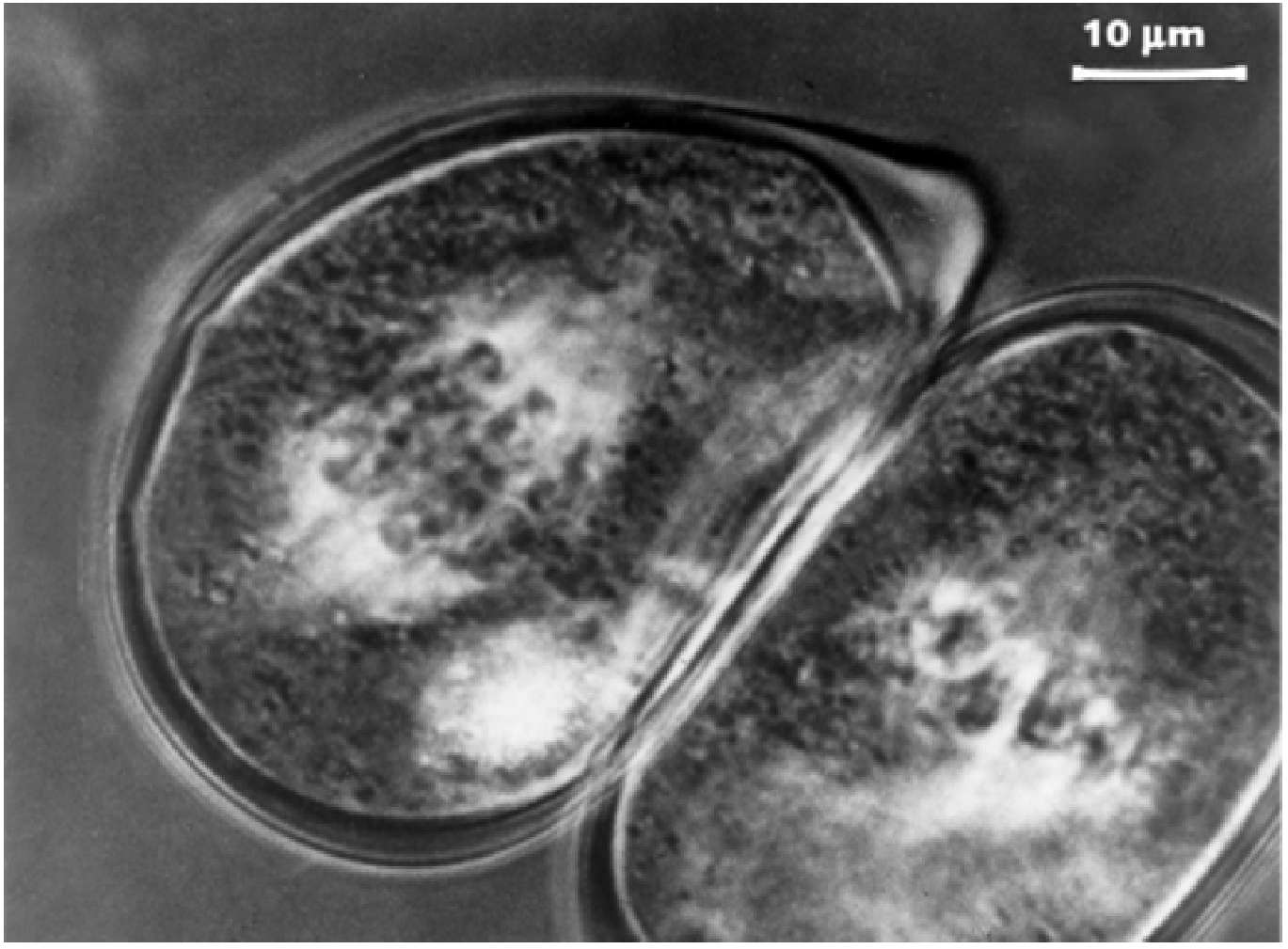
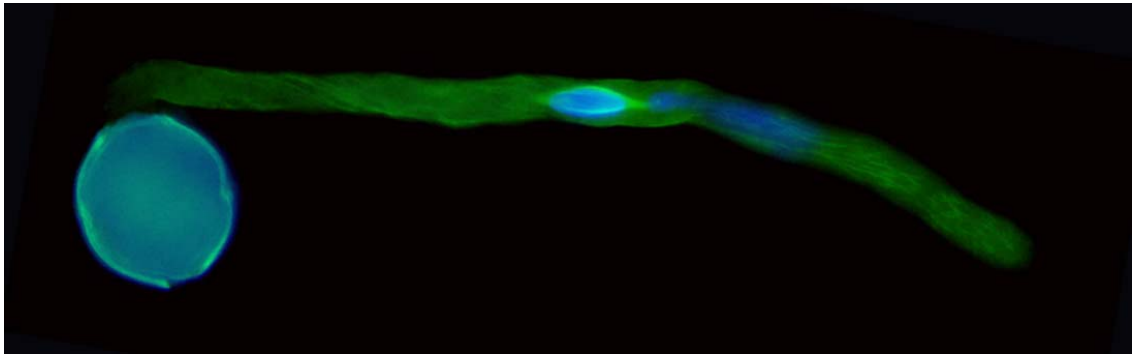
Cytoskelet

Co je vidět v buňkách?

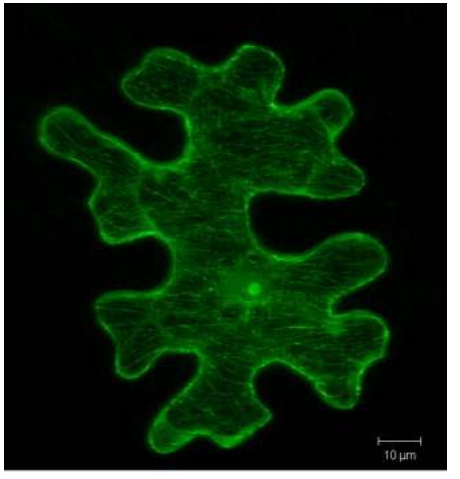
Inoue, 50. léta: „vláknité struktury“ (meióza mikrospor *Lilium longiflorum*)



Aktin a tubulin

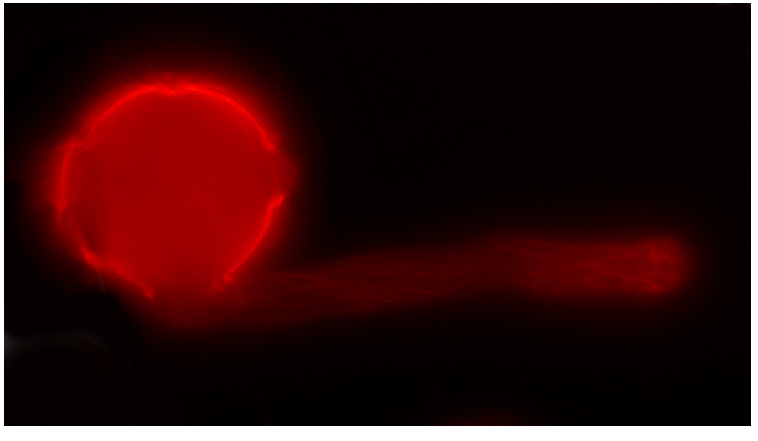


tubulin (a DNA) v pylové láčce tabáku

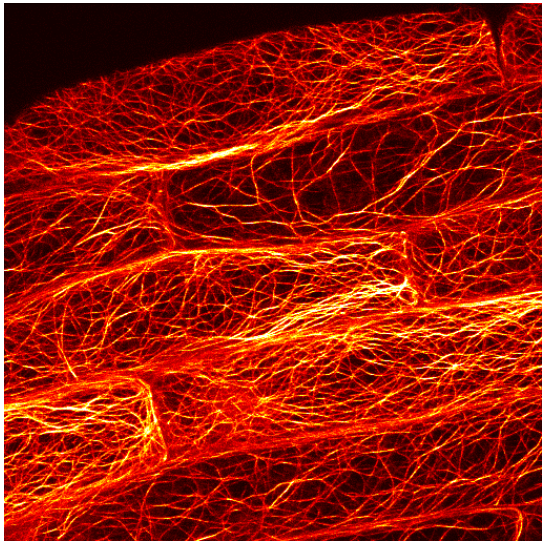


tubulin v epidermis
listů tabáku

aktin v pylové láčce tabáku

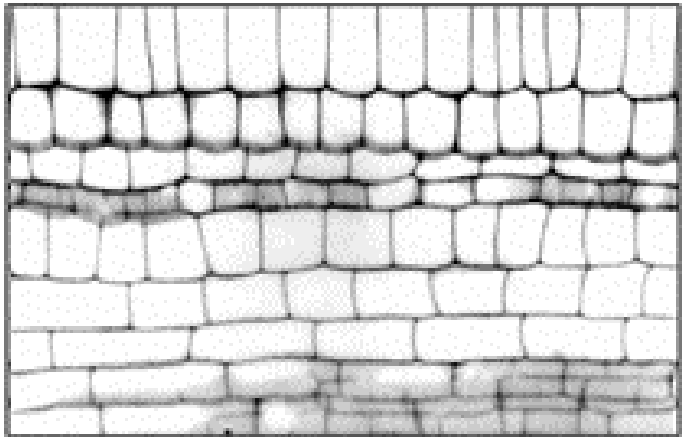


aktin v rhizodermis
Arabidopsis



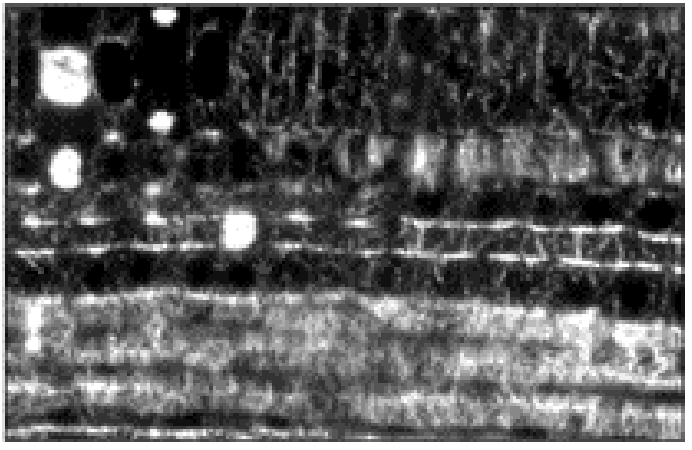
Co je vidět v buňkách?

(A)



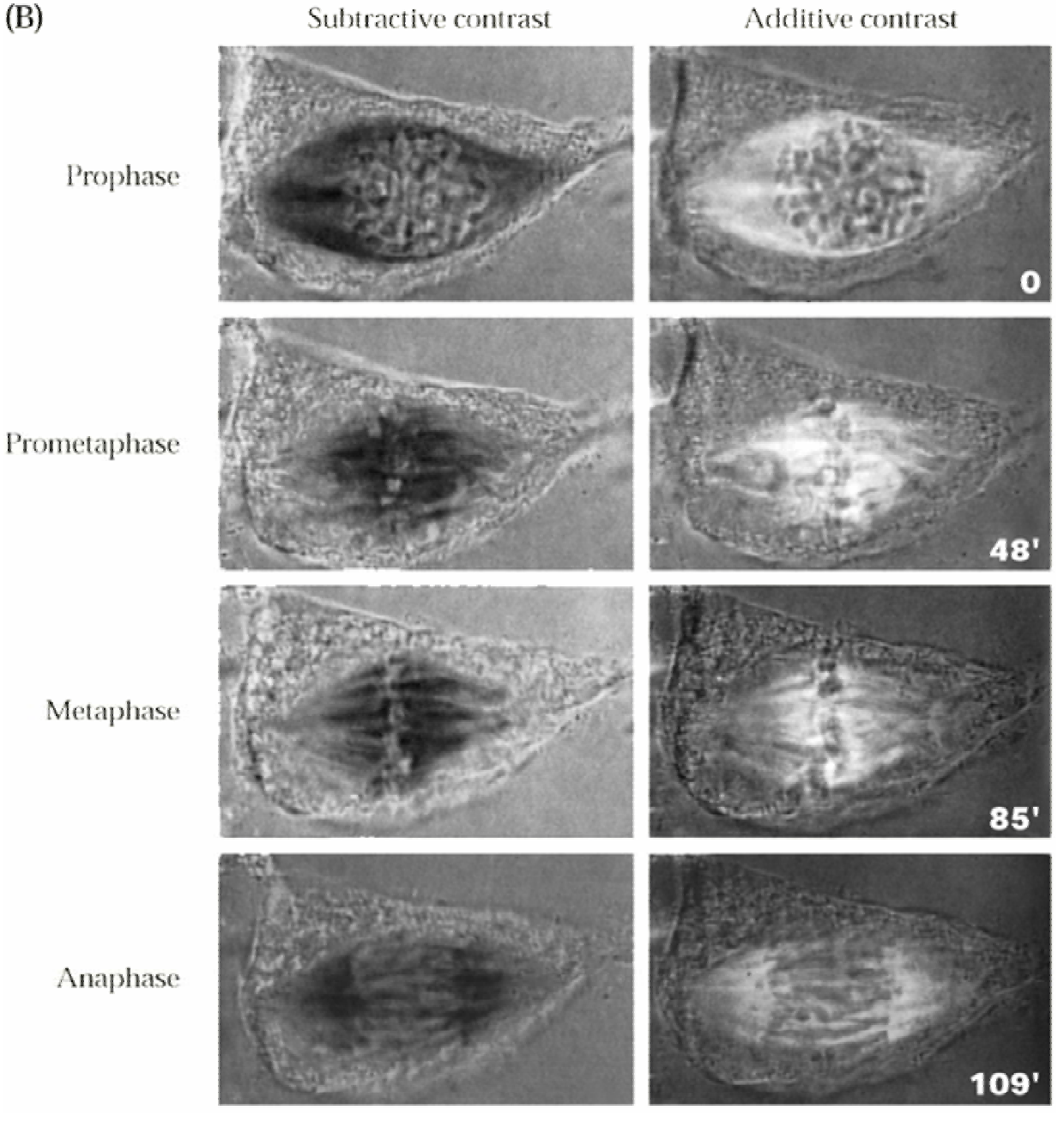
(B)

(C)



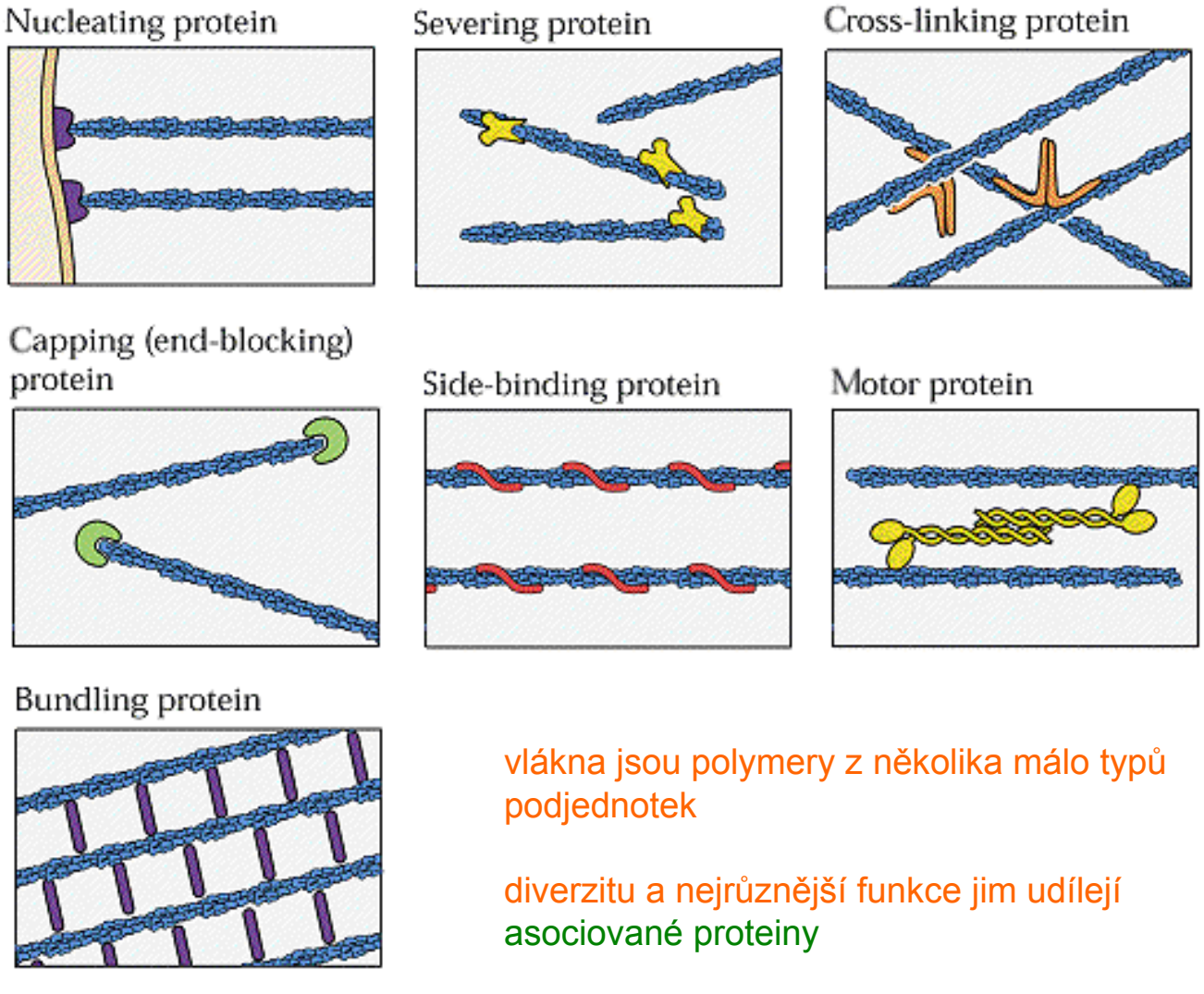
kořen Arabidopsis

Ledbetter MC, Porter KR (1964):
Morphology of Microtubules of Plant Cell.



Endosperm, *Haemanthus* sp.

Obecné rysy cytoskeletu

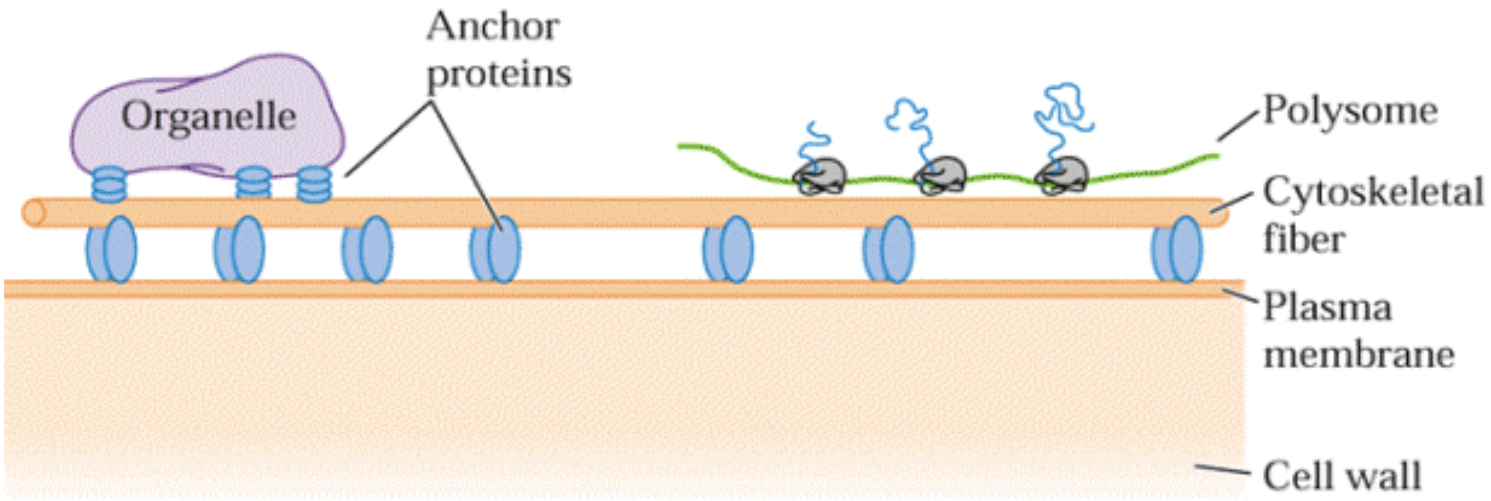


vlákna jsou polymery z několika málo typů podjednotek

diverzitu a nejrůznější funkce jim udílejí asociované proteiny

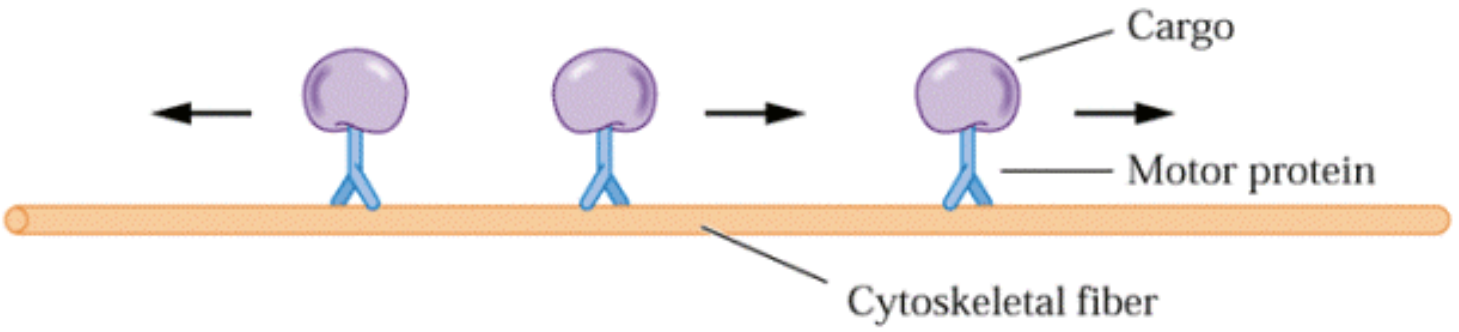
Obecné funkce cytoskeletu

vazba organel a multimolekulárních komplexů

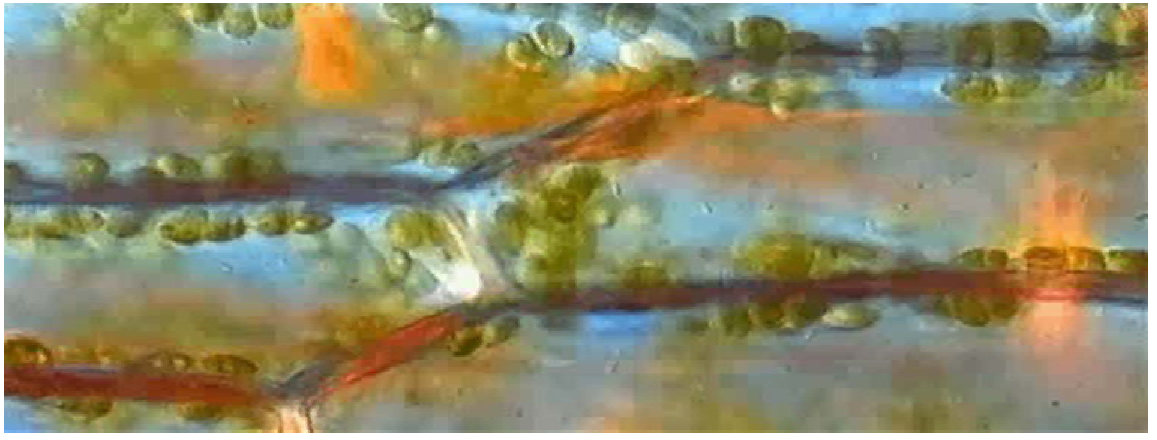


Obecné funkce cytoskeletu

řízený vnitrobuněčný pohyb

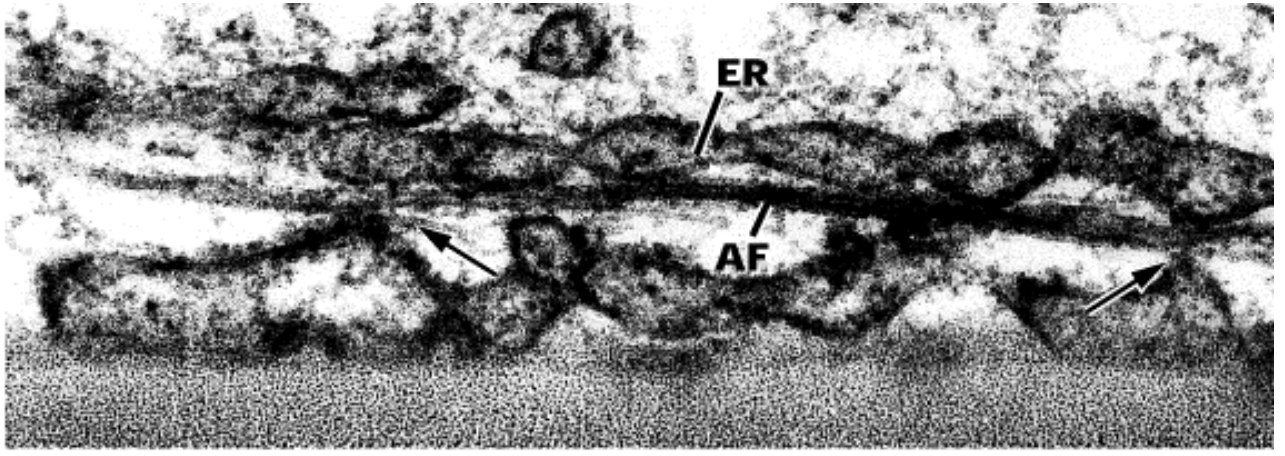


proudění cytoplasmy – *cytoplasmic streaming*

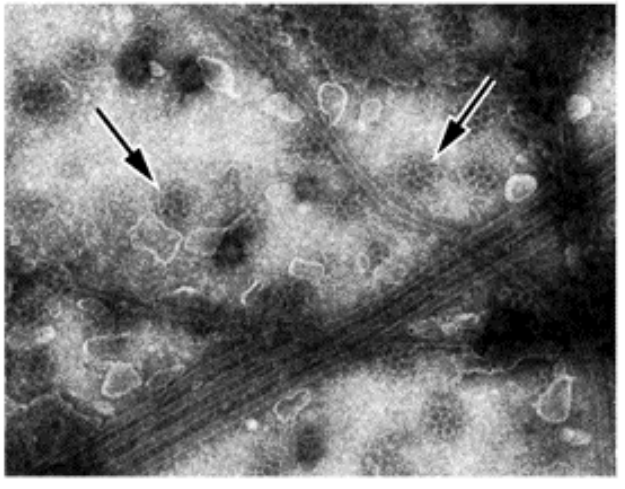


řízený vnitrobuněčný pohyb

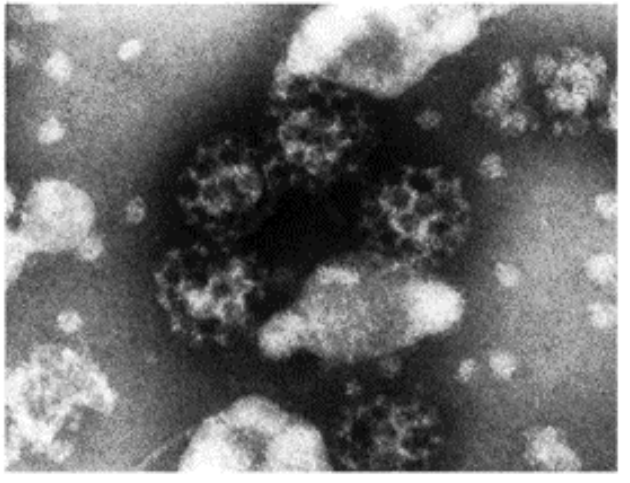
ER and clathrin-coated vesicles associated with AF and MT



(A)

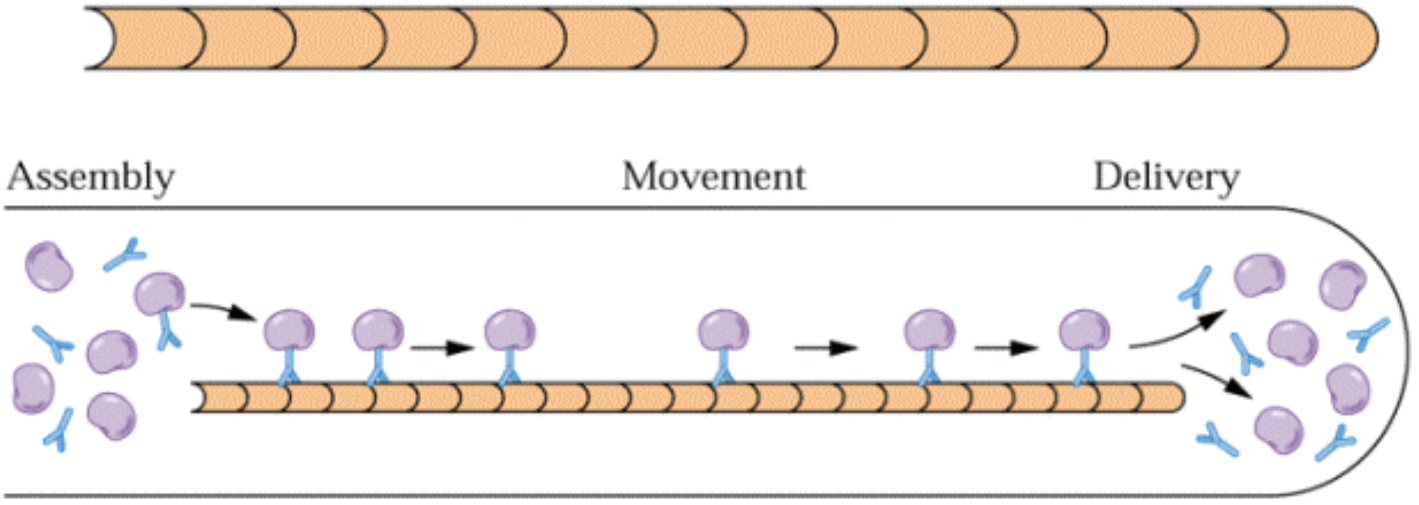


(B)



