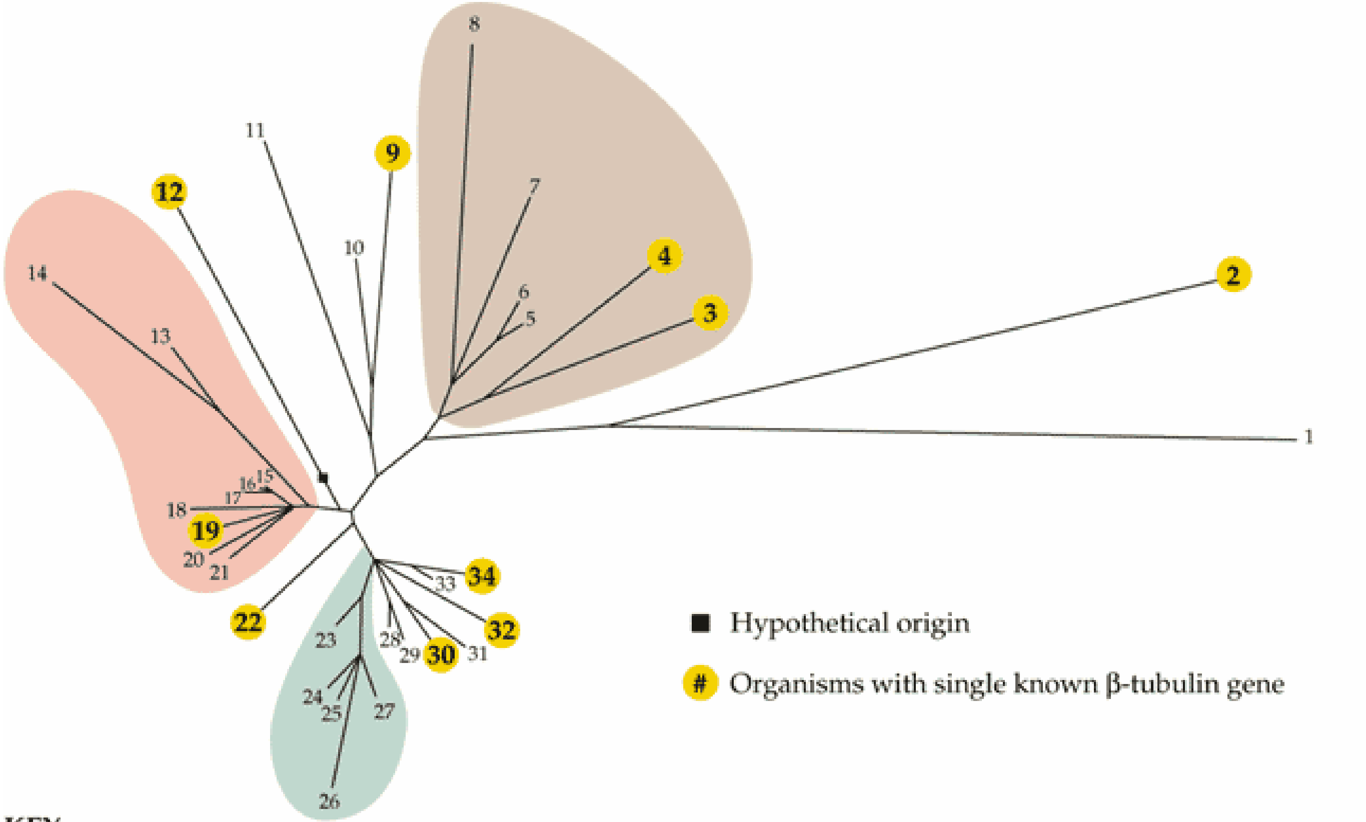
Dynamika přestaveb mikrotubulů





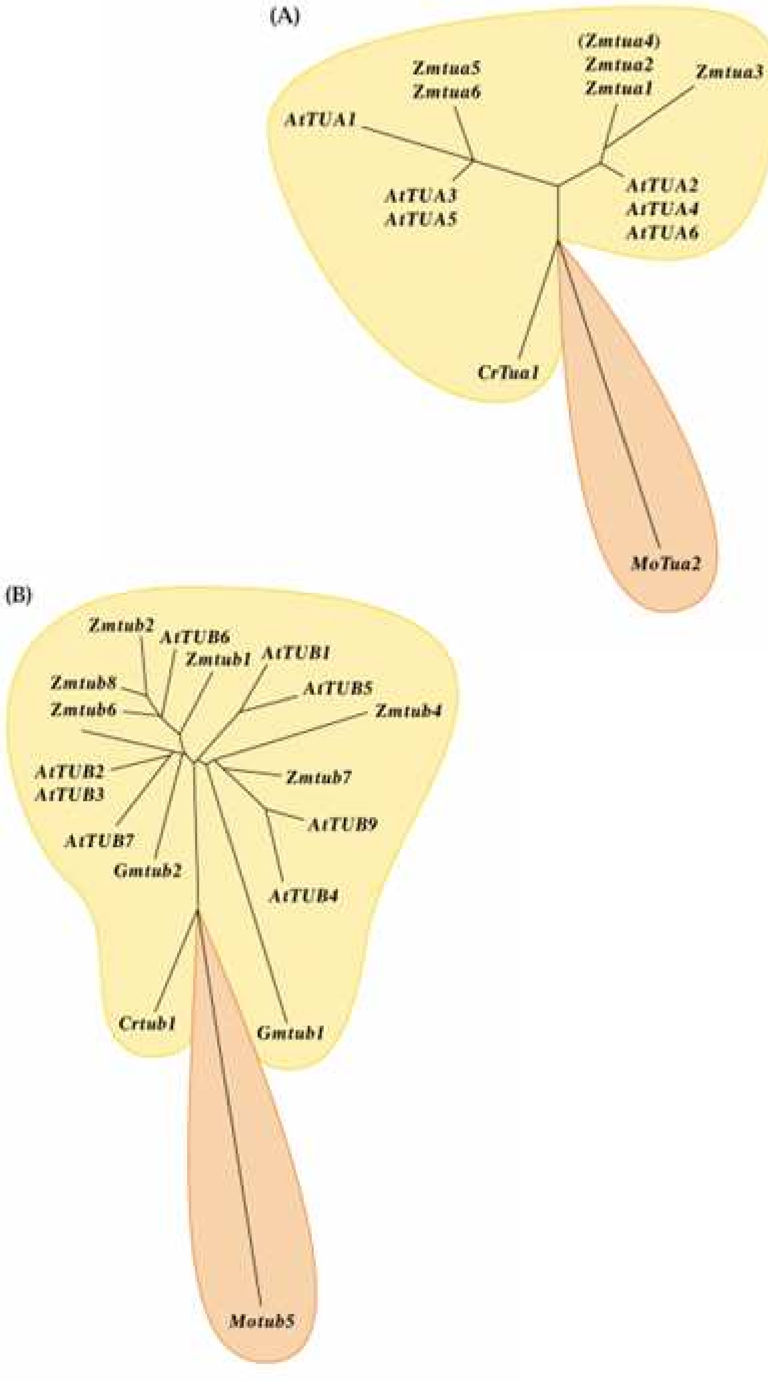
tubulinové „háčky“ v isol. fragmoplastech v nadbytku tubulinu

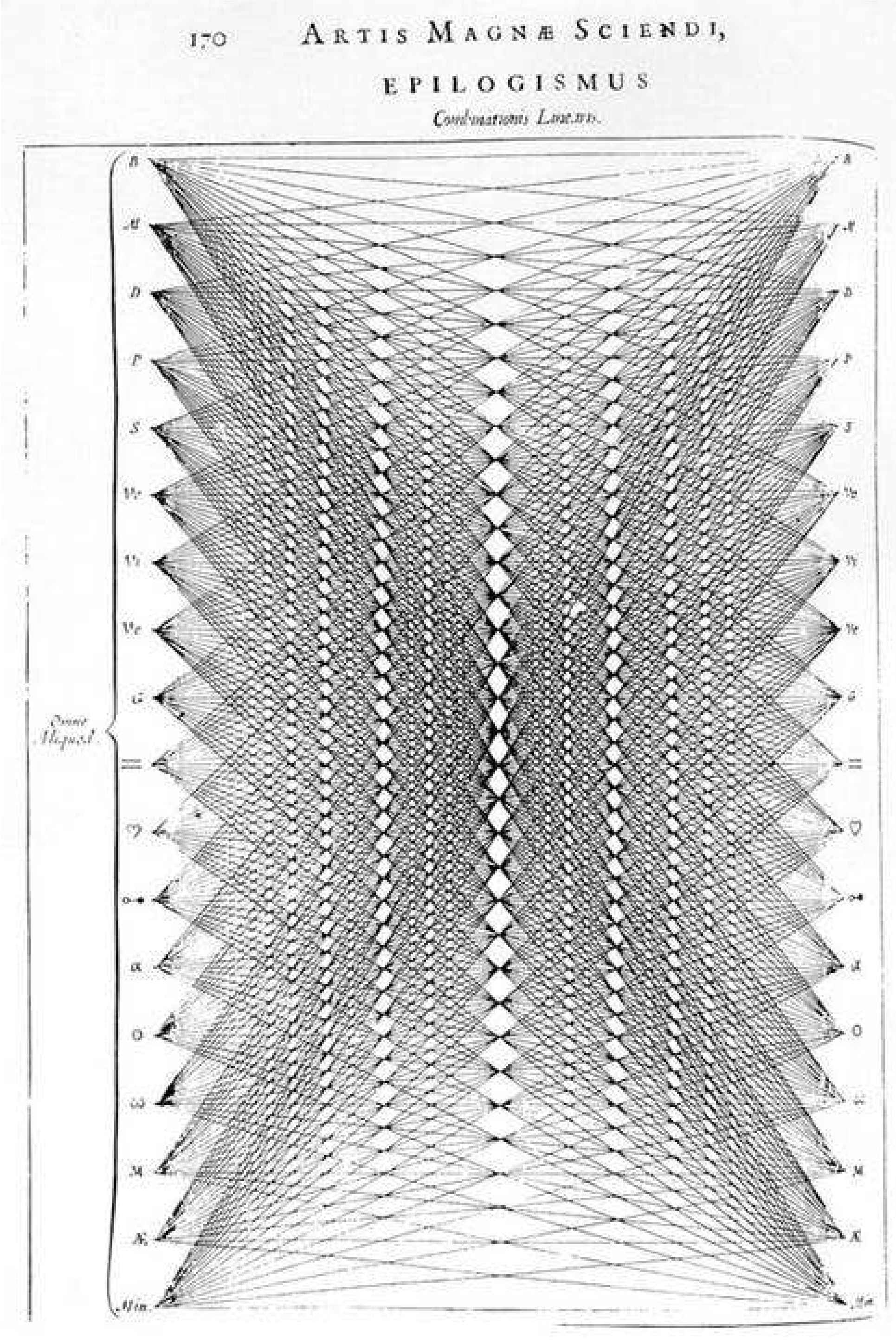
Rodiny tubulinových genů

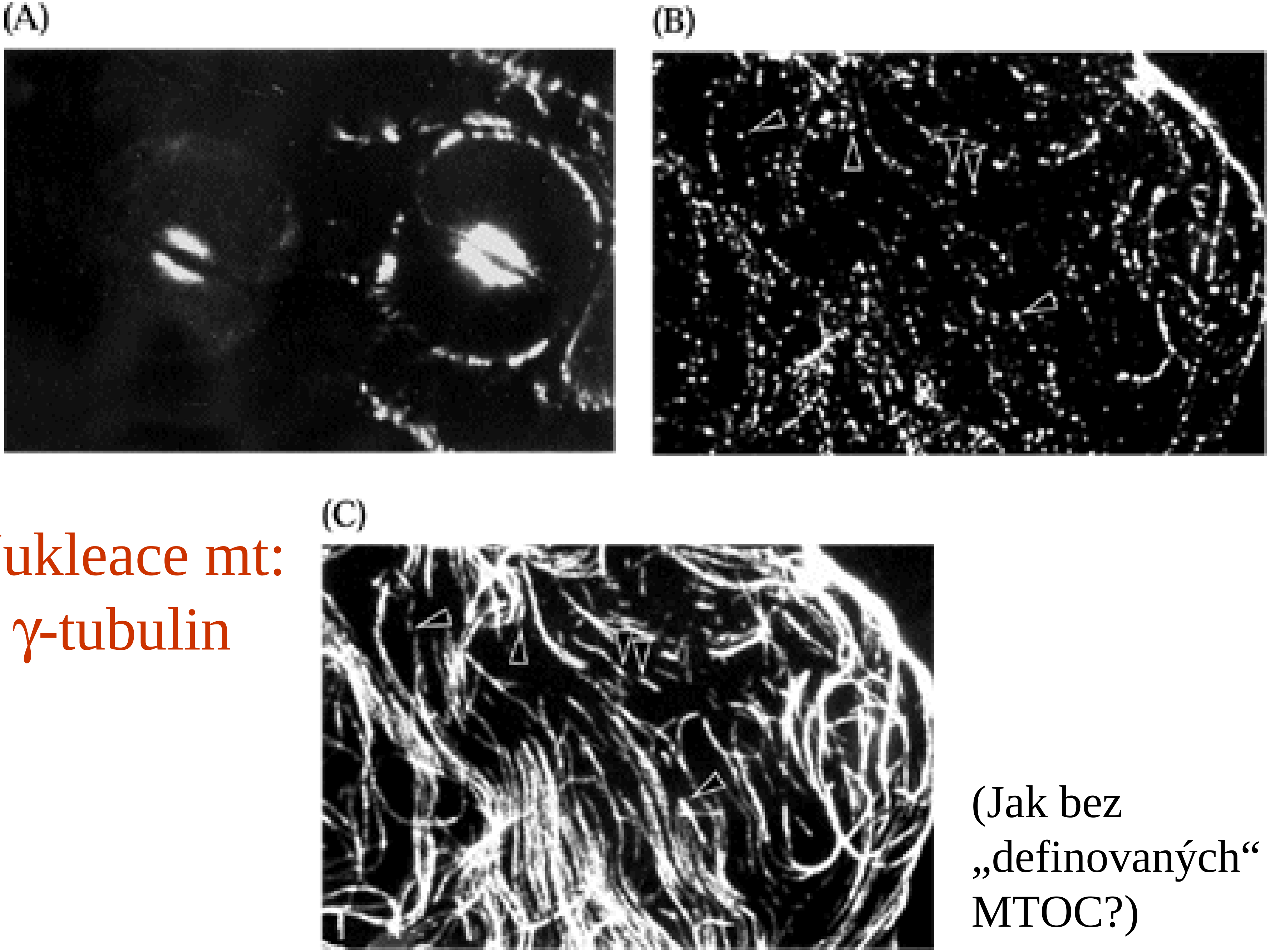


KEY:

Fungi	Metazoa	Plants + green algae	Protozoa	Primitive eukaryotes
3: *S. pombe*	13: Chicken β6	23: *Chlamydomonas* β1	1: *Reticulomyxa* β2	2: *Entamoeba*
4: *S. cerevisiae*	14: Mouse β1	24: *Arabidopsis* β1	9: *Dictyostelium*	12: *Trichomonas*
5: *Colletotrichum* β2	15: *Xenopus* β2	25: Maize β2	10: *Physarum* β2	22: *Giardia*
6: *Aspergillus* BenA	16: Human β5	26: Soybean β1	11: *Reticulomyxa* β1	
7: *Aspergillus* TubC	17: Hamster β1	27: Pea β2	28: *Tetrahymena* β1	
8: *Colletotrichum* β1	18: *Haemonchus* β8		29: *Plasmodium* βA	
	19: *Caenorhabditis*		30: *Achlya*	
	20: *Drosophila* β3		31: *Ectocarpus* βb	
	21: Chicken β5		32: *Trypanosoma*	
			33: *Physarum* β1	
			34: *Euglena*	


(A)
Zmtua5
Zmtua6
(Zmtua4)
Zmtua2
Zmtua1
Zmtua3
AtTUA1
AtTUA3
AtTUA5
AtTUA2
AtTUA4
AtTUA6
CrTua1
MoTua2
(B)
Zmtub2
AtTUB6
Zmtub1
AtTUB1
Zmtub8
AtTUB5
Zmtub6
Zmtub4
AtTUB2
AtTUB3
Zmtub7
AtTUB9
AtTUB7
Gmtub2
AtTUB4
Crtub1
Gmtub1
Motub5

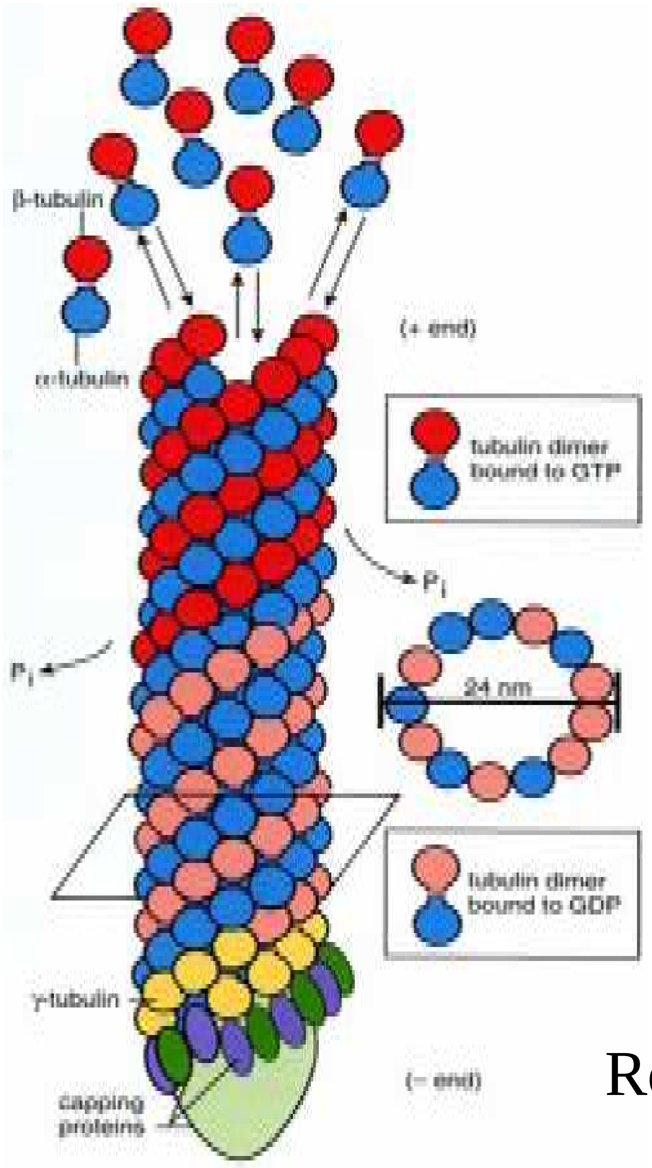

170 ARTIS MAGNÆ SCIENDI,
EPILOGISMUS
Combinationis Linearis.

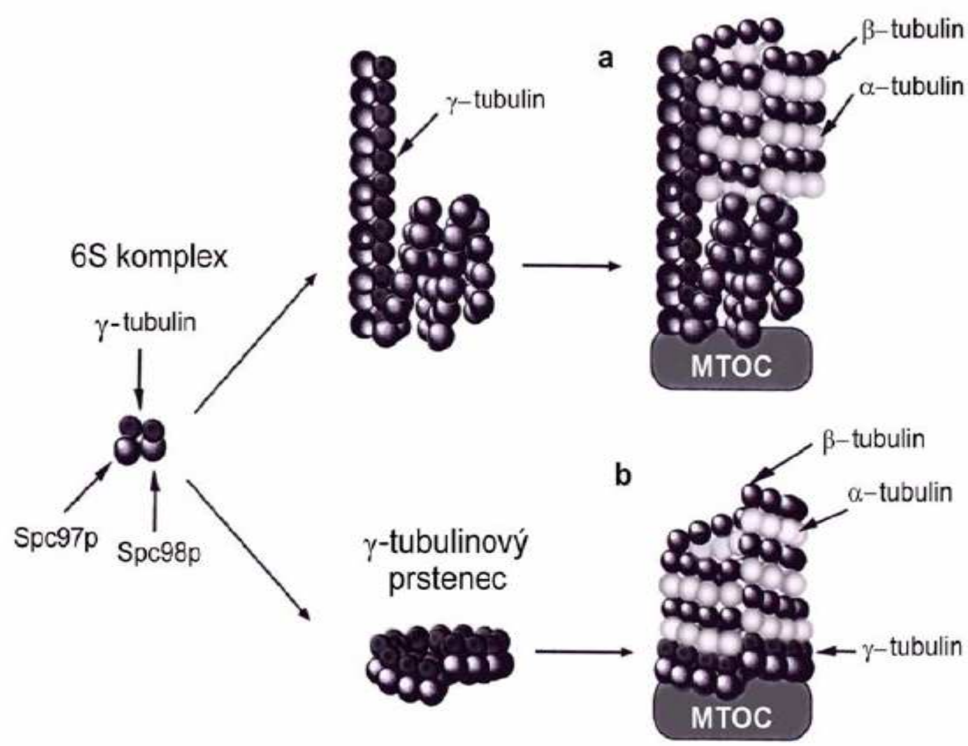


Nukleace mt: γ-tubulin

(Jak bez „definovaných“ MTOC?)

Nukleace mikrotubulů – gamma-tubulin





Rostliny nemají strukturně definovaná MTOC

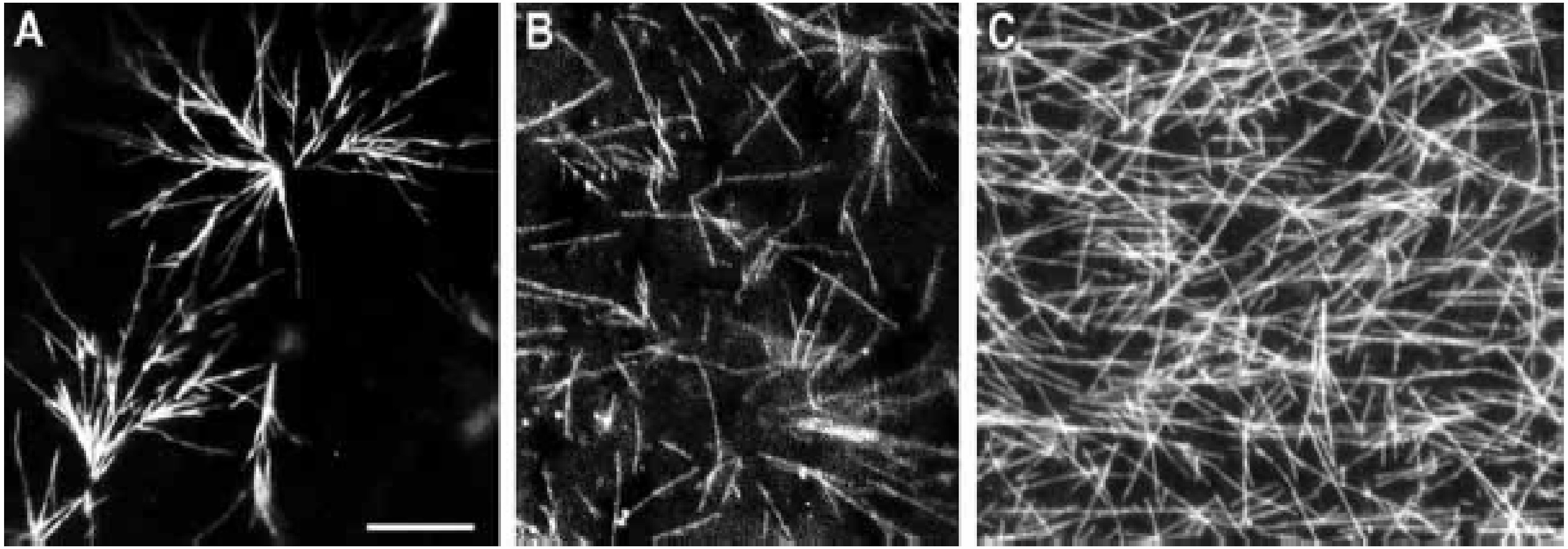
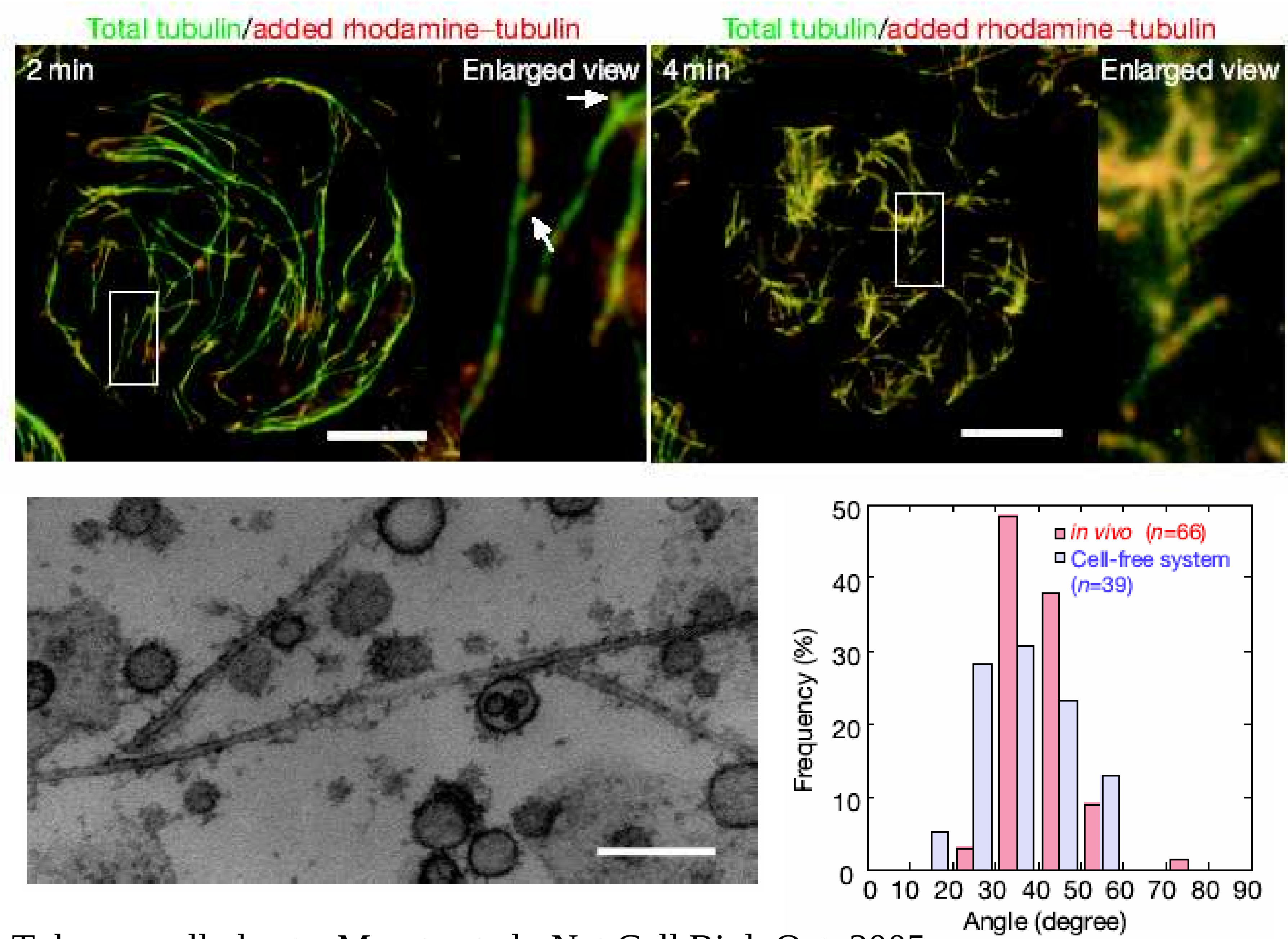


Fig. 2. Cortical microtubule recovery patterns after drug-induced microtubule disassembly. (A) Microtubules appear to diverge from the initial assembly site, forming fractal tree-shaped clusters, with microtubules diverging from each other at acute angles (figure adapted from Wasteneys and Williamson, 1989b). (B) Clusters eventually break up. (C) Later in recovery, parallel microtubule order begins to consolidate but some branching configurations and discordant microtubules persist. Bar, 10 µm.