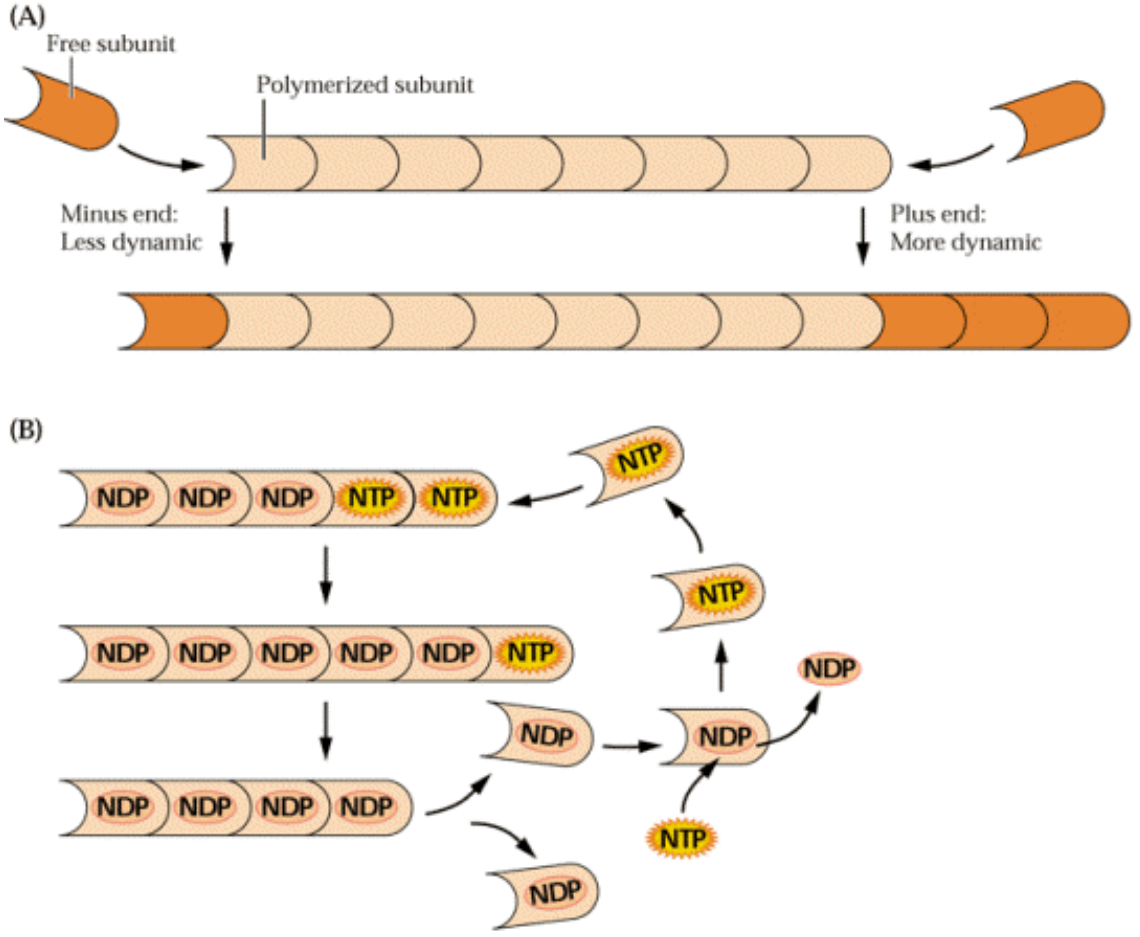
Obecné funkce cytoskeletu

buněčná polarita

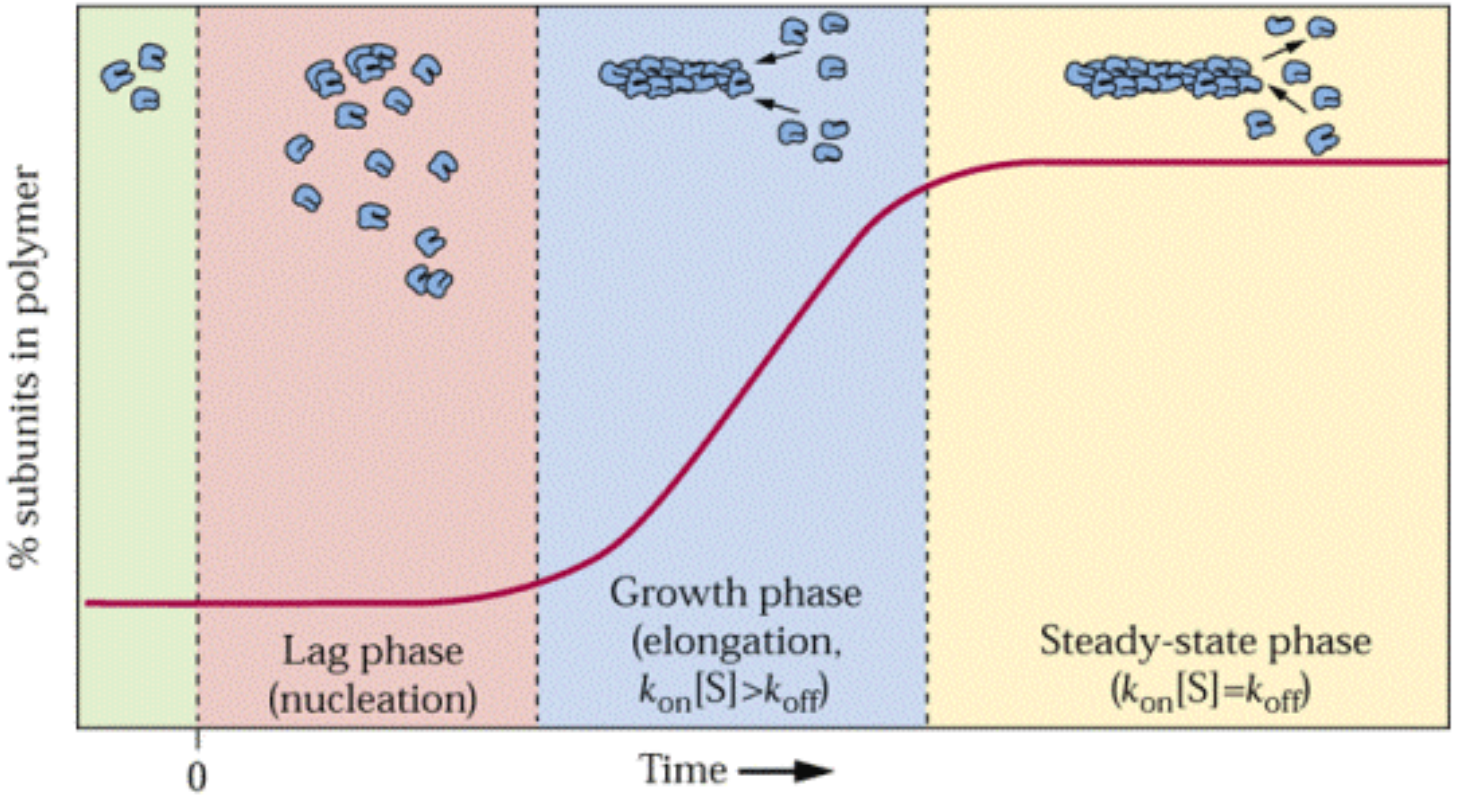


Vznik polárního vlákna z monomerů

obecný princip u aktinu i tubulinu



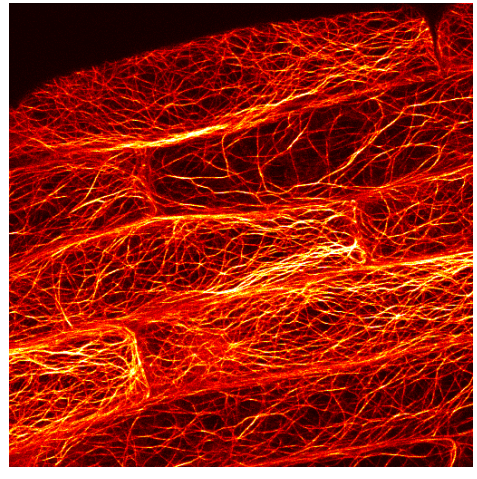
Vznik polárního vlákna z monomerů



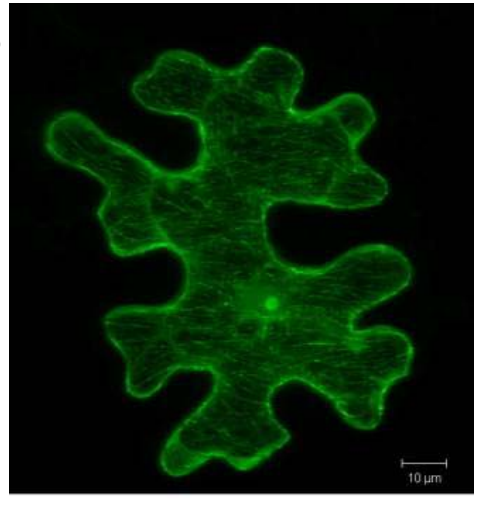
Základní cytoskeletální systémy eukaryot

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

talin-RFP

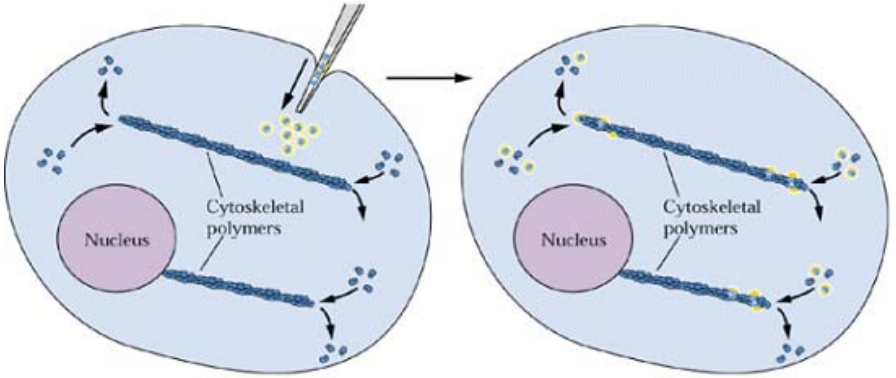


MAP4-GFP



Metody studia cytoskeletu

mikroinjekce



nepřímá fluorescence

infiltrace agrobakterií do listů tabáku

exprese fúzních fluorescenčních proteinů →

přímá fluorescence (faloidin)

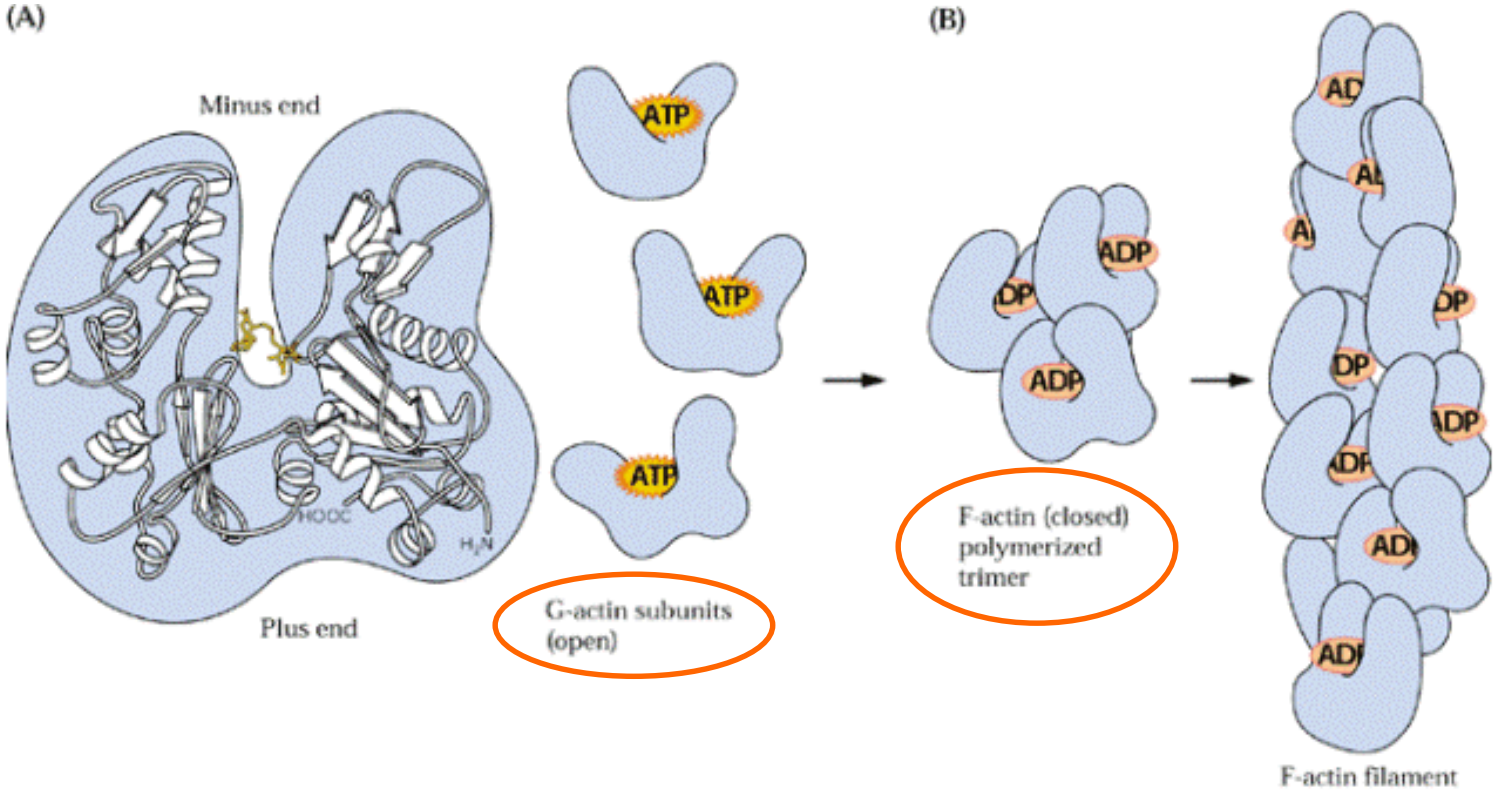
farmakologické působení (stabilizující x destabilizující látky)

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

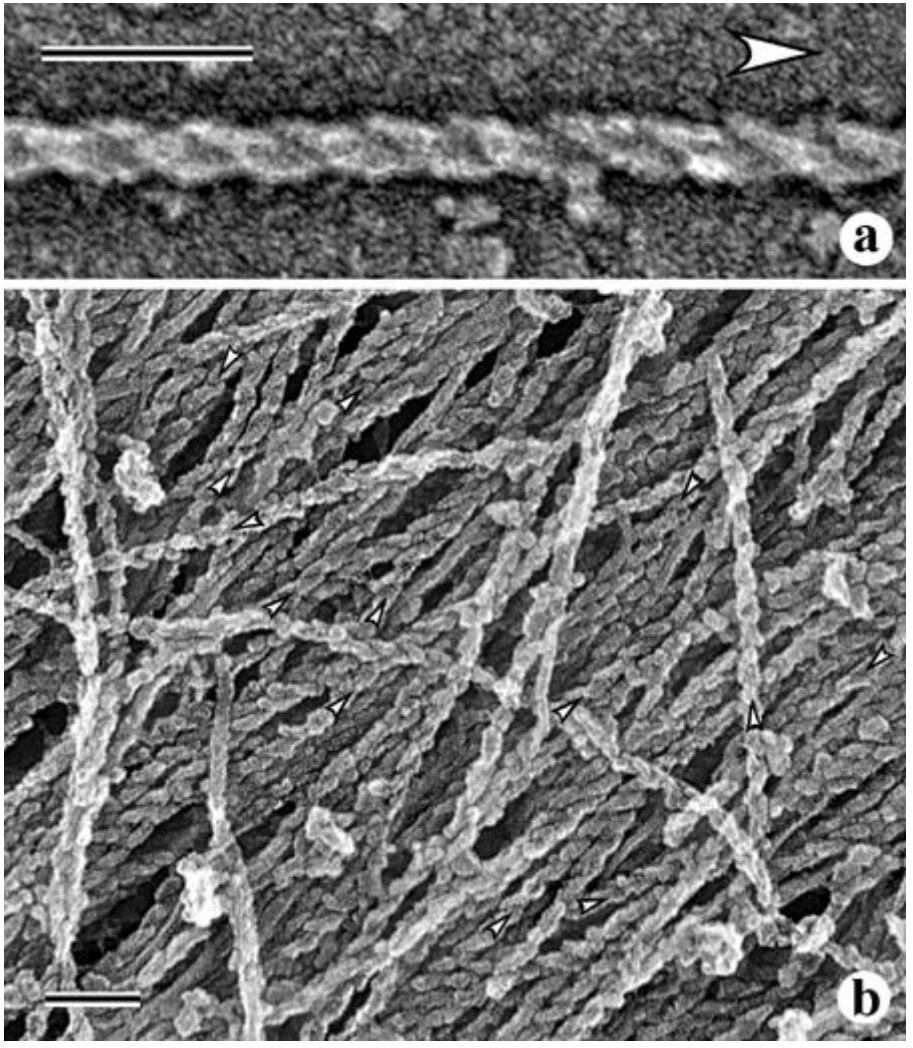
Aktinový cytoskelet

Vznik polárního vlákna aktinu

aktin



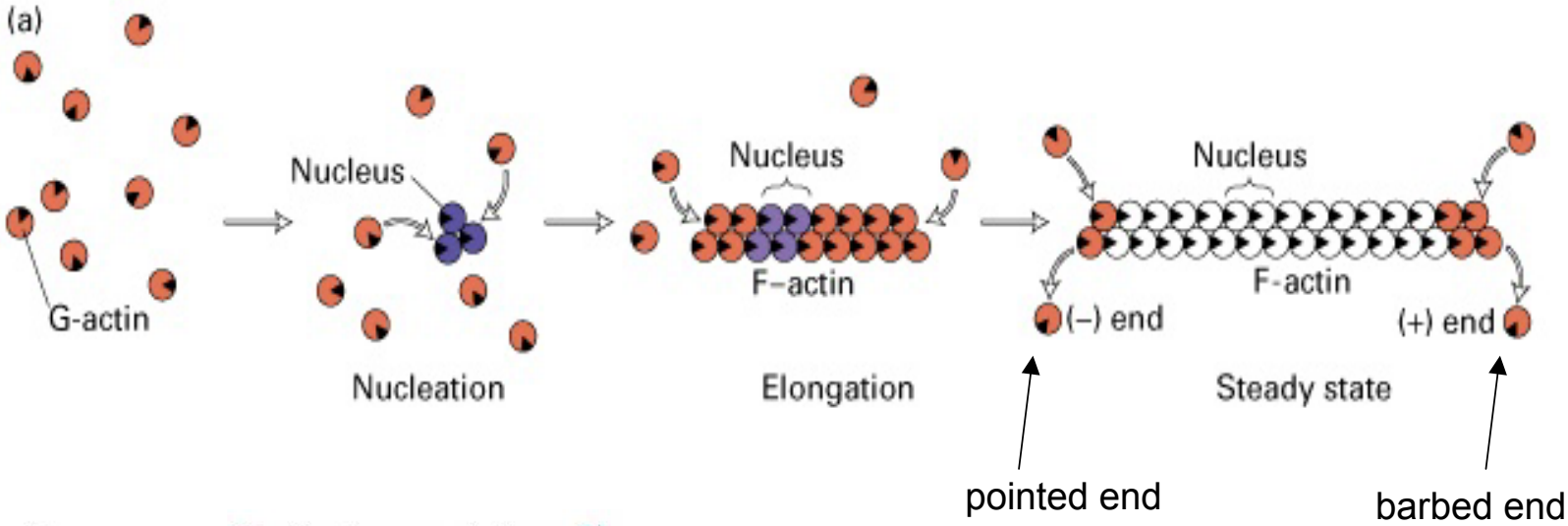
Vznik polárního vlákna z monomerů

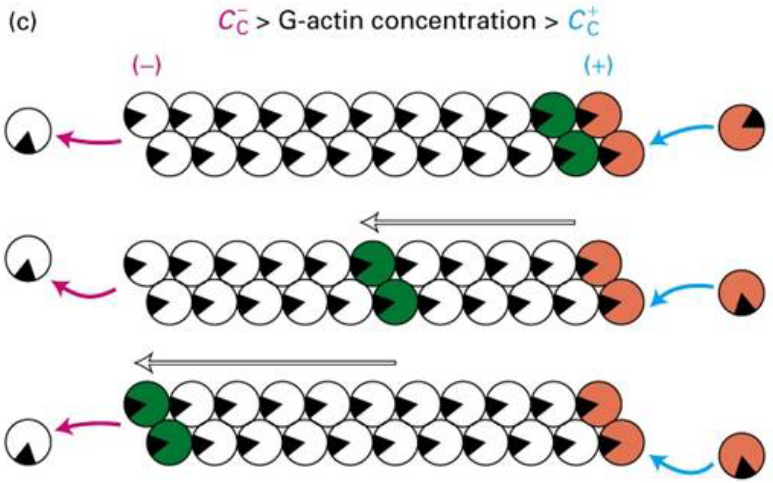


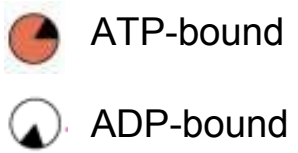
(a). S1 (myosin fragment)-decorated actin filament displays a helical rope-like appearance; the thicker part of a turn is directed to the pointed end of a filament (direction of the arrowhead). (b), part of an actin filament bundle from a REF-52 fibroblast after S1 decoration. Bars, 0.1 um

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

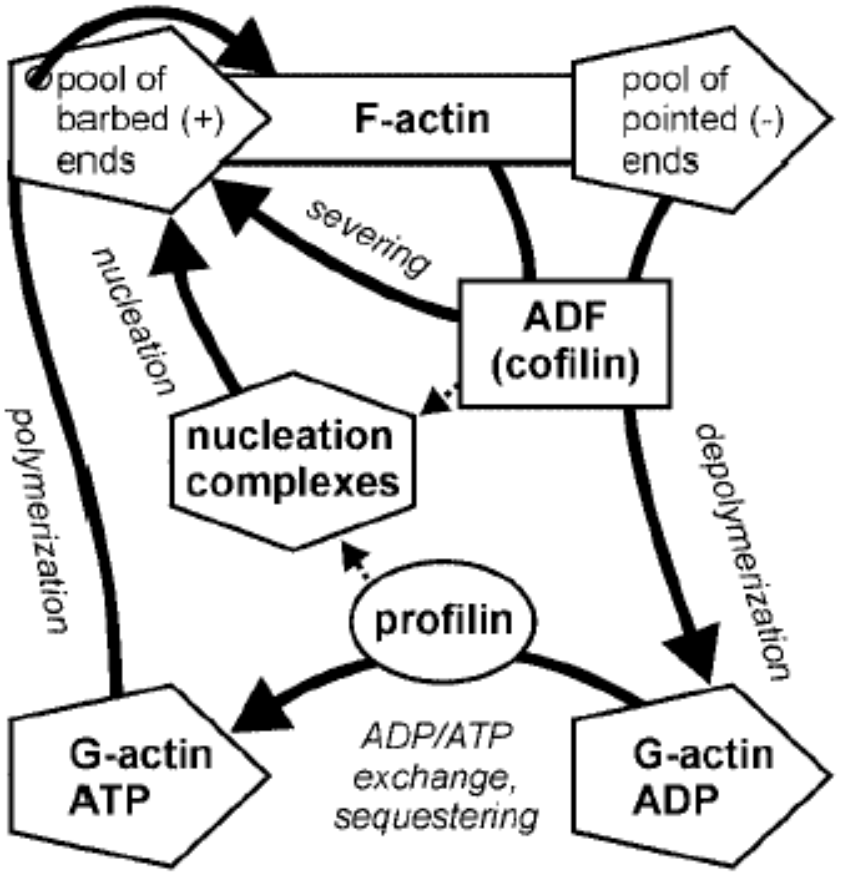
Dynamika aktinových vláken: polymerace a treadmilling



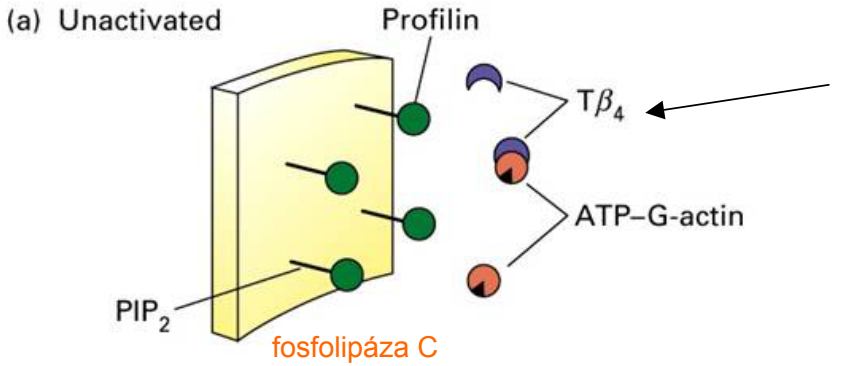




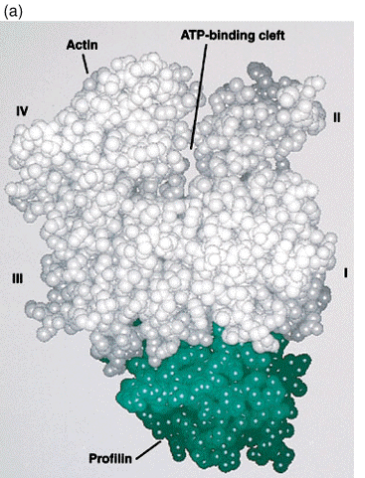
Proteiny ovlivňující dynamiku aktinu



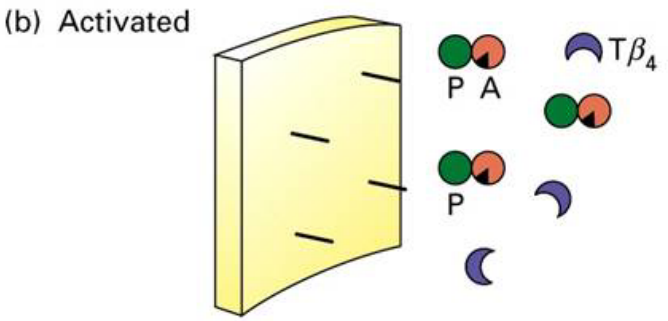
Proteiny vázající G-aktin: regulace dostupnosti podjednotek

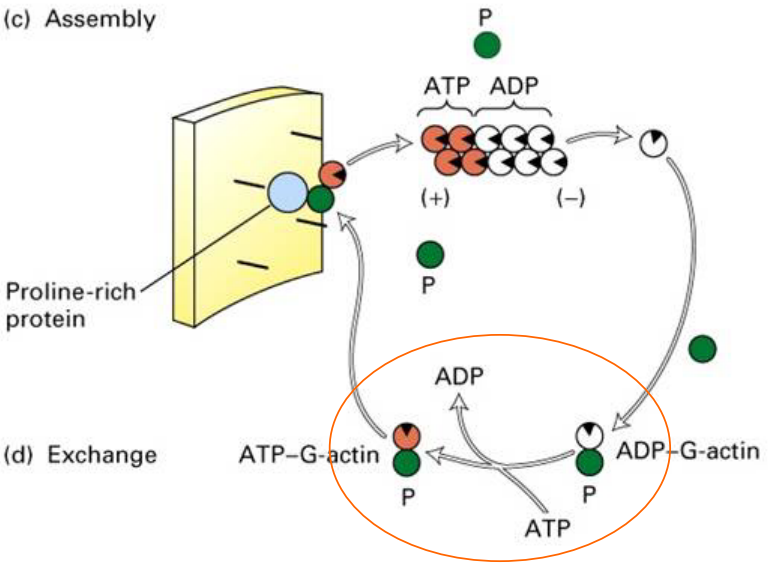


sequestered G-actin here:
thymosine β4 may be vertebrate-specific?



Profilin

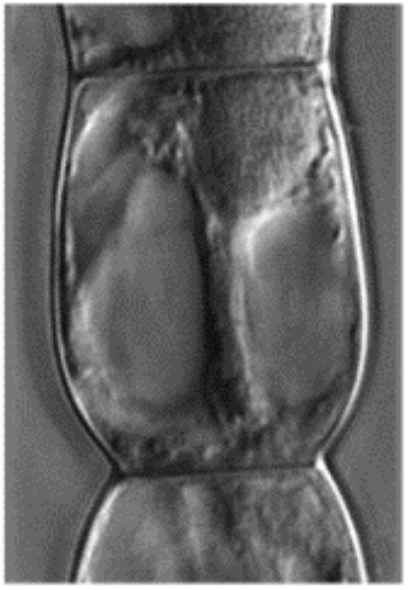




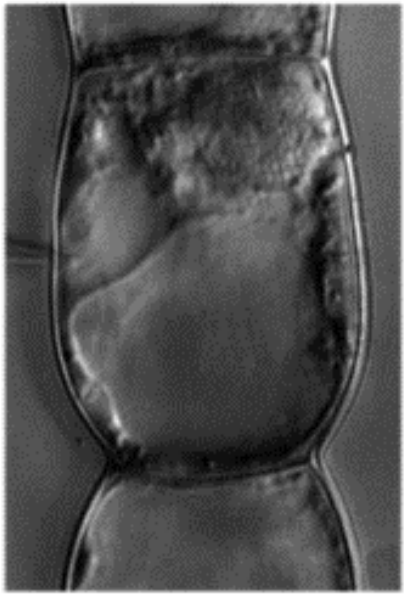
Tβ4 a profilin vážou přednostně ATP-G-aktin – udržují vysokou pohotovostní hladinu aktinu pro rychlou polymeraci

Profilin

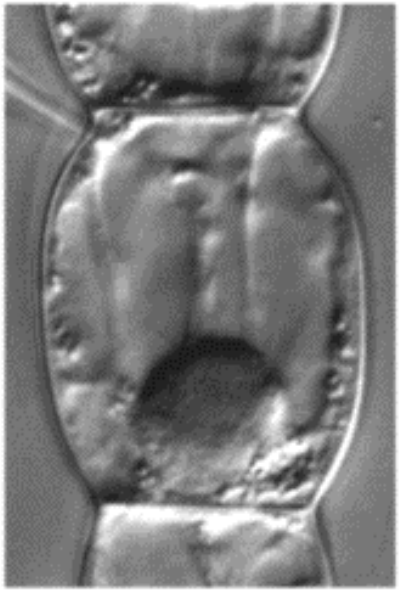
(A)



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.

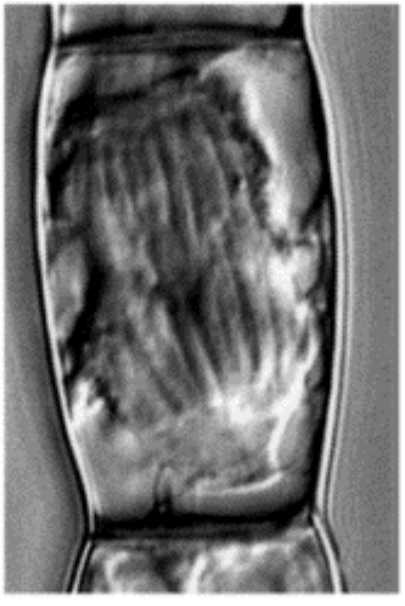


Streaming continues unabated 10 minutes after bovine serum albumin (BSA) is microinjected into a control cell.

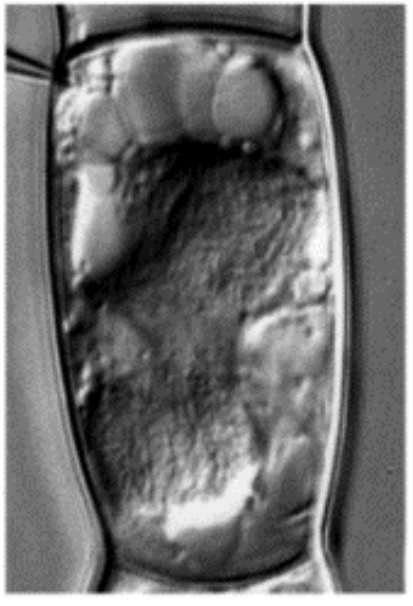
Tradescantia stamen hairs

Profilin

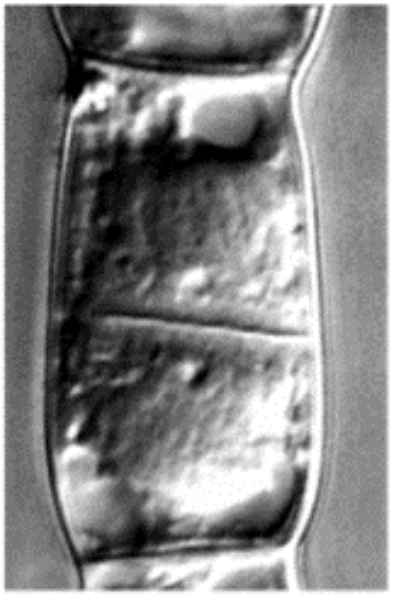
(B)



Anaphase cell undergoing chromosomal separation in an uninjected cell.

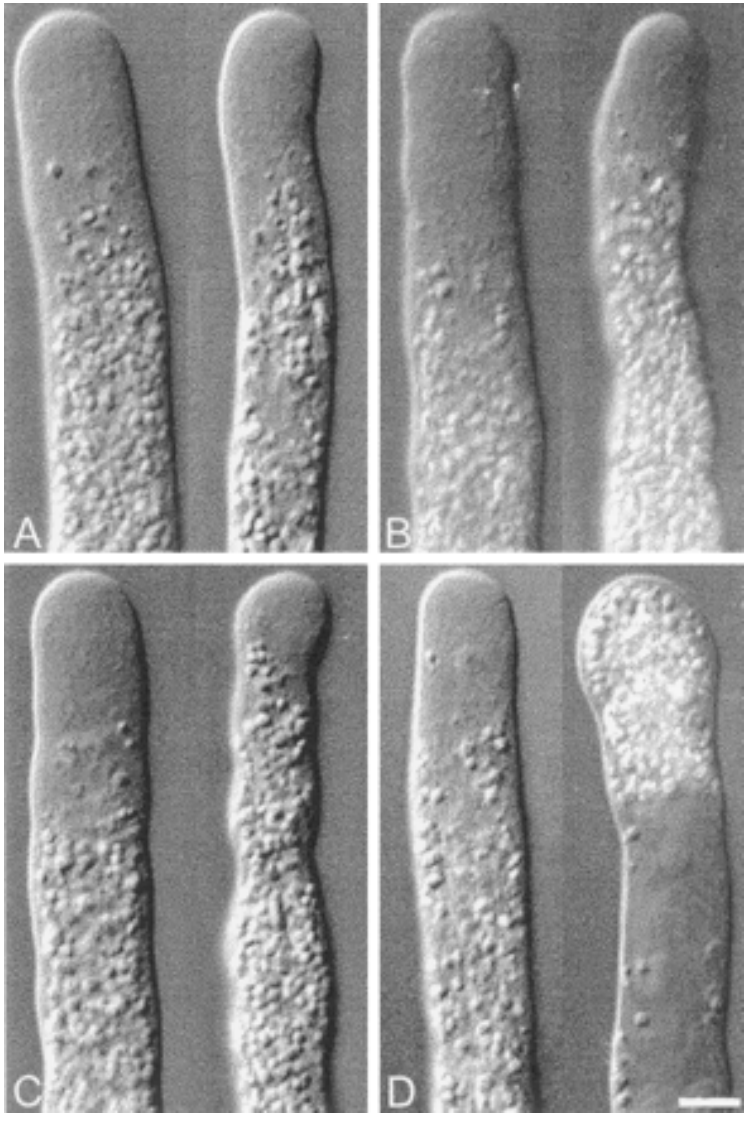


Fifty minutes after profilin is microinjected, chromosomal separation is complete, but a cell plate has not formed.

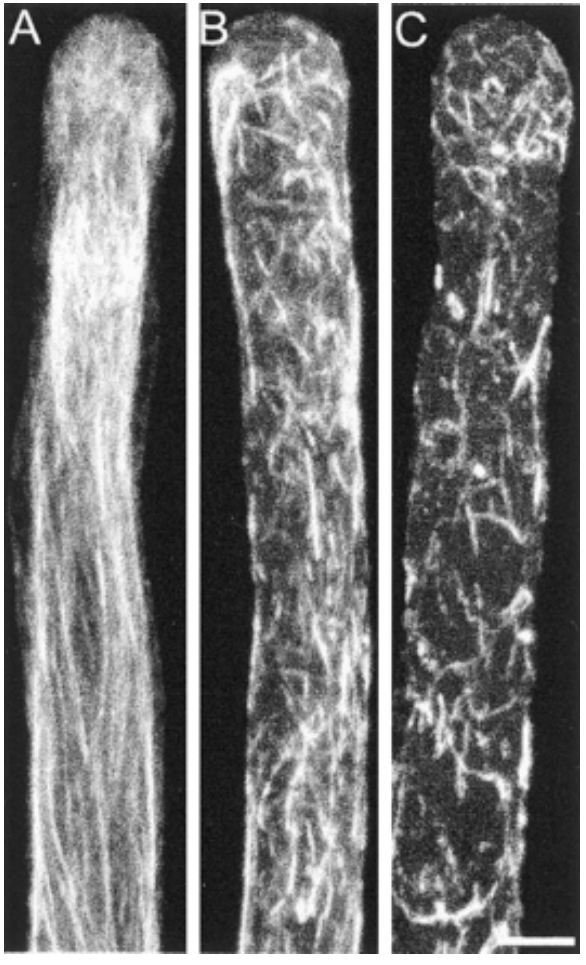


Twenty-five minutes after BSA is microinjected, a cell plate separates the control cell into two daughter cells.

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.

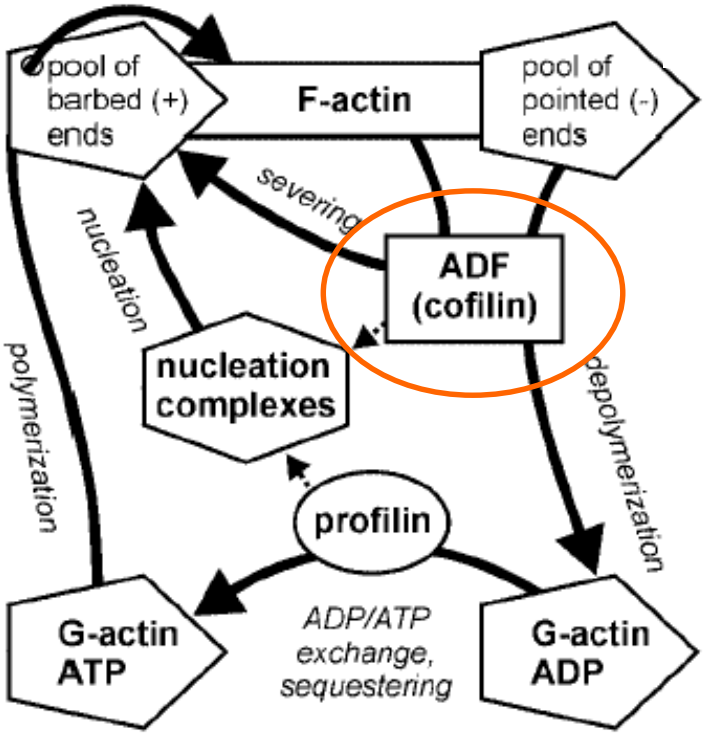


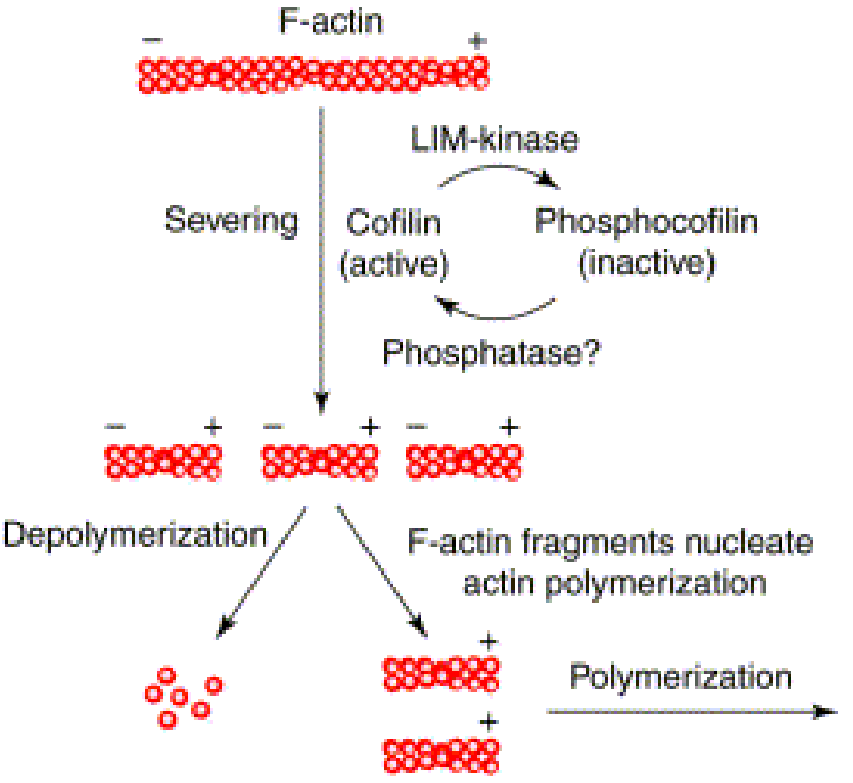
F-actin distribution of profilin-injected cells after chemical fixation and Alexa-phalloidin staining. Twenty minutes after injection cells were chemically fixed and stained with 0.3 μM Alexa-phalloidin. (A) Control cell. (B) Cell injected with a low concentration of profilin (~30 μM). (C) Cell injected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

Vidali et al. 2001

„Stříhání“ mikrofilament: ADF/cofilin

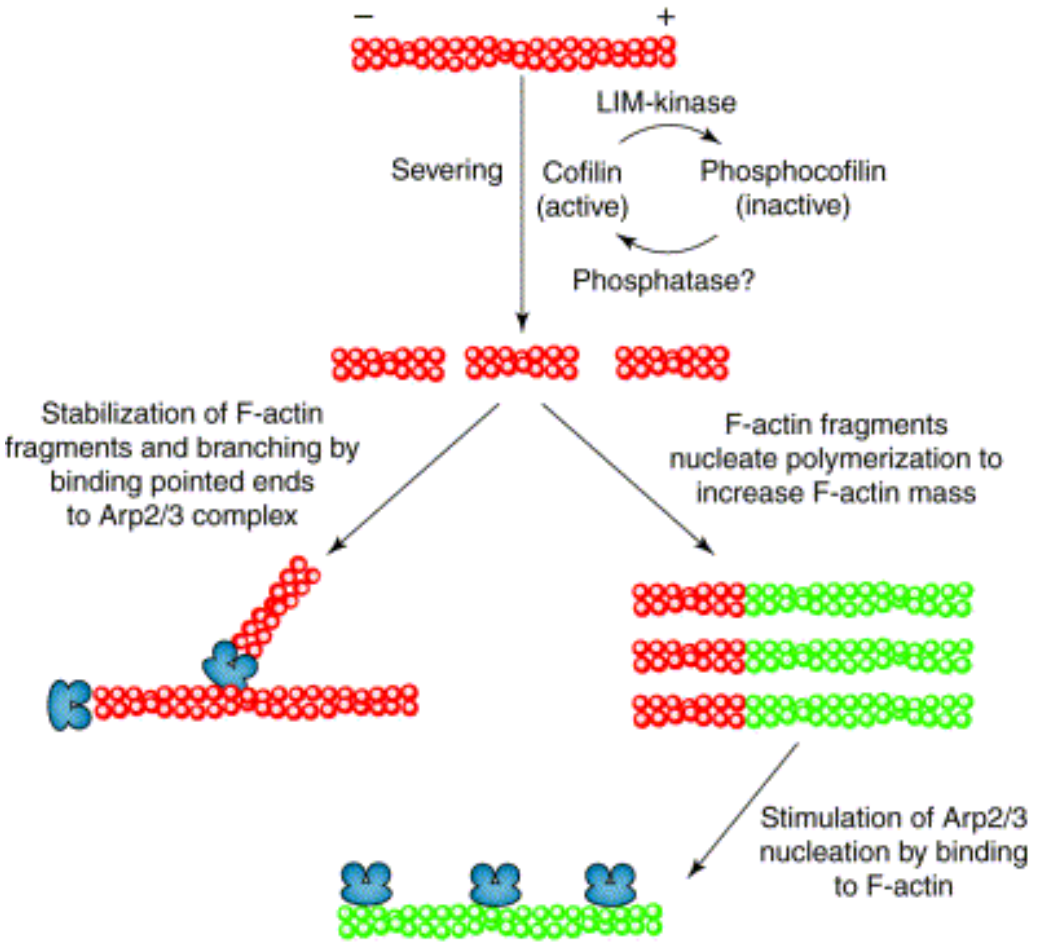
cofilin = Actin Depolymerizing Factor (ADF)





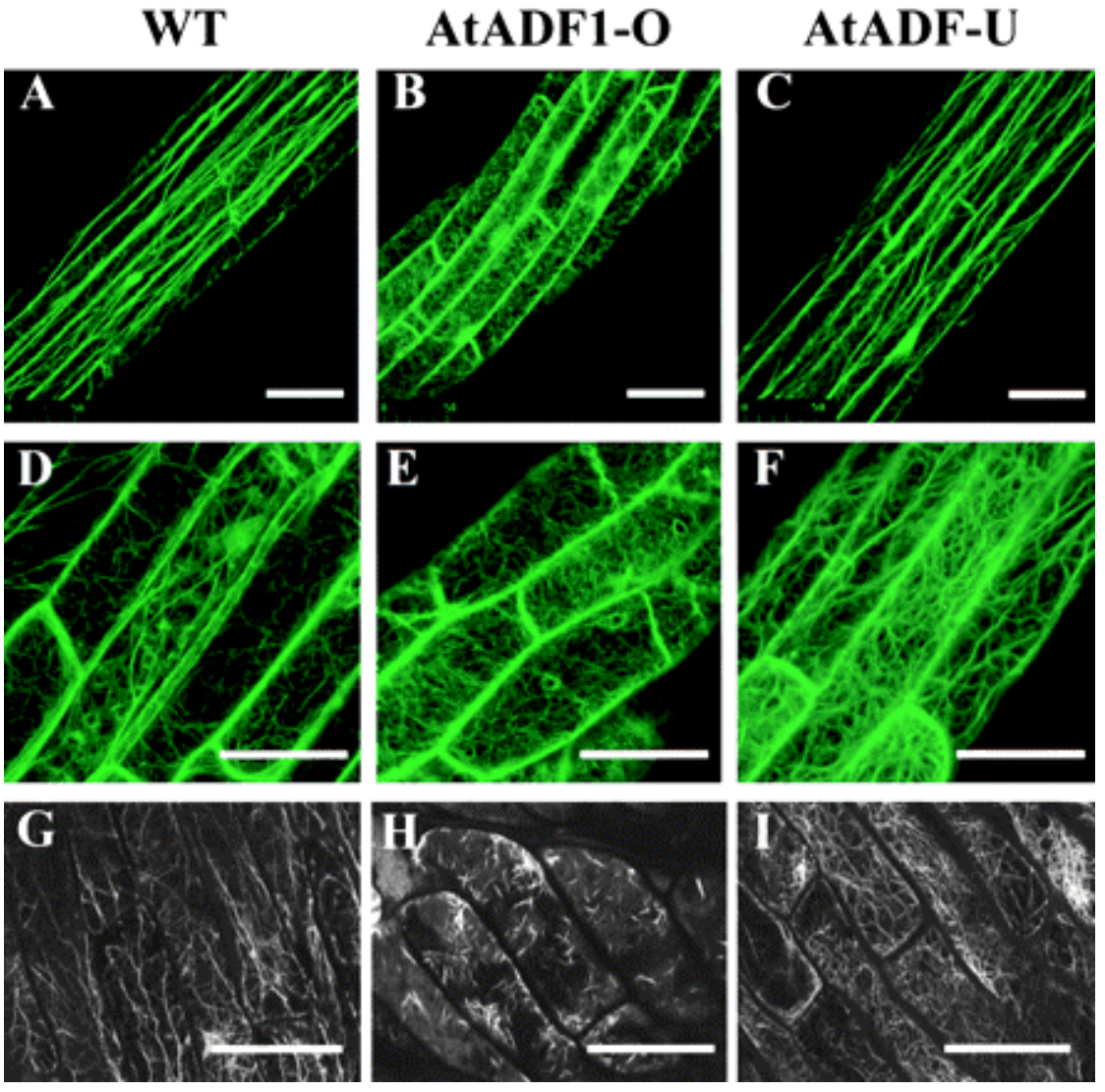
Condeelis 2001

„Stříhání“ mikrofilament: ADF/cofilin



Condeelis 2001

Změny hladiny ADF1 u Arabidopsis



overexprese:

vymizení svazků mikrofilament (cables)

menší buňky

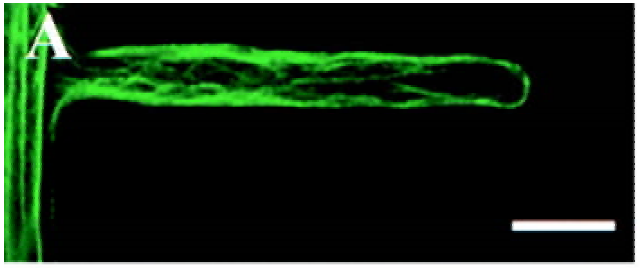
inhibice:

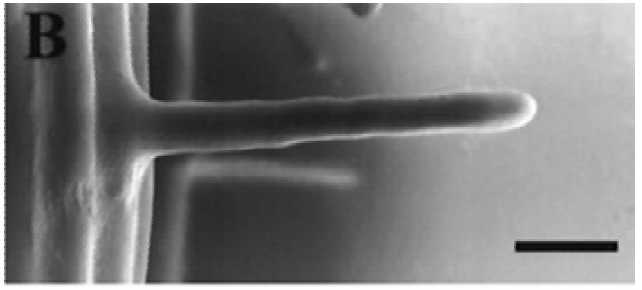
více svazků mikrofilament

Dong et al. 2001

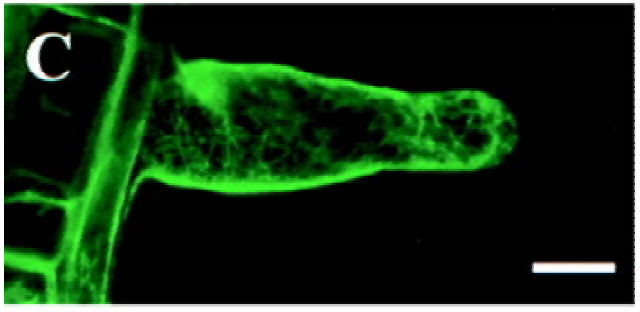
sense cDNA anti-sense cDNA ADF1

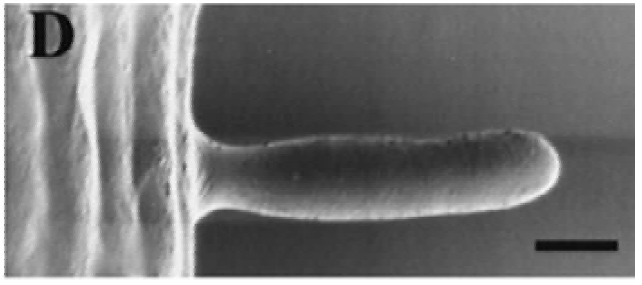
Změny hladiny ADF1 u Arabidopsis



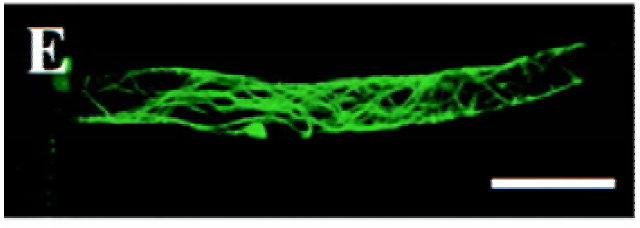


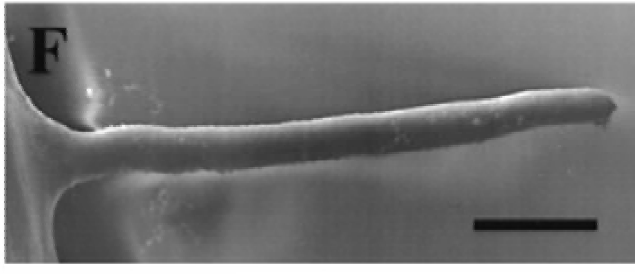
WT





AtADF1-O

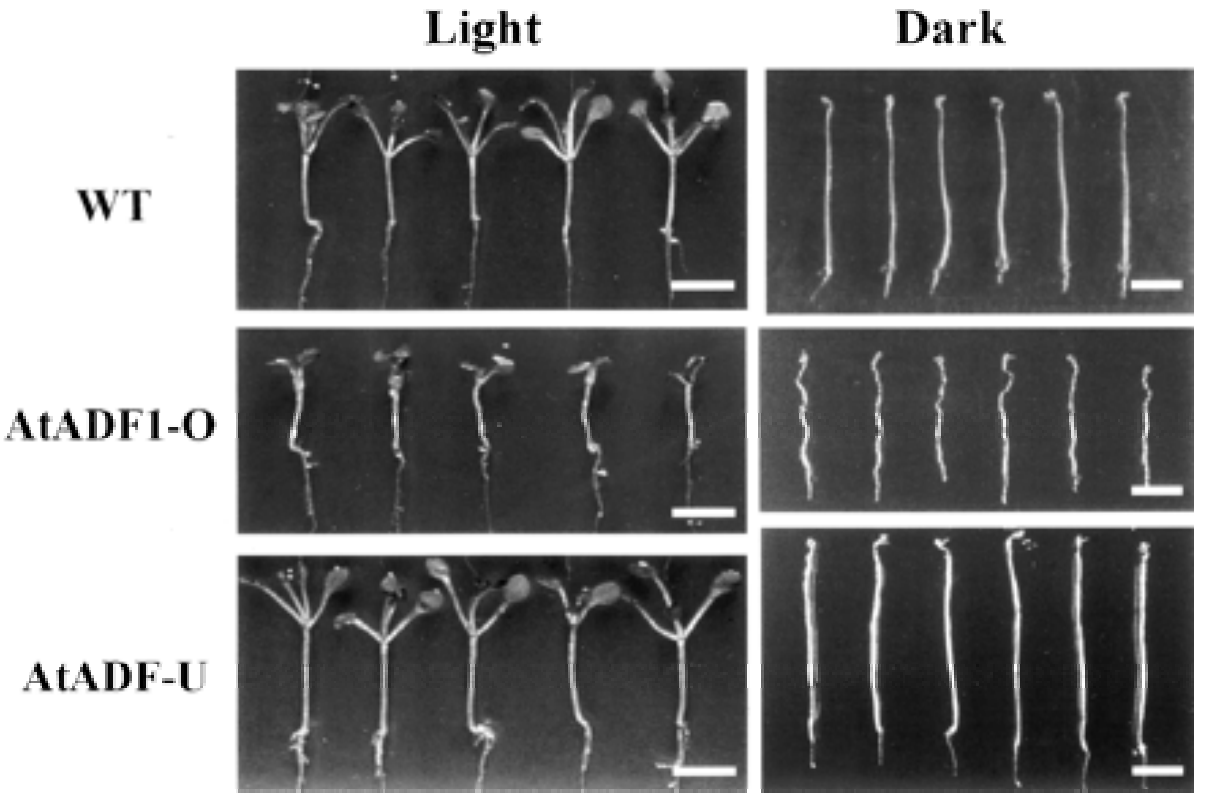




AtADF1-U

Dong et al. 2001

Změny hladiny ADF1 u Arabidopsis



overexprese:

omezení růstu buněk/ogánů

inhibice:

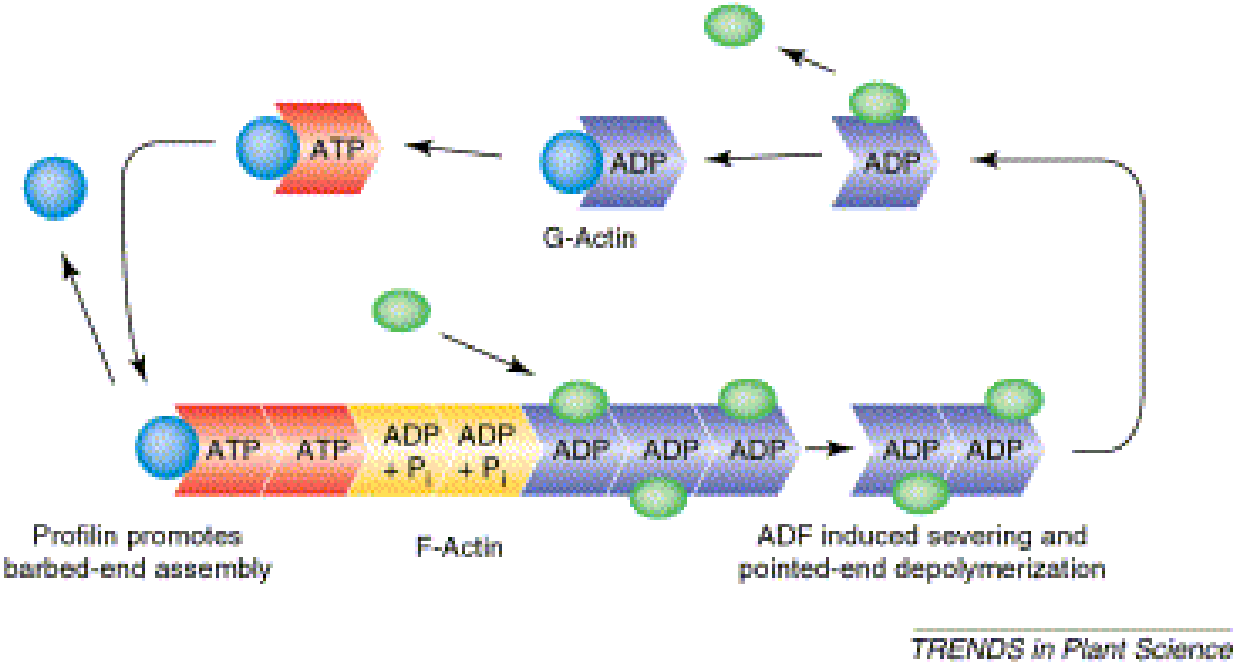
podpora růstu buněk/ogánů

Počet listů v růžici před vykvetením (vliv na indukci kvetení):
WT: 16,57 ± 0,95 ADF1-O: 16,46 ± 1,10 AtADF1-U: 25,61 ± 1,90

Dong et al. 2001

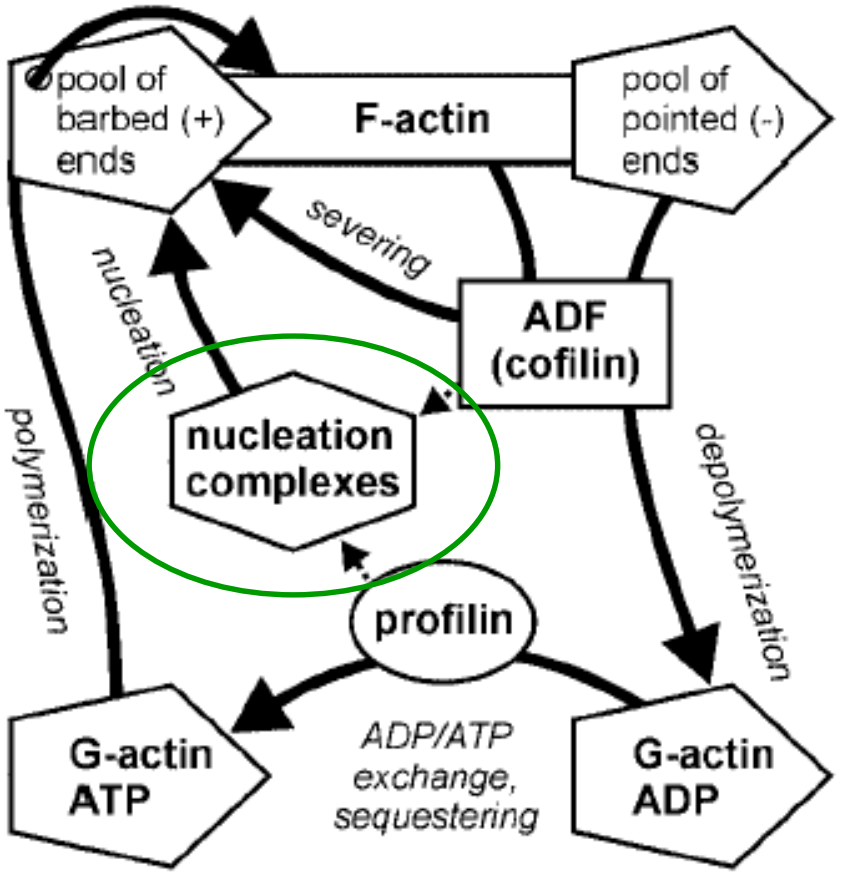
ADF je jedním z klíčových regulátorů aktinového cytoskeletu!