Tobacco cell ghosts; Murata et al., Nat.Cell.Biol. Oct. 2005

Nukleace γ-tubulinem - model

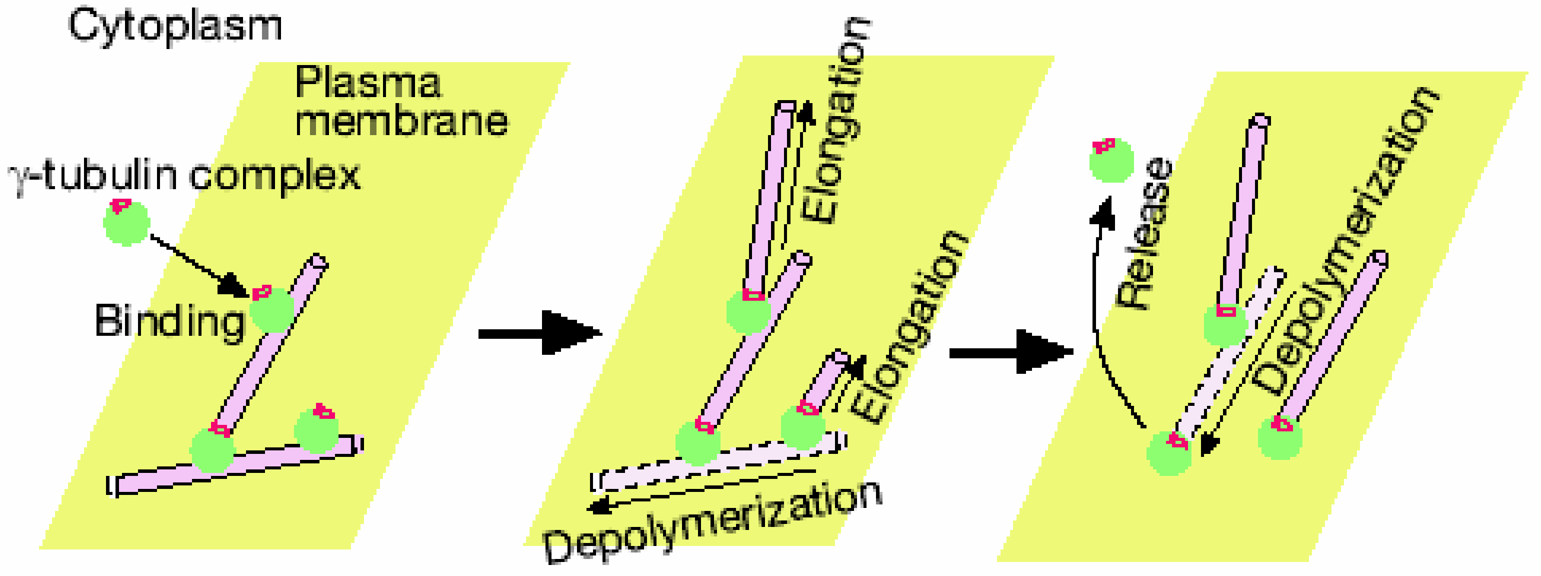
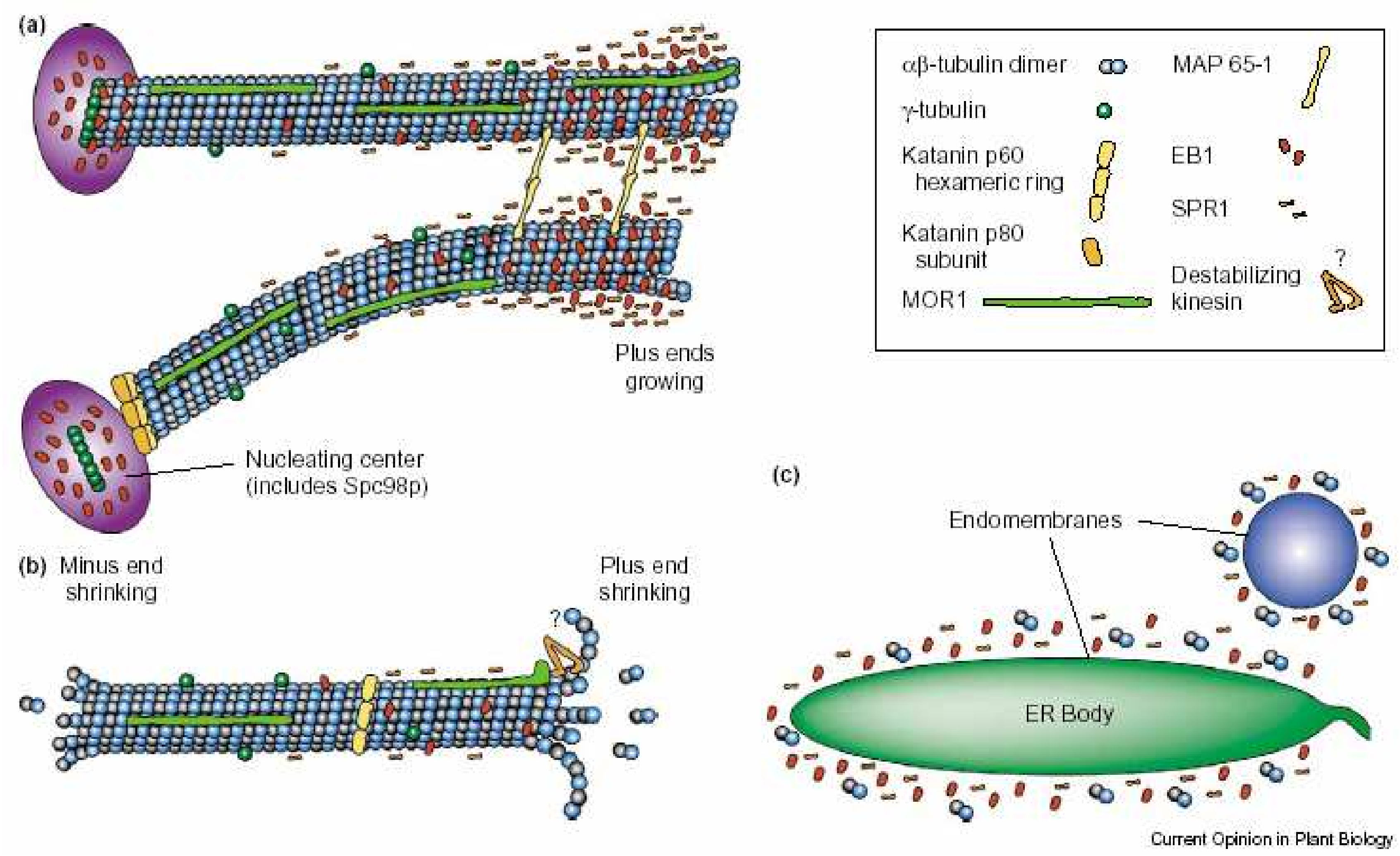


Figure 5 Model for nucleation of cortical microtubules in plants. Cytosolic γ-tubulin complexes bind onto pre-existing cortical microtubules (left) and nucleate microtubules as branches (centre). The original microtubules frequently depolymerize (centre) but γ-tubulin complexes are not released until depolymerization of newly formed microtubules occurs (right).

Modulace dynamiky mikrotubulů - MAPs



MAPS predicted to affect microtubule dynamics and organization.

Predicted MAP (species)	Family size	Intracellular localization	Mutant name	Mutant phenotype	Predicted cellular function
AtEB1a, AtEB1b (*Arabidopsis*)	3	Cortical MT plus ends, spindle poles, cortical MT nucleating sites, around endomembranes?	ND	ND	MT polymerization, recruiting MAPs to MTs
Katanin P60 subunit (AtKSS, AtKTN1) (*Arabidopsis*)	1[a]	Spindle poles; cortical MT nucleating sites, punctate along cortical MTs, perinuclear regions	*botero*, *fra2*, *frc2*, *erh3*, *lue1*	Disorganized cortical MTs, isotropic growth, cell wall composition/organization, ectopic root hairs, gibberellin signaling, trichome branching	ATP-dependent MT severing
Katanin P80 subunit (*Arabidopsis*)	4	ND (presumed to colocalize with katanin P60)	ND	ND	Targeting and regulation of katanin P60?
MAP-65 (main isoform in carrot suspensions)	ND	Along MTs in interphase cells	ND	ND	Cortical MT bundling and organization in interphase cells
NtMAP65-1b (tobacco)	ND	ND (presumed to be along MTs)	ND	ND	MT bundling and organization, stabilize MTs against cold
AtMAP65-1 (*Arabidopsis*)	9	Along MTs in preprophase band, at the phragmoplast midline, along a subset of cortical MTs	ND	ND	Crosslinking MTs
AtMAP65-3 (PLEIADE) (*Arabidopsis*)	9	Along MTs in preprophase band, at phragmoplast midline where antiparallel MTs overlap	*ple*	Cytokinesis defect in roots, distorted phragmoplasts	Crosslinks MTs and/or promotes MT polymerization in phragmoplast

EB1 a jiné (+)TIPs

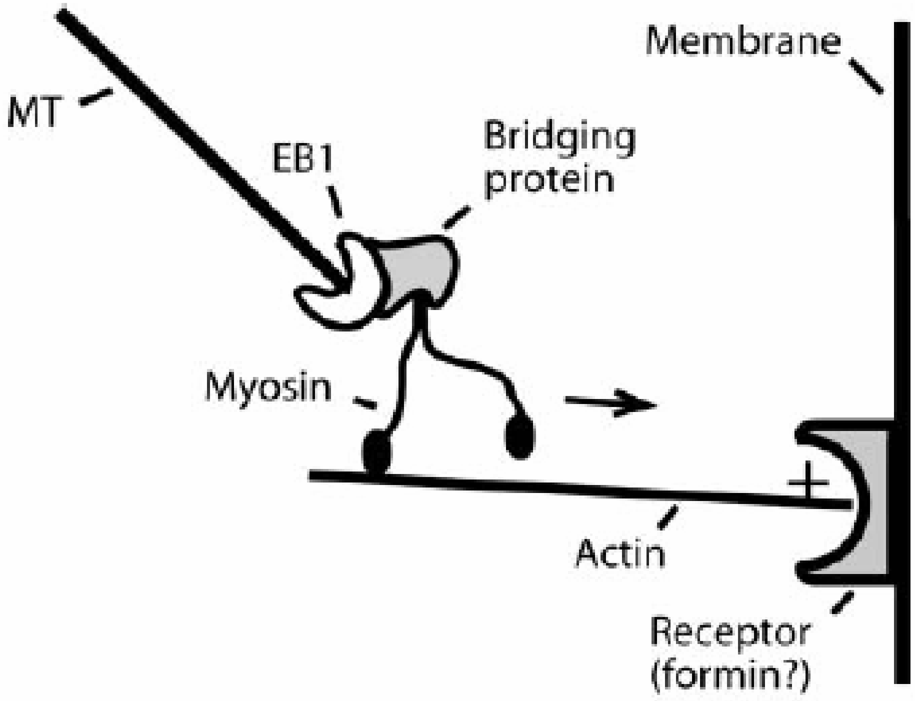
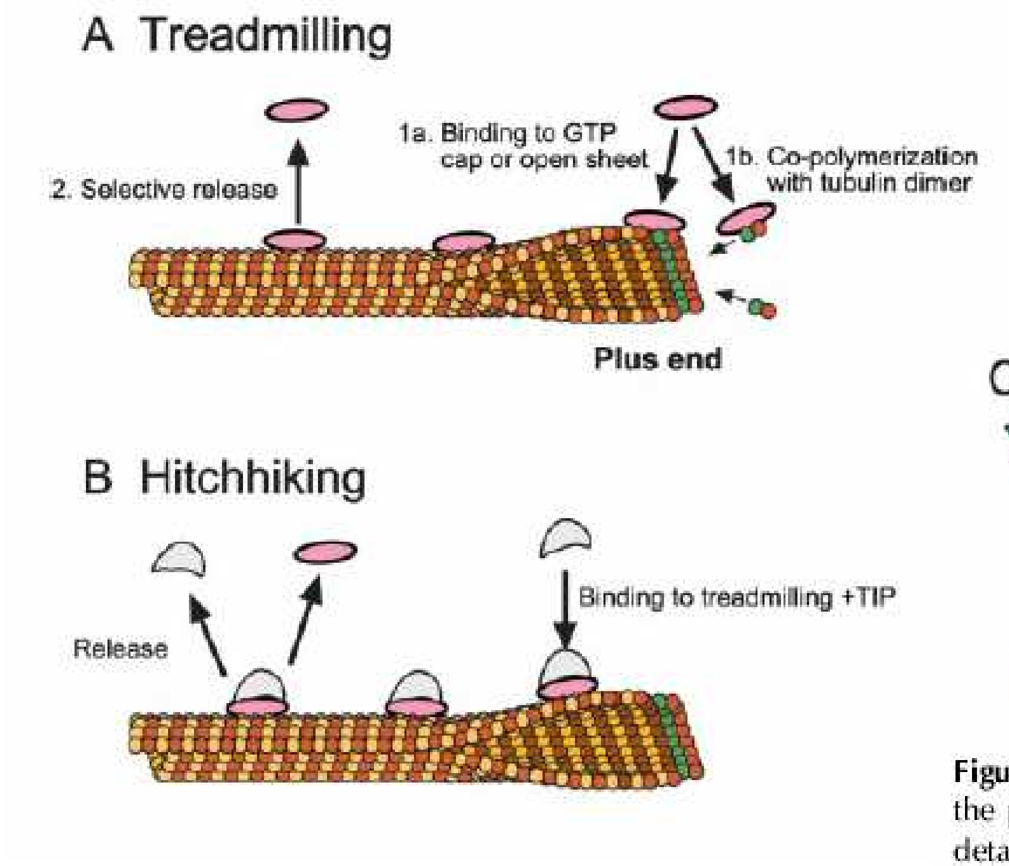


Figure 2. Hypothetical model for delivery of a plant MT to a cellular receptor at a specific site (e.g. PPB, phragmoplast, root hair tip) along actin filaments, based on models from yeast and fibroblasts (Gundersen et al., 2004). EB1 binds a bridging protein associated with myosin, which translocates toward the barbed (plus) end of the actin filament. Genome analysis identifies EB1, myosins, and formins in Arabidopsis.

Jak se proteiny mohou dostat na + konec mt?



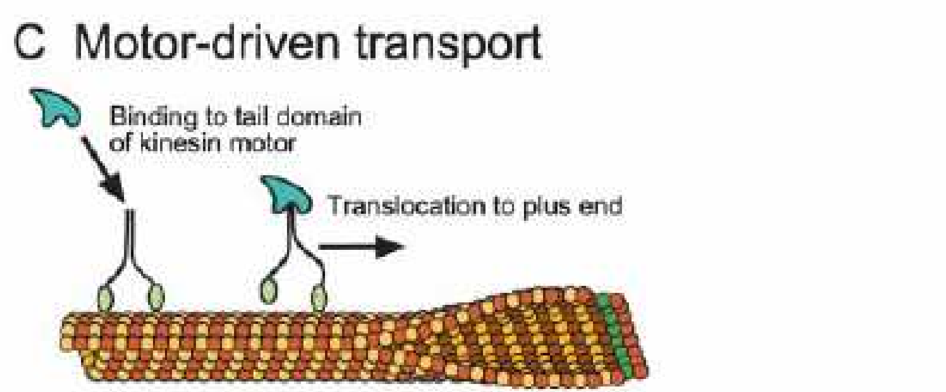
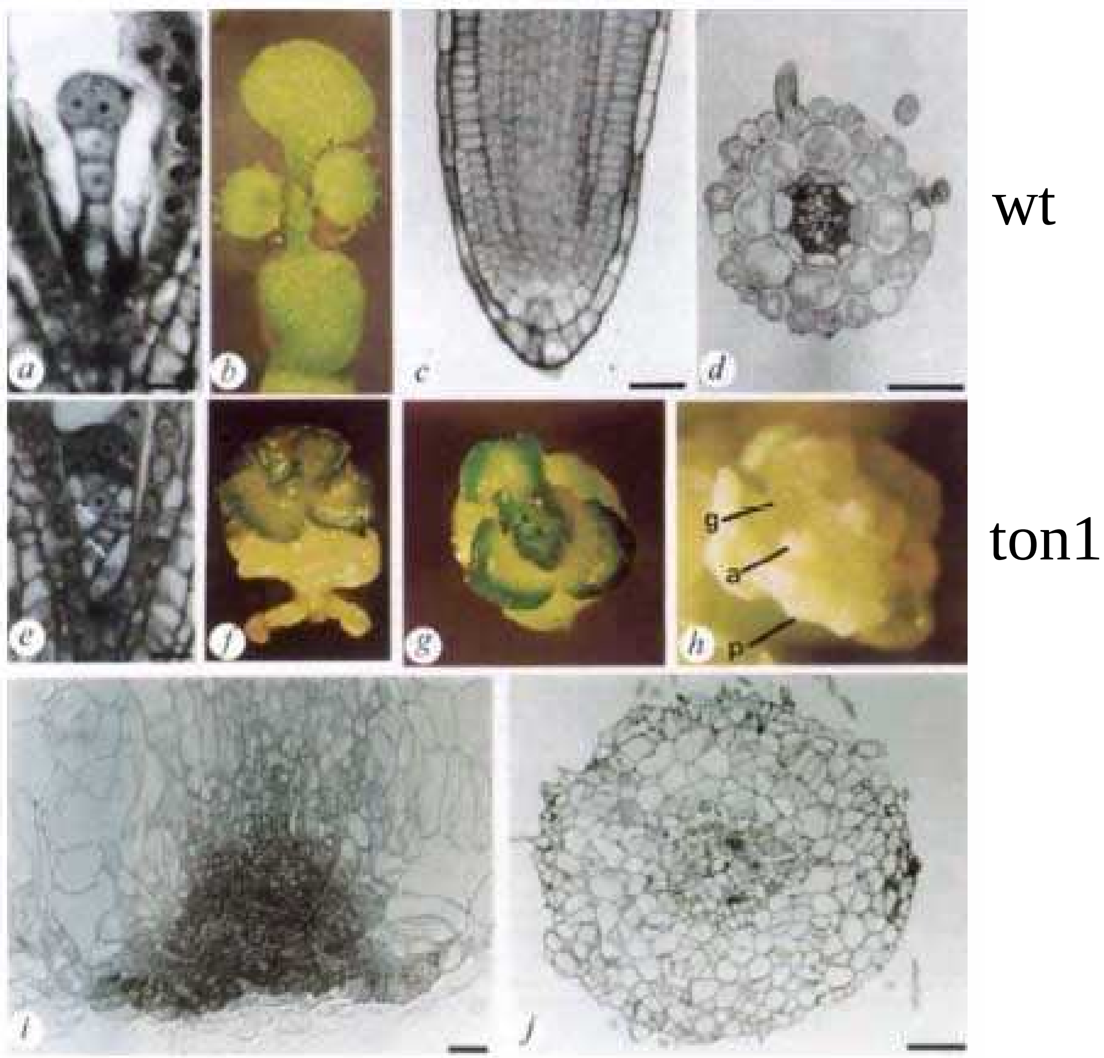


Figure 1. Proposed mechanisms for plus-end binding. Green circles at the plus end of the MT denote α-tubulin bound to GTP. See text for details.

TON1 (*tonneau*)

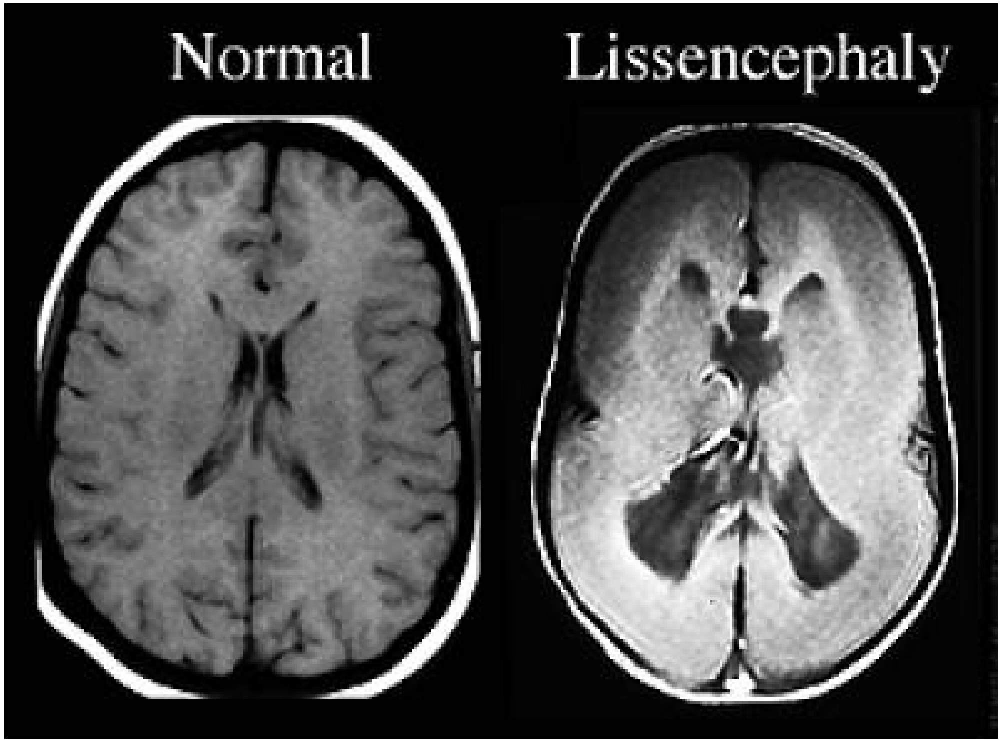
- defekt PPB a obec. mt organizace
- rodina genů



(Traas et al. 1995)

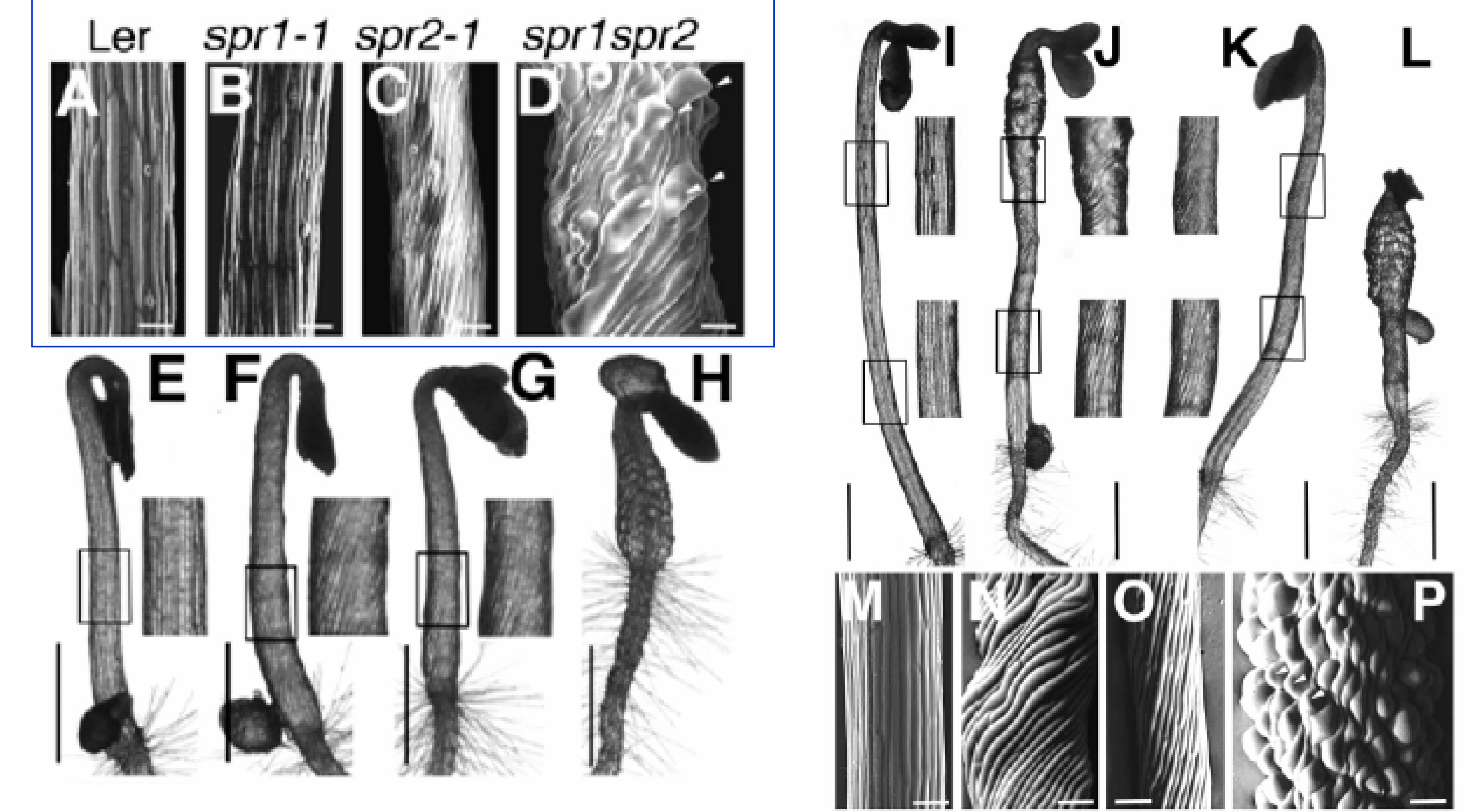
TON1 (*tonneau*)

- homolog *LISSENCEPHALY1* (H.s. LIS1) - defekt migrace neuronů
- LIS1 váže + konce mt, interakce s dyneinem
- ale rostliny nemají dynein



SPR1, SPR2 (spiral)

světlo



etiol.

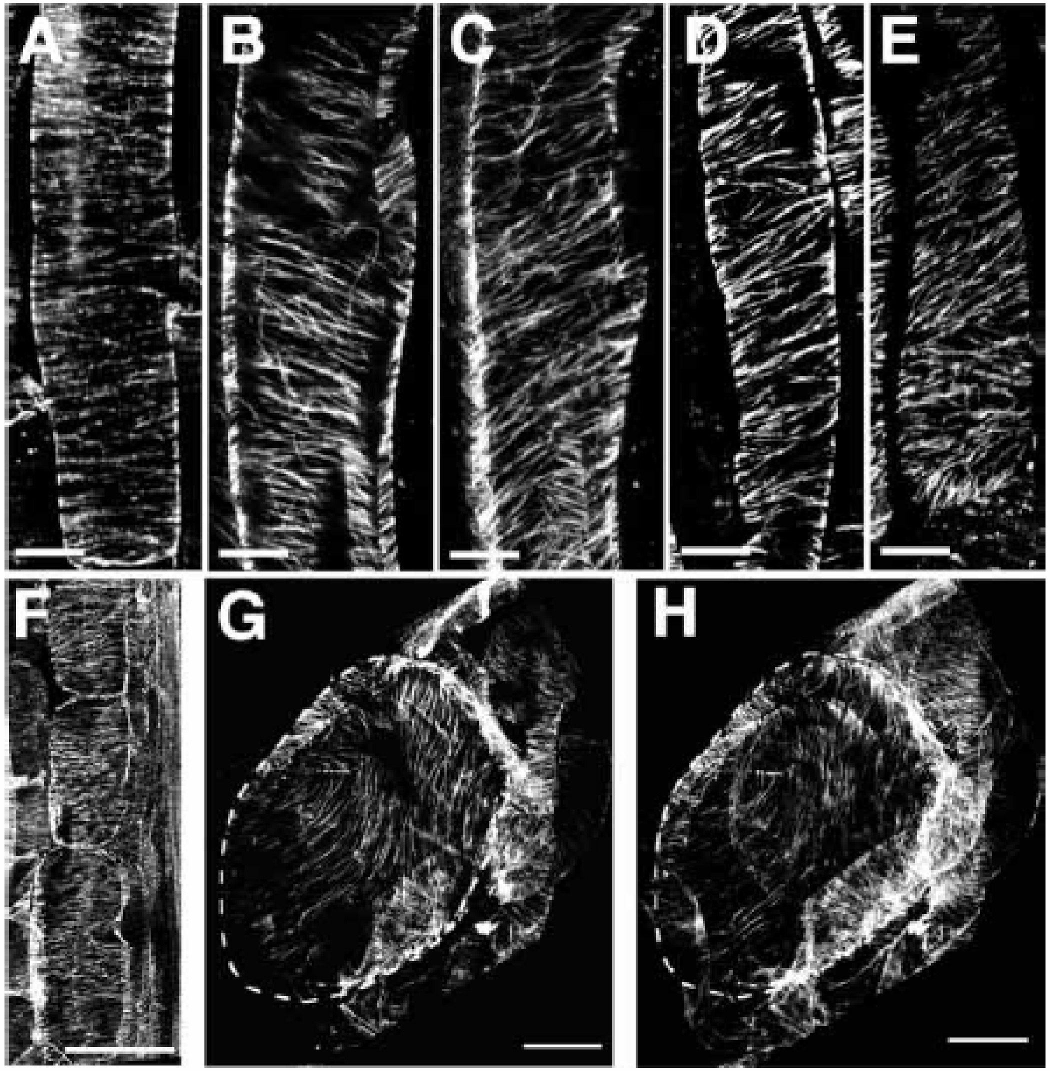
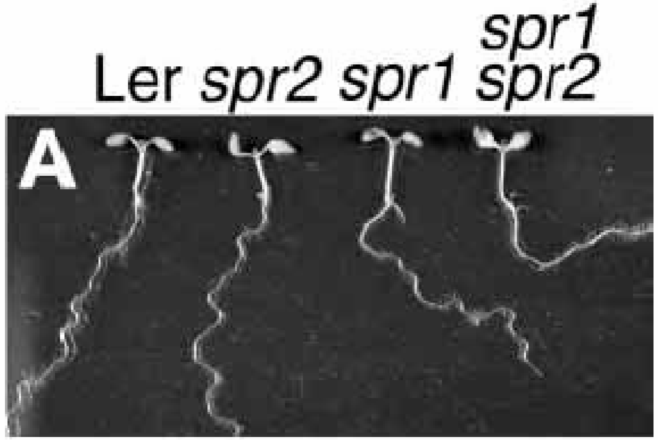
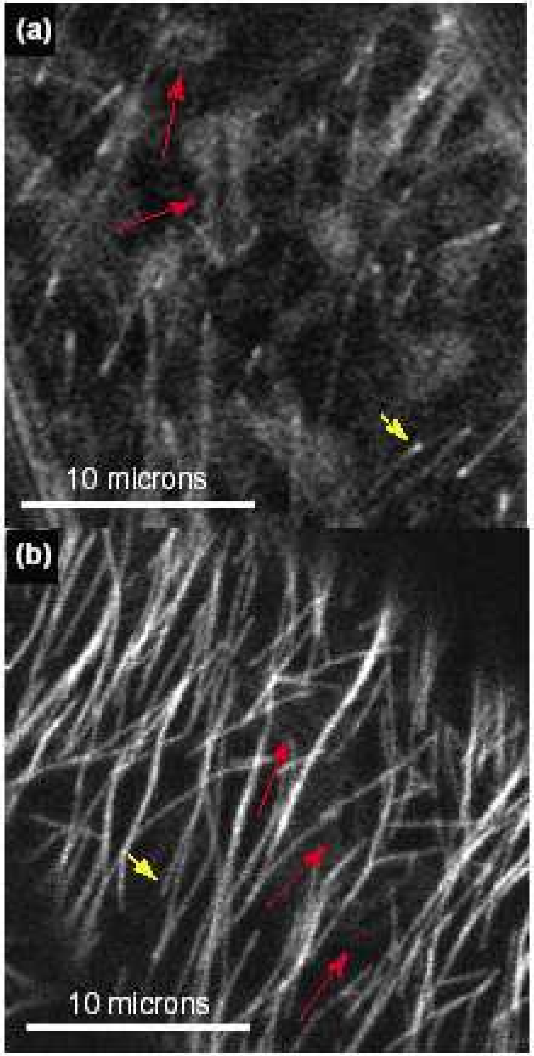


Fig. 9. Micrographs of cortical MTs in seedlings grown at 23°C. (A-E) Root epidermal cells at the basal elongation zone. (A) Ler, (B, C) *spr1-1*, (D) Ler grown on 1 μM taxol and (E) Ler grown on 3 μM propyzamide. Cortical MT arrays were located underneath the outer cell wall (A,B,D,E) or the inner cell wall (C). Panels B and C were from the same *spr1* cell. (F-H) Inner cortex cells at the upper region of 5-day-old etiolated hypocotyls. (F) Ler, and (G,H) *spr1-1*. G and H were from the same *spr1* hypocotyl optically sectioned at different focal planes. Broken line indicates shape of a cell in G and H. All images are positioned in their correct orientation relative to the long axis of the organ. Scale bars, 10 μm in A-E; 50 μm in F-H.



SPR1: malý protein na + koncích mt



Localization of SPR–GFP and YFP–TUBULIN in the hypocotyl cells of transgenic *Arabidopsis* seedlings. GFP fluorescence from **(a)** an SPR1–GFP fusion protein and **(b)** an YFP–TUBULIN fusion protein (single confocal microscope slices). Note that the SPR1–GFP localizes preferentially to the MT plus ends (yellow arrowhead) as well as in the cytoplasm surrounding endomembranes (delineated by red arrows). By contrast, YFP–TUBULIN evenly labels MTs, yet is also found around endomembranes (delineated by red arrows). For a better view, please watch supplemental movies S1 and S3 in [35**]. (b) is reproduced with permission from [35**], copyright American Society of Plant Biologists (2004).

„Točivost“ orgánů mutantů: směr nemusí souhlasit se směrem mt spirály

- Pravotočivé:
  - spr1
- Levotočivé:
  - inhibitory (taxol)
  - MAP (*mor1*)
  - *lefty1*, *lefty2*: mutace tubulinu

Točí se rostlina bez mikrotubulů doleva? A jak to dělá??

Katanin - p60 a p80

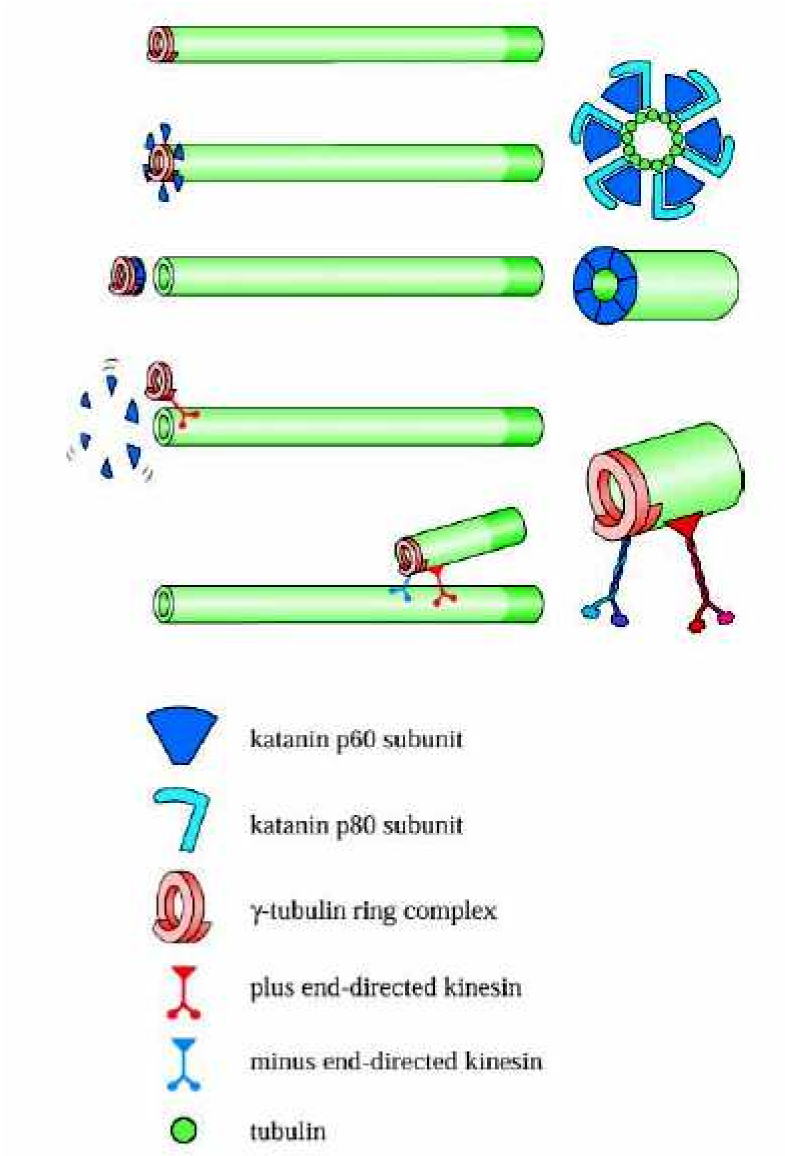
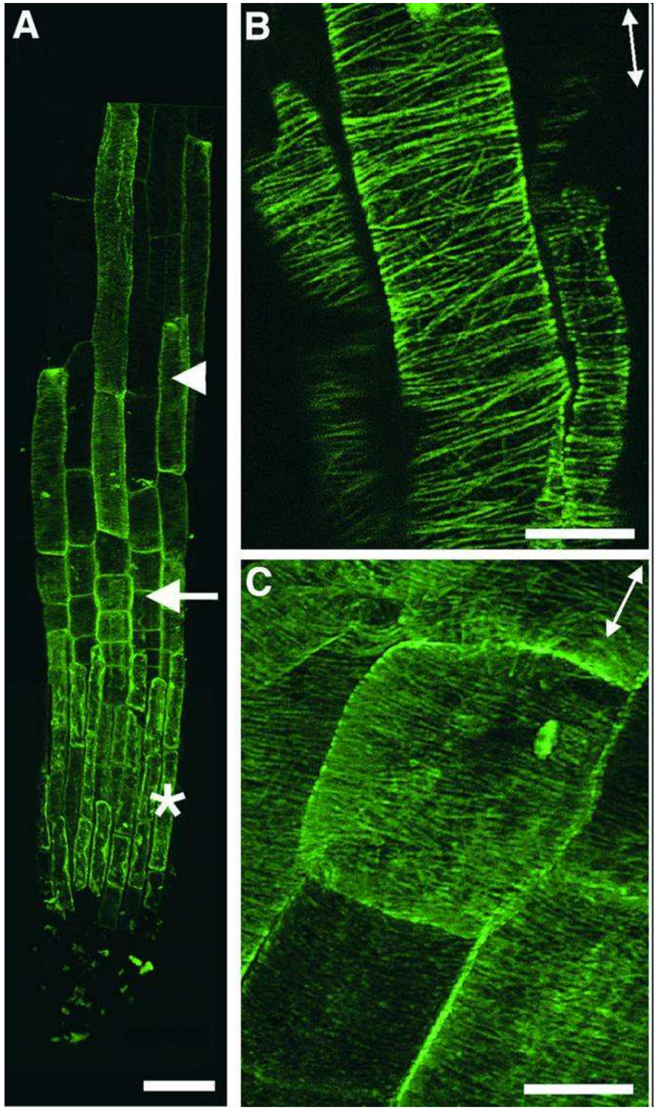


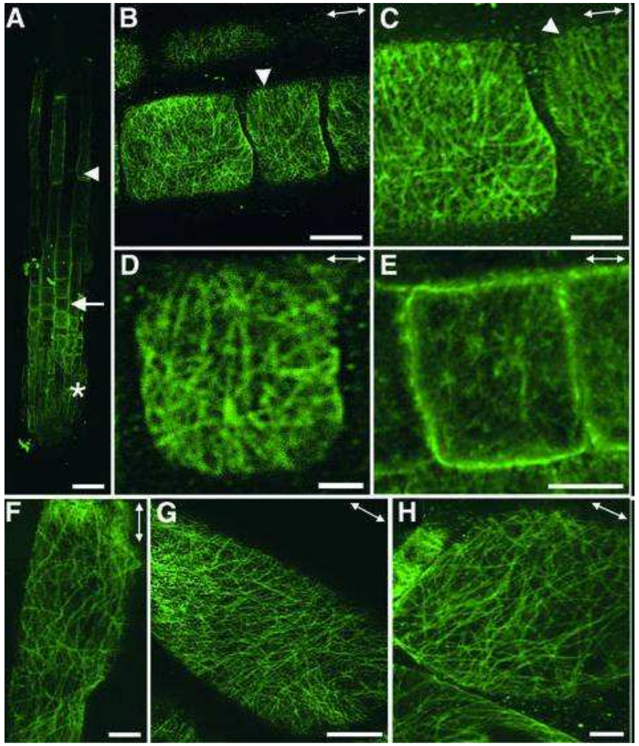
Fig. 3. Model for microtubule assembly by severing and transport of nucleating templates. In this model, a γ-tubulin ring complex associates with the minus end of a microtubule, while the microtubule extends by the addition of tubulin subunits at the fast-growing, GTP-tubulin-containing plus end (dark green). Severing of the minus end is achieved by the formation of a hexamer of katanin p60 subunits, whose association with the microtubule wall is coordinated by the larger p80 subunit, which may transiently dimerize with the p60 subunits. Microtubule-mediated ATPase activity results in inward movement of the p60 subunits, an action that cleaves the ring complex from the microtubule minus end. Katanin subunits dissociate but the lock-washer-shaped ring complex is transported along the microtubule by a plus-end-directed kinesin. The extent of transport along the microtubule may be regulated by the relative activities of plus- and minus-end-directed kinesins. The ring complex serves as a template for the assembly of additional microtubules. Repeated generation, severing and transport of nucleating templates at the minus end of the original microtubule may explain how the fractal tree complexes shown in Fig. 2A develop.

Mutace *fragile fibre2 (fra2), botero:*
brittle swollen semi-dwarf,
disordered cortical mt

fra2 (katanin subunit p60)



wt kořen

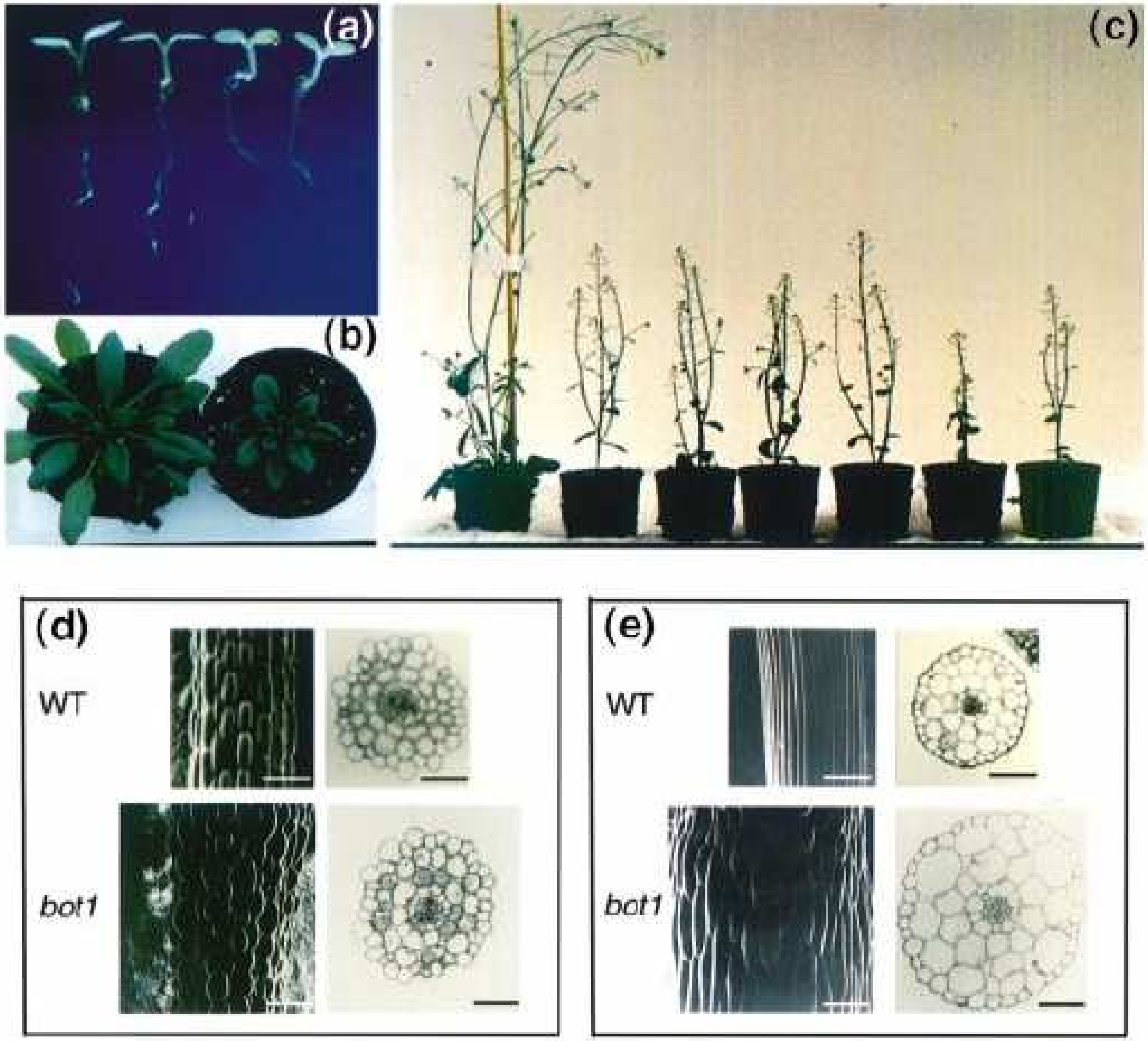


fra2

Důsledek: defektní biosyntéza a organizace stěny!

Burk and Ye 2002

fra2 - alela *botero* (p60)

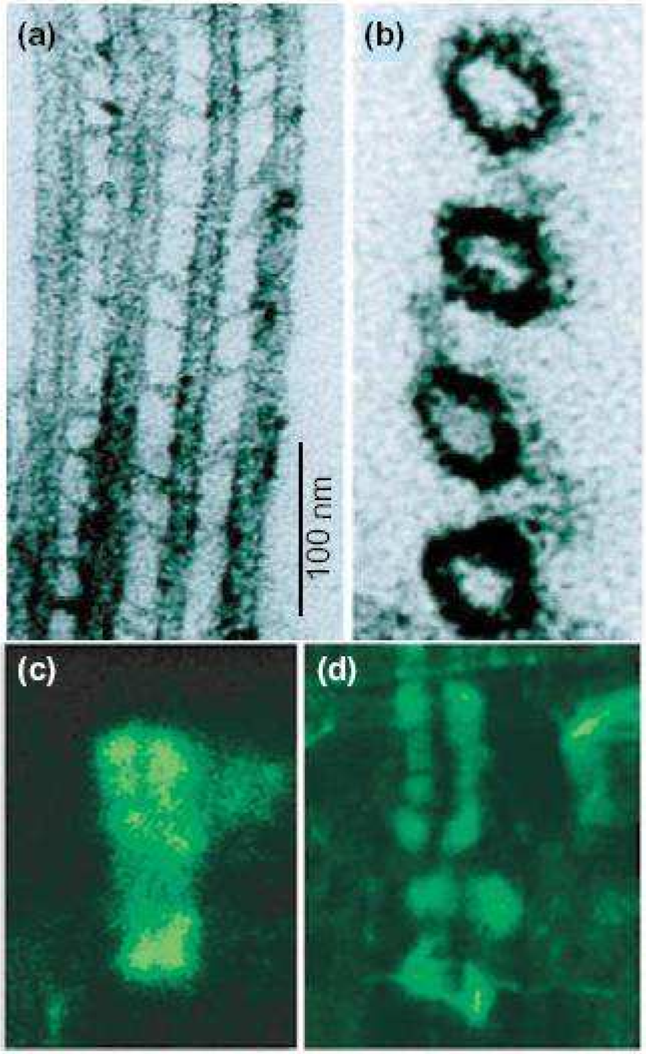


hypokotyl - světlo

tma

(Fernando Botero, Colombia)

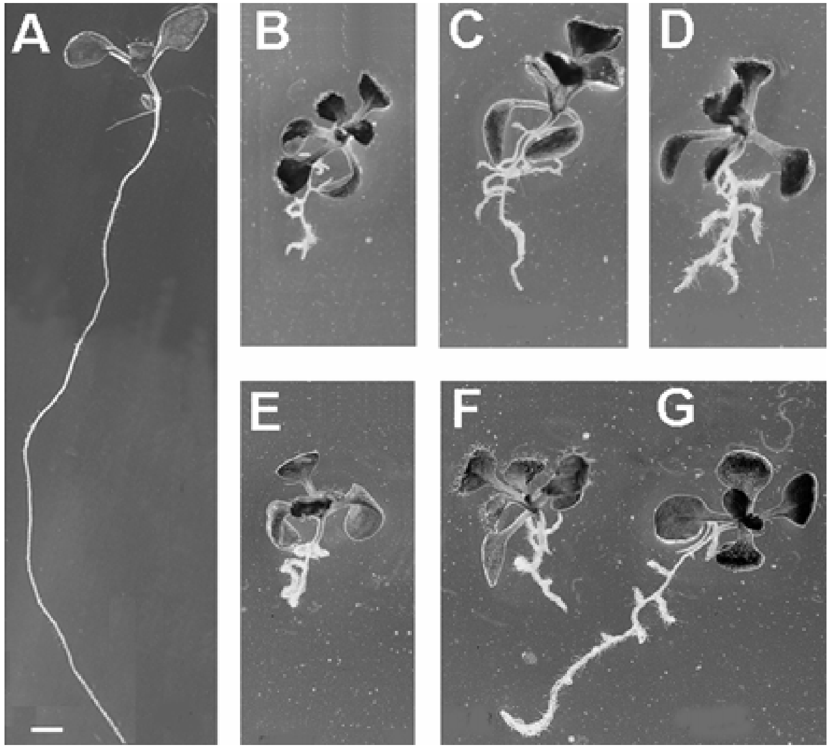
MAP65: crosslinking („rostlinná specialita“)

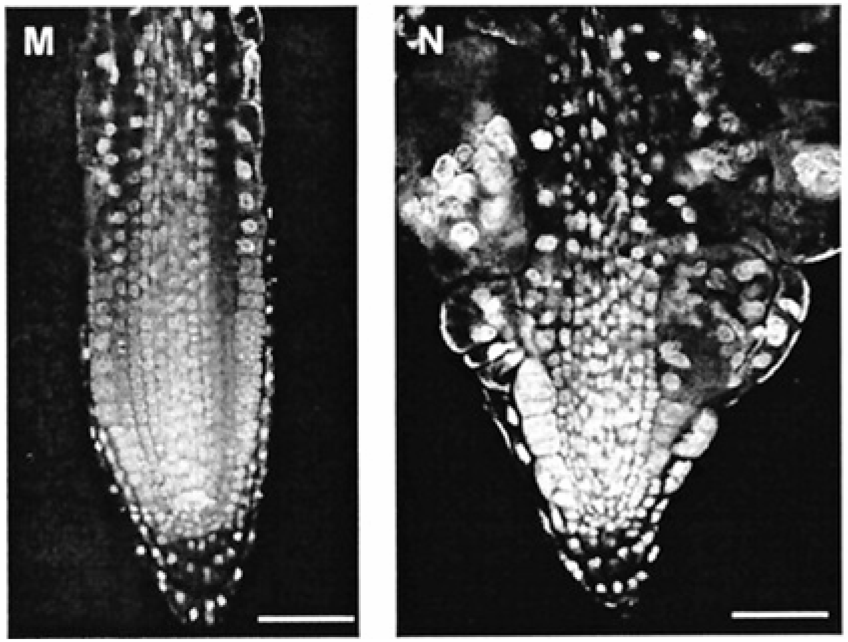


MAP-65 family members that cross-link MTs. **(a)** Longitudinal and **(b)** transverse electron micrograph sections of MTs cross-linked *in vitro* by purified carrot MAP-65. MAP-65 can be seen as the evenly spaced filamentous cross-bridges (reproduced with permission from [46], copyright National Academy of Sciences, USA [1999]). **(c)** Wildtype and **(d)** *ple-6* phragmoplasts visualized with MAP4–GFP in *Arabidopsis* (confocal microscopy). Note the larger clear zone in *ple-6*, which is mutated in the *AtMAP65-3* gene (reproduced with permission from [51••], copyright Cell Press [2004]).

Arabidopsis: rodina 9 genů

pleiade – mutace v MAP65



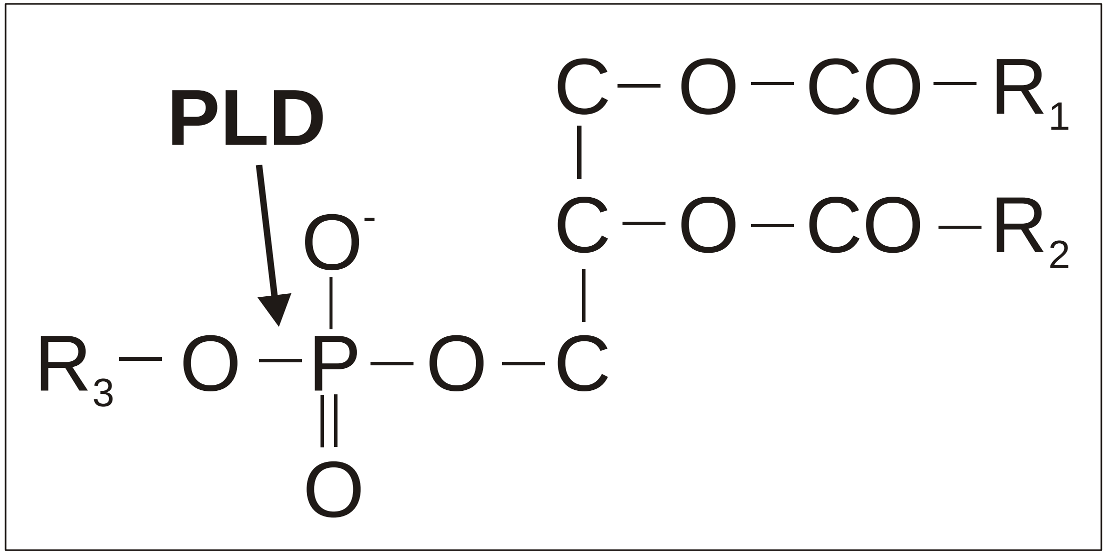


mnohojaderné buňky
(kořenově specifické!)

(Muller et al. 2002)

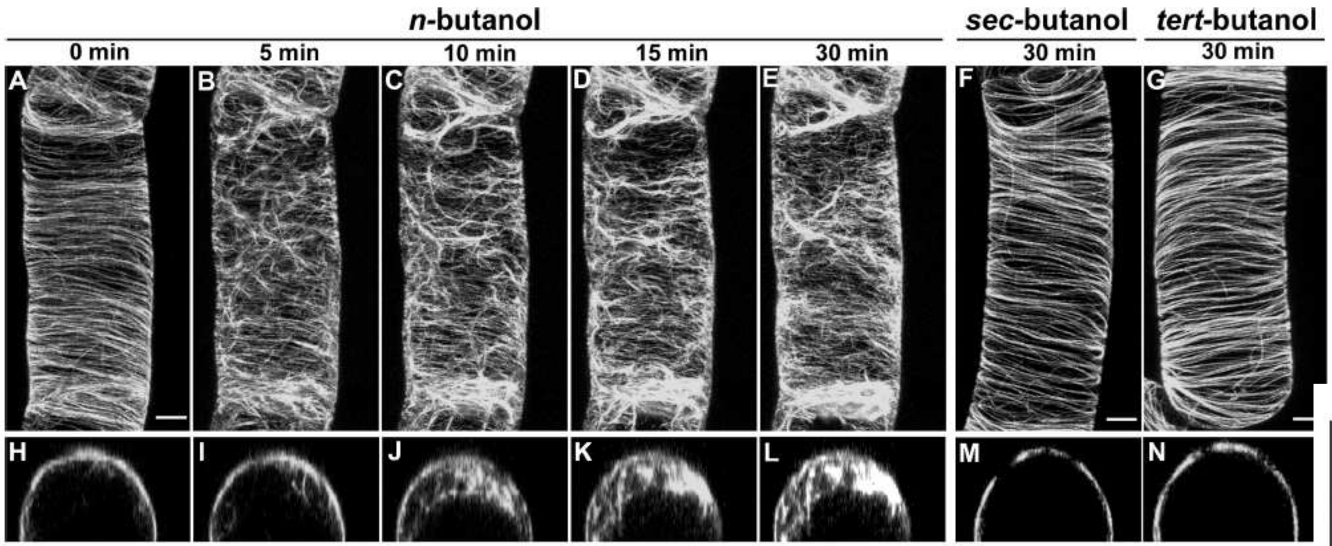
MOR1 (*Arabidopsis*)	1	Punctate along cortical MTs, at phragmoplast midline' spindle	*mor1*, *gem1*	*mor1* alleles are temperature sensitive with shortened/disorganized MTs and isotropic cell expansion, *gem1* alleles have cytokinesis defects and are homozygous lethal	Stabilize MTs, MT organization
TMBP200 (MOR1-like) (tobacco)	ND	ND	ND	ND	MT bundling?
p90 PHOSPHOLIPASE D (tobacco)	ND	Along cortical MTs and linking them to the plasma membrane	ND	ND	Conveys hormonal and environmental signals to cortical MTs?
PHOSPHOLIPASE Dδ (PLDδ) (*Arabidopsis*)	12	Along cortical MTs and linking them to the plasma membrane?	PLDδ null	Increased sensitivity to H_2O_2-induced cell death	Oleate stimulated phospholipase activity linked to H_2O_2 signaling
Spc98p (*Arabidopsis*)	1[a]	Punctate along nuclear surface and at cortex (presumably at MT nucleating centers)	ND	ND	MT nucleation?
SPIRAL1 (SPR1) (*Arabidopsis*)[b]	6	Cortical MT plus ends and uniformly covers MTs in other arrays, around endomembranes?	*spr1*, *sku6*	Organ axial twisting, root right skewing on agar surfaces, obliquely oriented cortical MTs	MT polymerization? Links proteins to MTs? Directional cell expansion
TANGLED1 (TAN1) (maize)	ND	Punctate throughout cytoplasm and along MTs in all four arrays	*tan1*	Abnormally oriented cell divisions	Orienting MT structures during cell division
WAVE-DAMPENED2 (WVD2) (*Arabidopsis*)	8	Along MTs?	*wvd2*	Overexpression causes twisted, stockier organs, right-skewing of roots on agar surfaces and obliquely oriented cortical MTs	MT bundling?