Profilin versus Cofilin



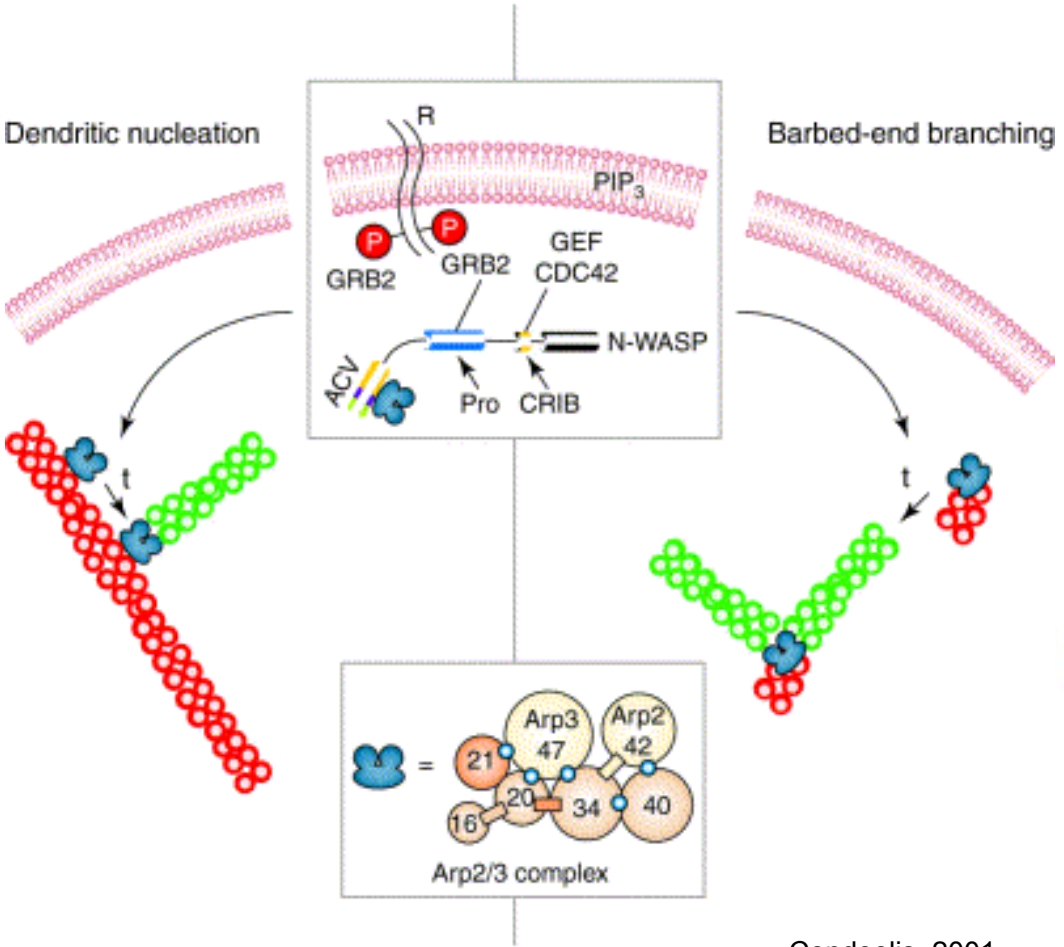
Deeks et al. 2002

Nukleace aktinových filament *de novo*



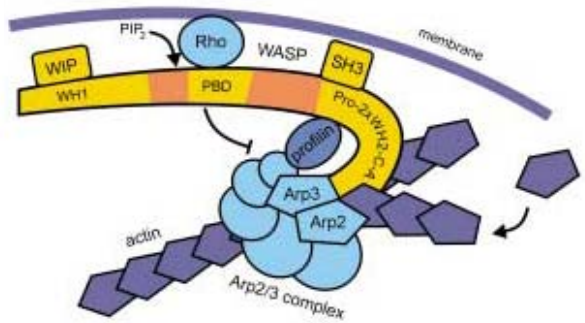
Nukleace aktinových filament *de novo*

Arp2/3 komplex



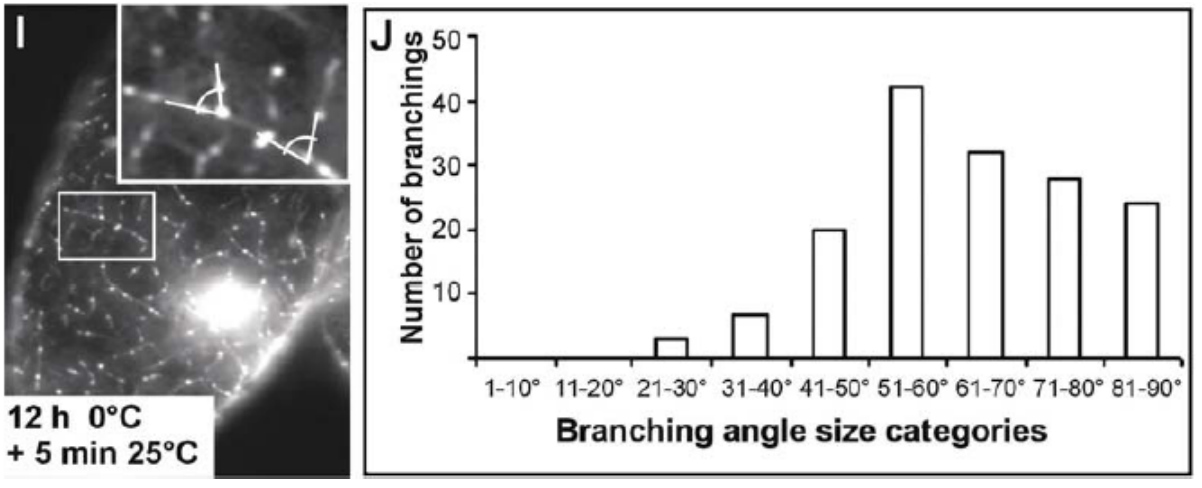
regulátory:

WASP
SCAR/WAWE



Condeelis, 2001

Nukleace aktinových filament *de novo*



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

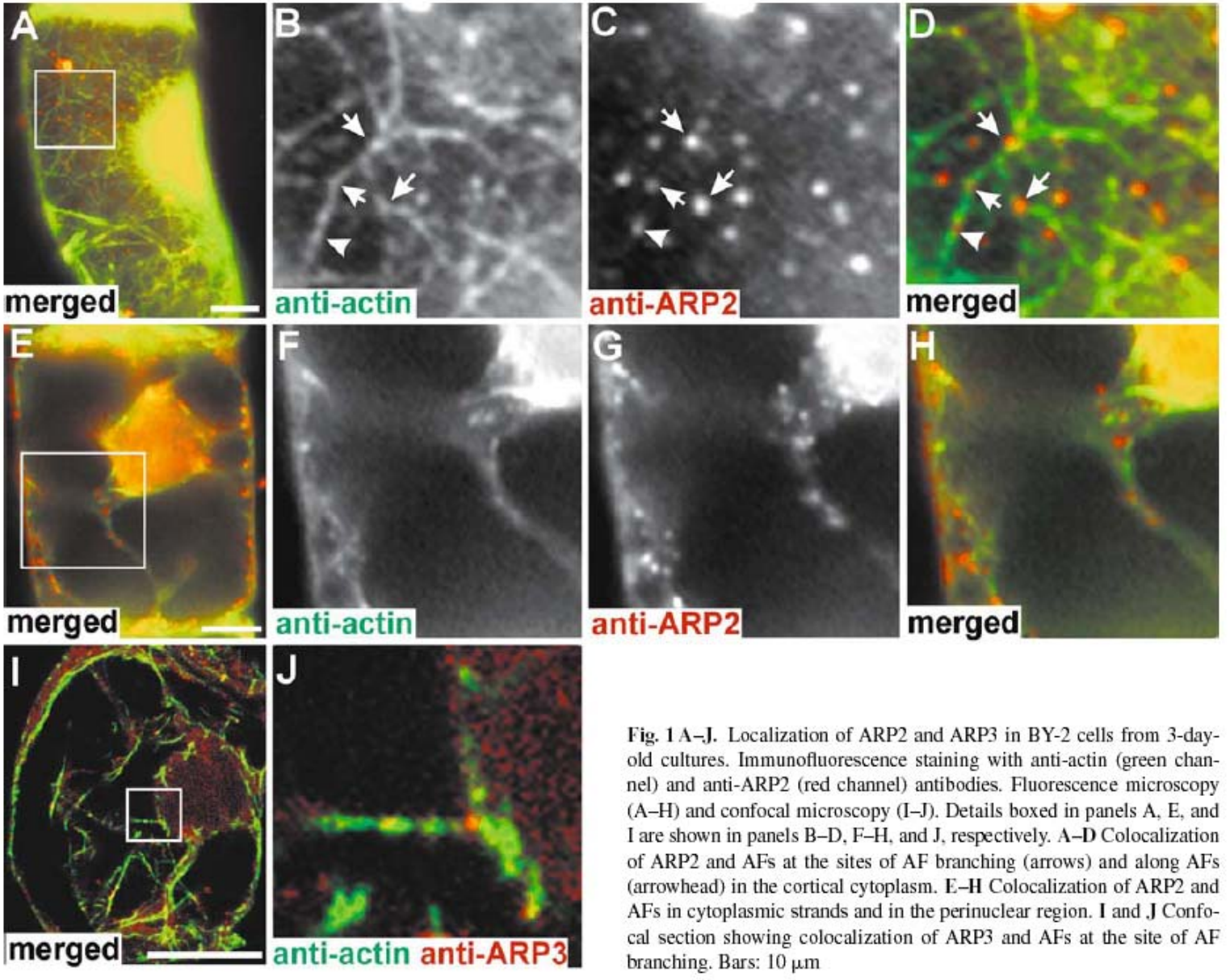
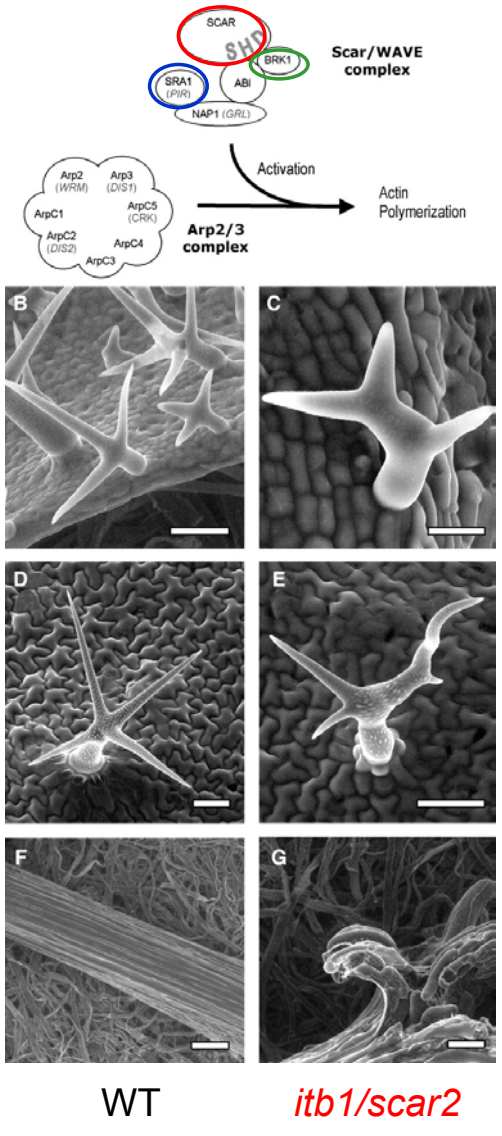


Fig. 1 A–J. Localization of ARP2 and ARP3 in BY-2 cells from 3-day-old cultures. Immunofluorescence staining with anti-actin (green channel) and anti-ARP2 (red channel) antibodies. Fluorescence microscopy (A–H) and confocal microscopy (I–J). Details boxed in panels A, E, and I are shown in panels B–D, 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

SCAR/WAWE: regulátory Arp2/3



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

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,
2) Nap1/NAP125,
3) Abi-1/Abi-2,
4) Brick1(Brk1)/HSPC300,
5) Scar/WAVE

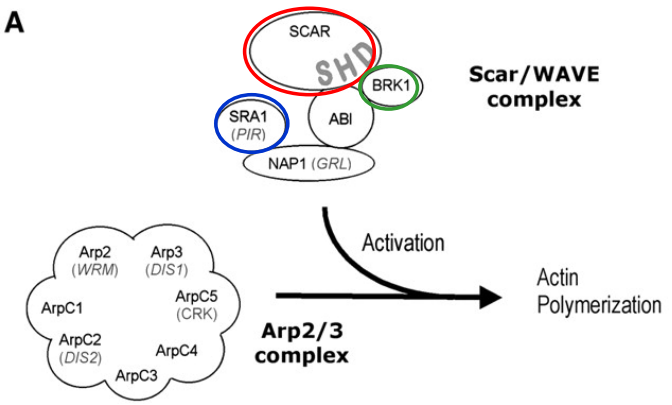
Arabidopsis BRICK1/HSPC300 Is an Essential WAVE-Complex Subunit that Selectively Stabilizes the Arp2/3 Activator SCAR2.
Current Biology, Volume 16, Issue 9, Pages 895-901
J. Le, E. Mallery, C. Zhang, S. Brankle, D. Szymanski

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§

WAVE-complex stabilizes the Arp2/3 activator SCAR2.

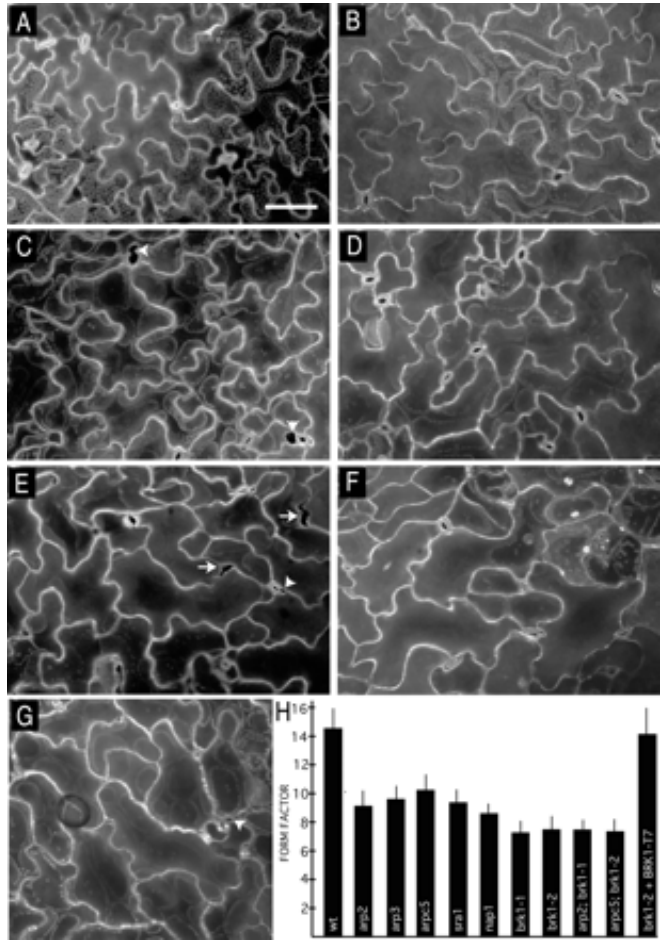


WT

arpc5

brk1
(BRICK)

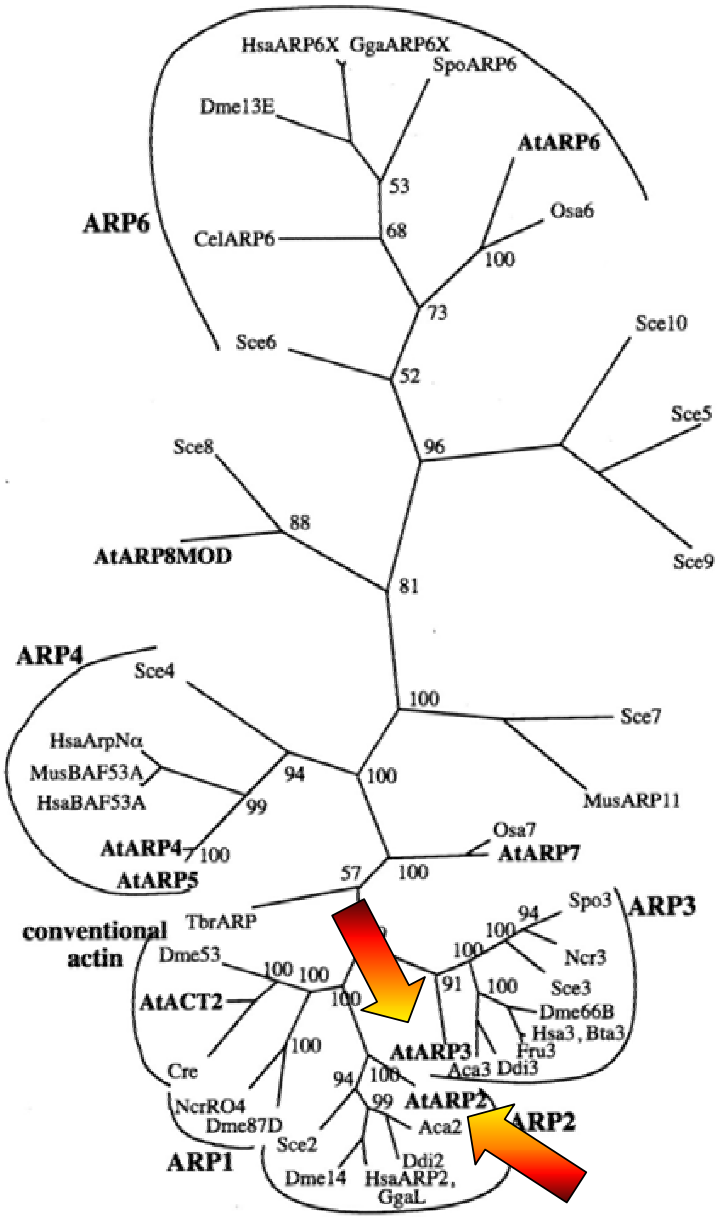
arpc5
brk1



arp2

sra1

arp2
brk1



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 distorted1

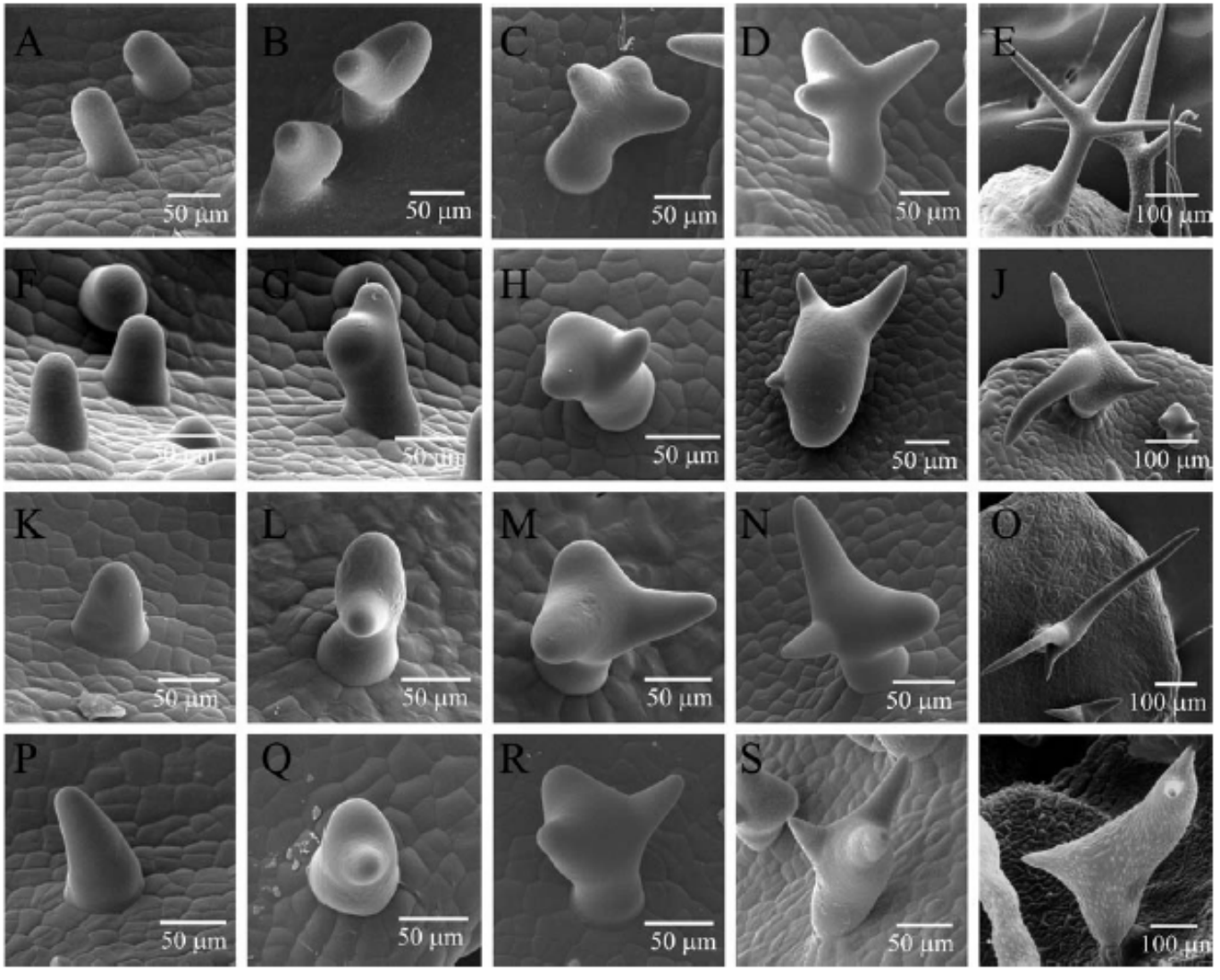
Table 3. The *DISTORTED* class of *Arabidopsis* genes

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
WURM	3	At3g27000	ARP2

Podjednotky a regulátory Arp2/3 komplexu!

(Mathur, 2005)

Arabidopsis: mutanti třídy *distorted*

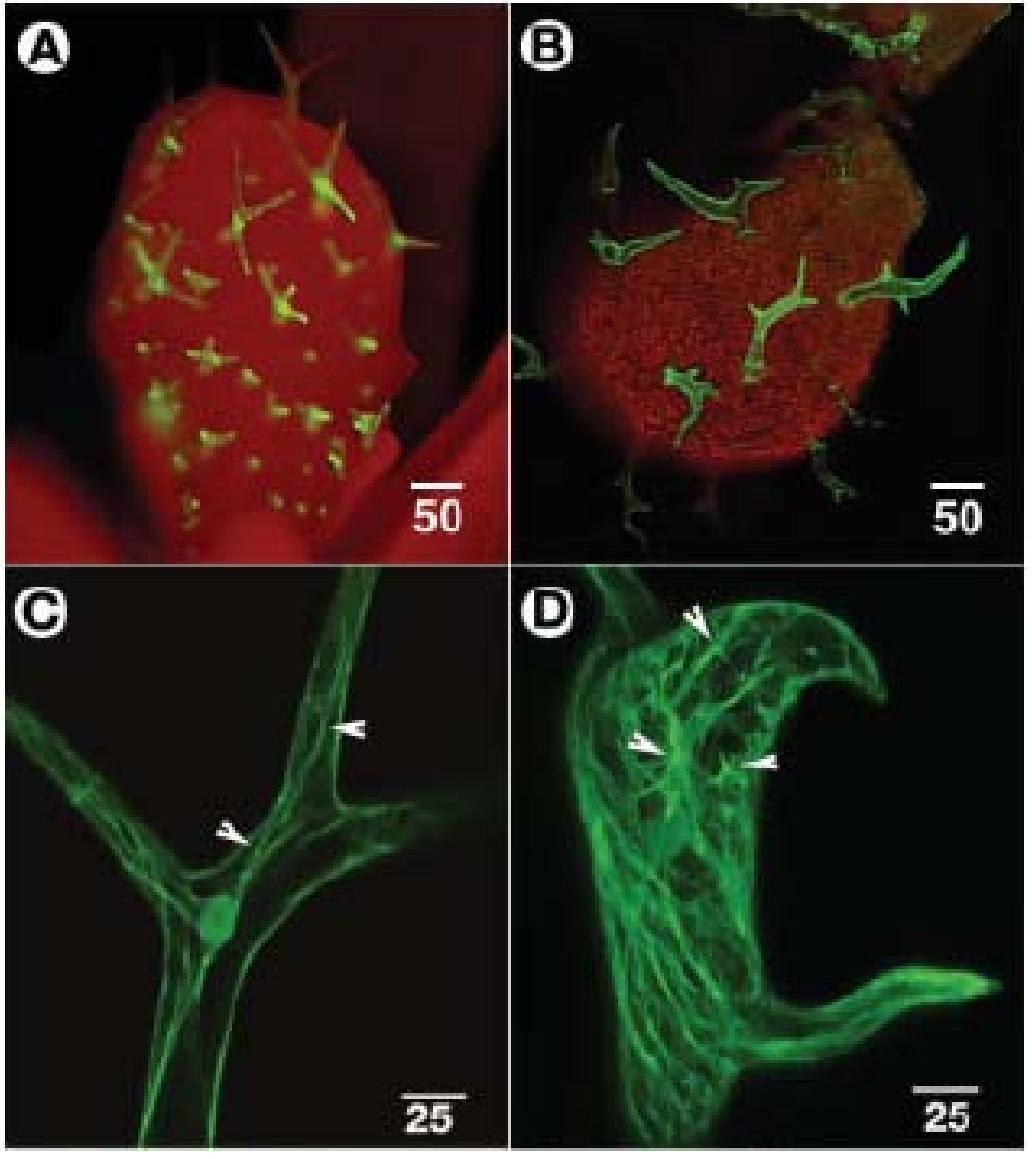


WT

crooked

spirrig

wurm



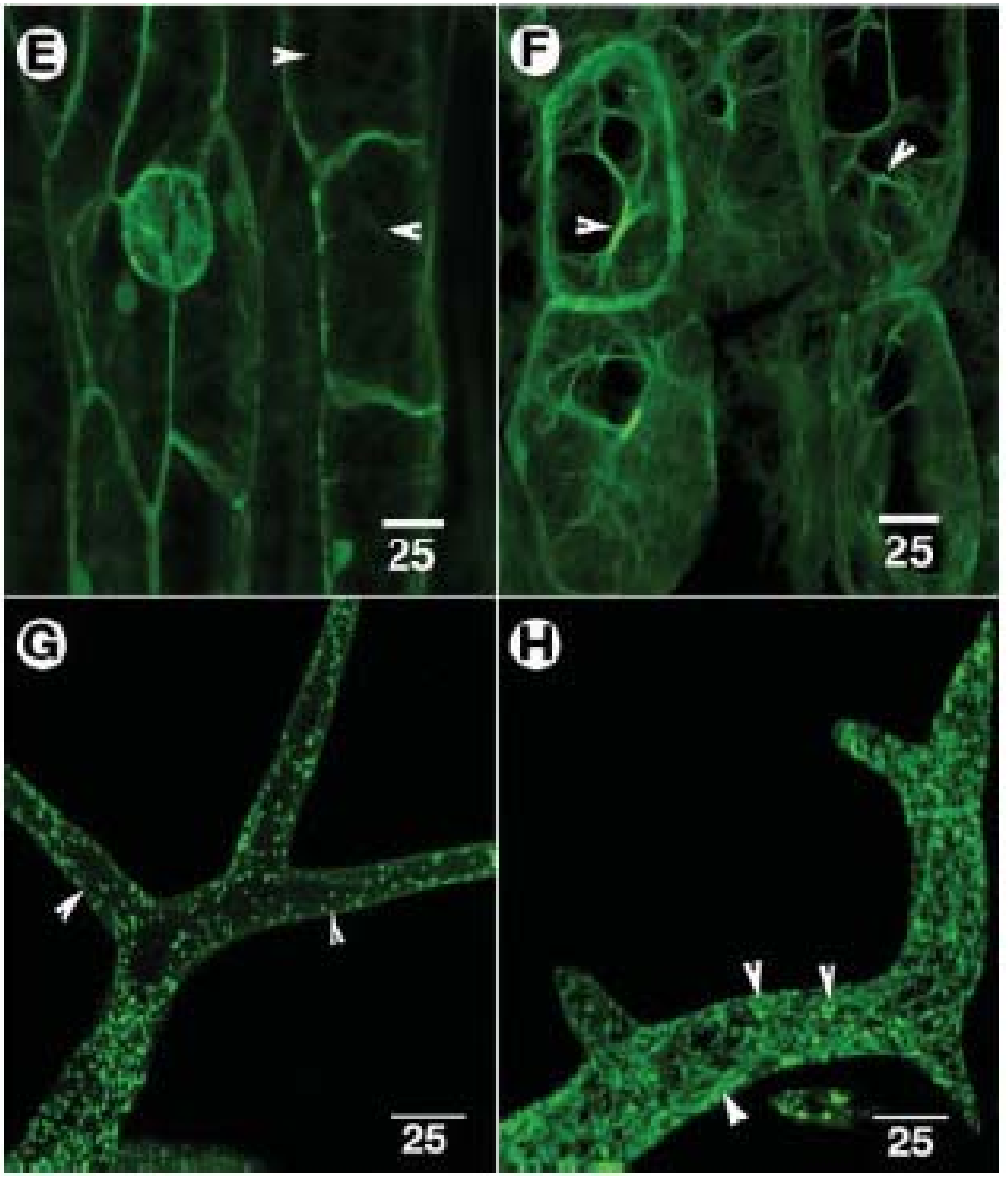
autofluorescence

GFP-mTalin

WT

wurm

hypokotyl
GFP-mTalin



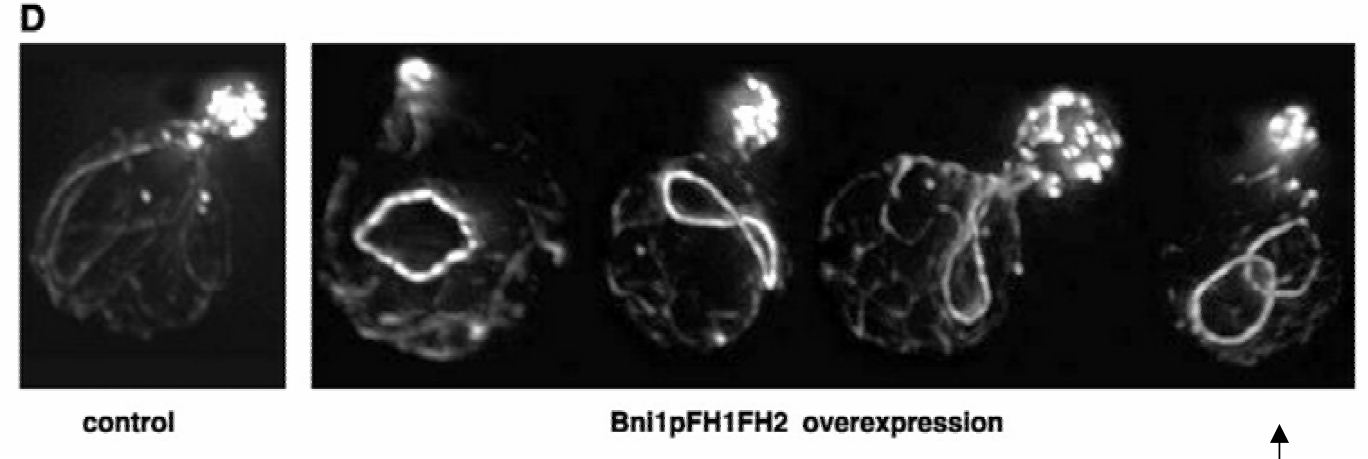
trichomy
Golgi

agregáty!

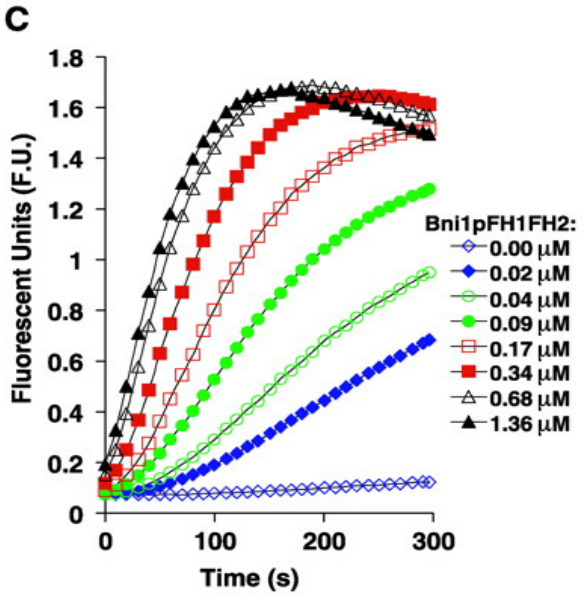
WT

crooked

Další způsoby nukleace: forminy (FH2 proteiny)

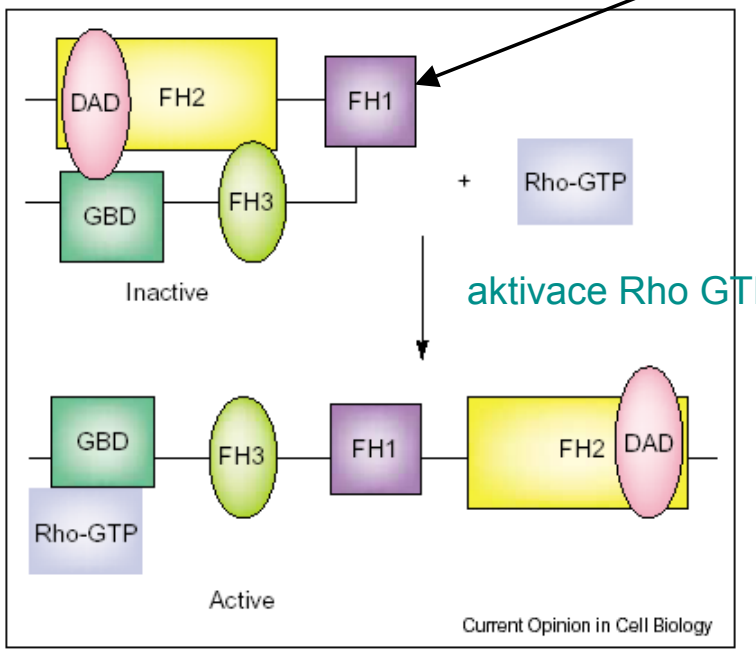


overexpression of a partial yeast formin
causes formation of ectopic actin structures

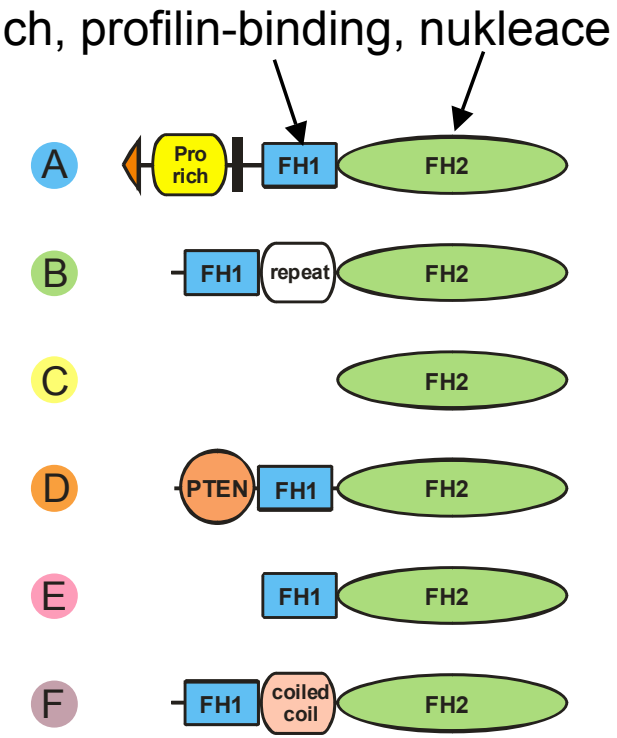


overexpression of formins
stimulates filament formation
in vitro

Doménová struktura forminů



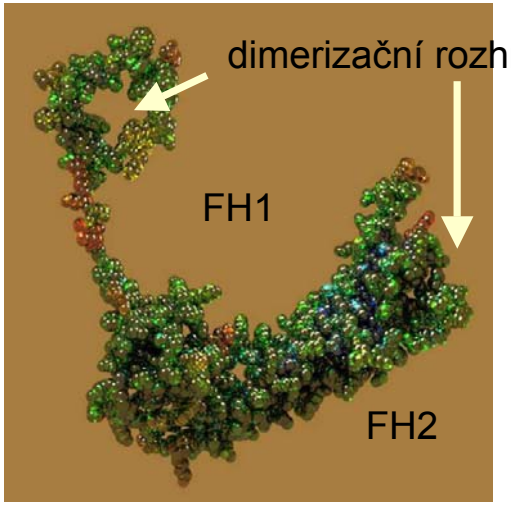
Activation of Diaphanous-related formin by Rho-GTPase. Diagram of domains present in Diaphanous-related proteins, illustrating the conformation change that occurs upon binding Rho-GTP.

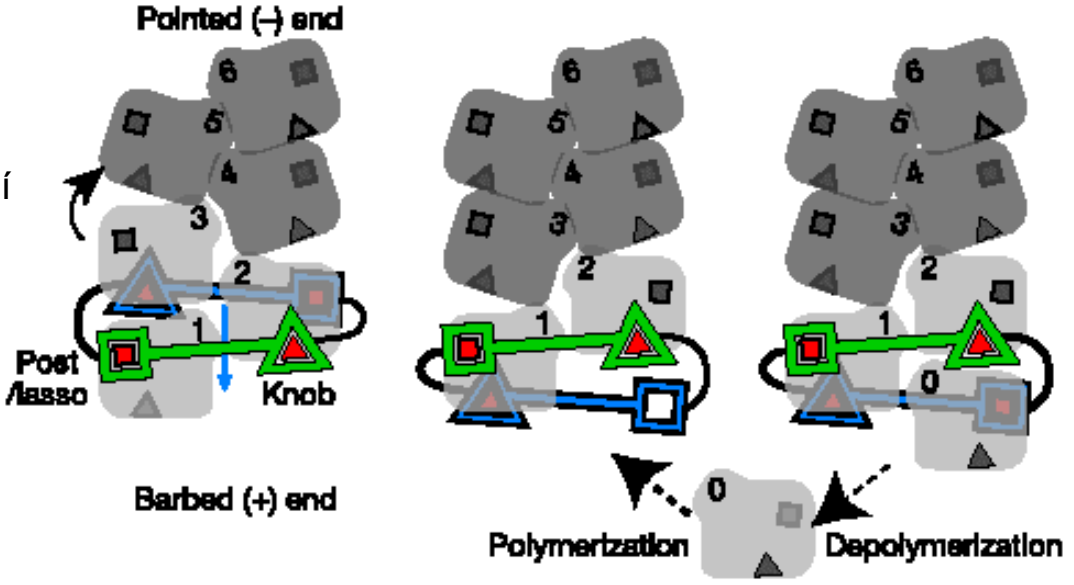


Arabidopsis: 21 genů!
FH1-FH11 transmembránová doména (Class I)
FH12-FH21 bez tm. domény (Class II)

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

„Kráčející nukleační komplex“



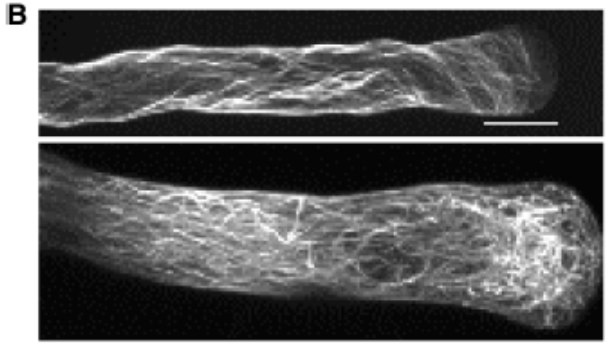


„leaky cap“ model - živočišný

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

u rostlin?
vazba na MT?

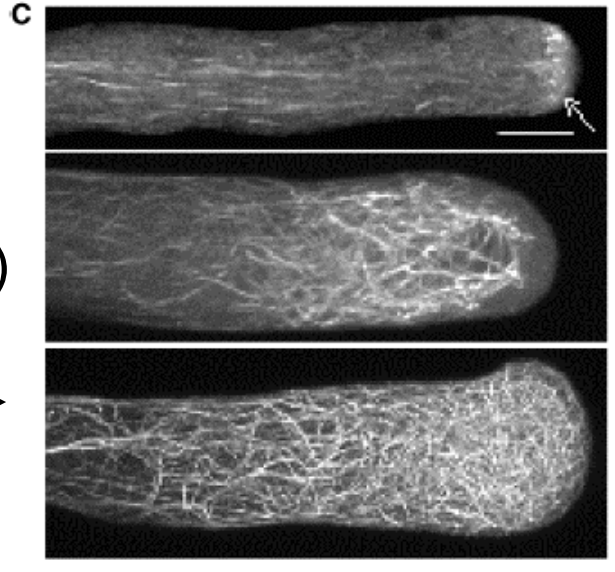
Overexprese AtFH1 v pylových láčkách

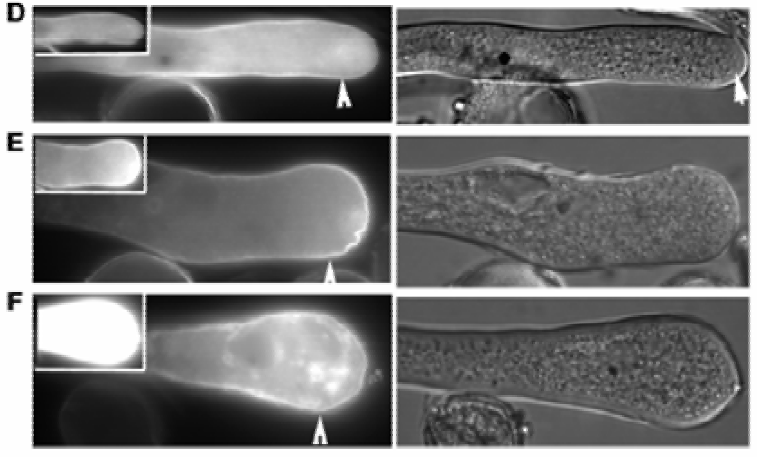


kontrola

AtFH1 (FH1/2)

talin





AtFH1:GFP

Membránová lokalizace forminů třídy I typická pro rostliny

(Cheung a Wu, 2004)

Proč mají rostliny právě tyto geny v tolika kopiích?

- Kromě 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)

Specifické funkce isoforem forminů

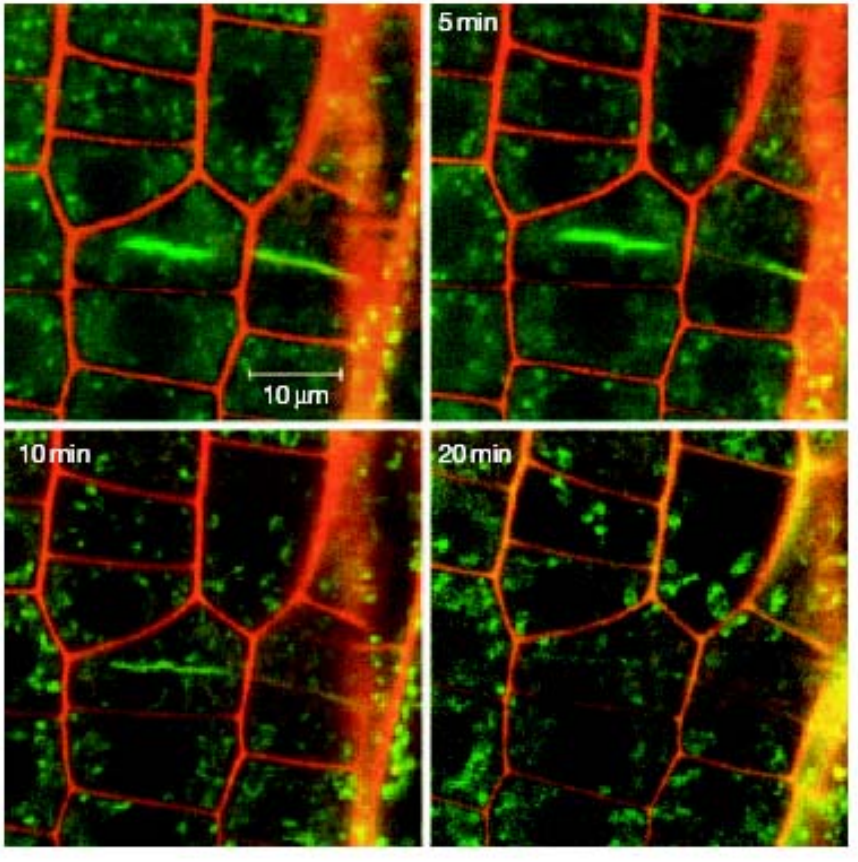
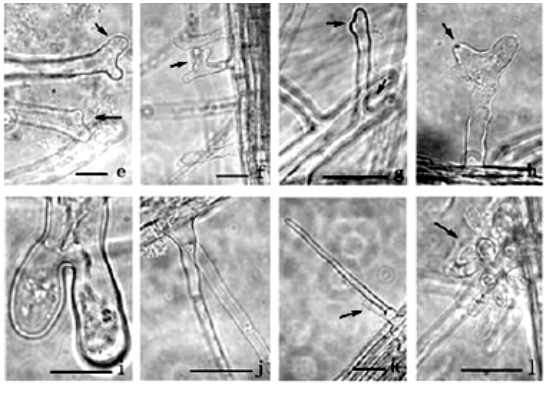


Figure 3 Formin AtFH5–GFP is targeted to the developing cell plate.

- AtFH1: bundling
- AtFH5: cytokineze endospermu
- AtFH6: zvýšená exprese v hálkách indukovaných hlísticemi,
- suprimuje mutaci *bni1* u kvasinky
- AtFH4/AtFH8: kořenově specifické, slabý defekt na kořenových vláscích



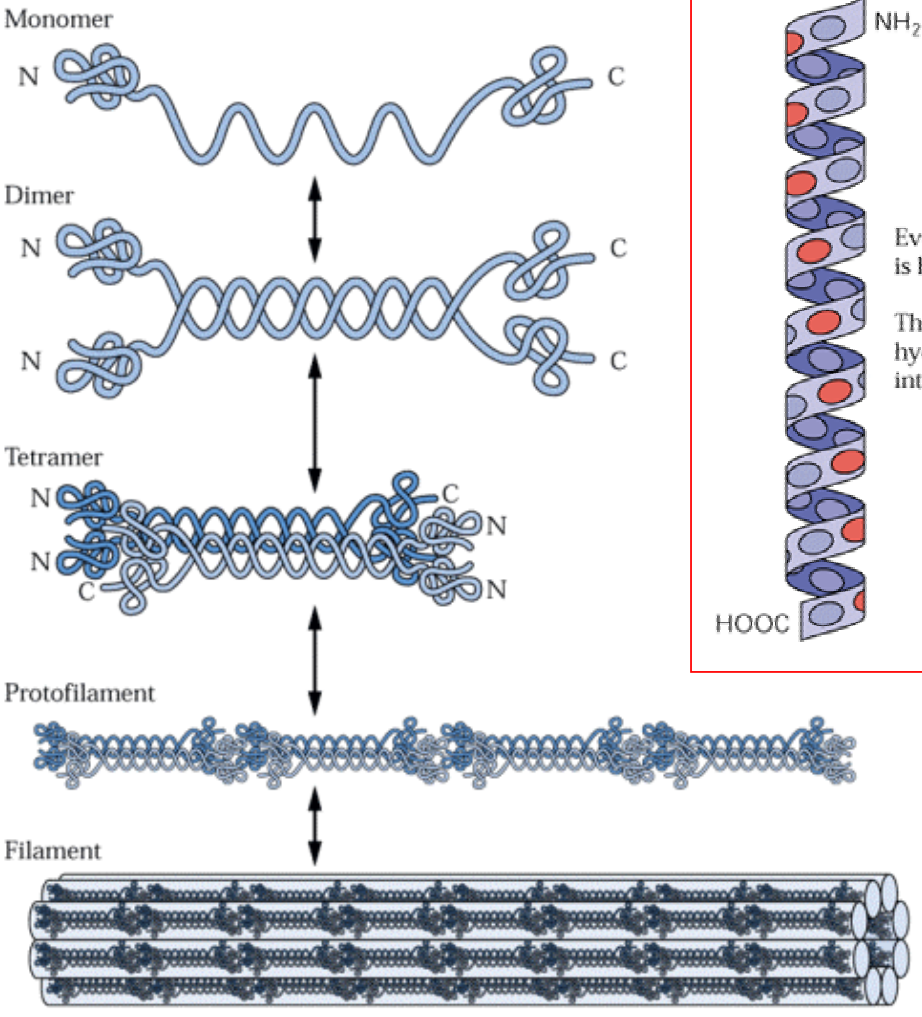
mutant *fh5* má zpožděnou celularizaci endospermu (jinde redundantní)

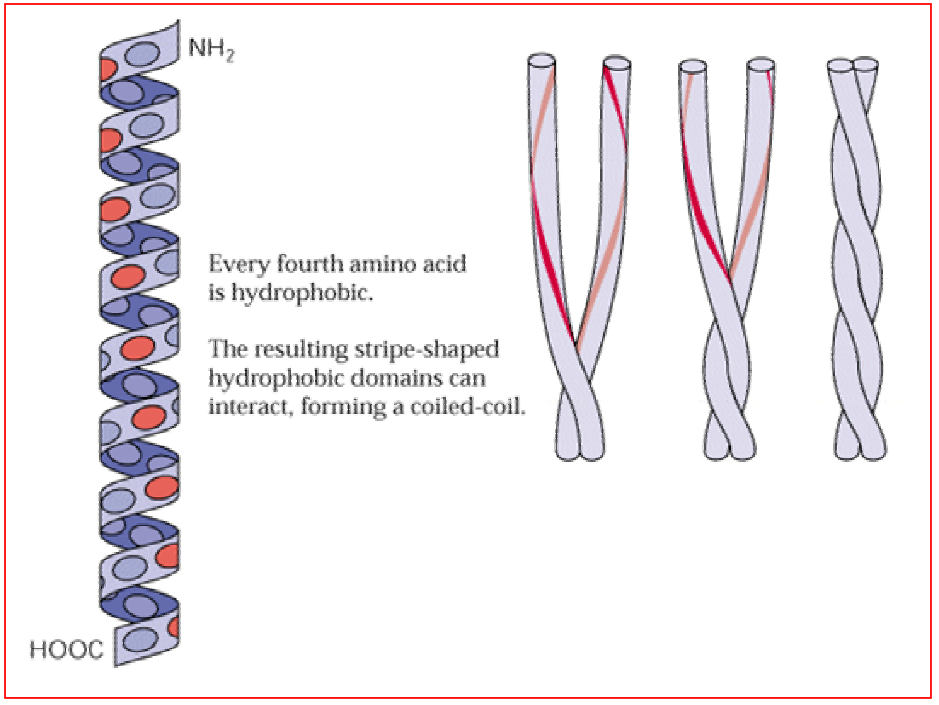
(Ingouff et al. 2005)

mutace ve forminech u různých organismů často vedou k defektům v cytokinezi

Intermediální filamenta

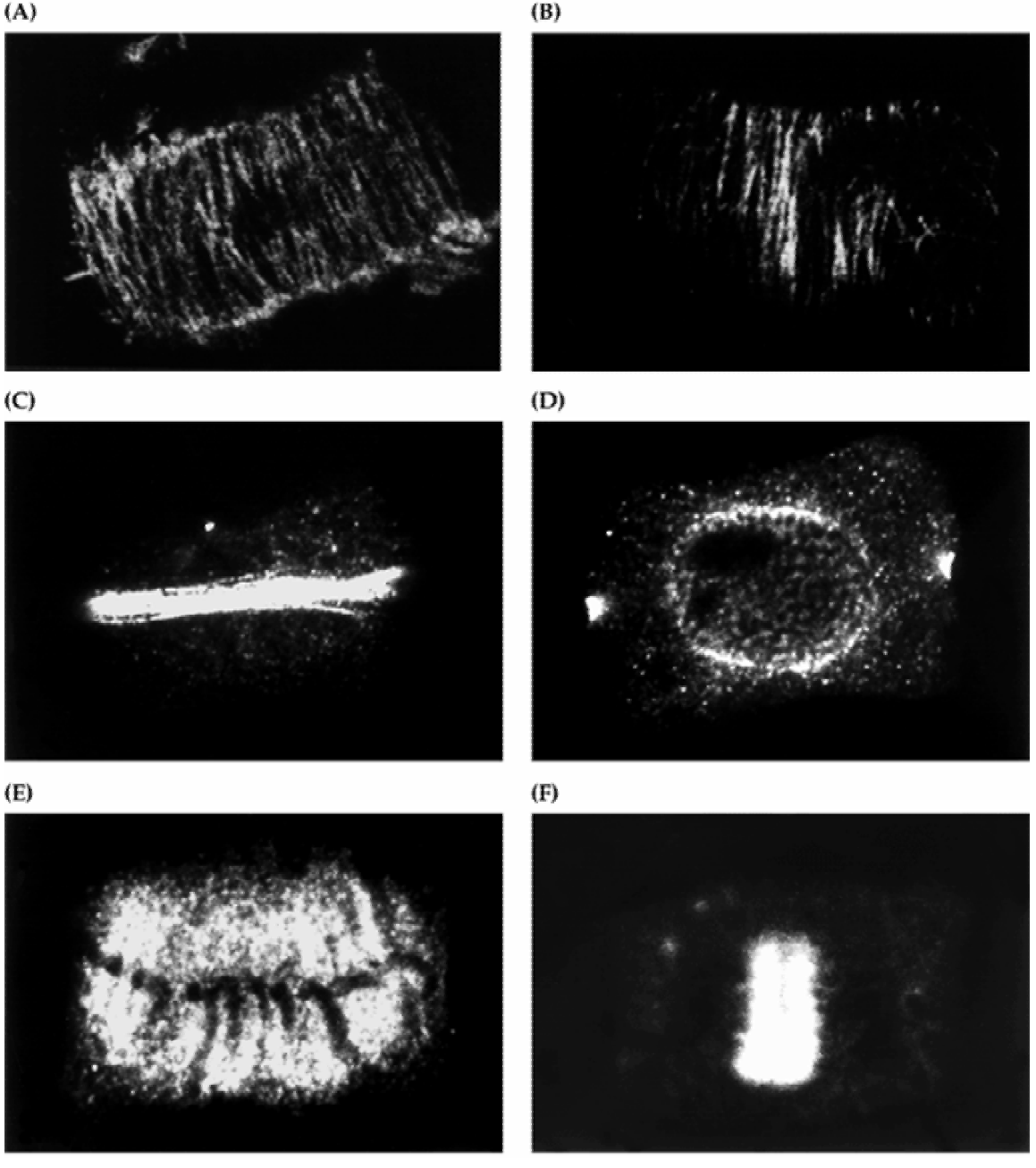
Intermediální filamenta: konvergence?





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

Výjimka z pravidla: rovnocenné konce!



Epitopy příbuzné IF v rostlinách?

interfáze

kolokalizace s MT?

profáze

mitóza a cytokineze

wheat root tip,
anti-keratin

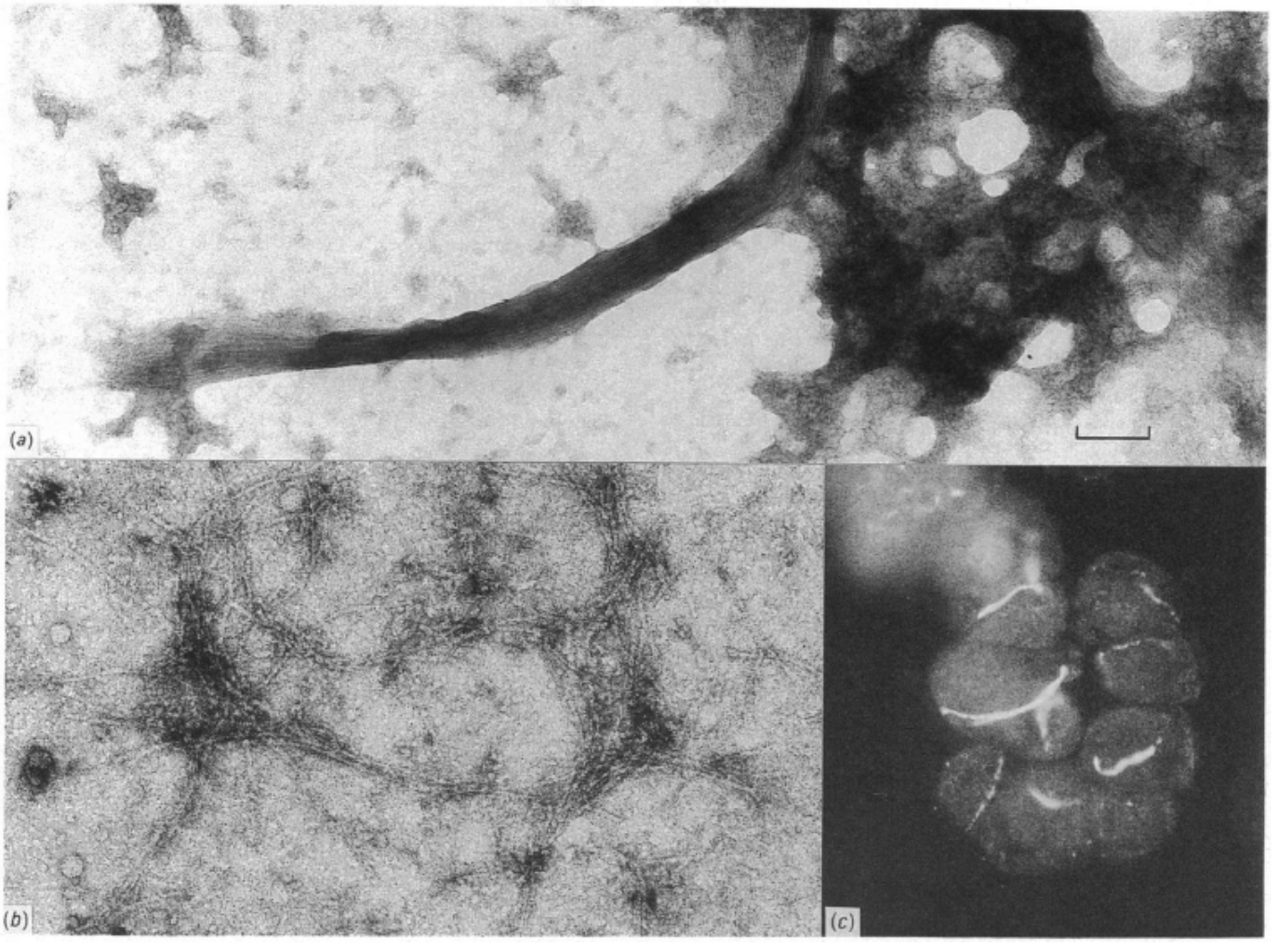


Fig. 1. Ultrastructural characterization of reconstituted 10 nm filaments and filament bundles

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*).

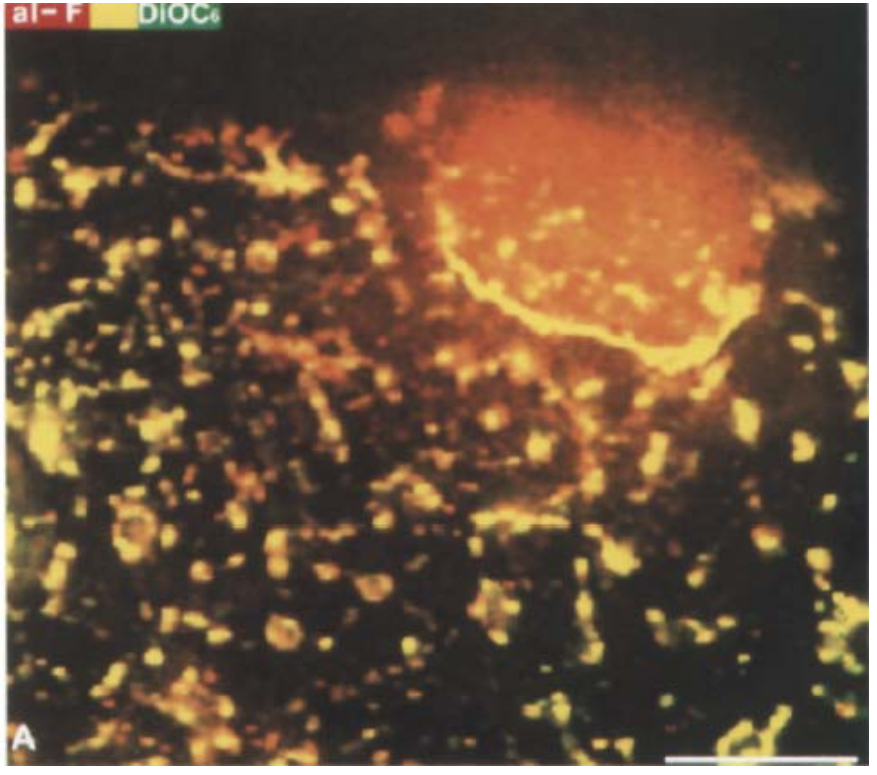
Biochem J. 1989 July 15; 261(2): 679–682.

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***

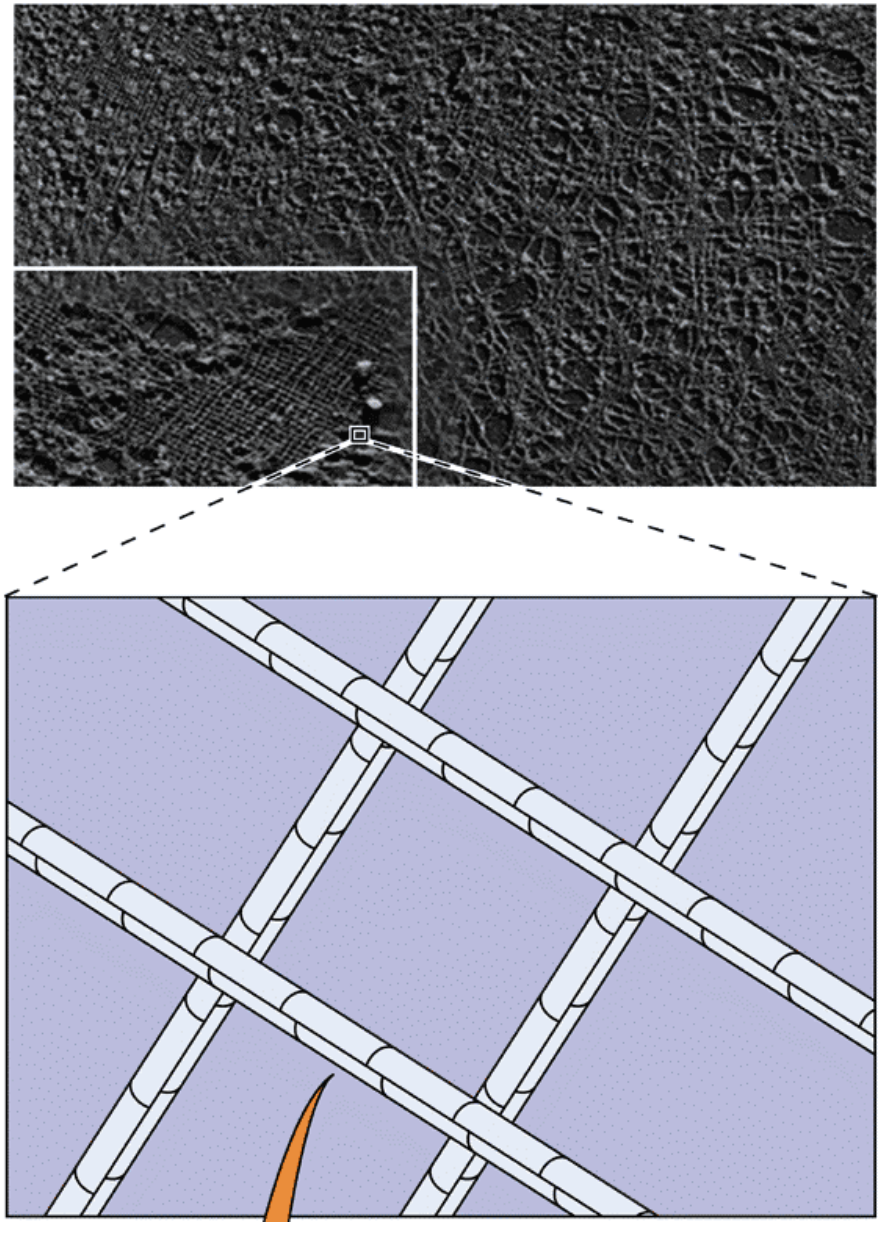


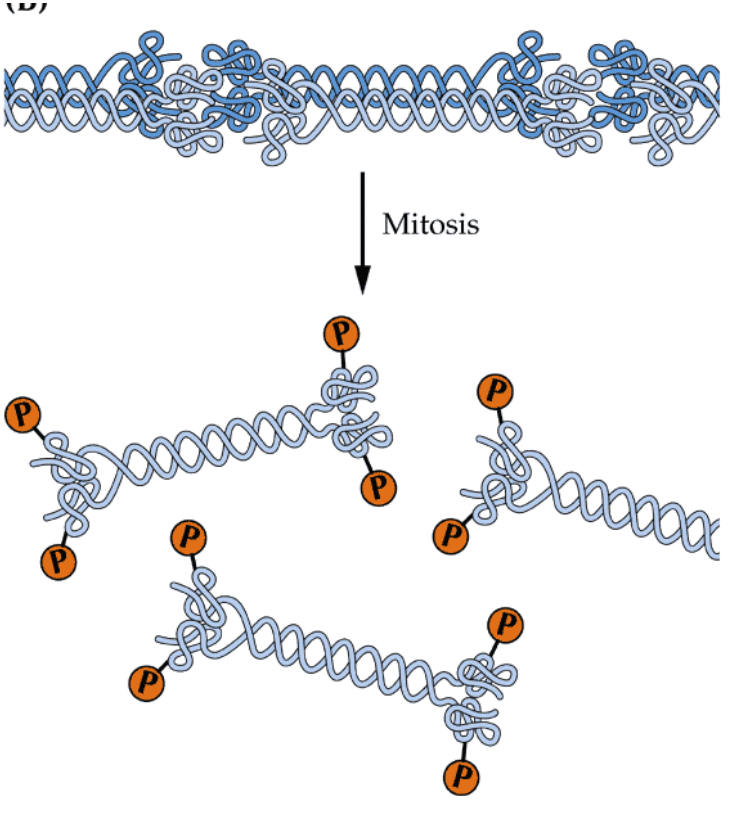
anti-IF

DIOC6 - membrány

Jaderná IF - laminy

Asi jen u živočichů?





Jak u rostlin?