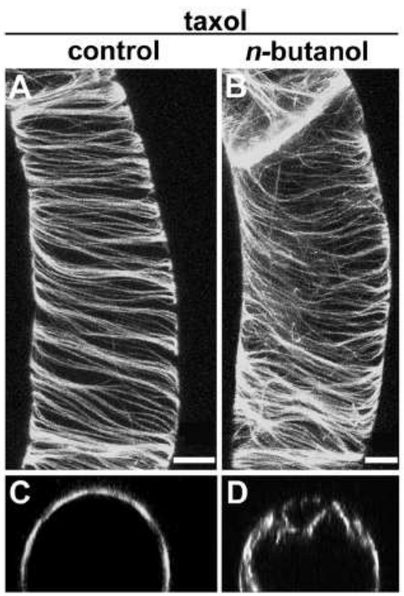
Fosfolipáza D



- Produkt: kys. fosfatidová (PA) – signální molekula
- Inhibitor: n-butanol (interferuje s produkcí PA)
- Přímá interakce s MT?

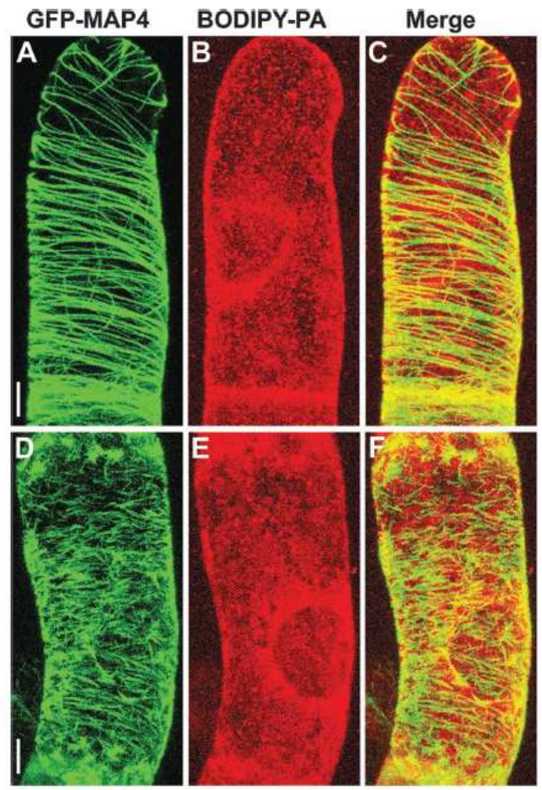
Fosfolipáza D a mikrotubuly





BY2 buňky
(Dhonukshe et al., Plant cell 2003)

(a není to díky PA)



Fosfolipáza D a mikrotubuly: ALE....

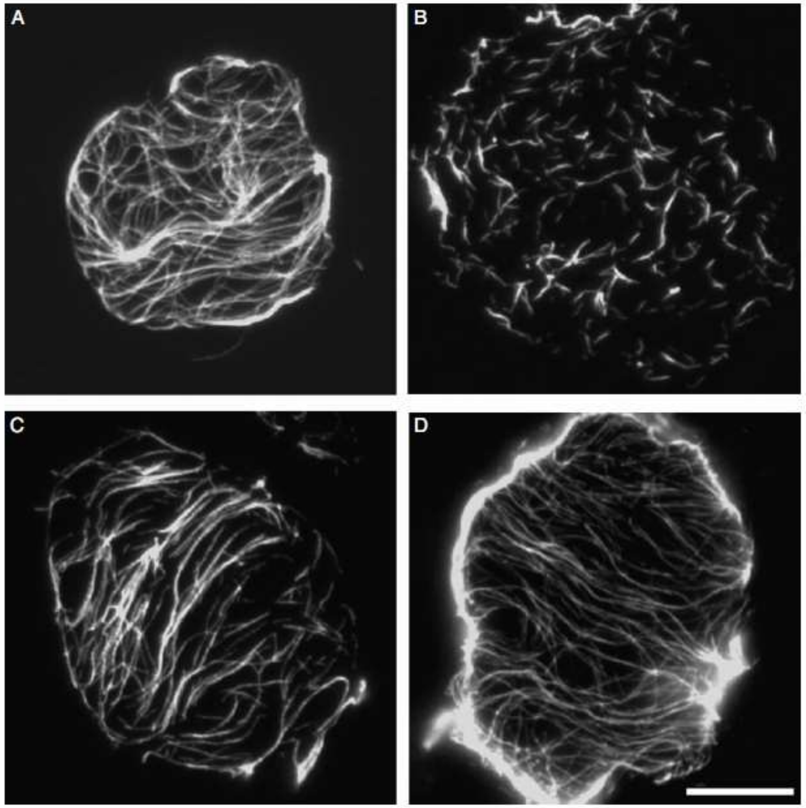


Fig. 2 MTs on membrane ghosts prepared from protoplasts treated with butanols. Membrane ghosts were prepared from treated with culture medium (A), or culture medium supplemented with 0.5% *n*-butanol (B), *t*-butanol (C) or *n*-butanol plus
(D) for 20 min. Bar = 5 µm.

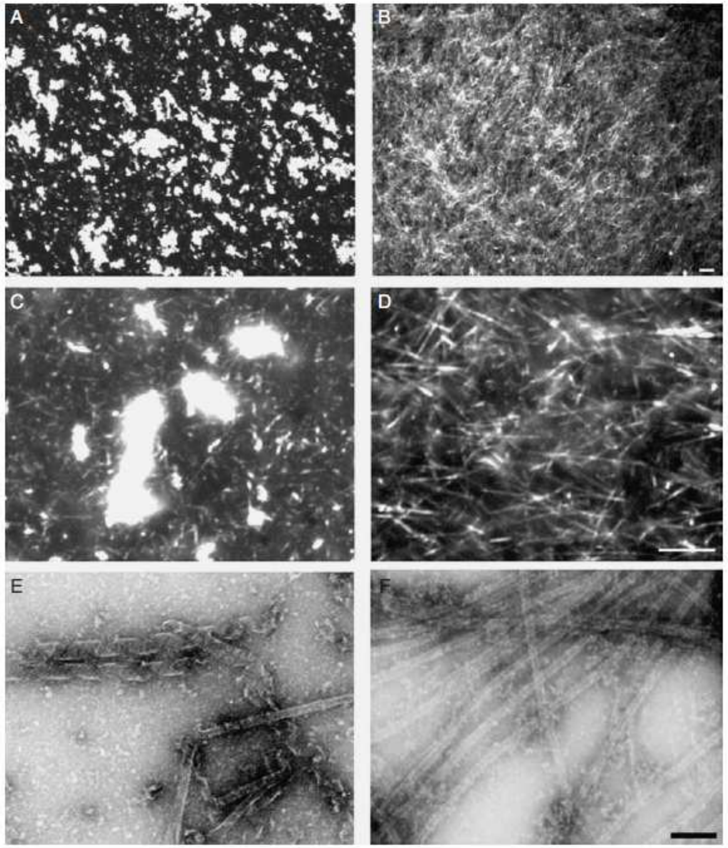


Fig. 3 Effect of butanols on MTs in vitro. MTs assembled from tubulin purified from BY-2 cells were incubated in PME buffer supplemented with 1% *n*-butanol (A, C, E) or *t*-butanol (B, D, F). MTs were examined by dark-field microscopy (A–D) and electron microscopy (E, F). Bars in b and d = 10 µm; f = 100 nm.

Plant Cell Physiol. 47(7): 1004–1009 (2006)
doi:10.1093/pcp/pcj055, available online at www.pcp.oxfordjournals.org

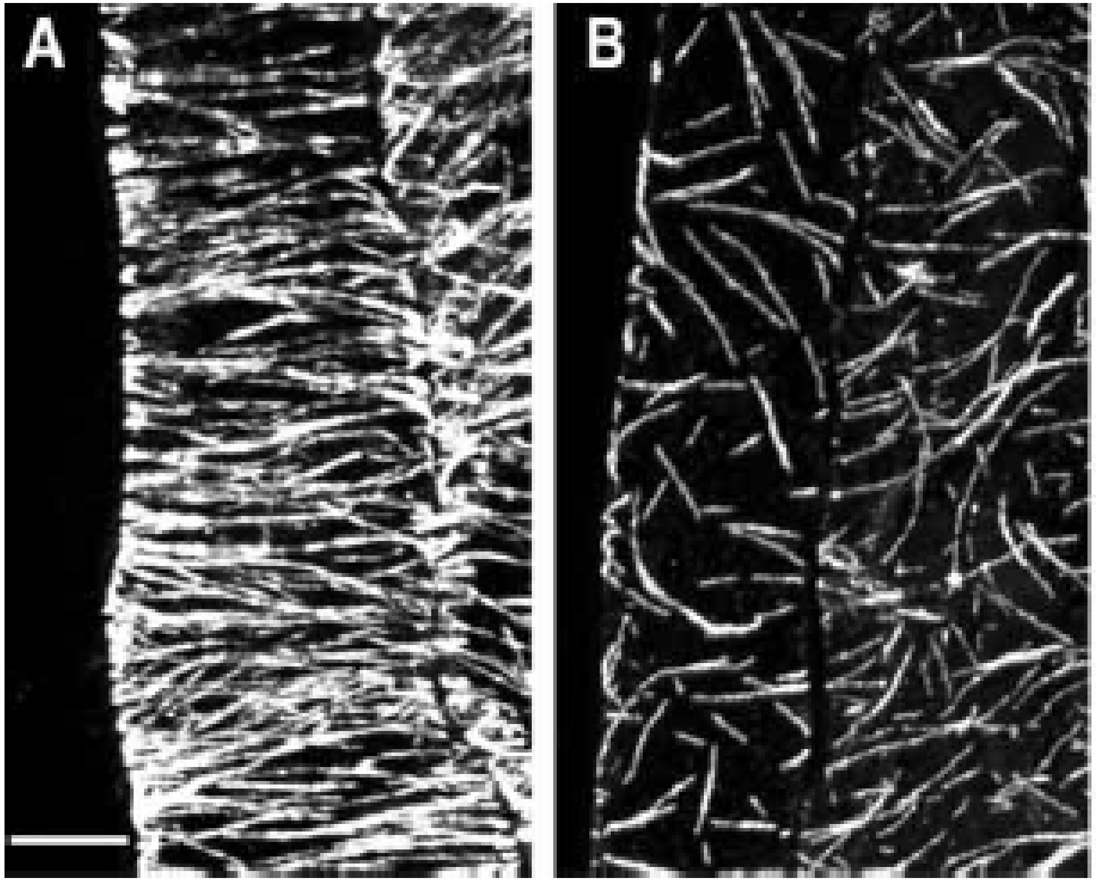
Short Communication

_n_-Butanol induces depolymerization of microtubules in vivo and in vitro

Ai Hirase [1], Takahiro Hamada [1], Tomohiko J. Itoh [2], Teruo Shimmen [1] and Seiji Sonobe [1, *]

(jak čistý byl preparát MT, pokud PLD = MAP?)

MOR1 (microtubule organization)



(MAP215)

Fig. 4. Microtubule patterns in the epidermis of *Arabidopsis thaliana* cotyledons after 4 hours at 29°C. (A) Cortical microtubules are abundant and transversely oriented in wildtype. (B) In the *mor1* mutant, microtubules appear short and disoriented. Bar, 10 µm.

Modely funkce MOR1

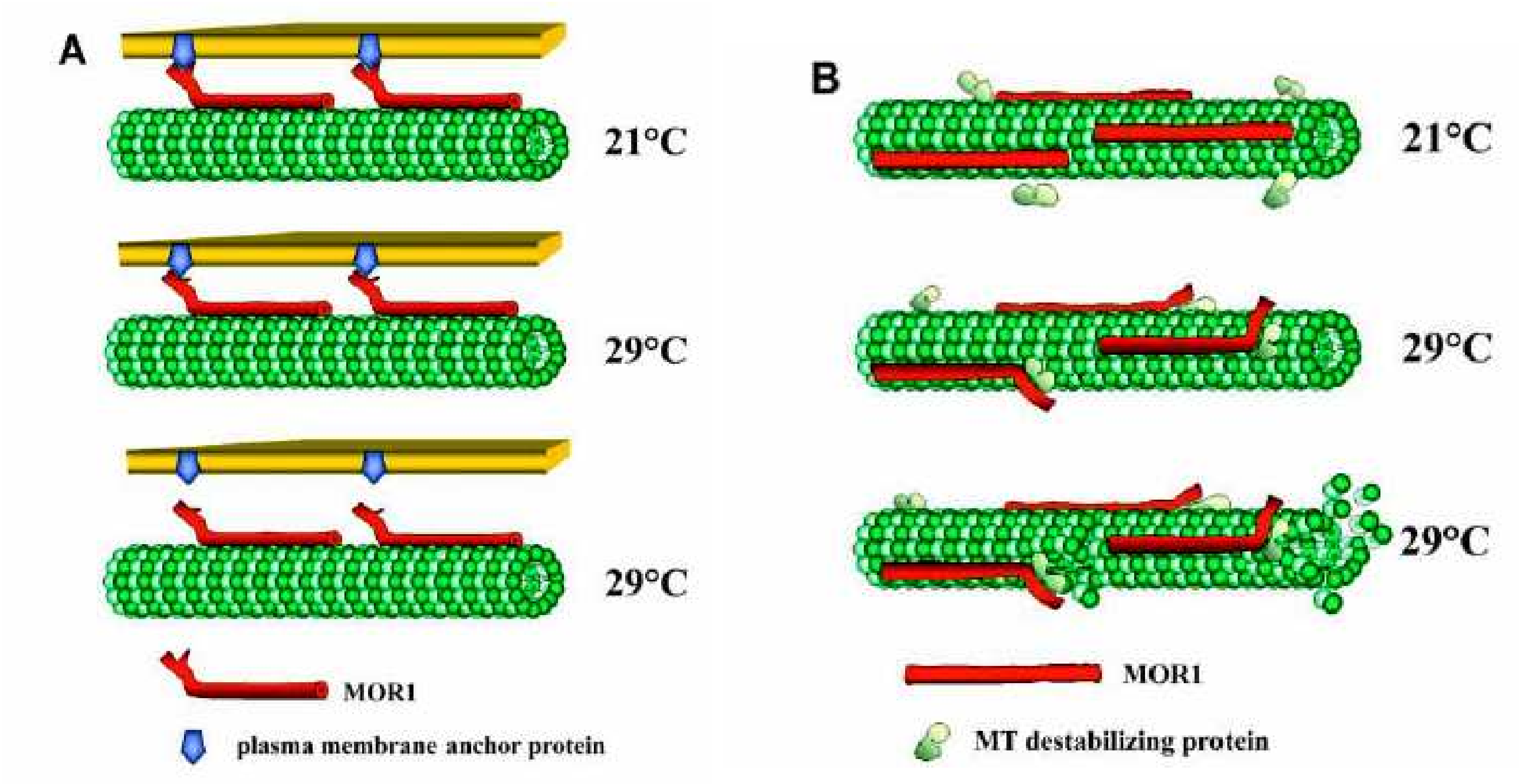
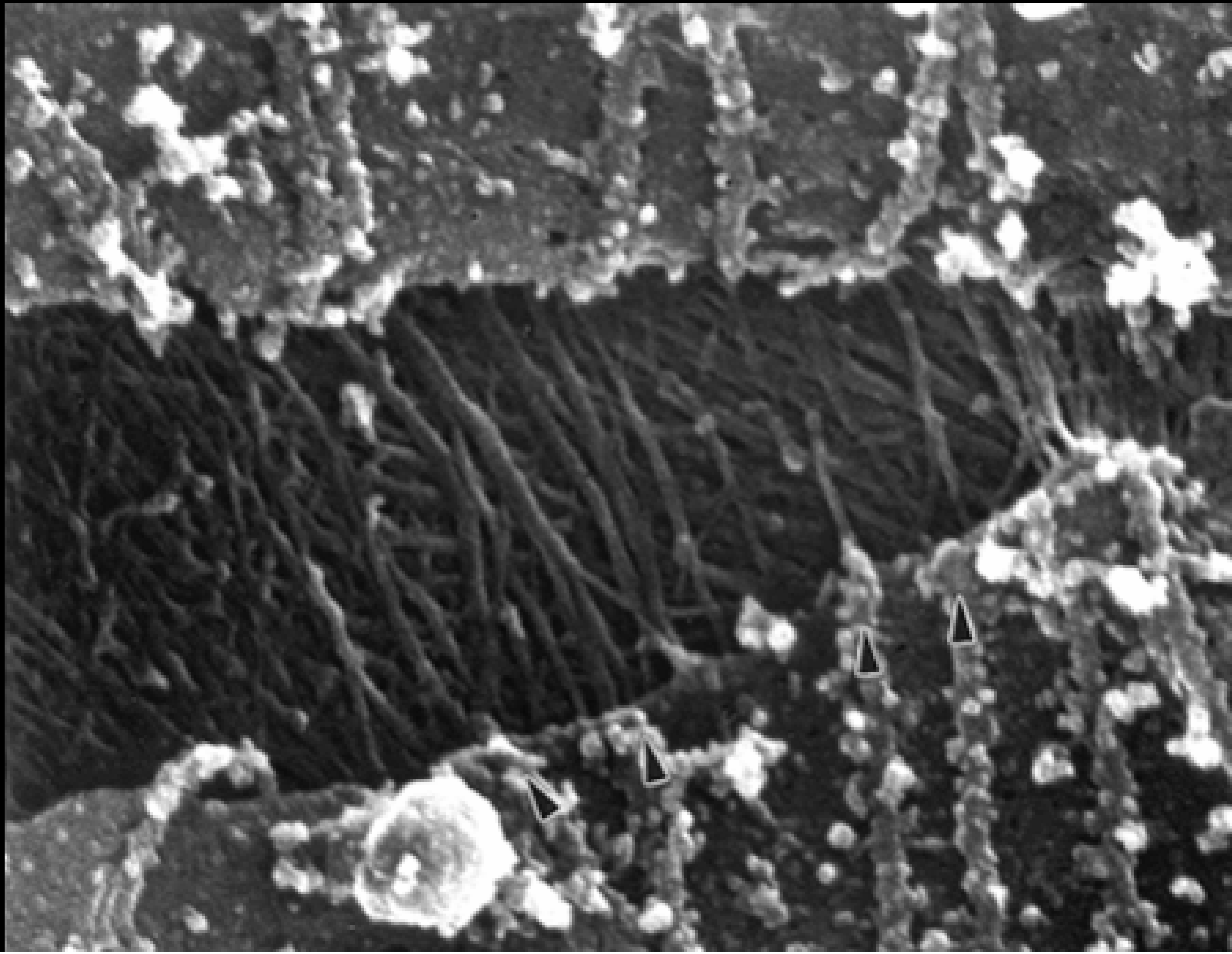


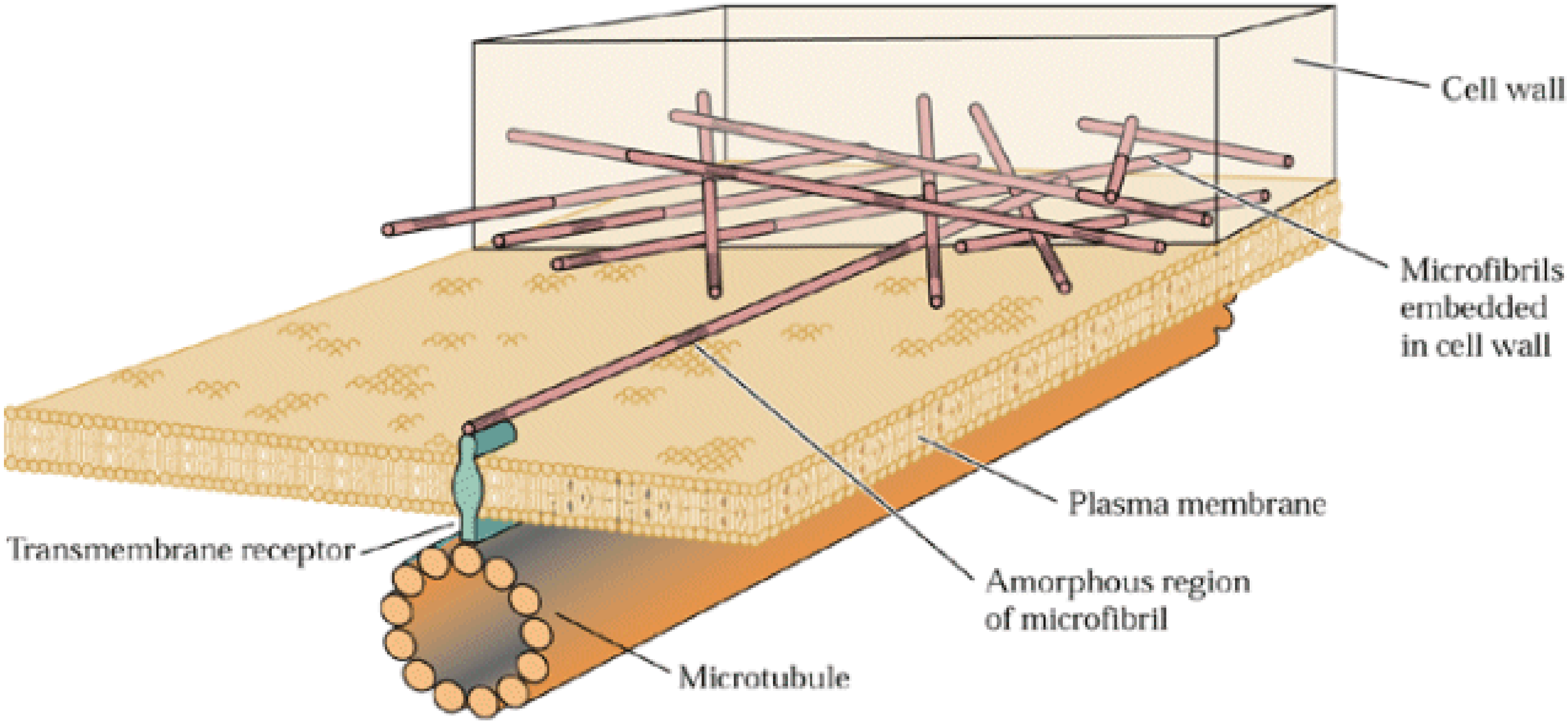
Fig. 5. Possible functions of the MOR1 HEAT repeat-1 (HR1) in microtubule stabilization. (A) HR1 links microtubules to the plasma membrane via a plasma-membrane-associated protein. At restrictive temperature, this loss of binding dissociates microtubules from the plasma membrane, promoting their destabilization. (B) HR1 competes with a destabilizing protein (probably a kin1-like kinesin) for binding. At permissive temperature, the high affinity of MOR1 for this site prevents destabilization. At 29°C, this affinity is lost, leading to kin1-dependent destabilization and microtubule shortening.

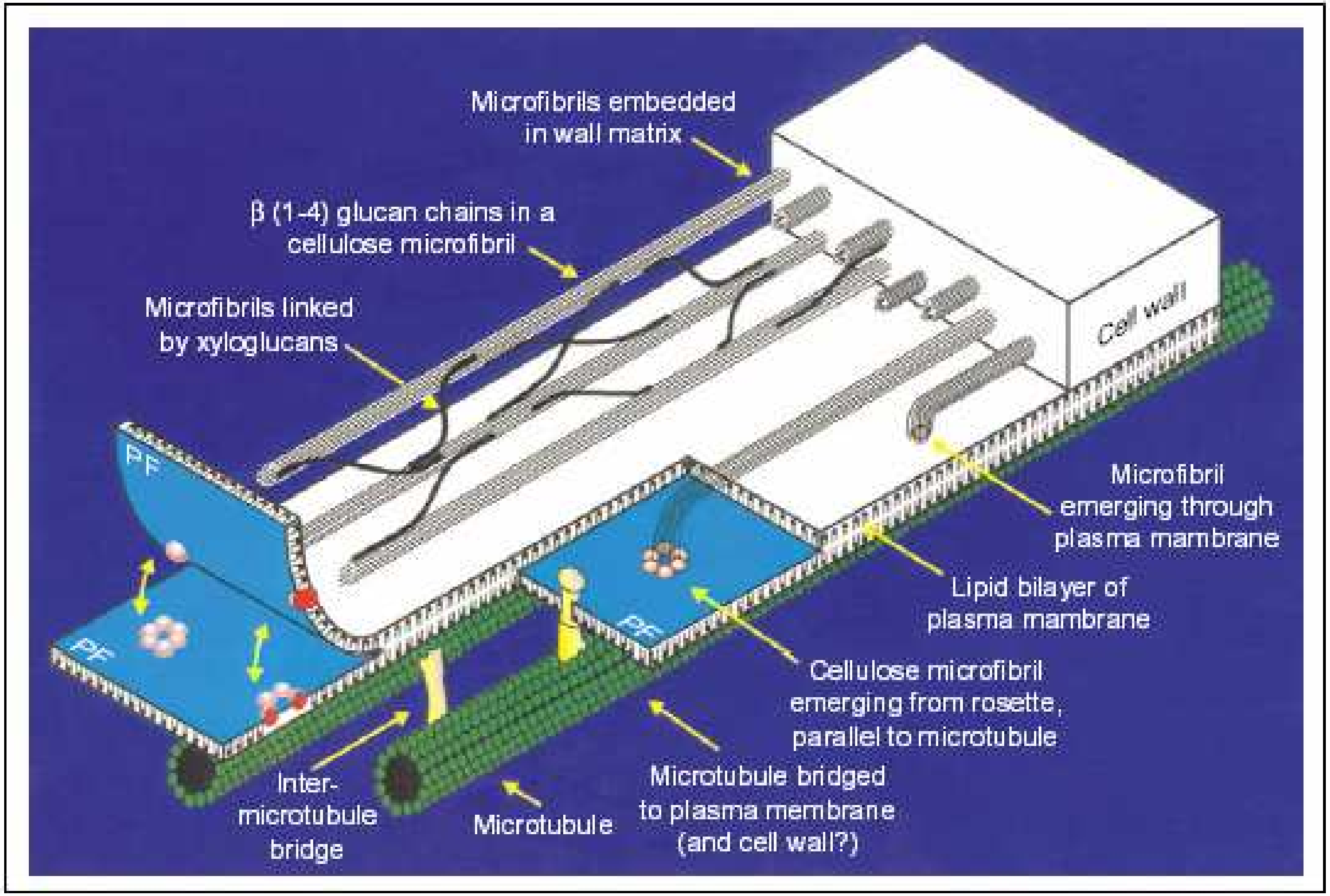
Asociace mezi kortikálními mt a stěnou



onion root

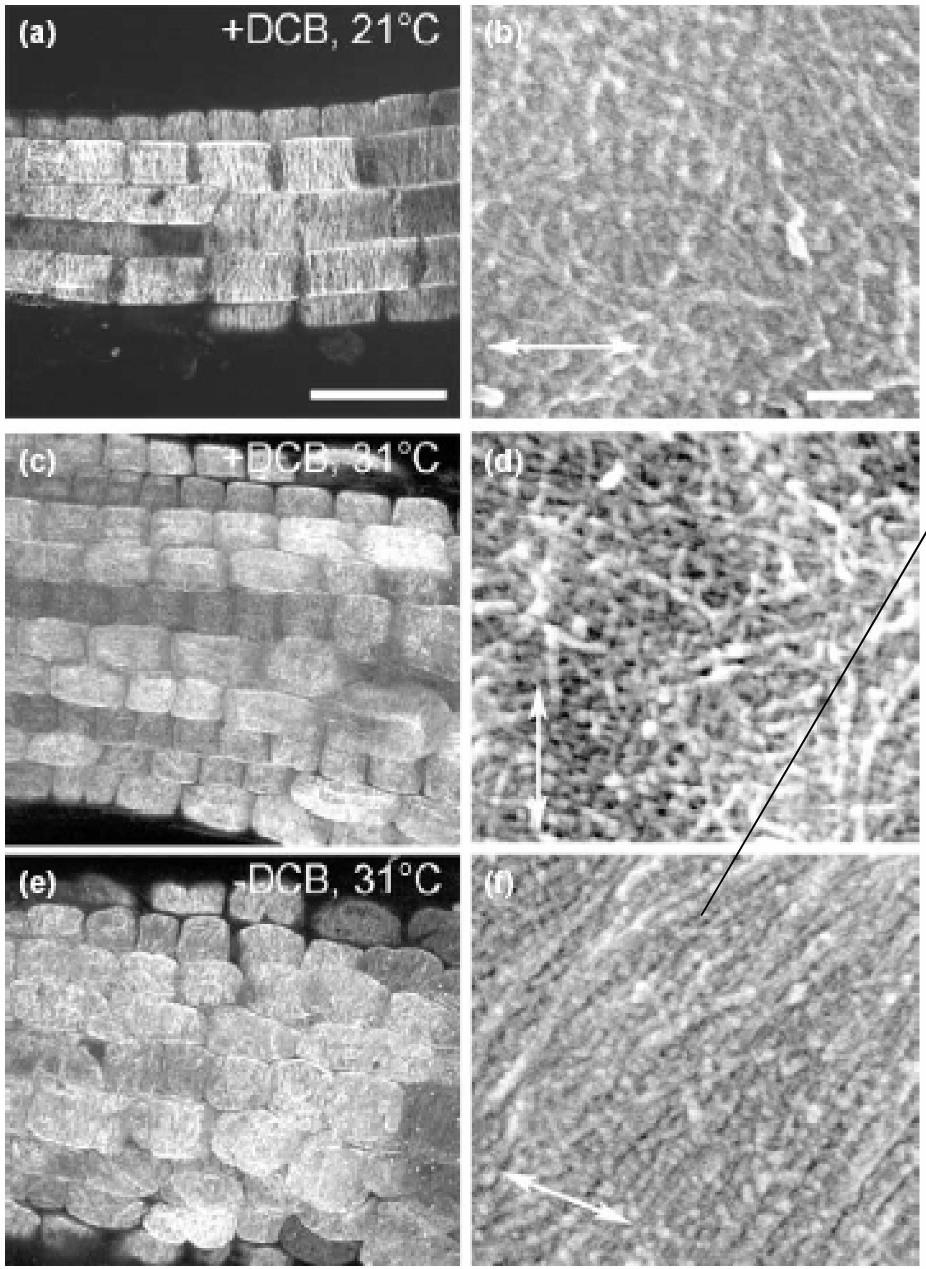
Co je příčina a co následek?

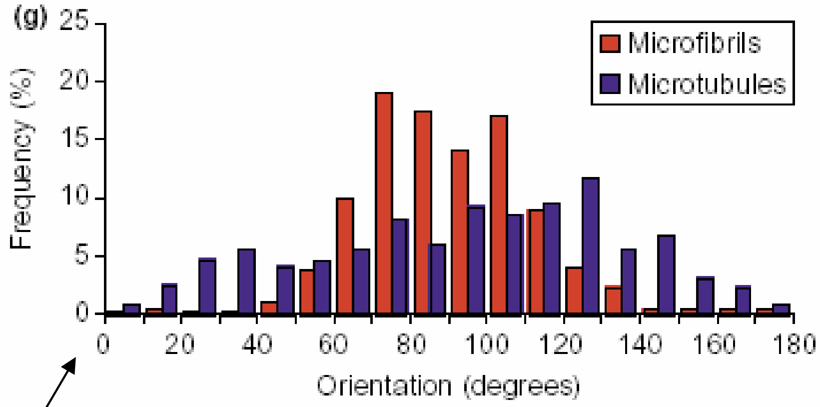




Relationships between cellulose-synthesizing complexes (rosette type), wall microfibrils, plasma membrane and microtubules. Diagram provided by Brian Gunning.

MOR1





An experiment to test whether microfibrils can re-establish parallel order when the existing microfibril template is disordered and cortical microtubules are disorganized. After DCB treatment for 4 h, **(a)** cortical microtubules remain transversely oriented in *mor1-1* root cells, whereas **(b)** the formerly transverse orientation of cellulose microfibrils has been disrupted by inhibiting cellulose synthesis. Shifting the temperature from 21 °C to 31 °C **(c)** disorganizes the microtubules while **(d)** the disordered microfibril texture is maintained. Maintaining the 31 °C temperature leads to significant lateral expansion of cells and **(e)** continued disorganization of the cortical microtubules, but 18 h after DCB's removal, **(f)** microfibrils are organized in parallel arrays. **(g)** At the time point shown in (e) and (f), microtubule orientation is widely dispersed about the transverse axis (blue bars), while microfibrils (red bars) show relatively tight distribution about the transverse axis. Microtubules were examined by immunofluorescence and cellulose microfibrils by field-emission scanning electron microscopy. Scale bars are 50 μm for (a), (c) and (e), and 200 nm for (b), (d) and (f). This figure is modified from Figures 2 and 3 of [14**].

Wasteneys 2003/2004: mikrofibrily se uspořádají i samy

2-6-dichlorobenzonitril

Cytoskelet a stěna jsou i MECHANICKÉ systémy...

Referát:

Science AAAS

Developmental Patterning by Mechanical Signals in Arabidopsis
Olivier Hamant, *et al.*
Science **322**, 1650 (2008);
DOI: 10.1126/science.1165594

Molekulární motory a buněčné pohyby

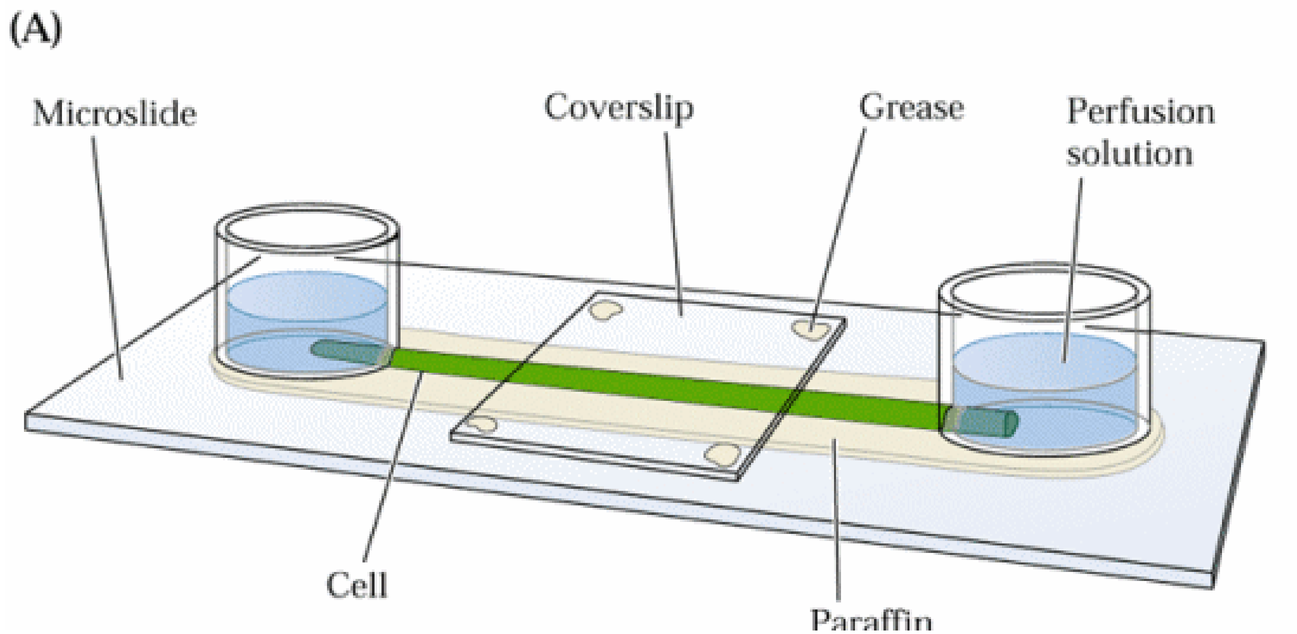
Molekulární motory

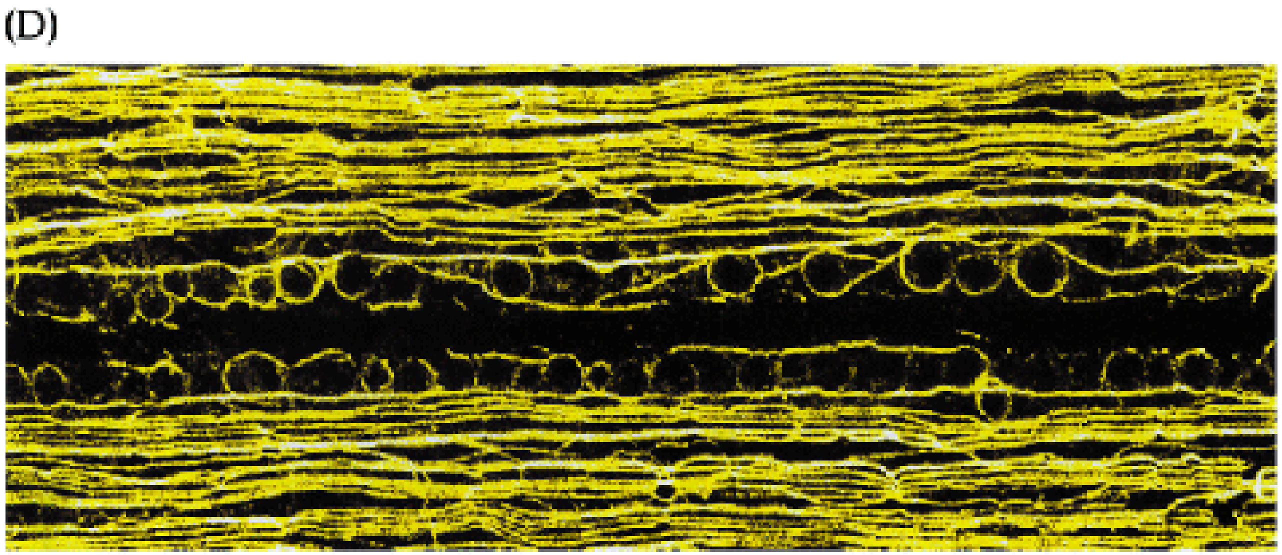
- Definice: pohon ATP
- Po aktin. filamentech:
  - až 100 μm/s (*Chara*), typicky jednotky μm/s
  - proudění cytoplasmy, pohyb organel
  - typický motor - myosin
- Po mikrotubulech:
  - cca 150 nm/s (řádově pomalejší)
  - pohyby chromosomů, vřeténka, membrán. váčků, organizace mikrotubulů ...
  - kinesiny (od - k +) a dyneiny (od + k -)

Přesun nákladu stojí energii

(L. Synek)

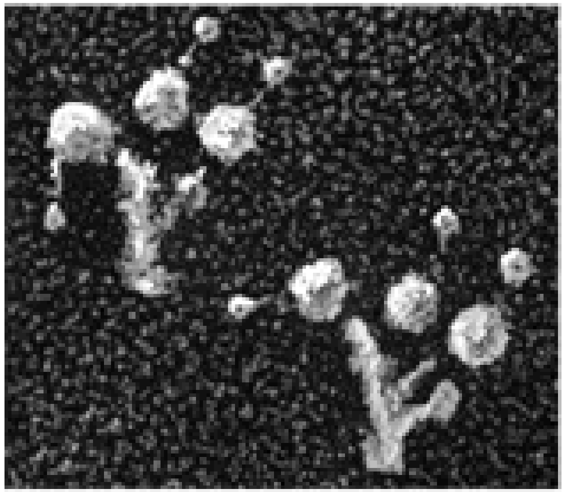
Chara: jak se to měřilo





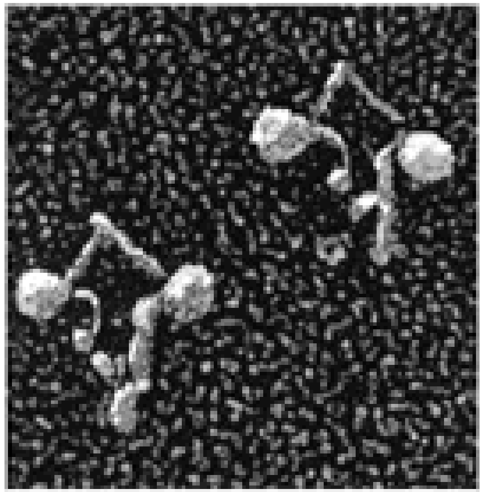
(aktin)

(A)



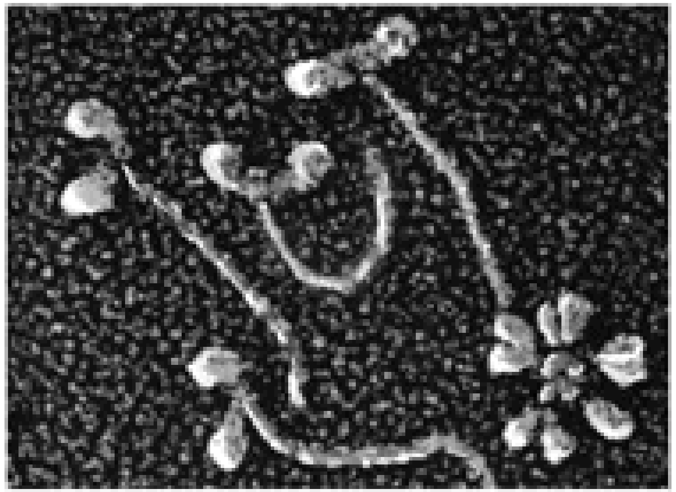
dynein

(B)



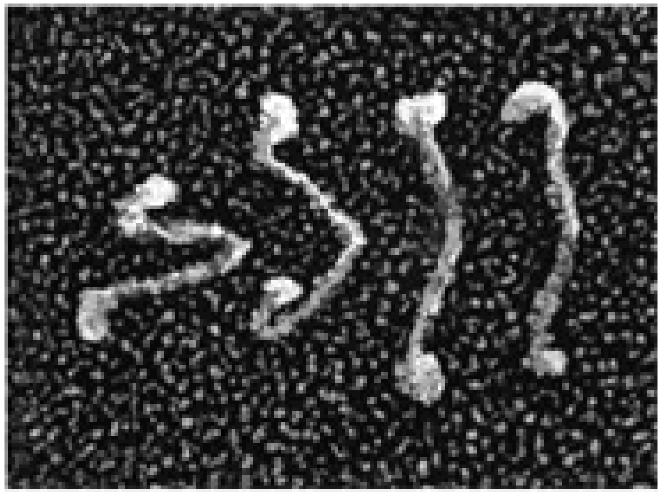
dynein

(C)



myosin

(D)

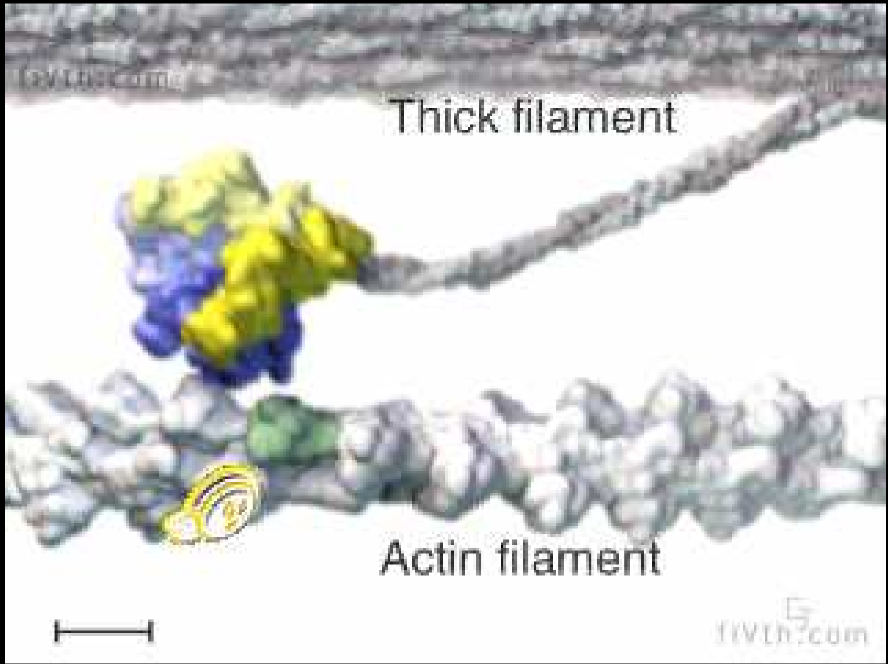


kinesin

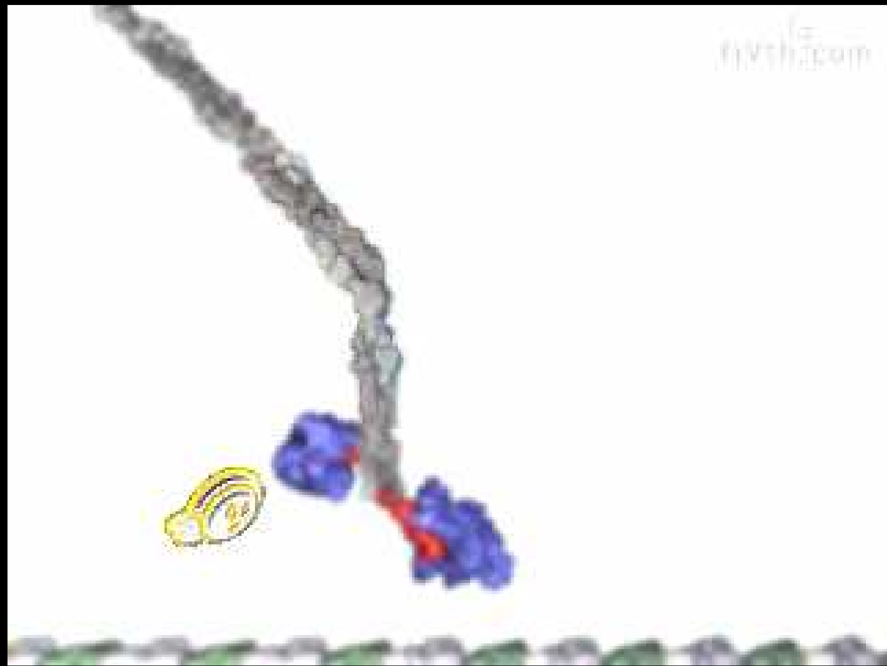
Nadrodiny molekulových motorů

	S. cer.	**A. th.**	**Počet tříd**
Myosiny	5	17	15
Kinesiny	6	61	10 a nezařaz.
Dyneiny	1	0	

Princip pohybu mol. motoru

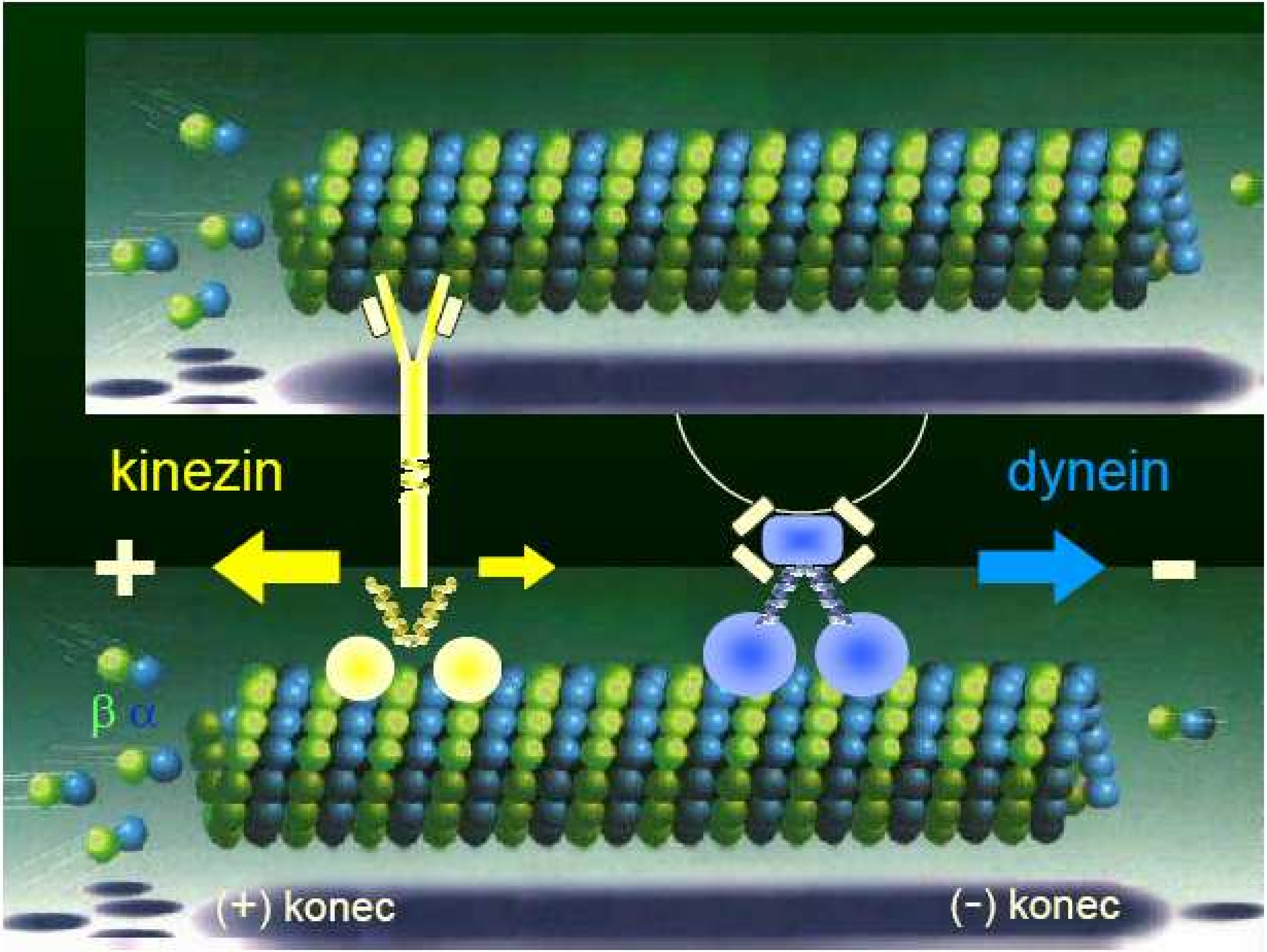


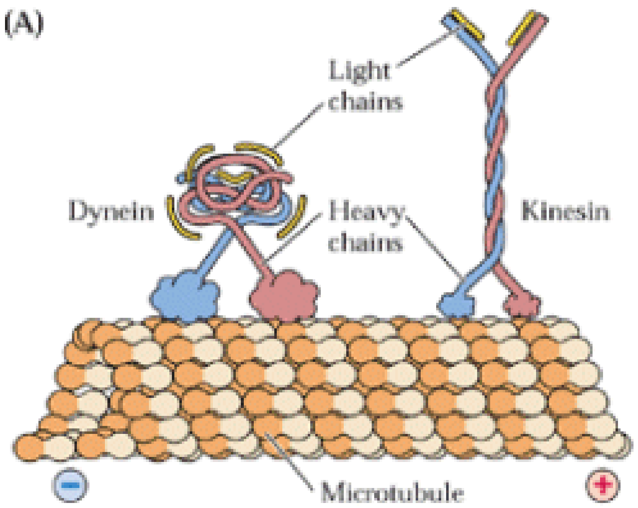
myosin

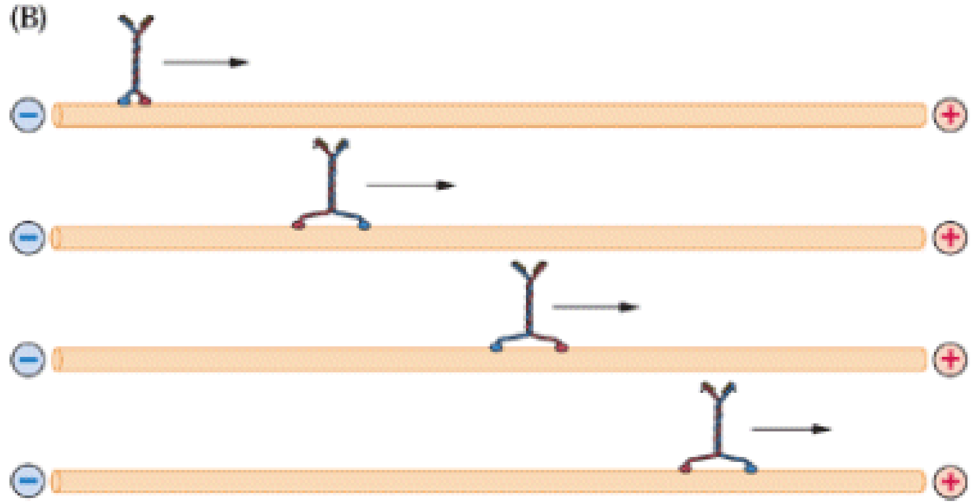


kinesin

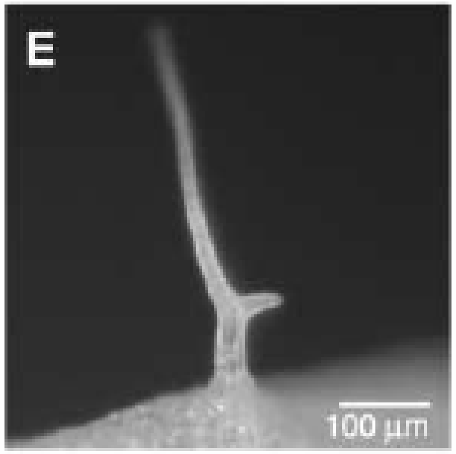
(R. Milligan, Scripps Inst.)


kinezin
dynein
+
-
β α
(+) konec
(-) konec

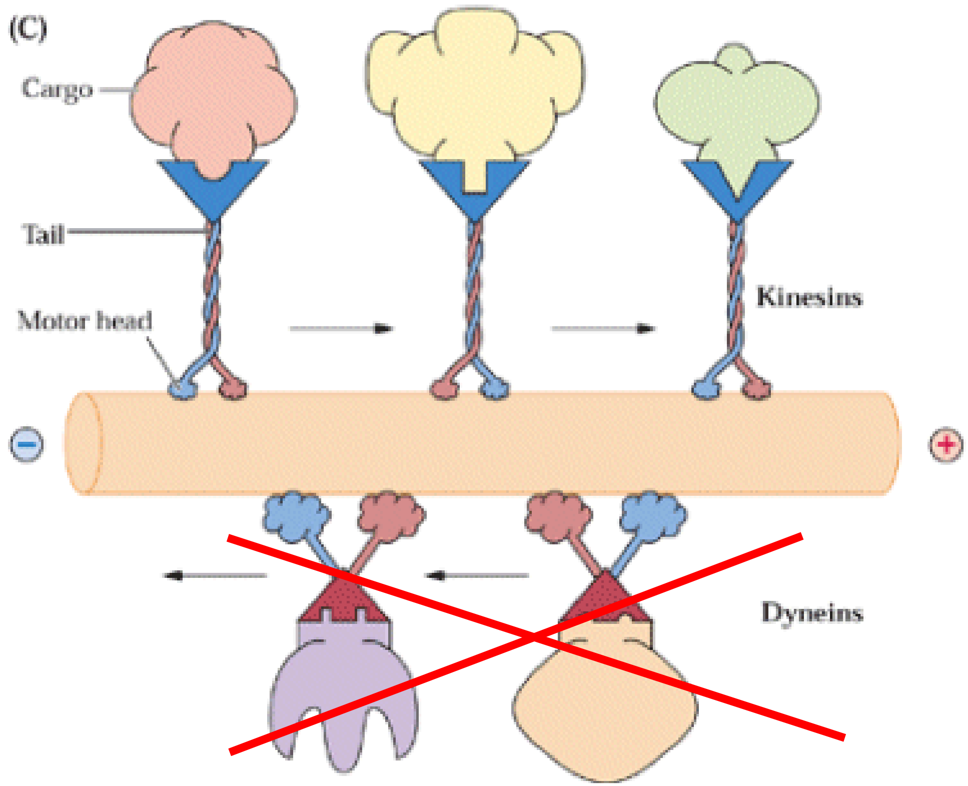




ZWICHEL kóduje kinesin!