- nacházíme „laminové“ epitopy, ale v genomu A. th. není ortolog
- rekonstituce jader Xenopus v bezbuněčných extraktech z tabáku (Lu and Zhai 2001)
- savčí LBR se lokalizuje do jaderné membrány v tabáku (Irons et al. 2003)

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

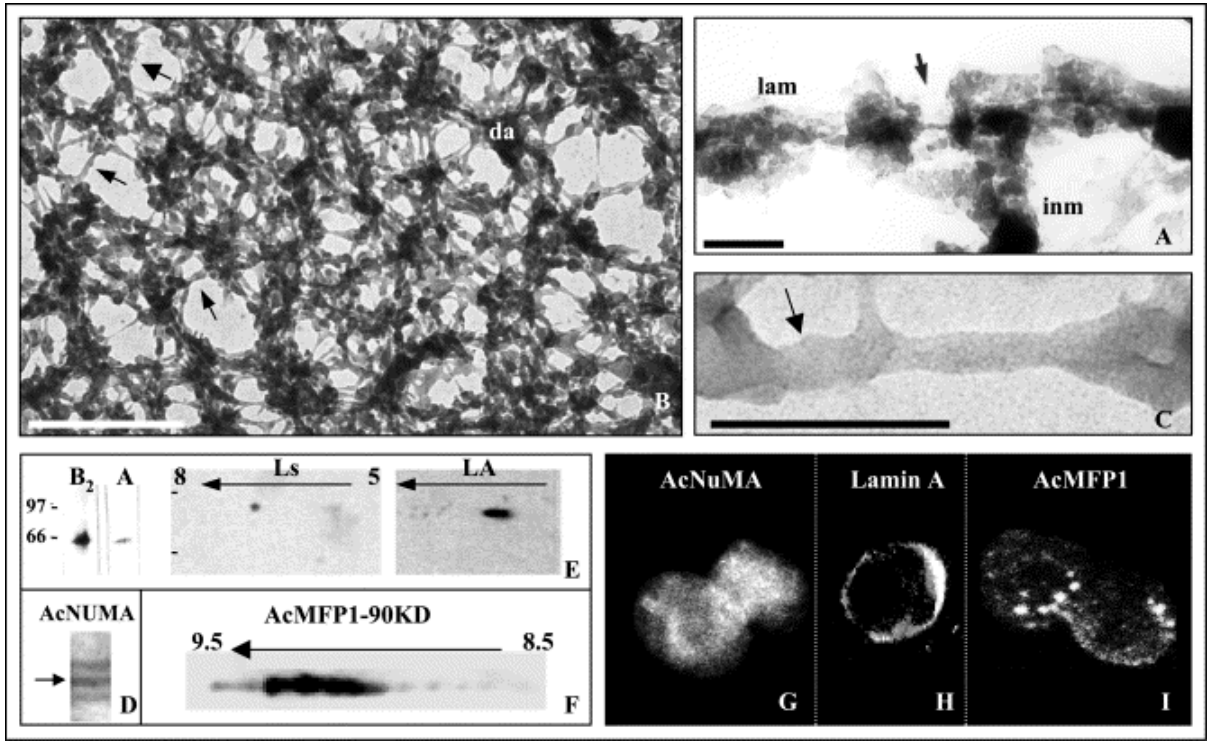


Fig. 1. Ultrastructural organisation of the plant NM in resinless sections. (A) Portion of the peripheral lamina with a complex structure (lam), connected with the internal matrix (inm). Individual filaments are observed (arrow). (B) A delicate anastomosed network of filaments forms the internal matrix: 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 ...

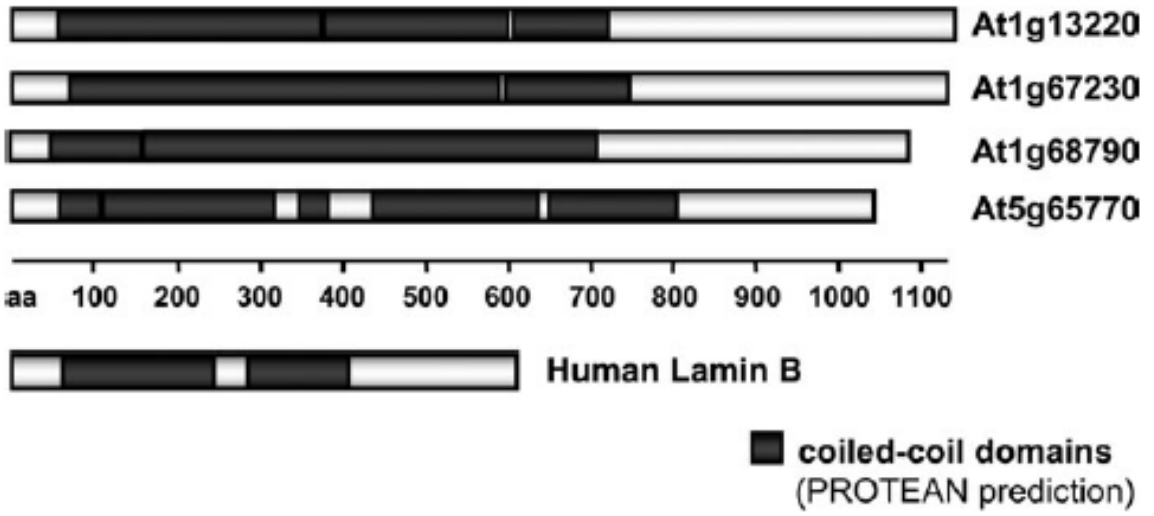


Fig. 1. Comparison of localization and structural organization of human lamin B and *Arabidopsis* nuclear matrix constituent proteins (NMCPs). While almost twice the size of lamins, NMCPs have a comparable domain organization of short head, coiled-coil centre, and longer tail domain. The gene identifiers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

Mikrotubulární cytoskelet

Mikrotubulární systémy rostlinné buňky

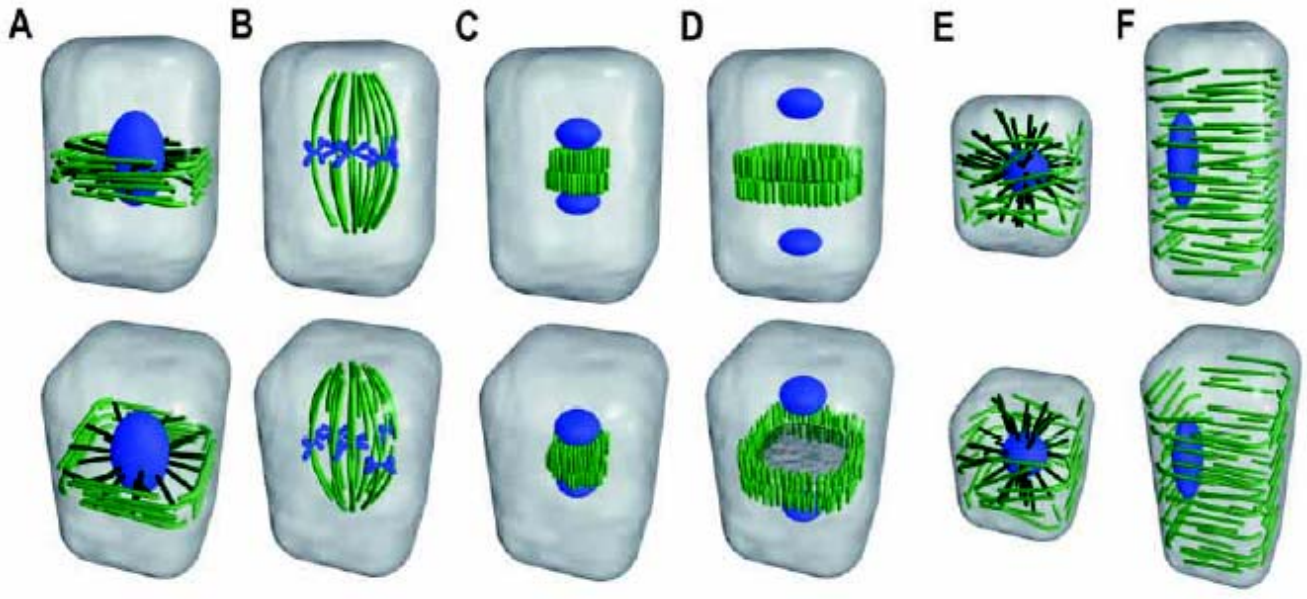
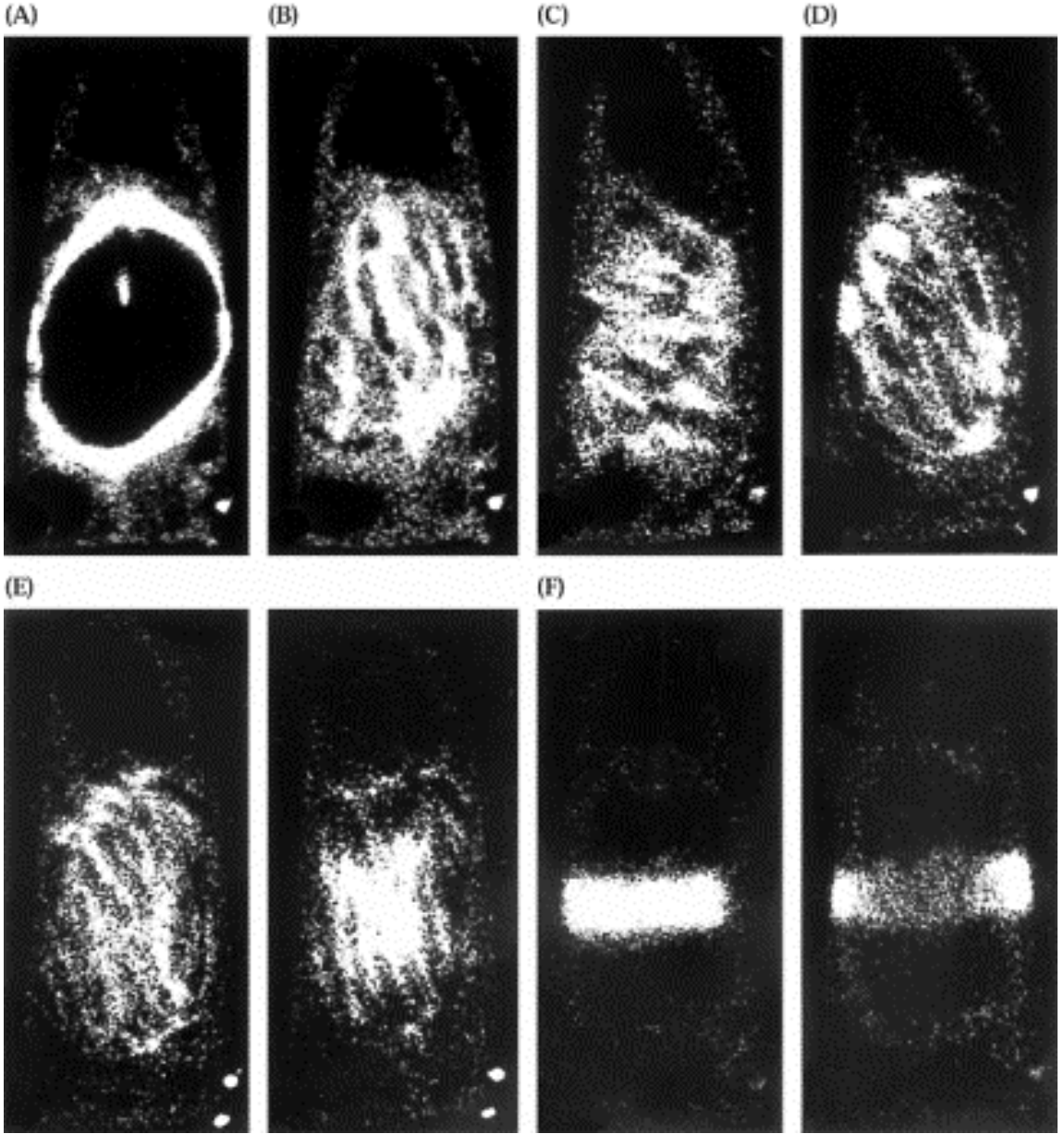


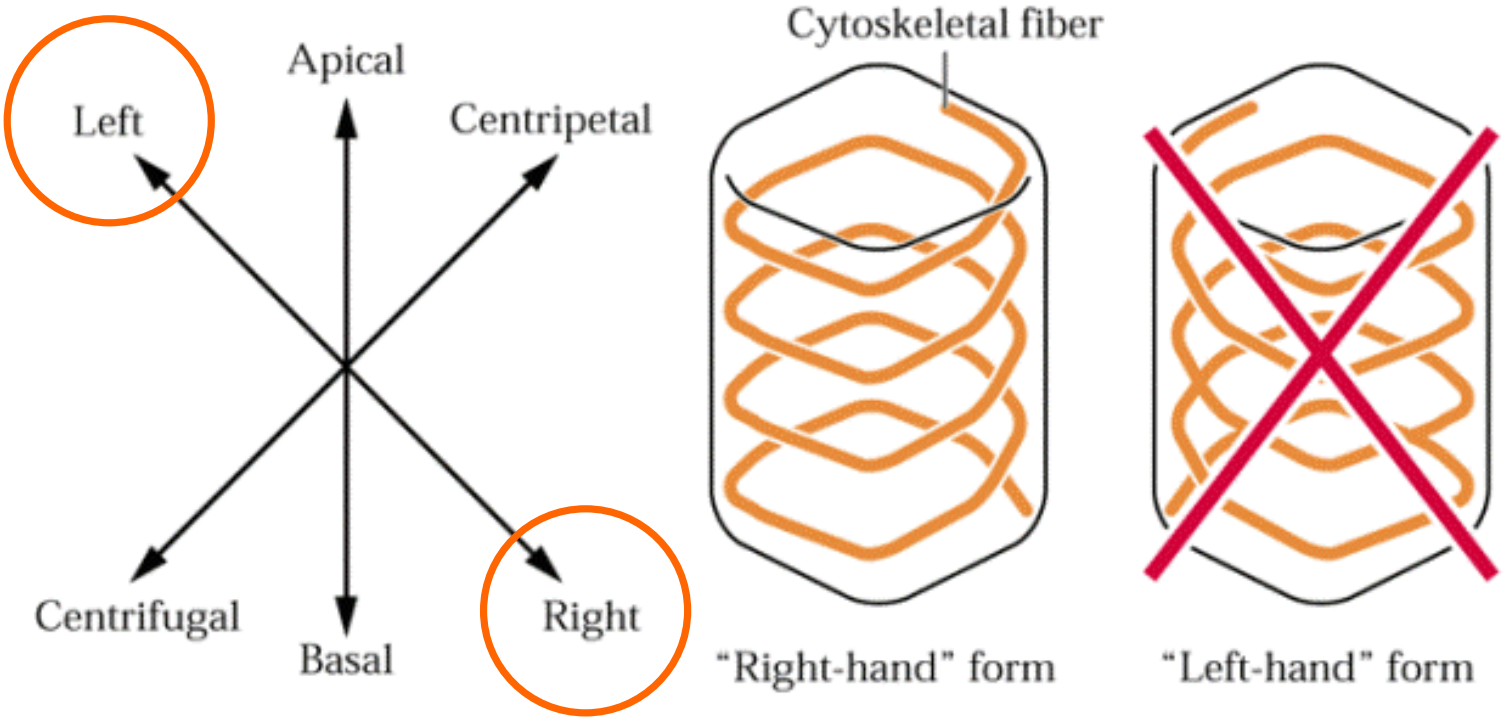
Fig. 1. These schematic illustrations, rendered in 3D at two aspects, show microtubule arrays through the plant cell cycle. (A) A preprophase band, linked to the nucleus by phragmosome microtubules, marks the future division site. (B) Metaphase spindle with a dispersed polar region. (C) In telophase,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

Helikální uspořádání MT

existuje preference směrů



Helikální uspořádání MT

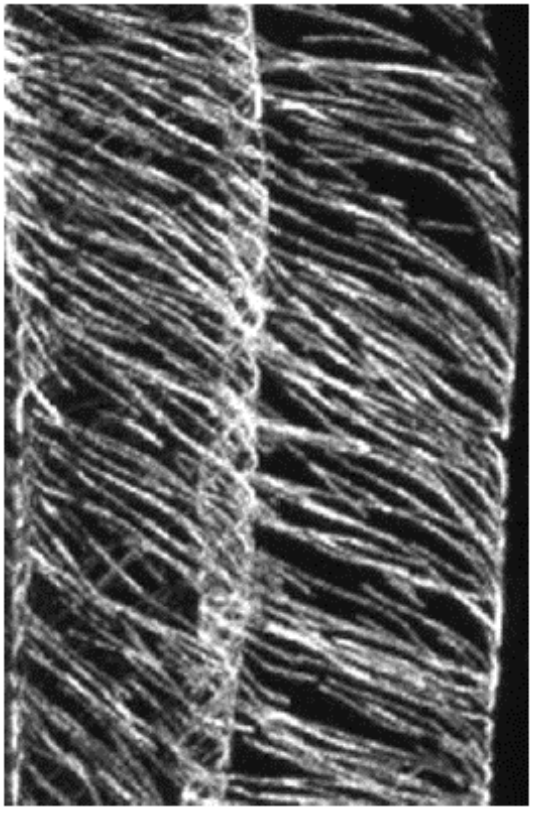
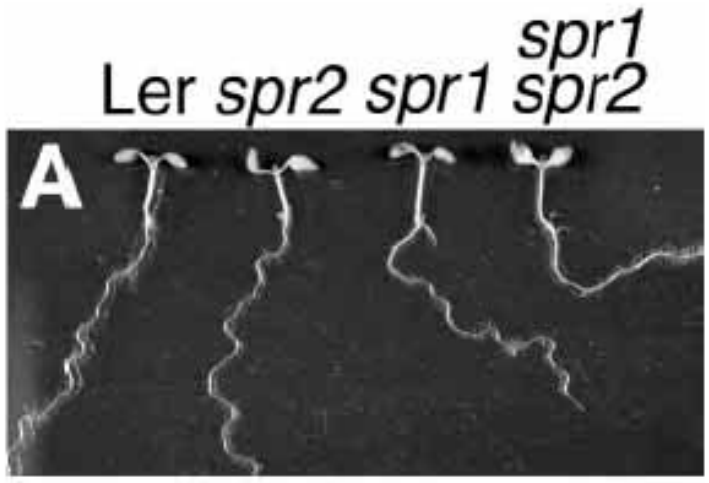
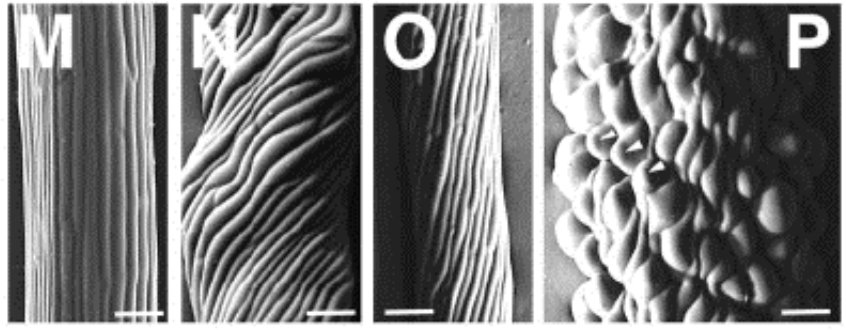


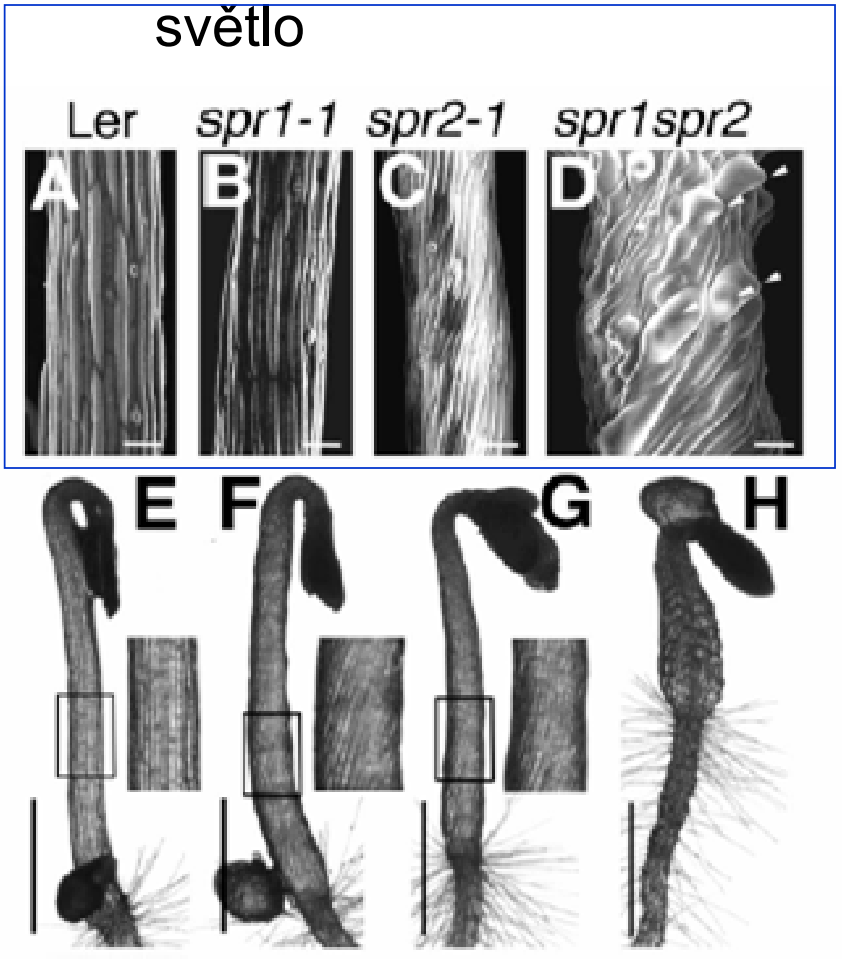
Figure 1. Microtubules in the *spiral* Mutant.

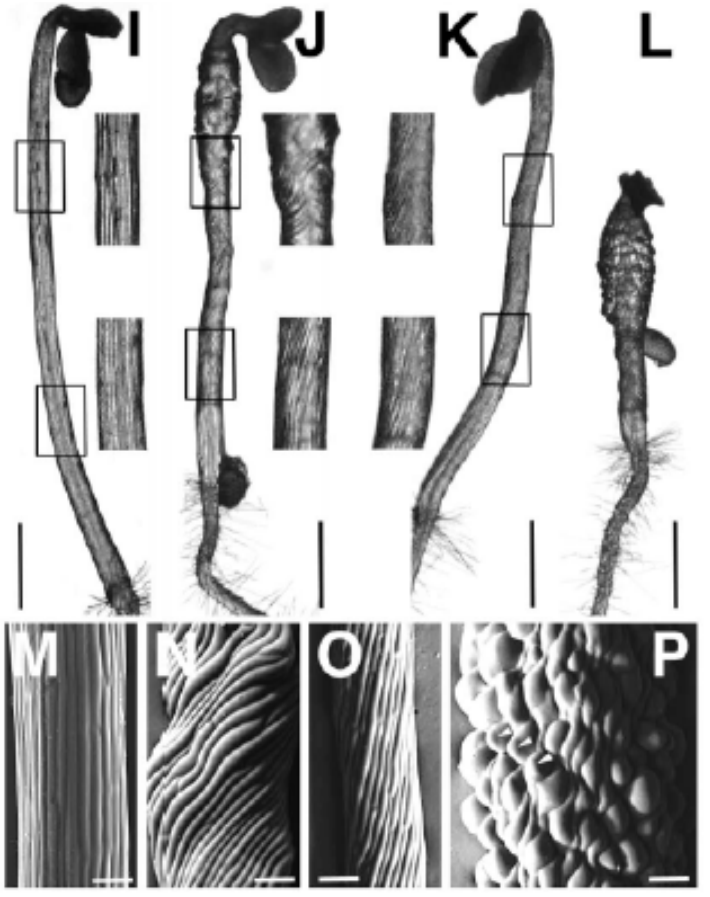
In the *spiral* mutant of Arabidopsis, the cortical microtubules of two epidermal cells wind around the cortex in left-handed S-shaped helices. Each single fluorescent strand is likely to represent a bundle of several microtubules. (*Figure courtesy of Keiko Sugimoto.*)





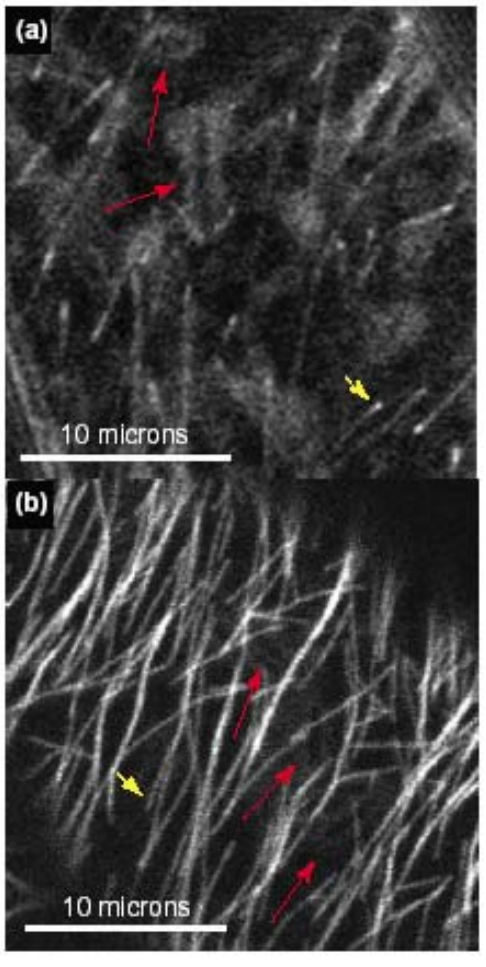
SPR1, SPR2 (spiral)





Arabidopsis *spiral1* (spr1) mutants show a right-handed helical growth in roots and etiolated hypocotyls due to impaired directional growth of rapidly expanding cells.

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).

SPR1 encodes a small protein. Its localization to cortical microtubules (MTs) suggests that SPR1 maintains directional cell expansion by regulating cortical MT functions.

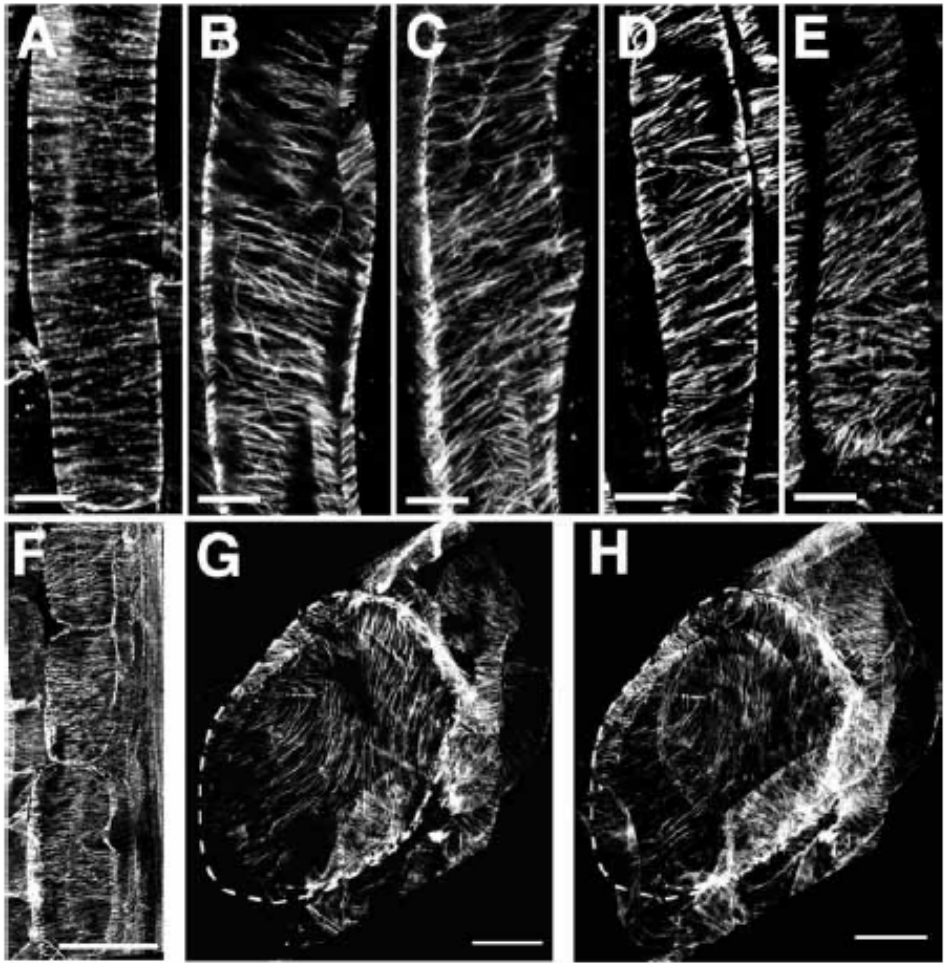
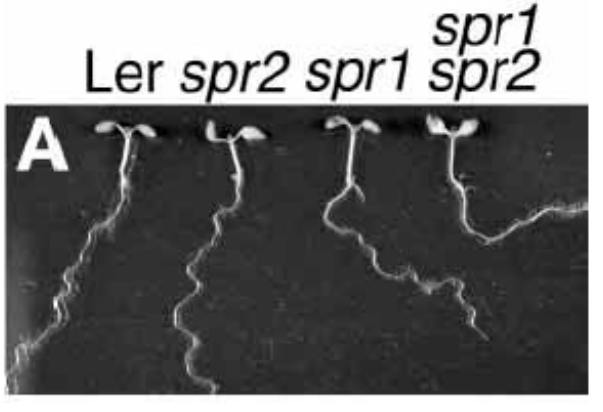


Fig. 9. Micrographs of cortical MTs in seedlings grown at 23°C. (A-E) Root epidermal cells at the basal elongation zone. (A) Ler, (B, C) *spr1-1*, (D) Ler grown on 1 μM taxol and (E) Ler grown on 3 μM propyzamide. Cortical MT arrays were located underneath the outer cell wall (A,B,D,E) or the inner cell 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.