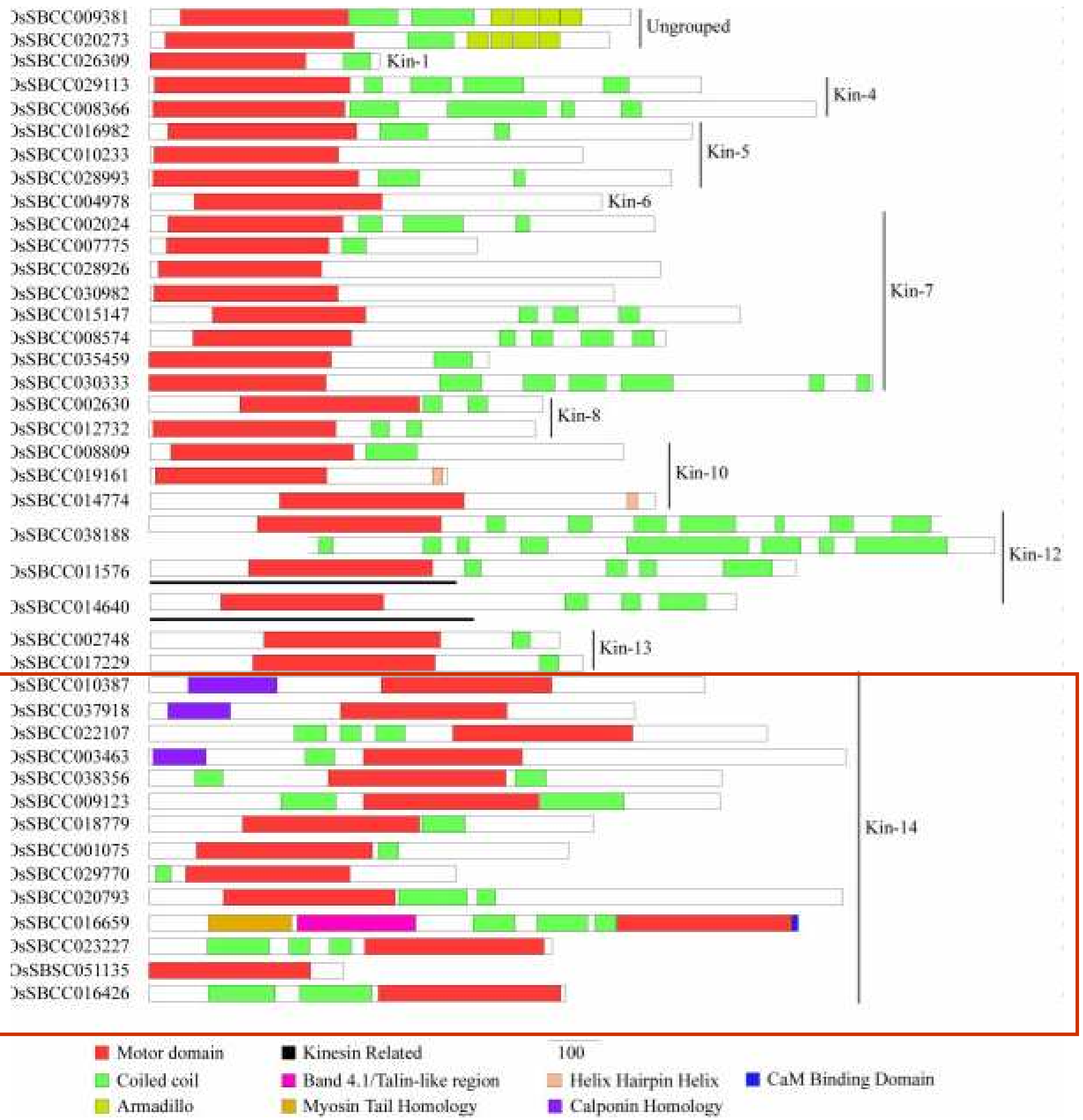


(jeden z mnoha!)



61 kinesinů Arabidopsis

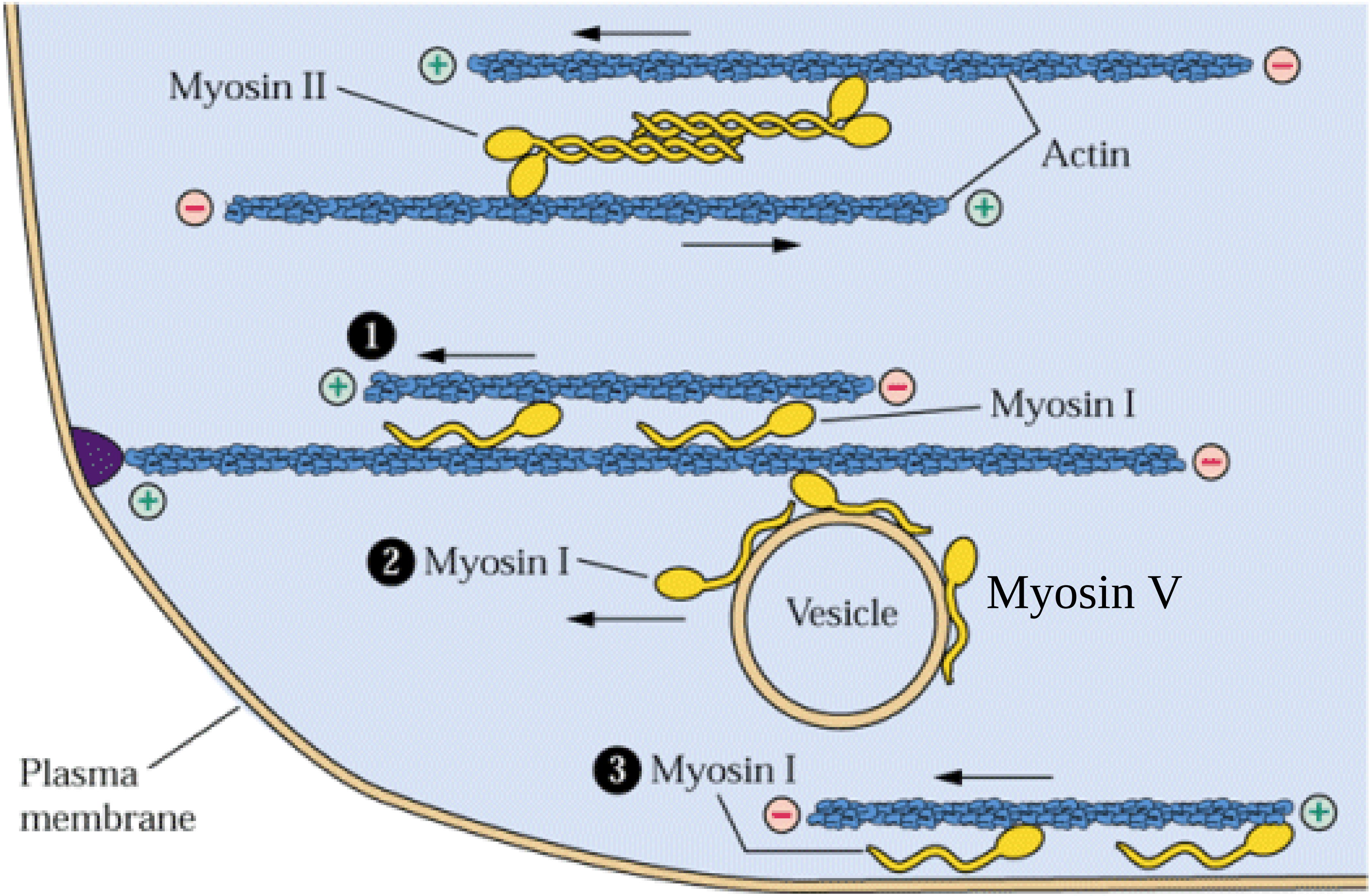
- pohyb po mikrotubulech oběma směry (zajišťován různými proteiny), směr určuje „krček“
- většina (živočišné a 40 u A.th.) + end-directed
  - často role v cytokinesi (PAKPs – phragmoplast-associated kinesin proteins)
- zbytek (21) – end-directed
  - ATK1 – funkce vřeténka
  - ZWI – regulace calmodulinem
  - KCH – calponin homology domain

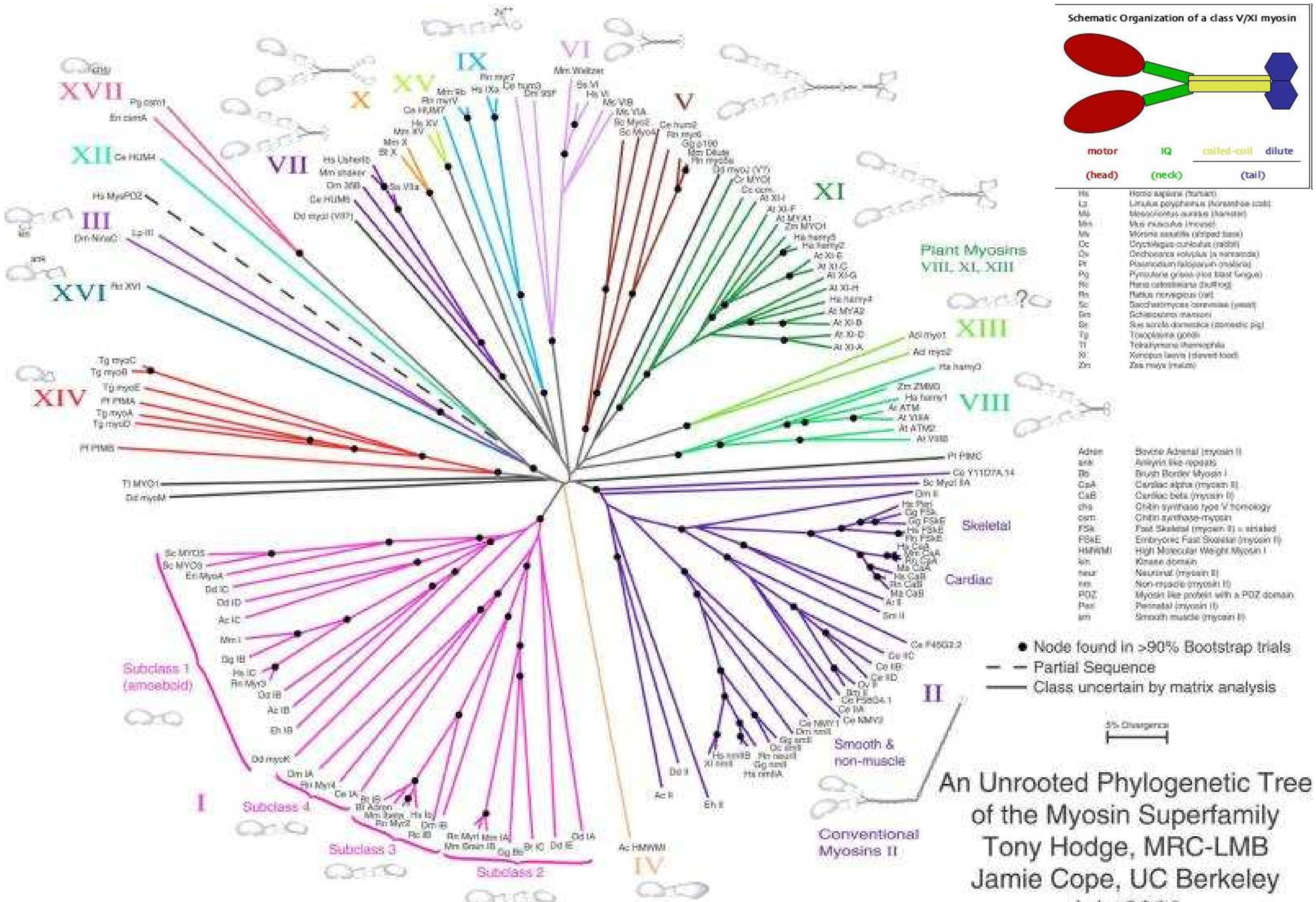


Rozmanitost doménové stavby rostlinných kinesinů (rýže)

(-end)

Myosiny - pohyb po aktinu

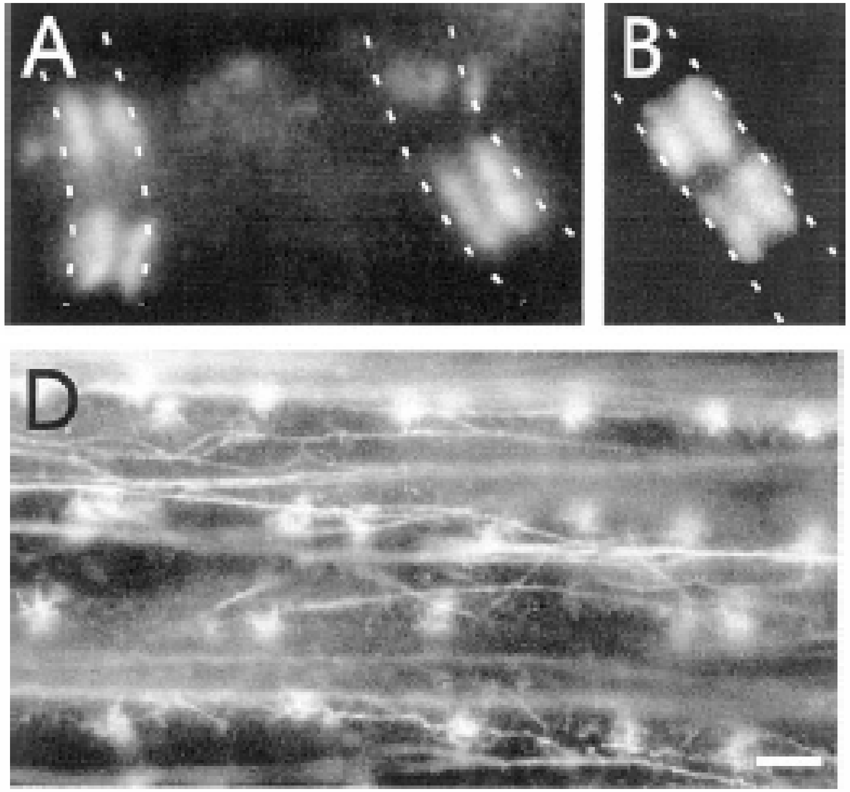




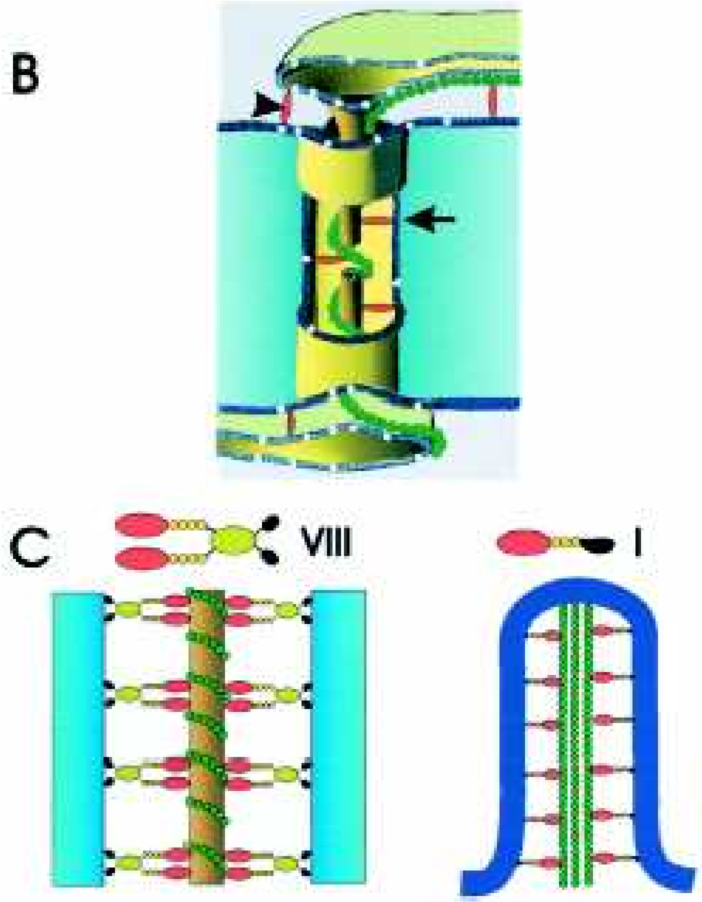
Arabidopsis: 17 myosinů

Myosin VIII v plasmodesmech

MyoVIII



aktin (pit fields)



„mikrovilus naruby“?

Baluška et al. 2001

Regulace molekulových motorů

- Ca2+ a calmodulin:
  - IQ motivy myosinů
  - KCBP kinesin
  - injekce Ca2+ urychluje pohyb chromosomů
- fosforylace

Organizace cytoskeletu:
její regulace a koordinace
s dalšími buněčnými ději

Multifunkční proteiny asociované s cytoskeletem

- KCBP, GhKCH2: „zčásti kinesin, zčásti myosin“
- Jinde:
  - forminy (mDia živ.) interagují i s mt ... etc ...

Conserved microtubule–actin interactions in cell movement and morphogenesis

Olga C. Rodriguez, Andrew W. Schaefer, Craig A. Mandato, Paul Forscher, William M. Bement and Clare M. Waterman-Storer

NATURE CELL BIOLOGY VOLUME 5 | NUMBER 7 | JULY 2003

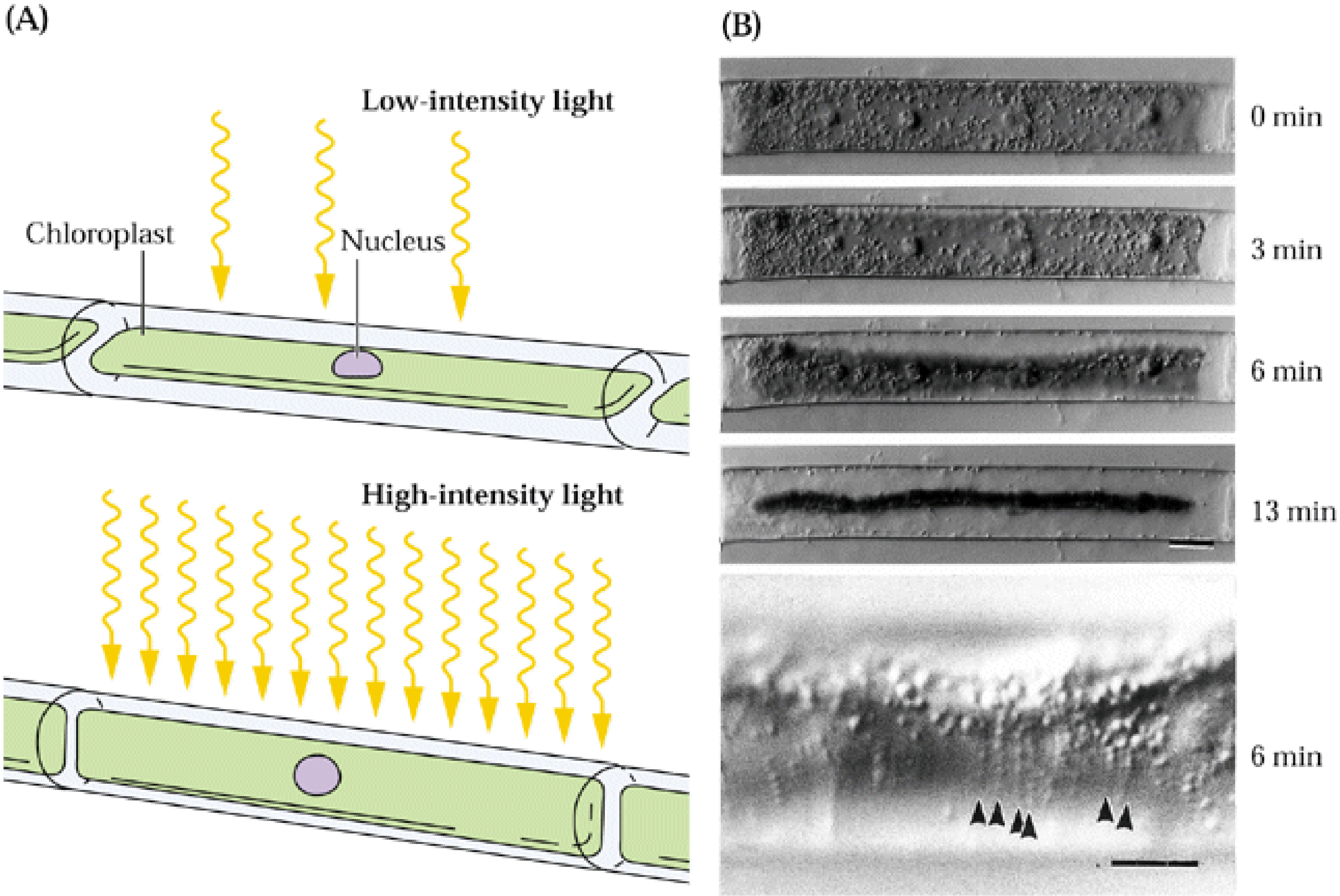
Table 1 Molecular candidates for mediating structural interactions between microtubules and actin.

Protein(s)	Cell type/system of characterization	Localization or association	Functions/roles
Adenomatous polyposis coli (APC) (refs 34–37, 44–45, 74)	*Drosophila* syncytial embryos, epithelial cells, human colorectal cancer cells	Actin caps, pseudocleavage furrows, adherens junctions and microtubule plus ends; binds EB1, binds β-catenin, binds the Rac GEF Asef	Mediates microtubule tip–actincortex interaction to anchor and orientate mitotic spindles; promotes microtubule polymerization and stabilization *in vitro*; in mice that have a mutation in the microtubule-binding domain of APC, intestinal cells fail to migrate out of the crypts
CHO1 (MLKP, family) (ref. 92)	Mammalian cells	CHO1 splice variant with actin-binding domain	Bundles microtubules; required for completion of cytokinesis
Coronin (ref. 101)	Budding yeast	Cortical actin patches; unique among coronin family for microtubule/actin binding	Promotes actin assembly and crosslinking
Cytoplasmic dynein/dynactin (refs 39, 98)	(1) Budding yeast (2) *C. elegans* (3) Mammalian fibroblasts, astrocytes, or epithelial cells	(1) Interacts with cortical protein Num1p (2) Cortex between the AB and P1 blastomeres (3) Colocalization to F-actin cortical spots and sites of cell–cell contact	(1) Microtubule capture by cortex to position mitotic spindle during cell division (2) Spindle orientation (3) Spindle orientation; MTOC reorientation during cell motility
IQGAP1/CLIP-170 (refs 38, 102–104)	Mammalian fibroblasts and epithelial cells	IQGAP1 binds actin; associates with microtubules via CLIP-170	IQGAP1 binds Rac and Cdc42; role in cell–cell compaction?; Cdc42-induced cell polarity
Bim1/Kar9/Myo2 (refs 42, 43)	Budding yeast	Bim1 on microtubule plus ends is linked to the myosin 5 homologue Myo2 via Kar9	Pulls astral microtubule along actin cables into the bud during spindle orientation

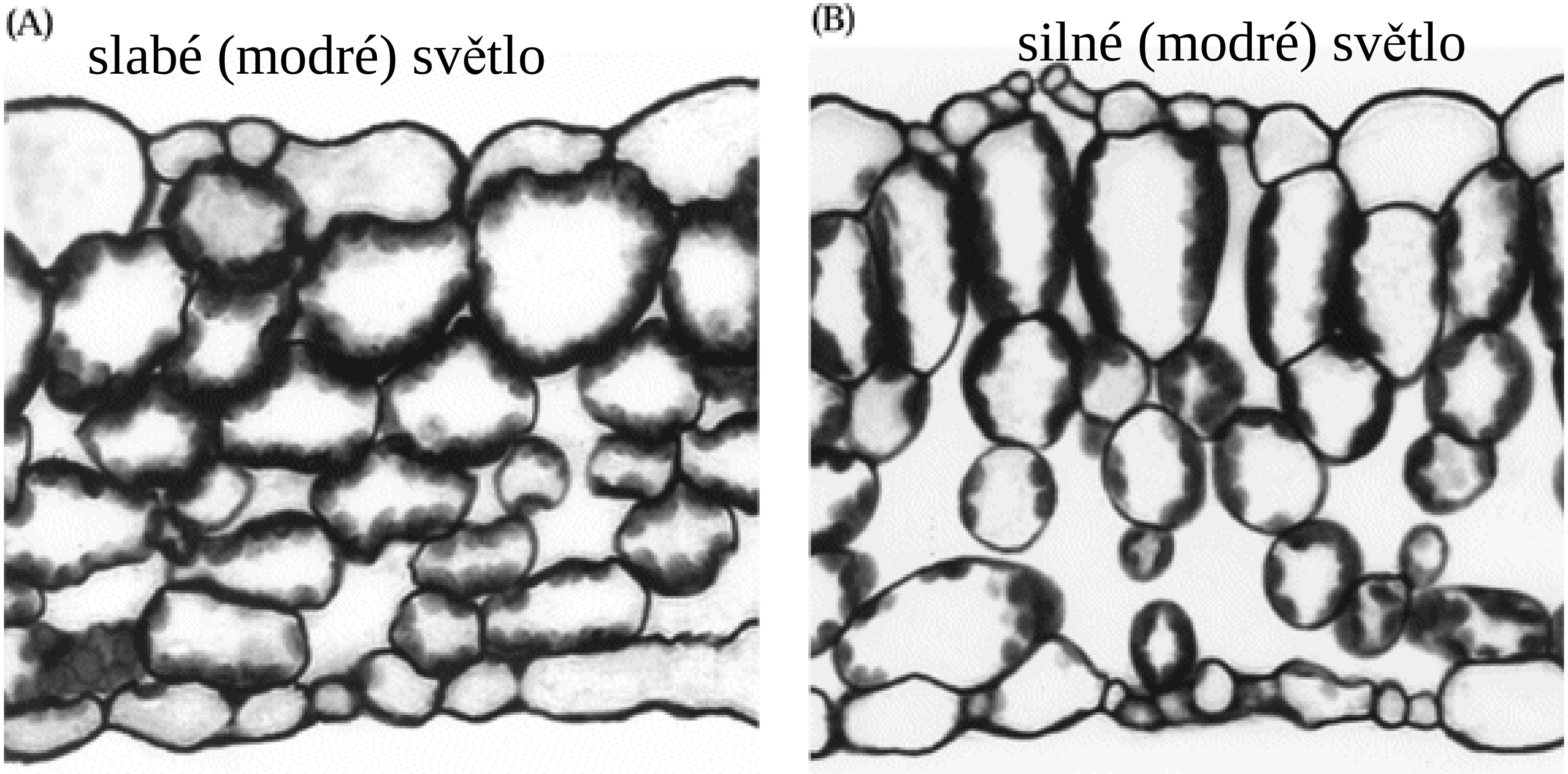
MAP2c (refs 61, 105)	Neurons, melanoma cells	Microtubule binding when unphosphorylated; phosphorylation enables actin localization and interaction	Promotes microtubule growth and actin bundling
Mip-90 (ref. 106)	Human fibroblasts	Colocalizes with actin and microtubules	Function unknown
Myo5a-kinesin complex (ref. 107)	Neurons, melanocytes	Myo5a and kinesin interact in yeast two-hybrid screen	May coordinate organelle transport along microtubules and actin
Myo6–D-CLIP190 complex (refs 108, 109)	*Drosophila* embryos	Colocalize in the nervous system and posterior pole of embryo	Mutation phenotype suggests Myo6 mediates membrane remodeling during embryogenesis and spermatogenesis
hGAR17β and hGAR22β (*Gas2*-related proteins) (ref. 110)	COS-7, NIH 3T3, and other cell lines	From overexpression and cosedimentation experiments, localizes to and binds actin and microtubules	Originally identified in a search for tumour suppressors; some evidence for upregulation in growth-arrested cells
(1) *kakapo/short stop* (2) MACF (MACF7), vertebrate homologue of *kakapo/shortstop* (ref. 18)	(1) *Drosophila* embryos: neurons and epidermal muscle attachment cells (2) COS-7 cells, human adrenal carcinoma cells, mouse keratinocytes	(1) Localizes to microtubule ends (2) Sites of cell–cell contact, colocalizes with and binds microtubules and actin	(1) Mutation phenotype suggests role in axon outgrowth; wing tissue integrity (2) Stabilizes microtubules; mediates actin–microtubule interactions at cell periphery
BPAG1a (neuronal) and BPAG1b (muscle) (ref. 18)	Mouse embryos and tissue	Hemidesmosomes; both actin-binding and microtubule-binding domains	Skin blistering phenotypes suggest a role in maintenance of tissue architecture; also results in disorganized intermediate filaments and microtubules in degenerating neurons
Plectin (ref. 19)	Vertebrate cell lines, explants and tissues	Links intermediate filaments to actin and microtubules; localizes to stress fibres, hemidesmosomes	Disease and mutation phenotypes of skin blistering suggest a role in maintenance of tissue integrity; regulates actin organization?

This list is not exhaustive, and some entries are not referred to in the text.

Pohyby organel: plastidy jdou za světlem



Mougeotia



Signalizace: receptor - phototropin

Böhm JA (1856) Beiträge zur näheren Kenntnis des Chlorophylls. S.B. Akad. Wiss. Wien, Math.-nat. Kl. 22: 479-498 (z disertace S. Schmidt von Braun)

SITZUNGSBERICHTE

DER KAISERLICHEN

AKADEMIE DER WISSENSCHAFTEN.

[...] Nach Mohl's [1]) Angabe sind in der mittleren Schichte des Blattes von *Orontium (Rhodea) japonicum* die Chlorophyllkörner in der Mitte der Zelle zu einem Haufen zusammengeballt. Ich habe diese Pflanze oft untersucht, fand aber immer die Chlorophyllkörner an der Zellwandung anliegen. Bei der grossen Aufmerksamkeit jedoch, mit der ich die Chlorophyllkörner der verschiedenen *Sedum*-Arten untersuchte, zeigte sich mir eine höchst interessante Erscheinung. Ich brachte nämlich mehrere Arten derselben mit cylindrischen Blättern ins warme Haus, dessen Fenster sich gegen Süden öffneten, um vielleicht in den Blättern der unter diesen Umständen sich rasch entwickelnden Triebe über die jugendlichen Zustände der Chlorophyllkörner einigen Aufschluss zu erhalten. Zufälliger Weise untersuchte ich sie längere Zeit hindurch täglich zur Mittagsstunde, und ward nicht wenig überrascht, stets sämmtliche Chlorophyllkörner zu einer Gruppe vereinigt irgend einer Stelle der Zellwandung anliegend zu finden [...]

BMC Plant Biology

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In vivo reorganization of the actin cytoskeleton in leaves of *Nicotiana tabacum* L. transformed with plastin-GFP. Correlation with light-activated chloroplast responses

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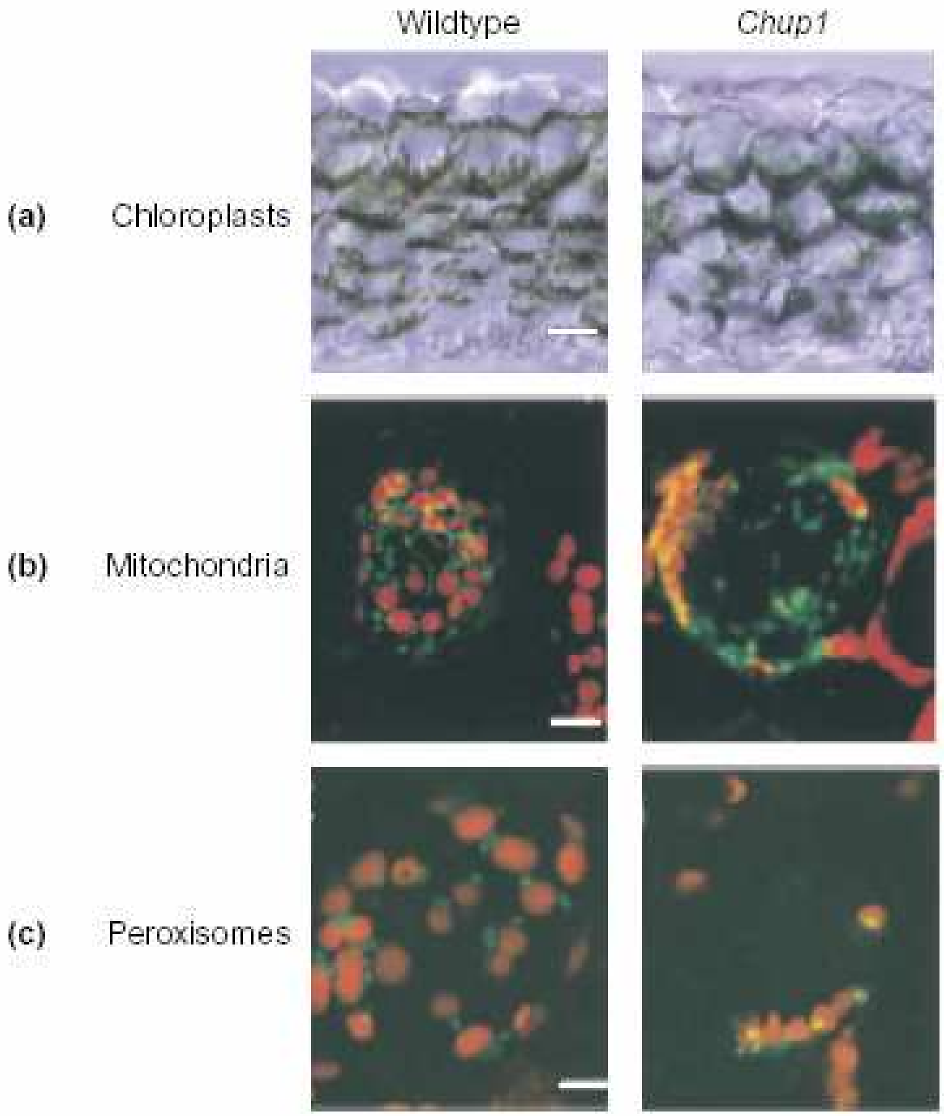
Mutant *chup1* (chloroplast unusual positioning)



Distribution of chloroplasts, mitochondria and peroxisomes in wildtype and *chup1* mutants. **(a)** Cross-section of a light-adapted leaf. In the wildtype, chloroplasts are positioned on the upper and lower cell surfaces, whereas in the cells of *chup1* mutants, chloroplasts are aggregated on cell bottom. Bar represents 30 μm. **(b)** Mitochondrial distribution viewed by transient expression of mitochondria-targeting GFP. The positioning of mitochondria is similar in wildtype and *chup1* mutants. Bar represents 10 μm. **(c)** Peroxisomal positioning viewed by transient expression of peroxisome-targeting GFP. Peroxisomes are localized close to chloroplasts. In *chup1* mutant cells, peroxisomes are closely associated with aggregated chloroplasts. Bar represents 10 μm.

CHUP1: actin binding!

Chup1: lokalizace na periferii plastidů

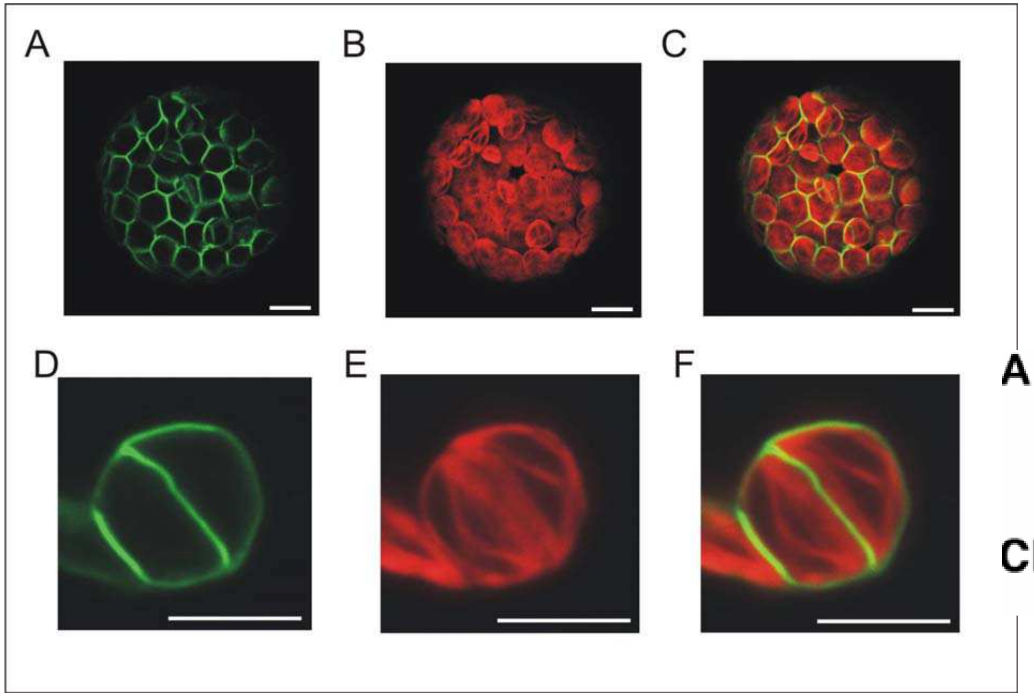
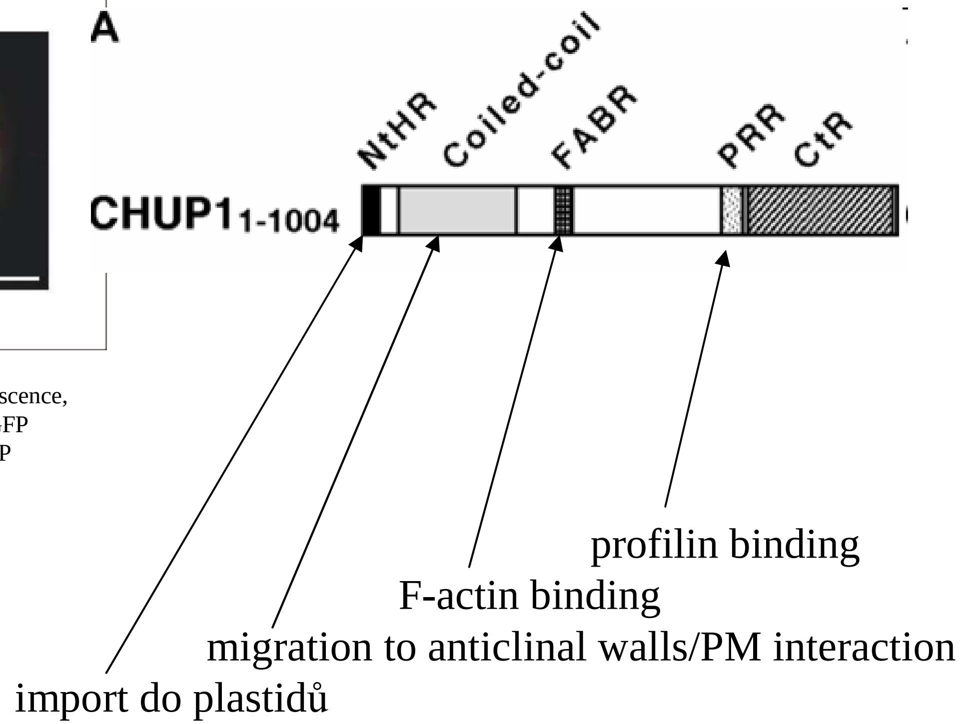
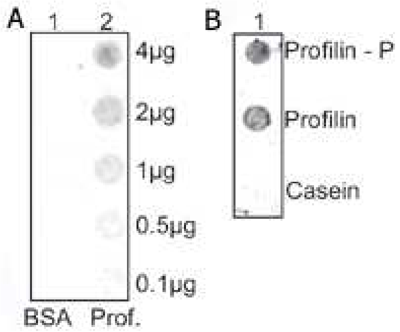


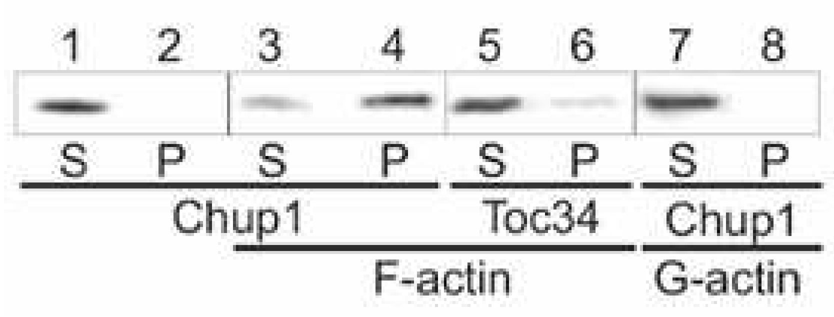
Figure 21 Expression of Chup1-GFP in A.thaliana protoplasts. Left panel: GFP fluorescence, middle panel chlorophyll autofluorescence, right panel: overlay picture. A-F Chup1-GFP expression in protoplasts. D-F Close-up of two chloroplasts surrounded by Chup1-GFP fluorescence. Bar = 10 µm. (Schmidt von Braun 2008)



(Oikawa et al. 2003, 2008)

Chup1 váže F-aktin a profilin

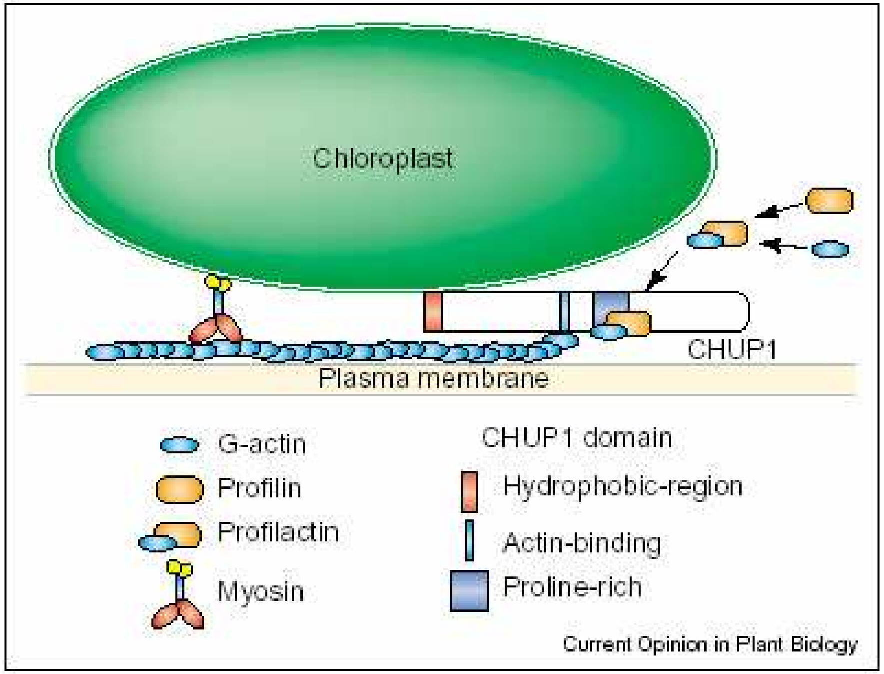




A In-vitro translated Chup1 was incubated with indicated amounts of BSA (lane 1) or profilin (lane 2) on an affinity matrix. The binding was visualized after extensive wash steps by autoradiography. B Chup1 in-vitro translation product was incubated with an affinity matrix coated with 20µg of casein or profilin treated with phosphatase (Profilin – P) or without treatment (Profilin). The binding was visualized after extensive wash steps by autoradiography.

Cosedimentation of Chup1 and F-actin. Radioactively labelled Chup1 (lane 1-4, 7, 8) or outer envelope protein Toc34 (lane 5, 6) were incubated with F-actin (lane 3-6) or G-actin (lane 7, 8) and supernatant (S) and pellet fraction (P) were separated by centrifugation, subjected to SDS-PAGE and autoradiography.

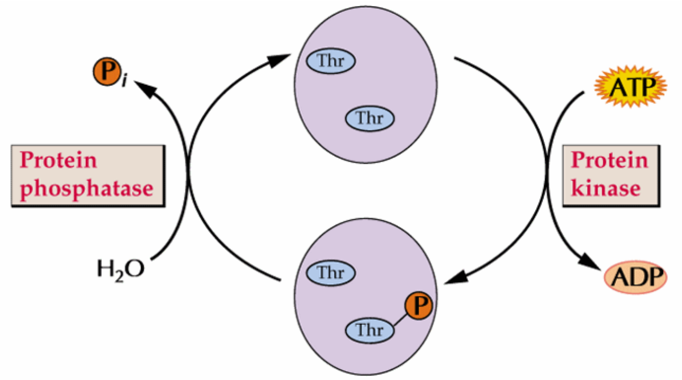
(Schmidt von Braun 2008)

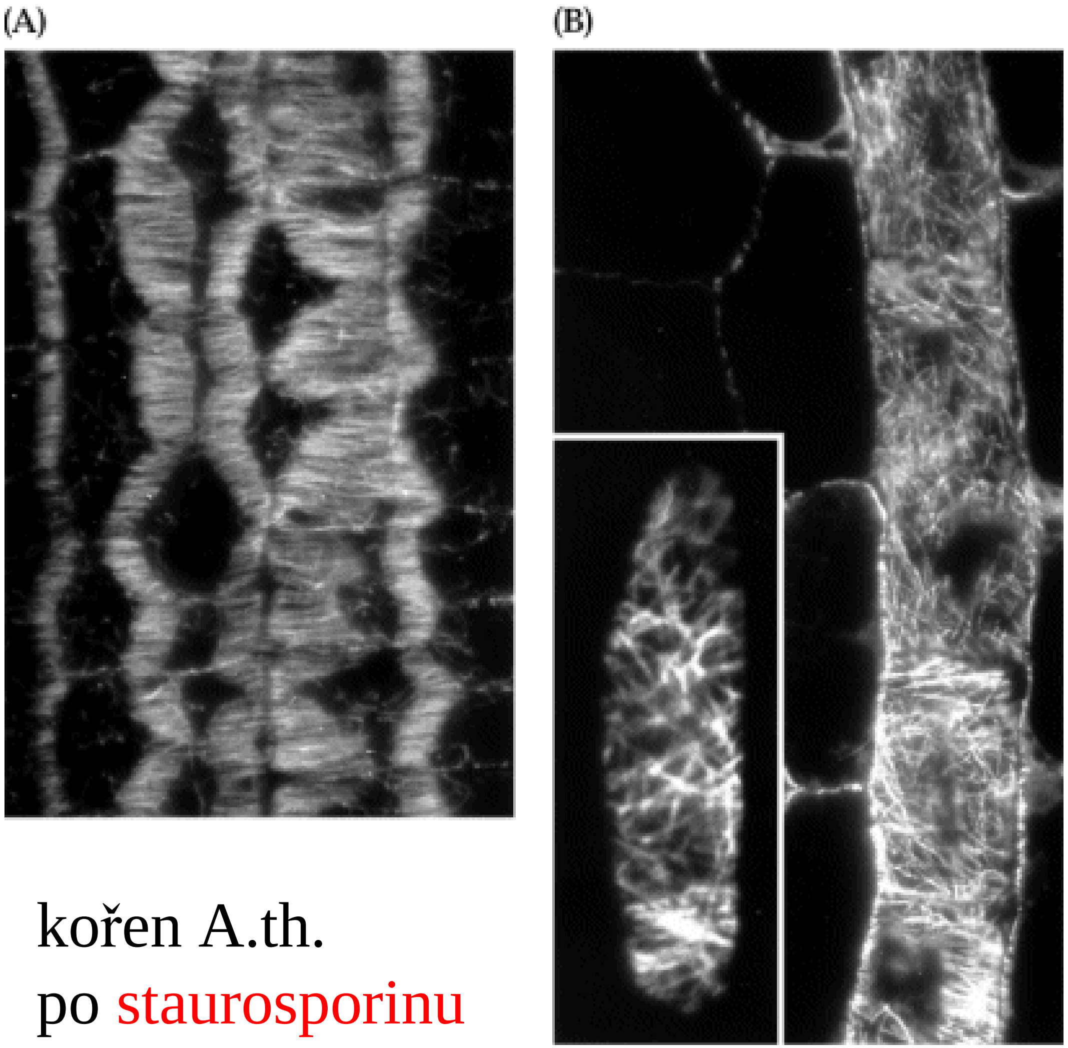


Working model of chloroplast positioning. The amino-terminal hydrophobic region of CHUP1 can target to the chloroplast outer membrane. The proline-rich motif of CHUP1 may serve in actin polymerization to recruit profilactin. CHUP1 binds polymerized F-actin through its actin-binding domain. These functions of CHUP1 may be important in anchoring chloroplasts to the plasma membrane. Myosin motor protein(s) may be necessary for chloroplast motility along actin filaments.

Regulace struktury a funkce cytoskeletu

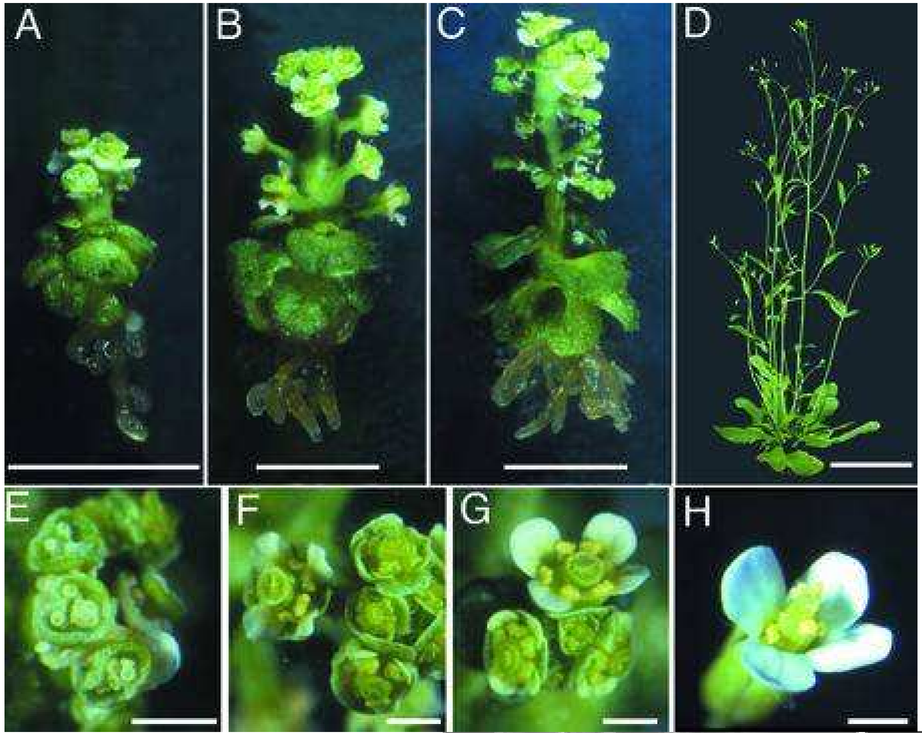
- Jako všude:
  proteinkinázy
  a fosfatázy





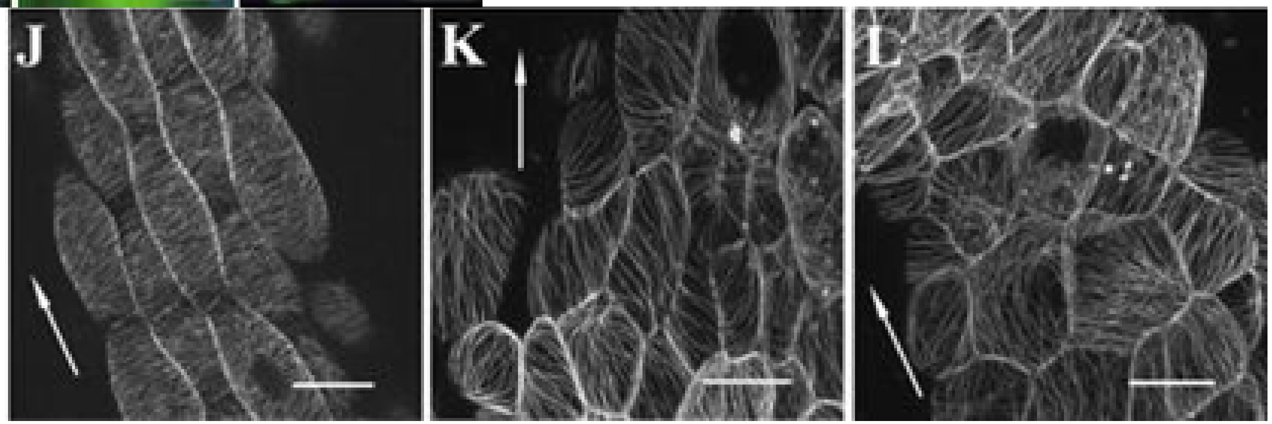
kořen A.th.
po staurosporinu
(mikrotubuly)

TON2 kóduje protein fosfatázu A



wt

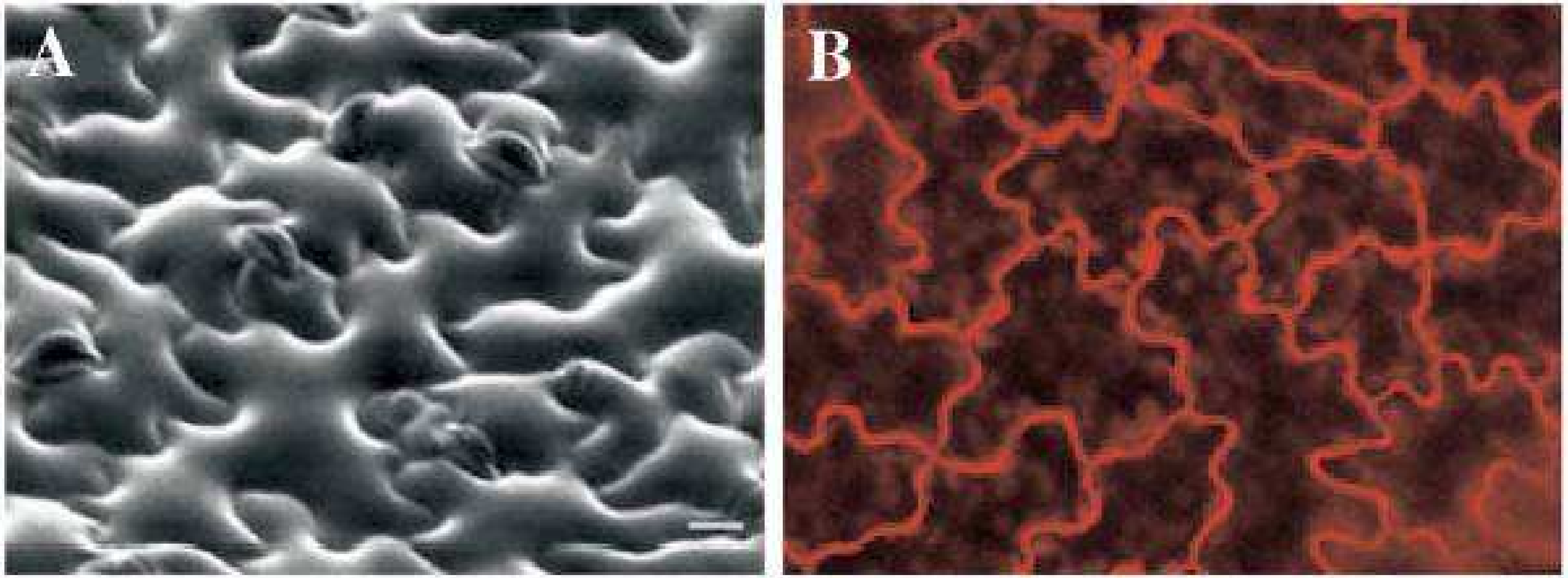
ton2

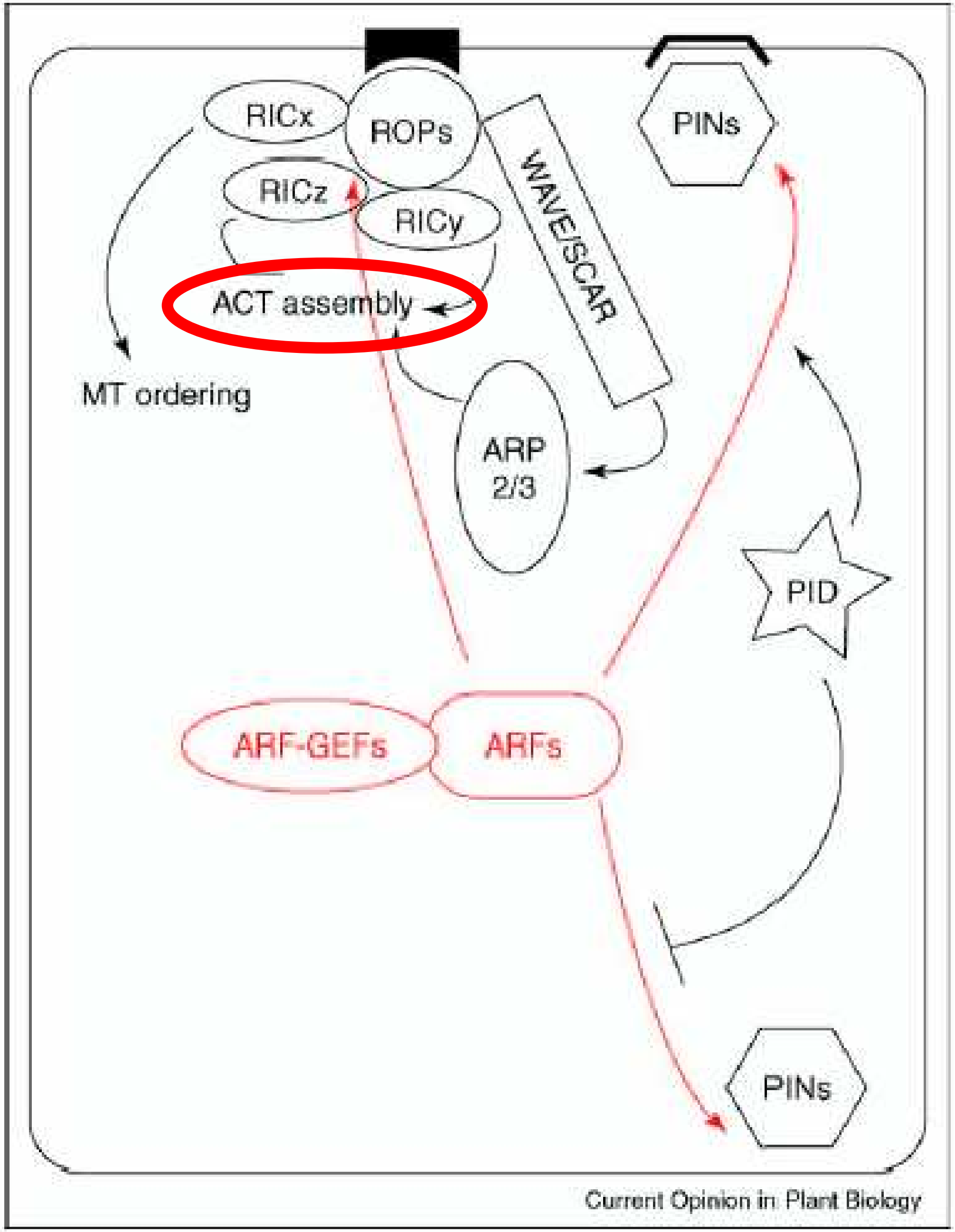


wt *ton*2-14 *ton*2-13

Regulační aspekty: ví se hlavně o aktinu

- *distorted* mutace (ARP2/3)
- *BRICK* (Z. mays)
- *SPIKE1* (multidomén. adaptor. protein)
- RICs/ROPs





Aktin: malé GTPázy (Rop, Arf)

Processes contributing to cell polarization. Vesicle trafficking that is mediated by Class 1 ARFs is required for the polar localization of ROP GTPases, which control actin (ACT) assembly through RICs and WAVE/SCAR–ARP2/3 pathways and microtubule (MT) bundling through other RICs. ARF-mediated vesicle trafficking and a specific ARF–GEF regulator of this process also control the localization of PIN proteins, and the polarity of this localization is controlled by the PID kinase, which functions as a binary switch. Polar localization cues for ROP localization or activation and for PIN localization (black bodies) remain unknown. Red arrows: vesicle trafficking control. Black arrows: protein activity control.