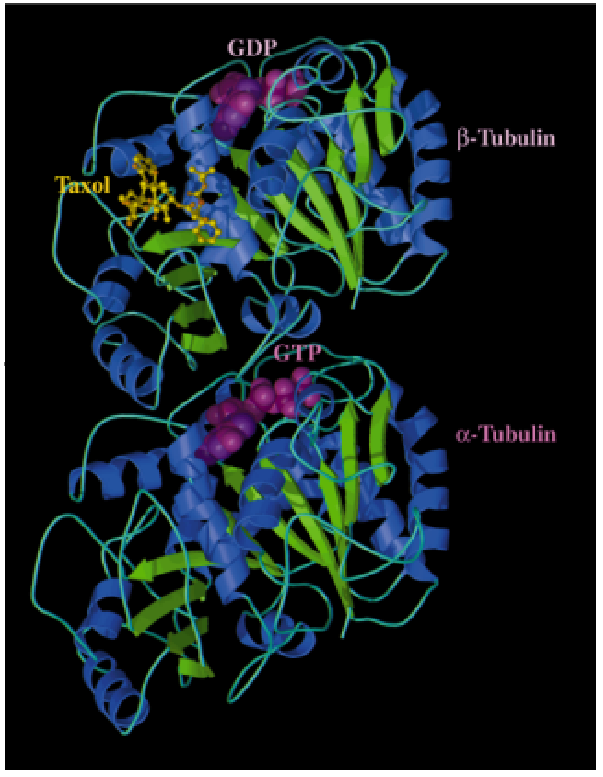


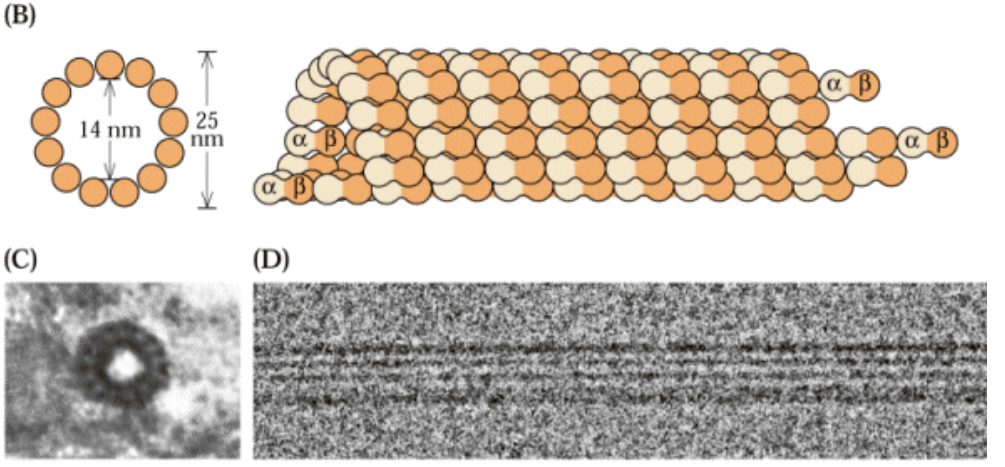
„Točivost“ orgánů mutantů: směr nemusí souhlasit se směrem mt spirály

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

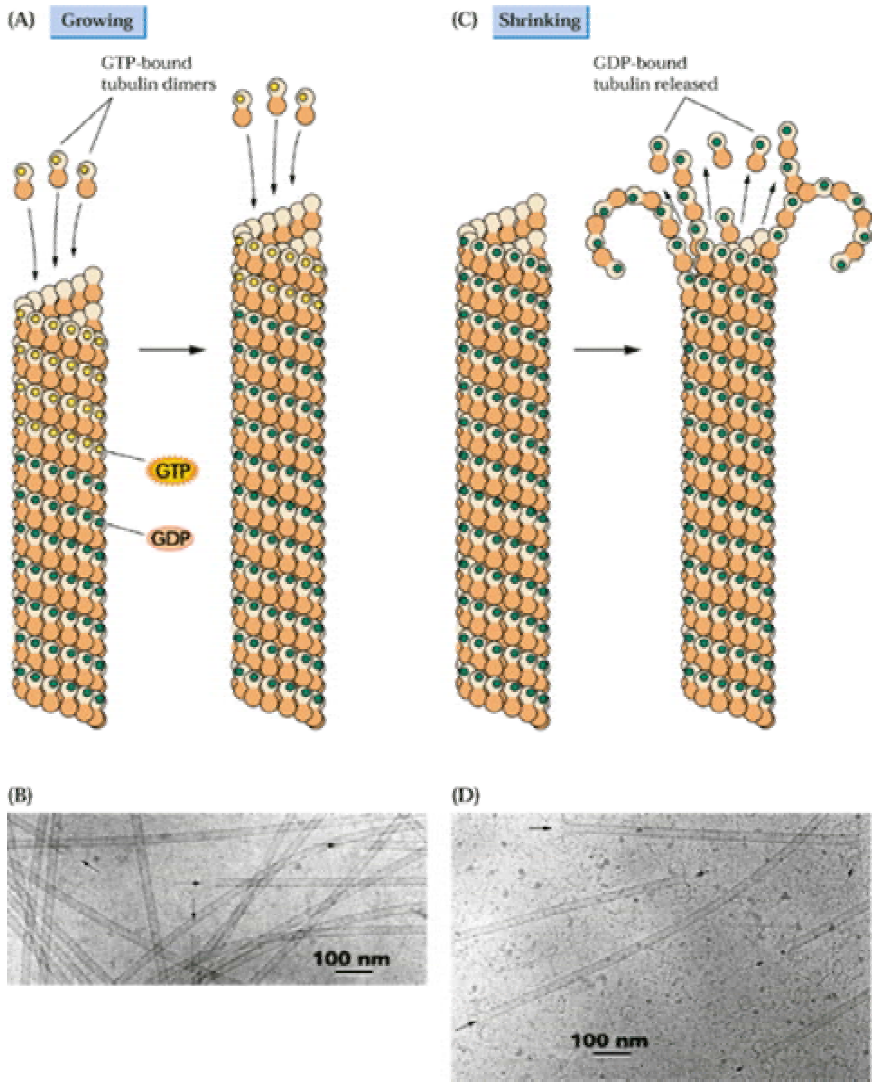
Točí se rostlina bez mikrotubulů doleva? A jak to dělá??

Struktura mikrotubulů



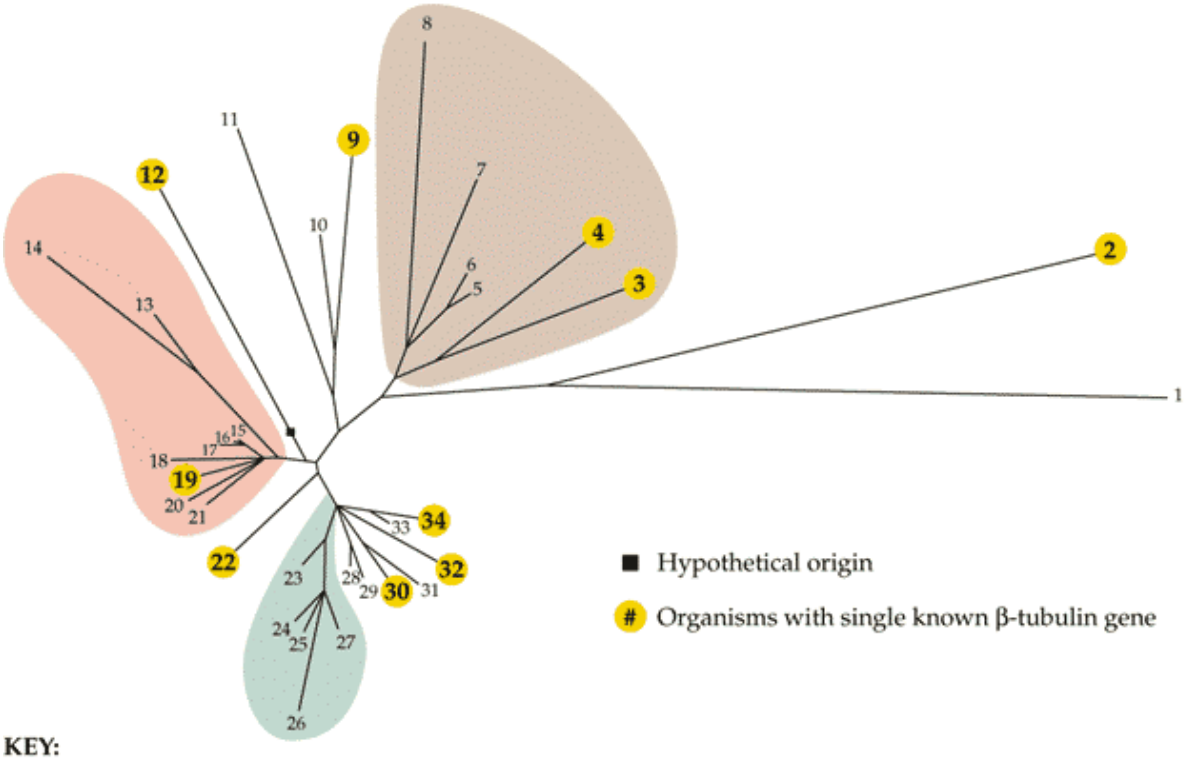


Dynamika přestaveb mikrotubulů



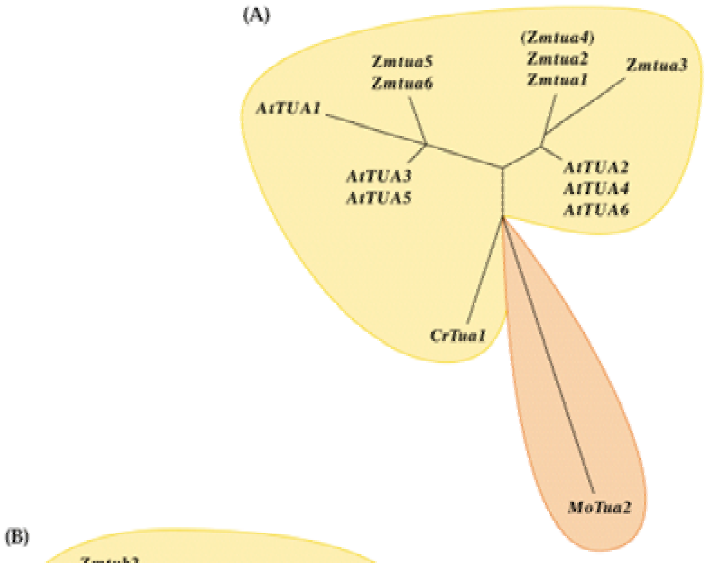
animace

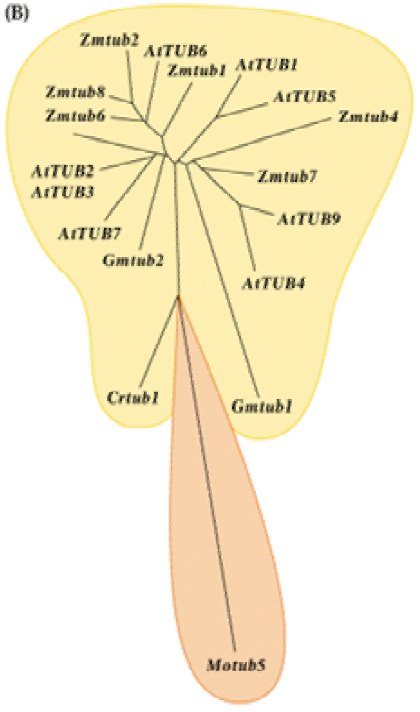
Rodiny tubulinových genů



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
Zmtub6
AtTUB5
Zmtub4
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Zmtub7
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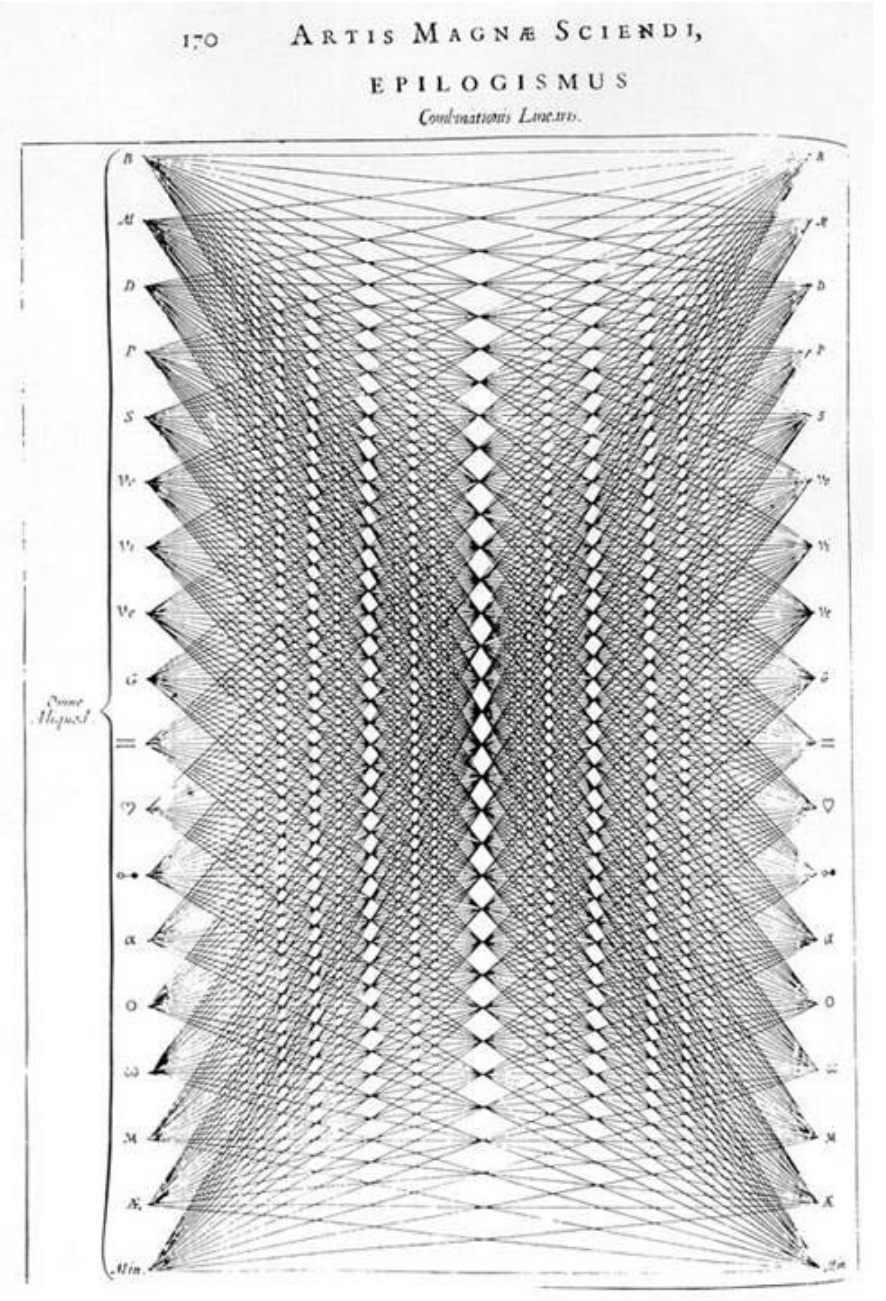
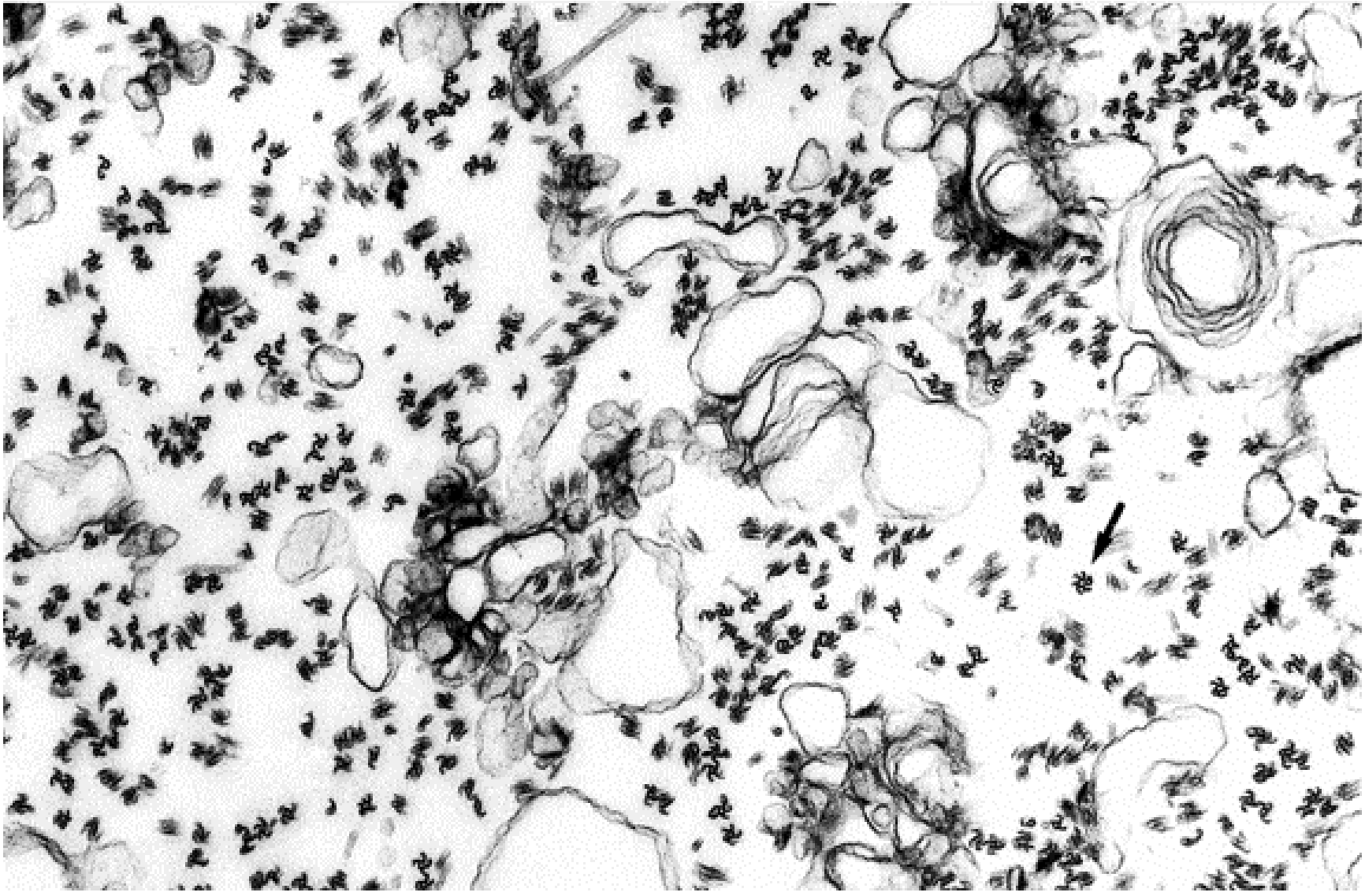
Gmtub2
AtTUB4
Crtub1
Gmtub1
Motub5


170 ARTIS MAGNÆ SCIENDI,
EPILOGISMUS
Combinationis Linearis.



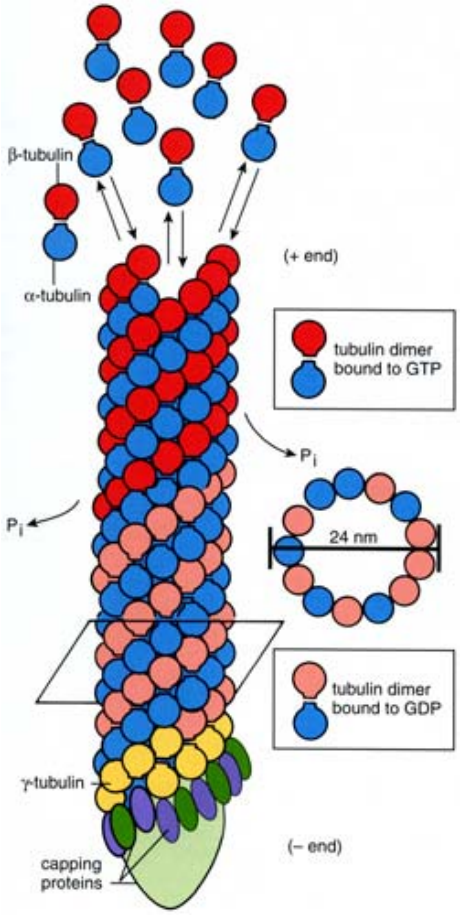
tubulinové „háčky“ v isol. fragmoplastech v nadbytku tubulinu

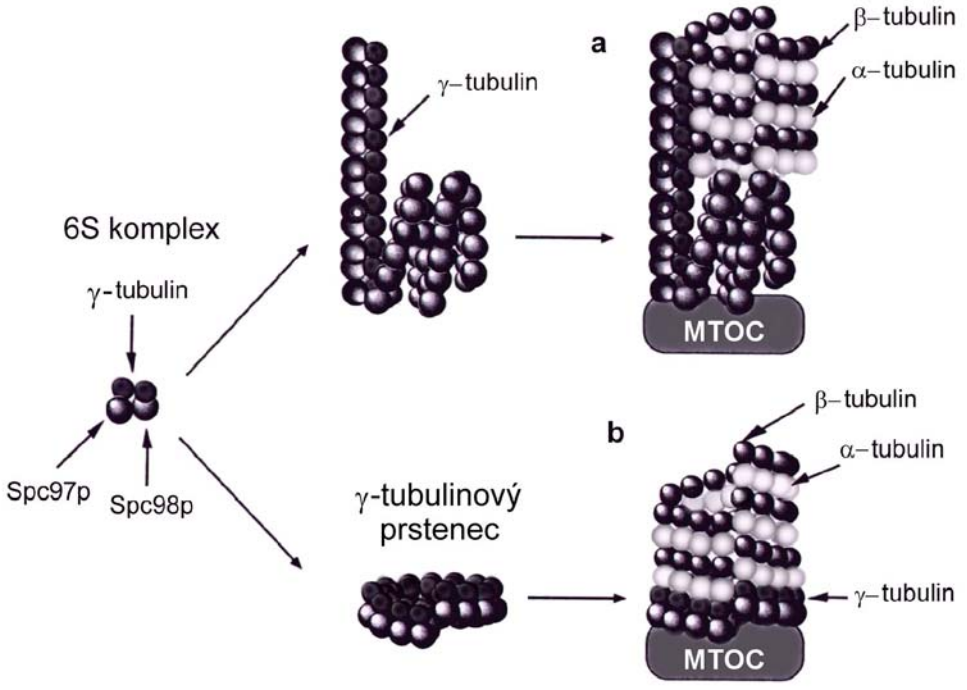
Nukleace mikrotubulů

γ-tubulin

mikrotubulární organizační centra (MTOC)

rostliny nemají žádná „definovaná“ MTOC





MT recovery after drug-induced disassembly

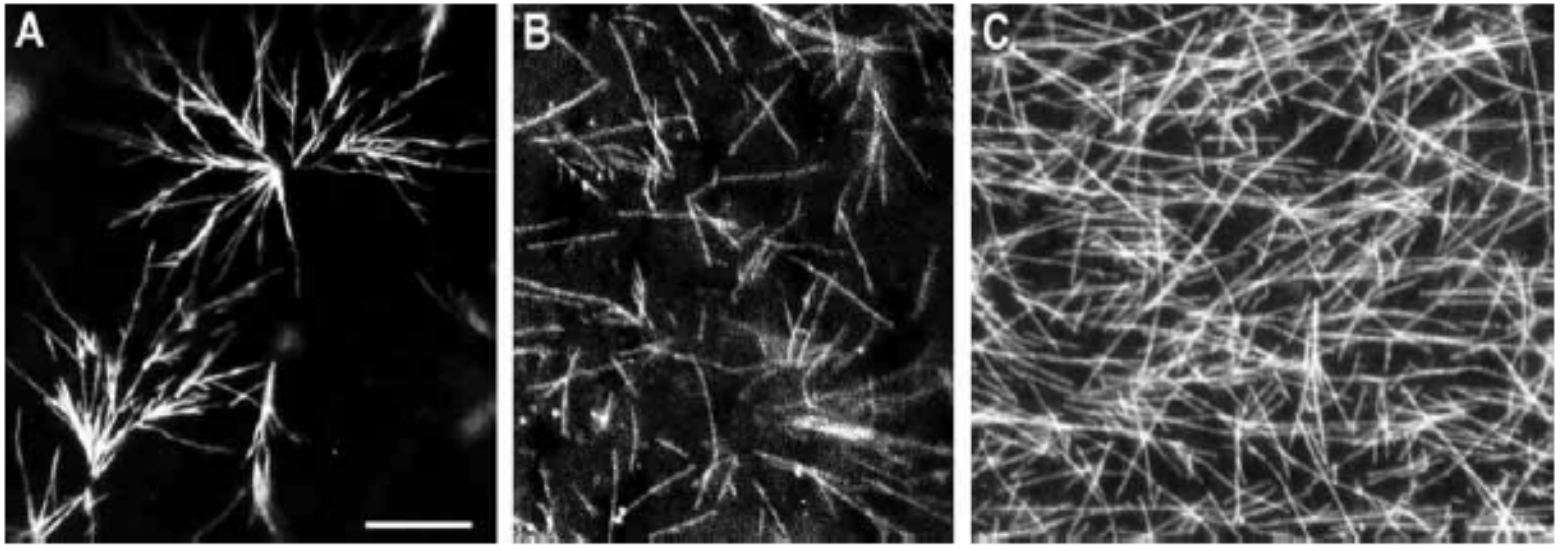
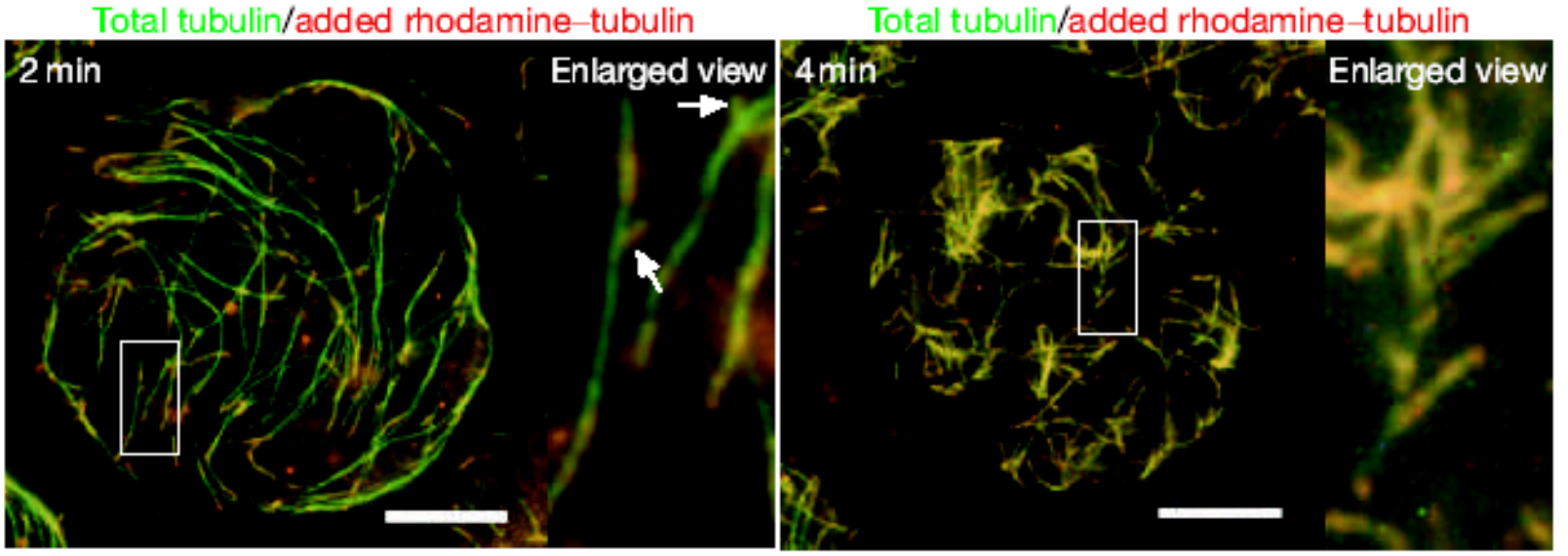
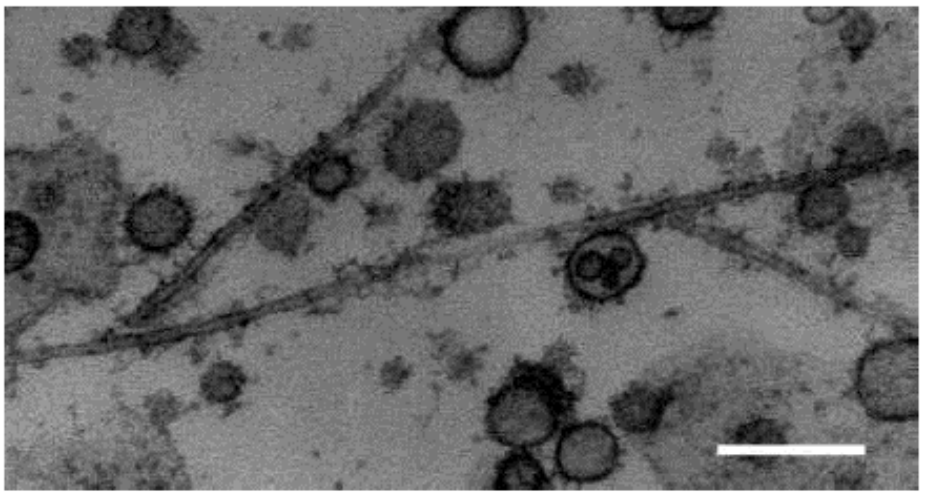
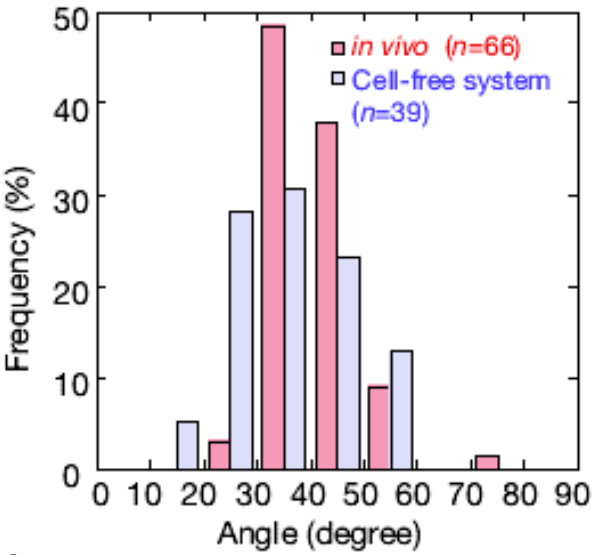


Fig. 2. Cortical microtubule recovery patterns after drug-induced microtubule disassembly. (A) Microtubules appear to diverge from the initial assembly site, forming fractal tree-shaped clusters, with microtubules diverging from each other at acute angles (figure adapted from Wasteneys and Williamson, 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

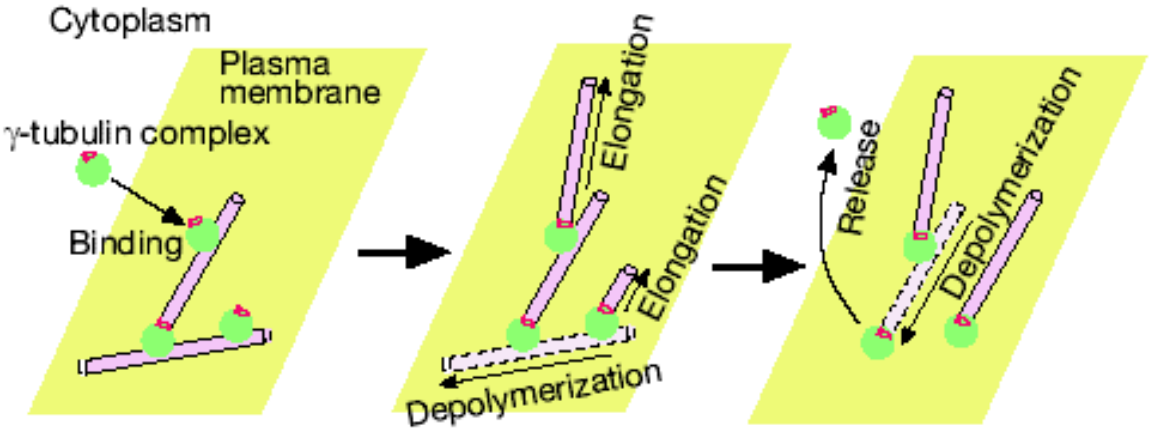
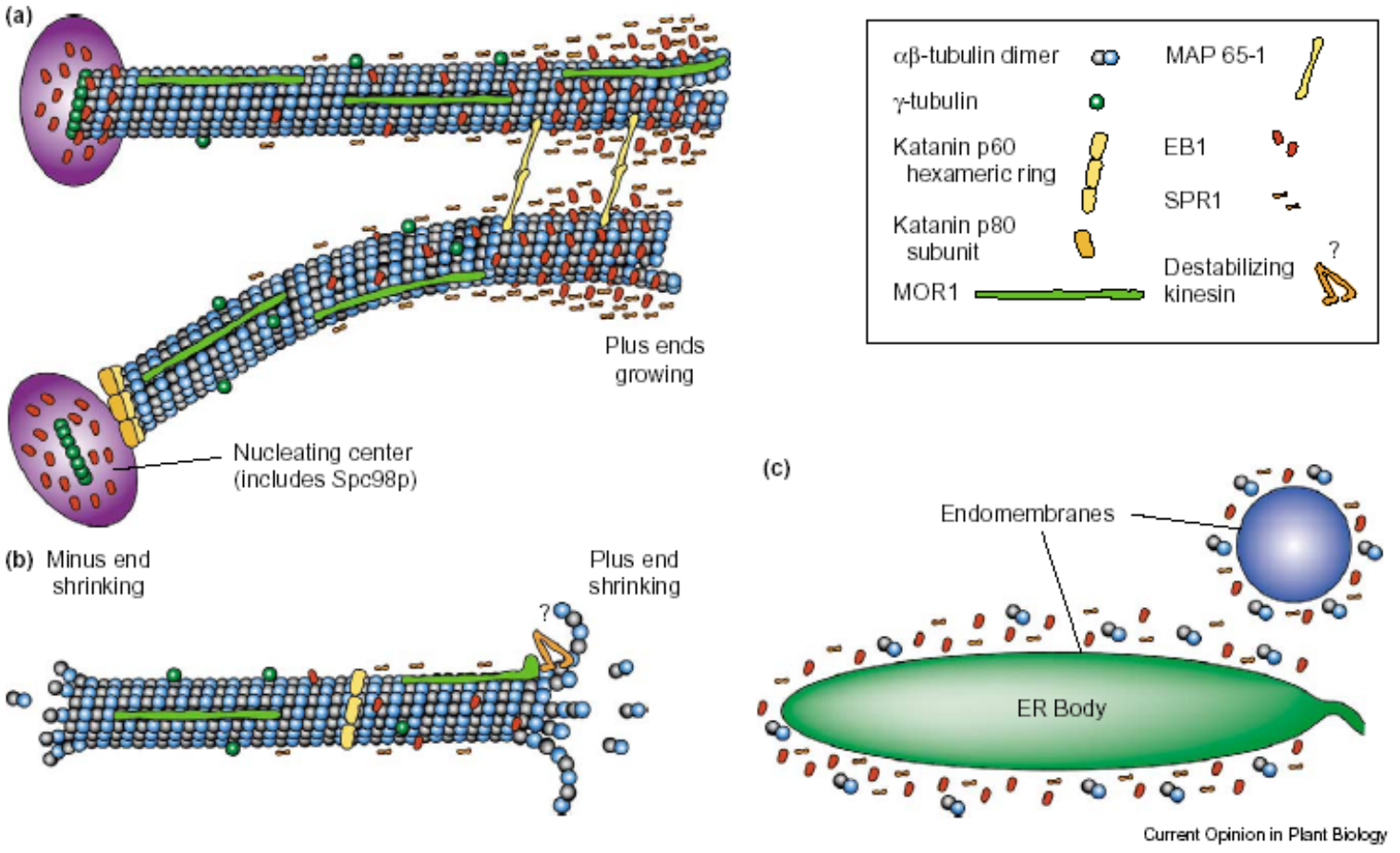


Figure 5 Model for nucleation of cortical microtubules in plants. Cytosolic γ-tubulin complexes bind onto pre-existing cortical microtubules (left) and nucleate microtubules as branches (centre). The original microtubules frequently depolymerize (centre) but γ-tubulin complexes are not released until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

End binding protein (EB1)

EB1 váže
+konce MT

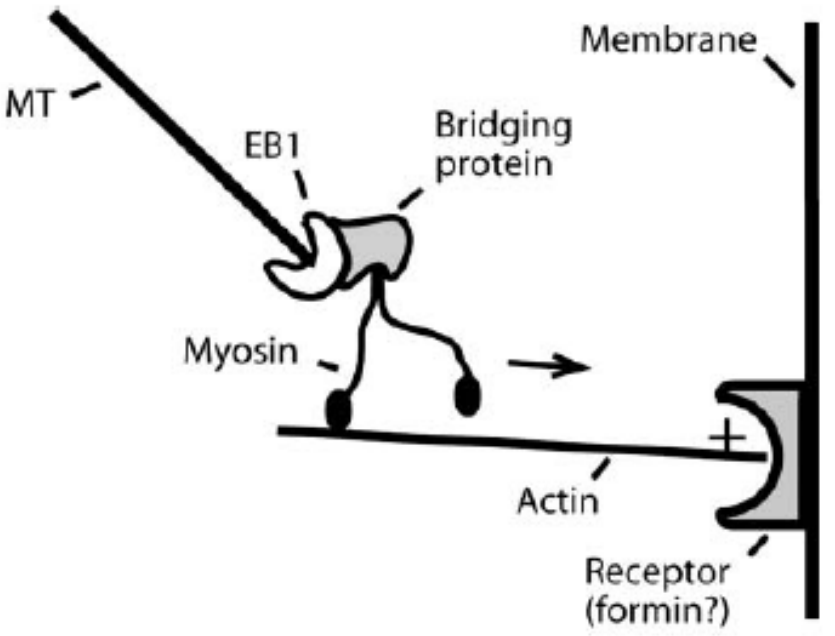


Figure 2. Hypothetical model for delivery of a plant MT to a cellular receptor at a specific site (e.g. PPB, phragmoplast, root hair tip) along actin filaments, based on models from yeast and fibroblasts (Gundersen et al., 2004). EB1 binds a bridging protein associated with myosin, which translocates 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?