Koordinace aktinu a mt: příklad diferenciace epidermálních buněk

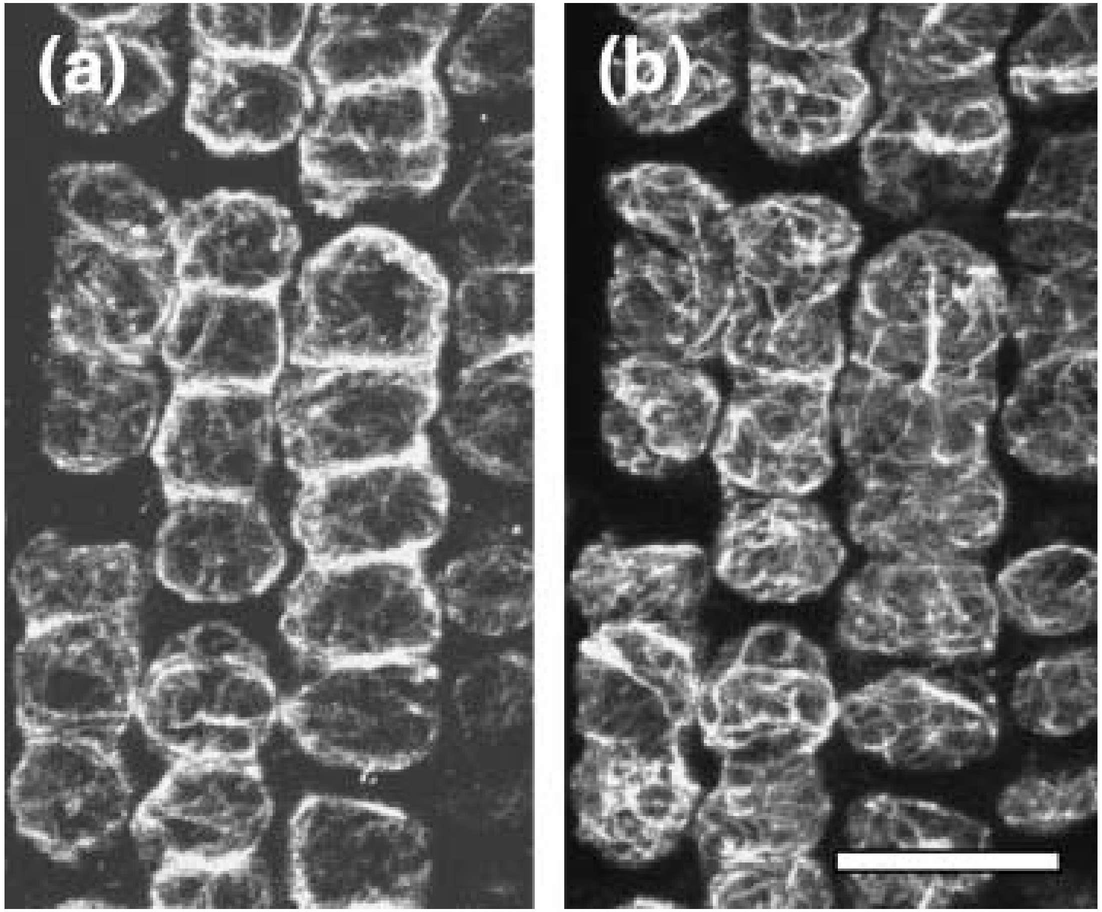
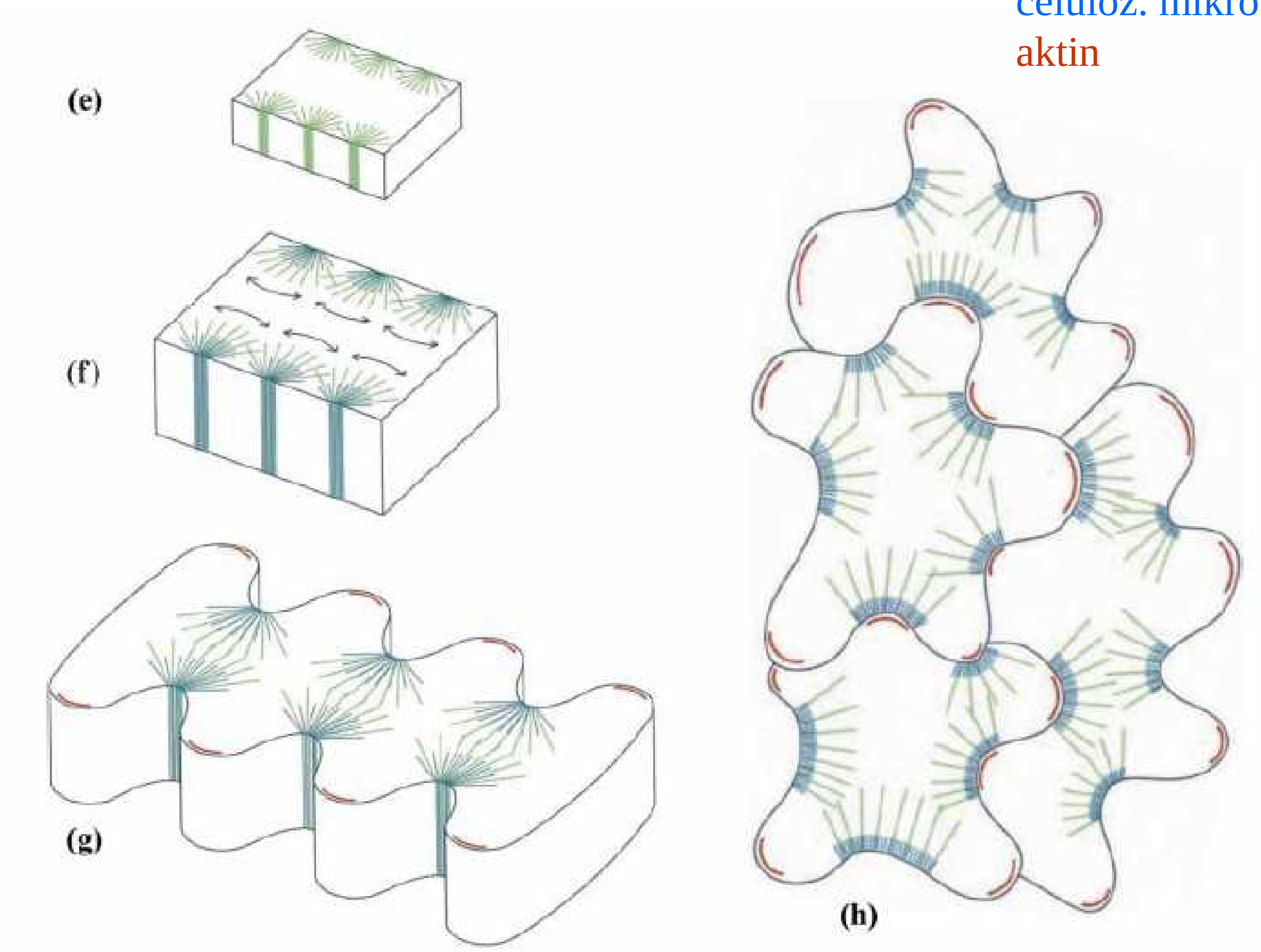


Fig. 1 Confocal-laser-scanning microscope (CLSM) images of lobed mesophyll cells of *Zea mays* after immunostaining of microtubules (MTs) (a) and visualization of actin filaments (AFs) with fluorescent phalloidin (b). Cortical MTs form ring-like bands (a), while AFs (b) exhibit diffuse arrangement. Bar, 20 µm.

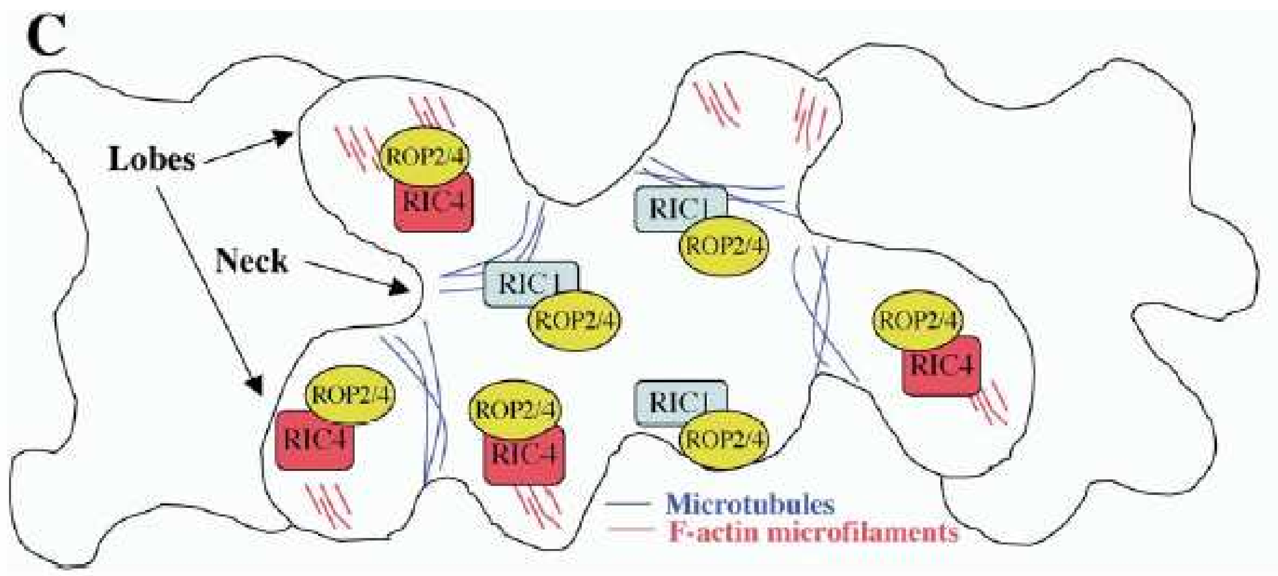
(Panteris a Galatis 2005)



(Panteris a Galatis 2005)

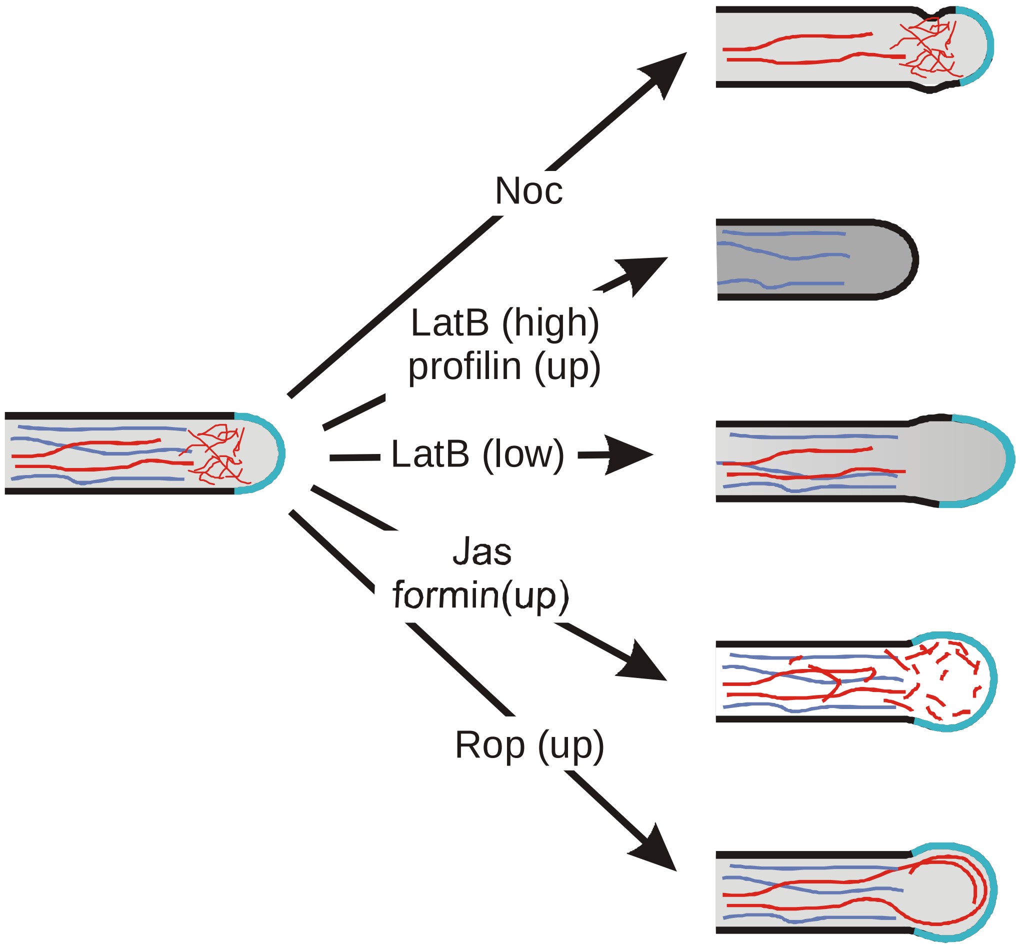
Regulace tvaru epidermálních buněk: RIC/ROP systém

- Rop Interacting CRIBs: efektory ROP, kontrolující aktin i tubulin!
- RIC1: mt bundling
- RIC4: actin polymerization
- ROP2/4:
  - inhibice RIC1
  - stimulace RIC4



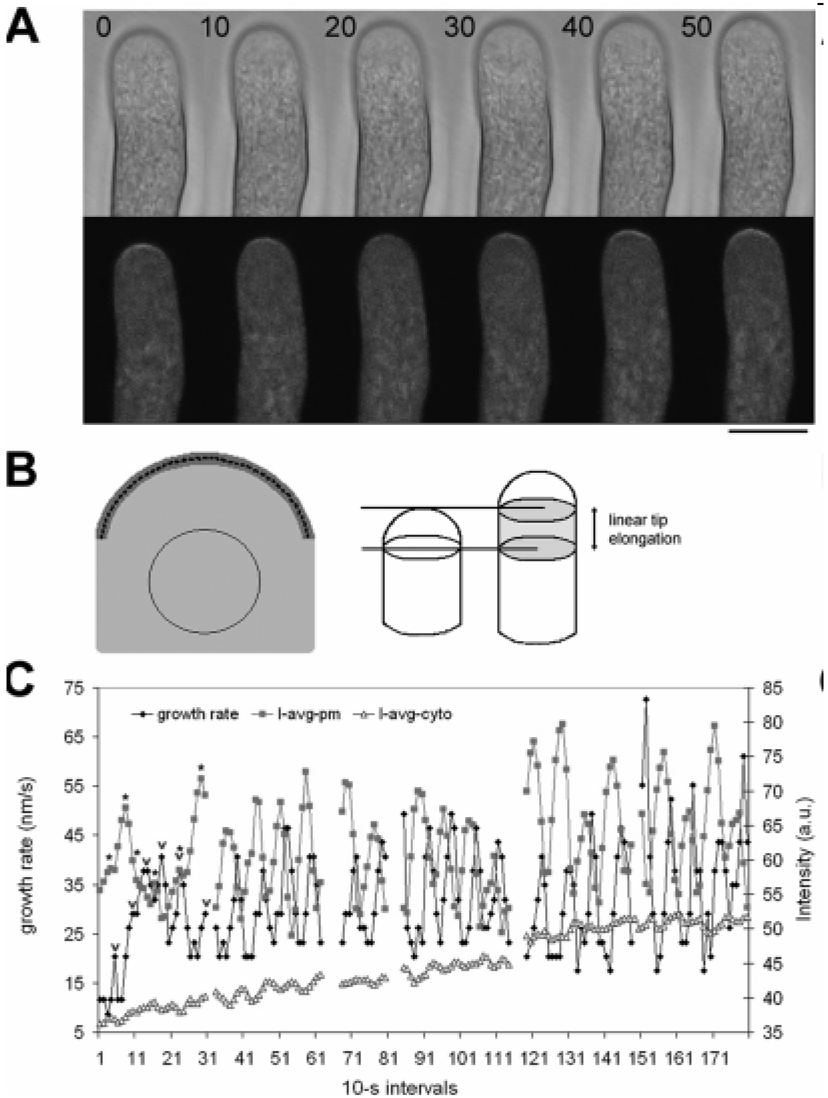
(C) Schematic representation of the model proposed by Fu et al. to explain the role of ROP GTPases and their effectors (RICs) in leaf morphogenesis. ROP2/4 GTPases, via activation of RIC4, promote actin microfilament formation in regions of growing lobes. At the same time, the ROPs, via RIC1 binding, promote microtubule bundling at neck regions to restrict widening and sequester RIC1 at the plasma membrane at sites of lobe initiation in order to prevent microtubules from organizing at those sites.

Cytoskelet a buněčná polarita: příklad vrcholového růstu

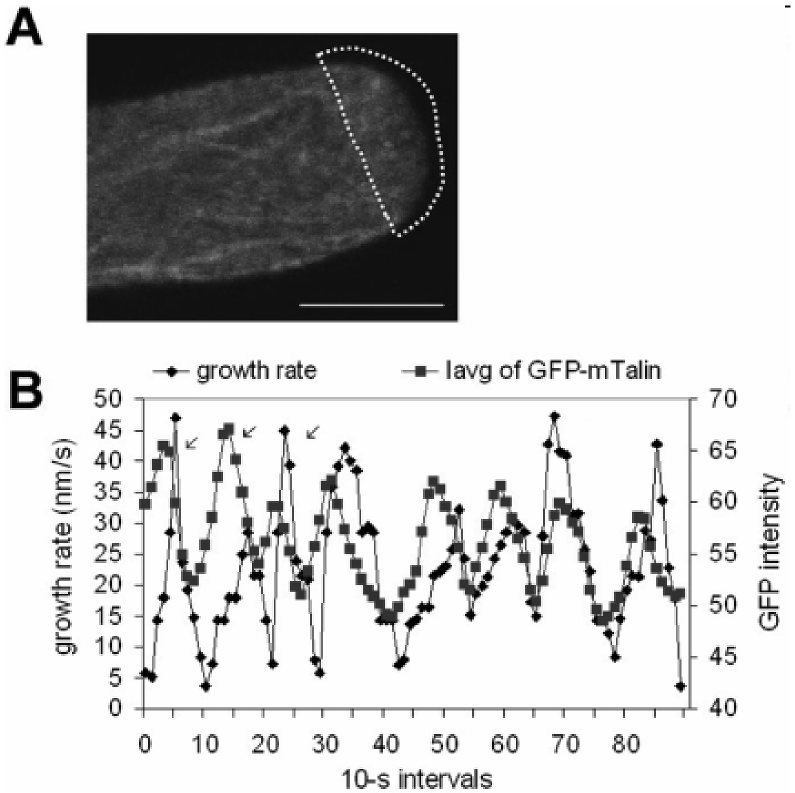


Jas = jasplakinolid (stabilizuje aktin)

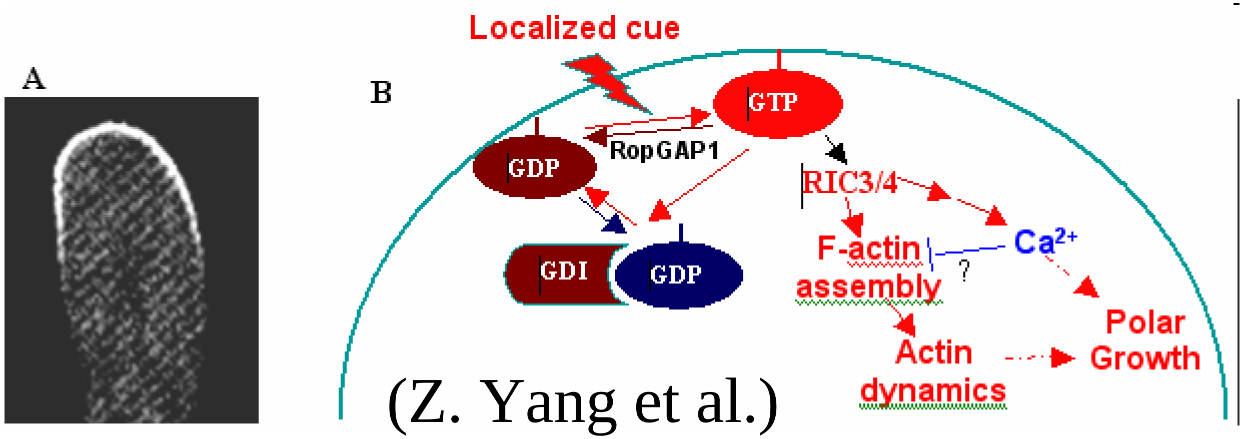
RIC/ROP systém se uplatňuje i jinde: vrcholový růst



Množství GFP-RIC4ΔC v apikální plasmalemě láčky tabáku osciluje souběžně s rychlostí růstu a vrcholovým F-aktinem (Hwang et al. 2005)

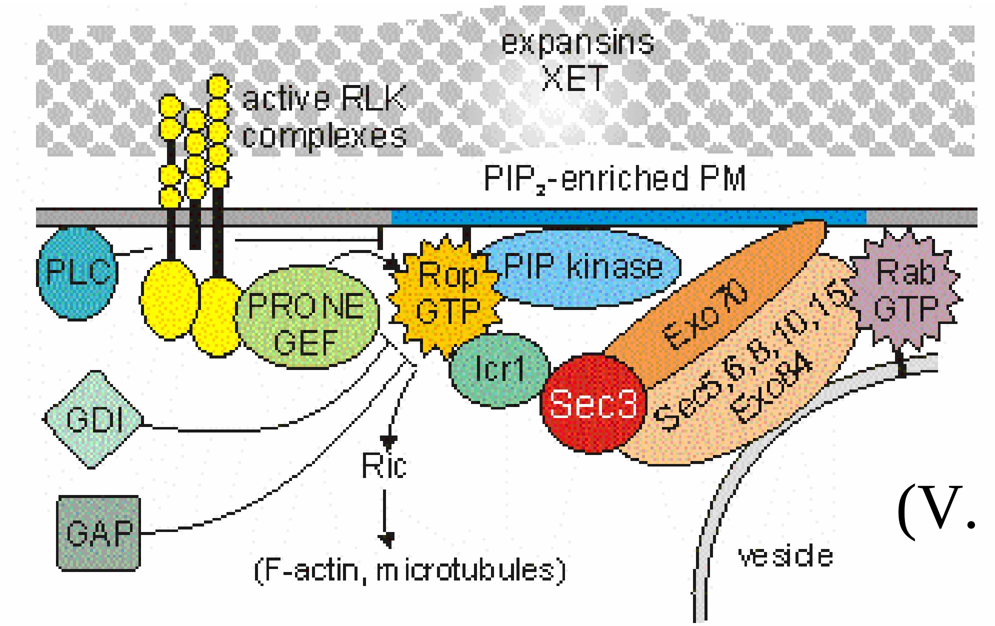


Rop-RIC modul



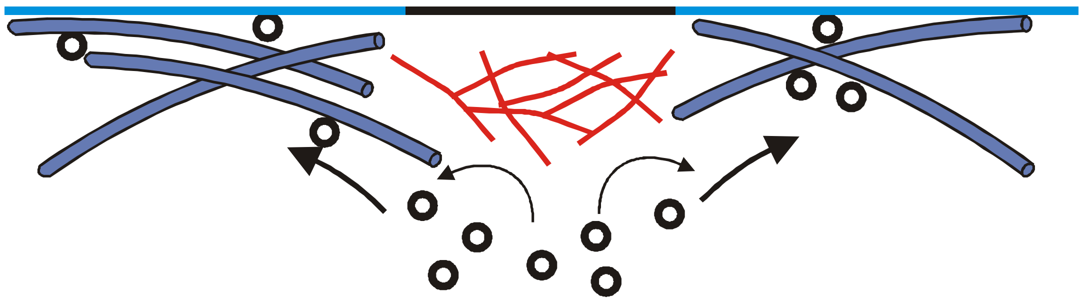
(Z. Yang et al.)

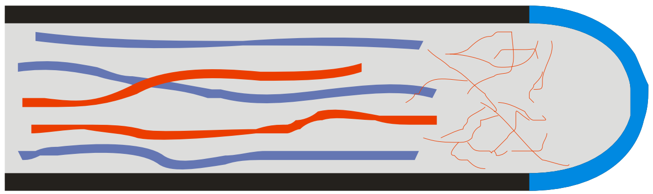
...v kontextu aktivované kortikální domény (ACD)



(V. Žárský et al., 2009)

Aktin, tubulin a sekrece





Tubulin jako „koleje pro přísun materiálu“, aktin coby „forma“, „plot“?

srv. neurony, lymfocyty...

Buněčný cyklus je (také) VELMI cytoskeletální záležitost …

… a také téma samo pro sebe!