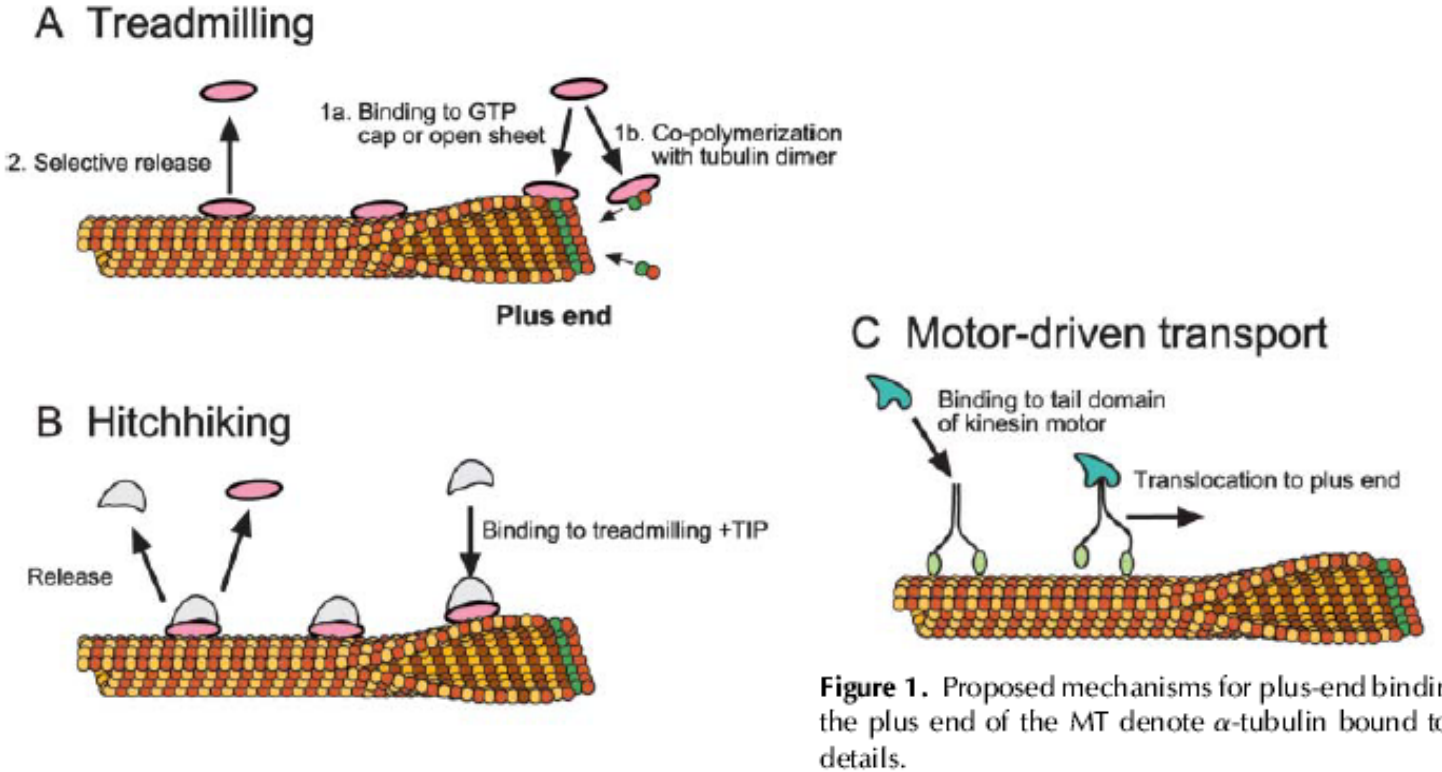


Figure 1. Proposed mechanisms for plus-end binding. Green circles at the plus end of the MT denote α-tubulin bound to GTP. See text for details.

Katanin - p60 a p80

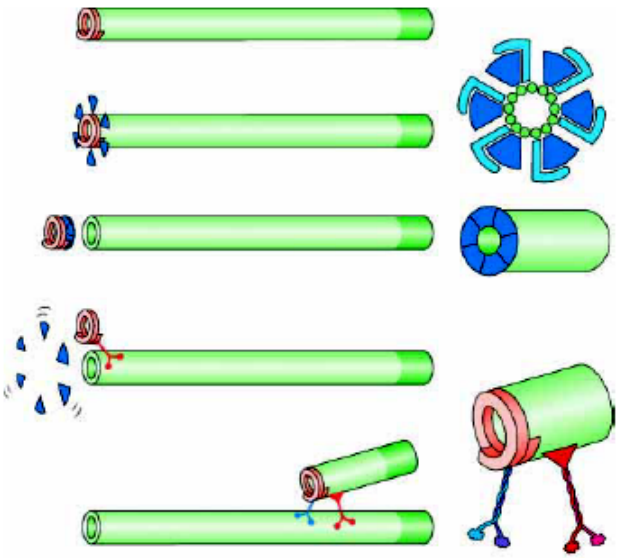
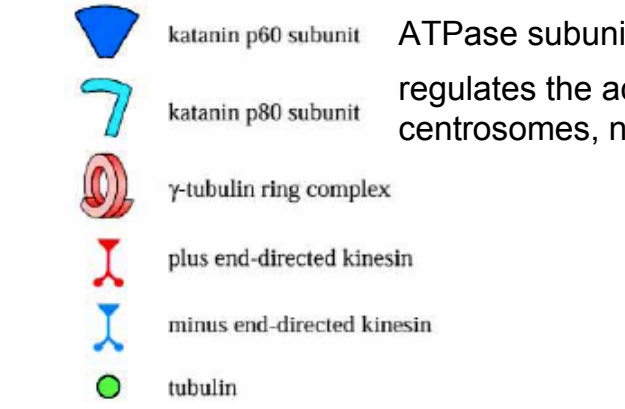


Fig. 3. Model for microtubule assembly by severing and transport of nucleating templates. In this model, a γ-tubulin ring complex associates with the minus end of a microtubule, while the microtubule extends by the addition of tubulin subunits at the fast-growing, GTP-tubulin-containing plus end (dark 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.

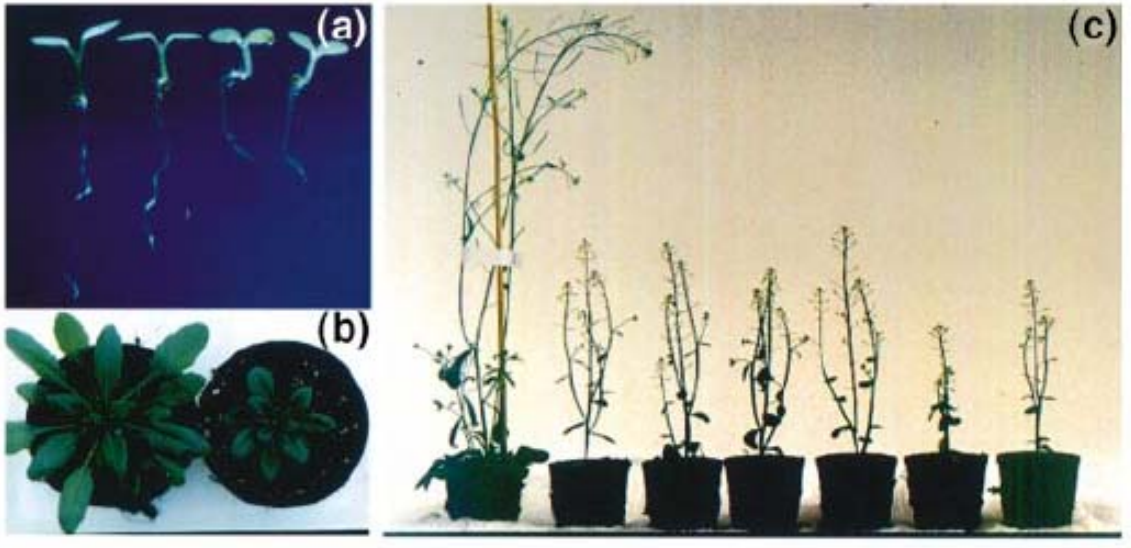


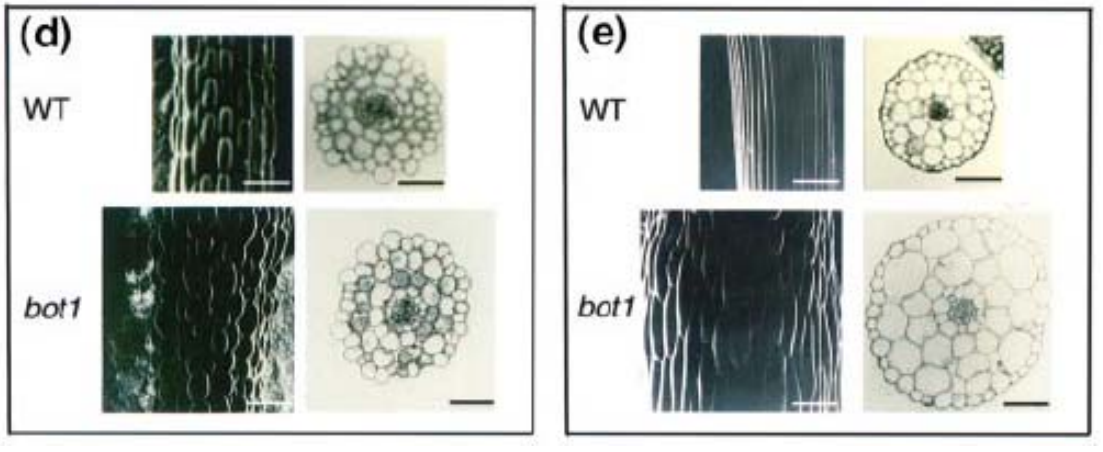
ATPase subunit, which functions to sever microtubules

regulates the activity of the ATPase and localizes the protein to the centrosomes, not present in plants

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

botero1 mutant

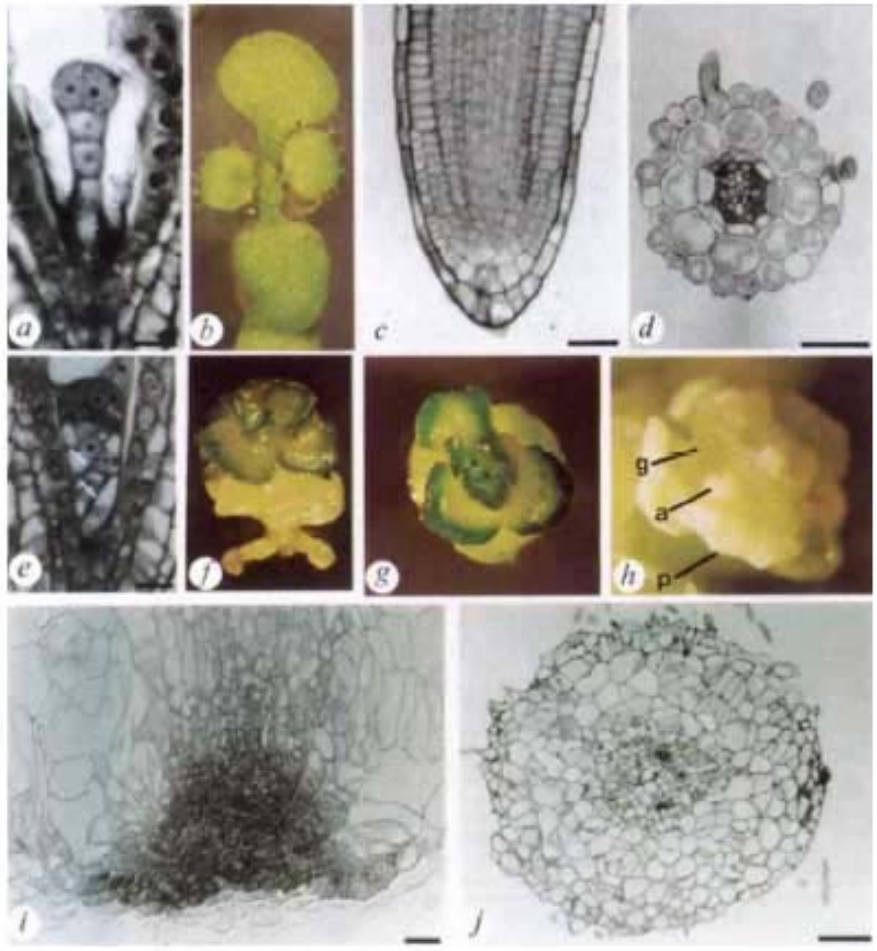




BOT1 is required for organizing cortical microtubules into transverse arrays in interphase cells, and that this organization is required for consolidating, rather than initiating, changes in the direction of cell expansion.

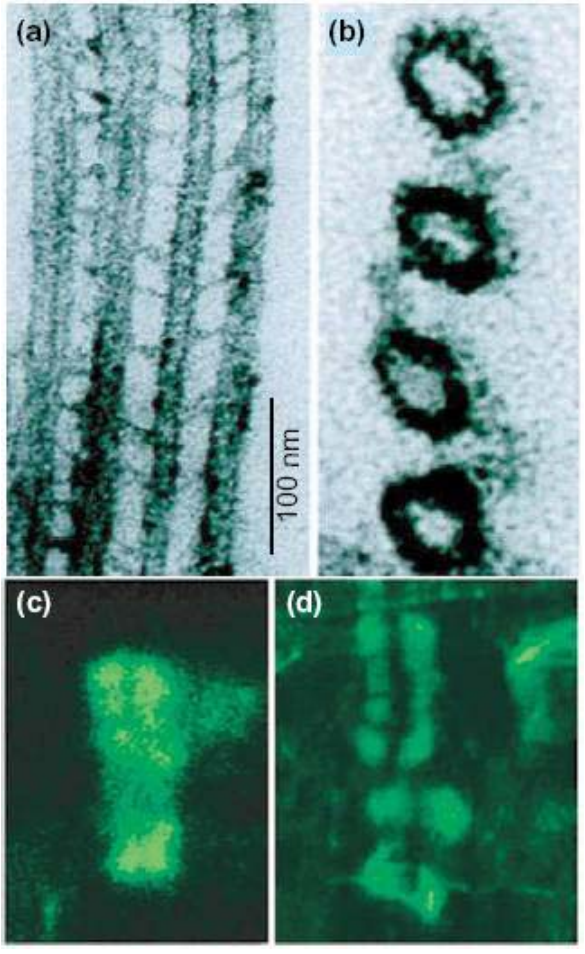
TON1 (*tonneau*)

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



(Traas et al. 1995)

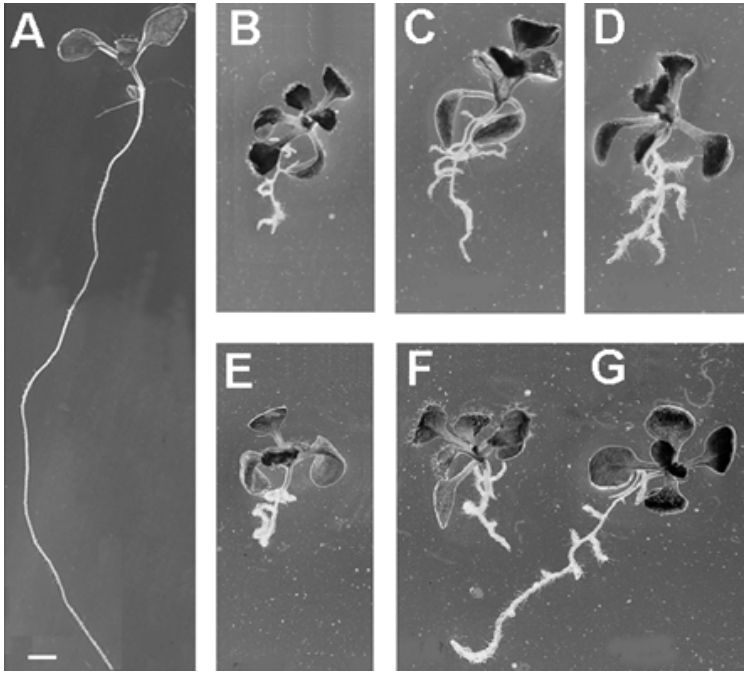
MAP65: crosslinking („rostlinná specialita“)

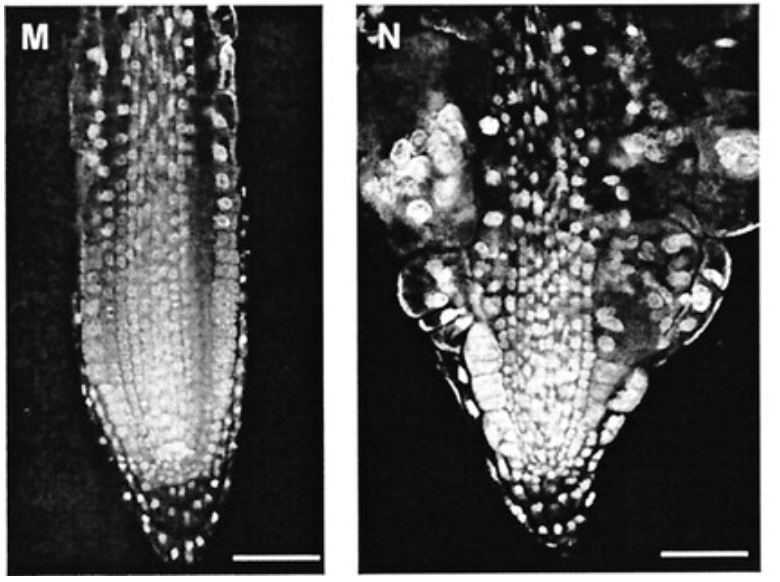


MAP-65 family members that cross-link MTs. **(a)** Longitudinal and **(b)** transverse electron micrograph sections of MTs cross-linked *in vitro* by purified carrot MAP-65. MAP-65 can be seen as the evenly spaced filamentous cross-bridges (reproduced with permission from [46], copyright National Academy 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 má 9 homologů MAP65

pleiade – mutace v MAP65



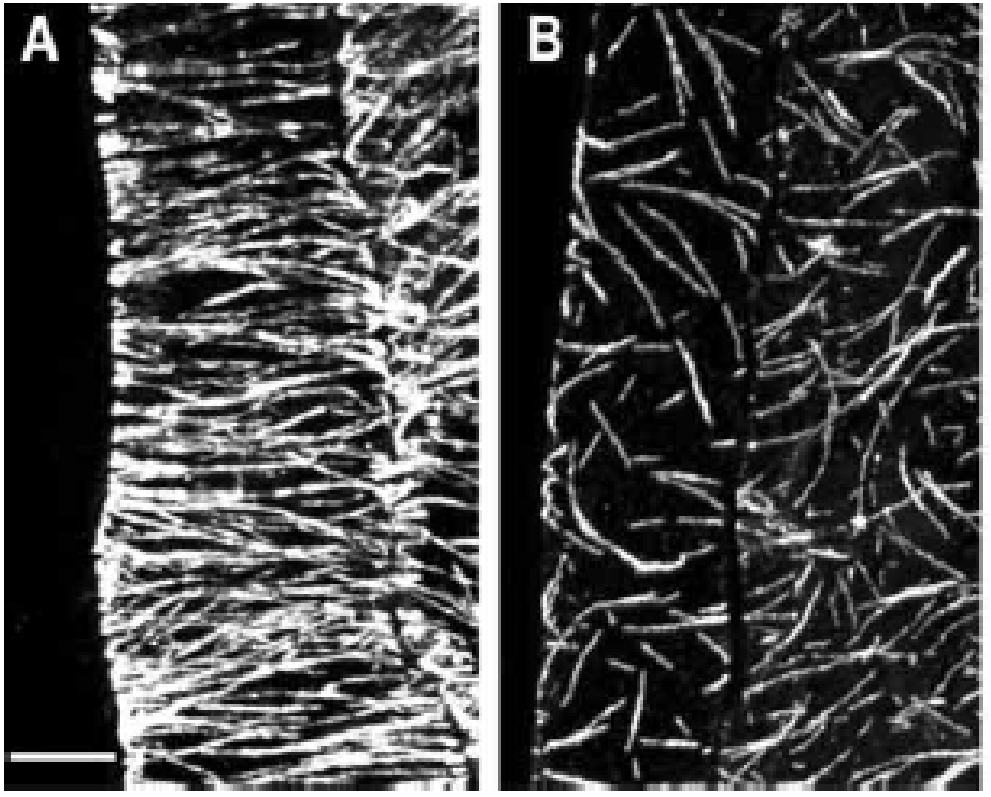


mnohojaderné buňky
(kořenově specifické!)

(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?

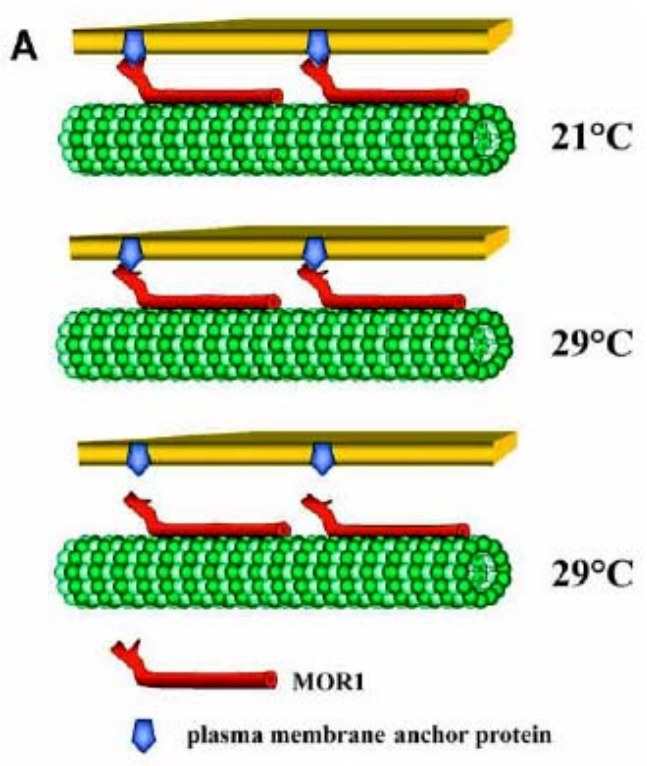
MOR1 (microtubule organization)



(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



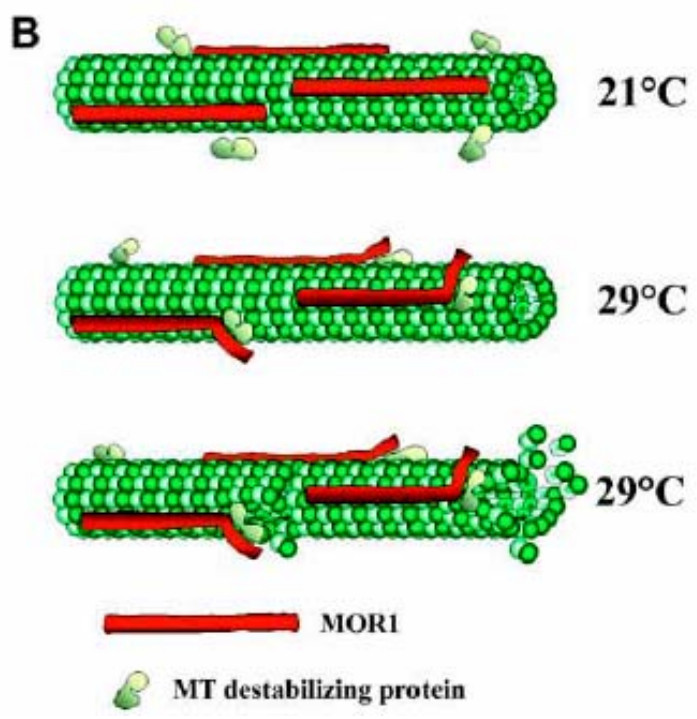
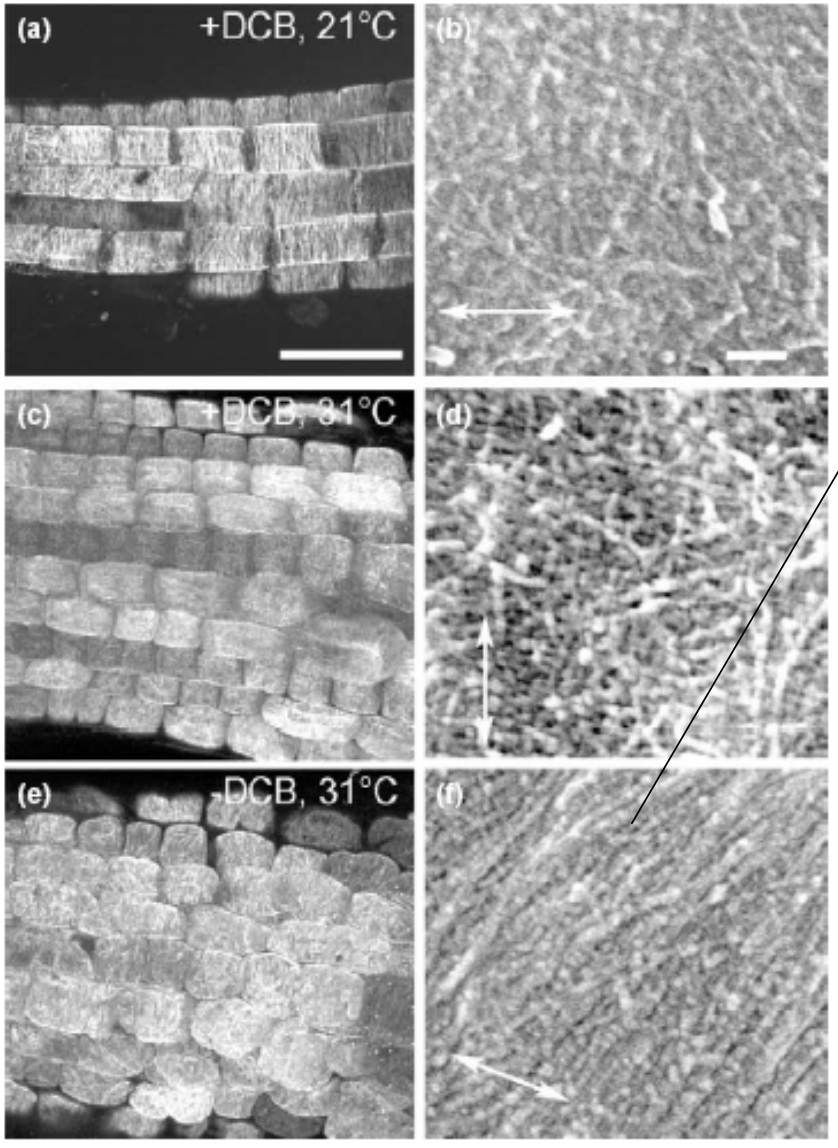
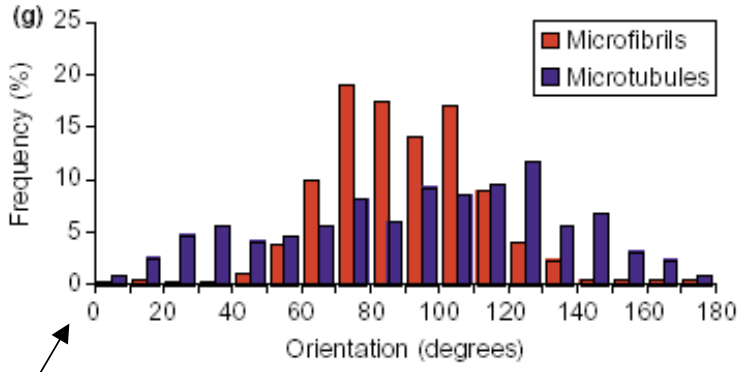


Fig. 5. Possible functions of the MOR1 HEAT repeat-1 (HR1) in microtubule stabilization. (A) HR1 links microtubules to the plasma membrane via a plasma-membrane-associated protein. At restrictive temperature, this loss of binding dissociates microtubules from the plasma membrane, promoting their destabilization. (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.

MOR1



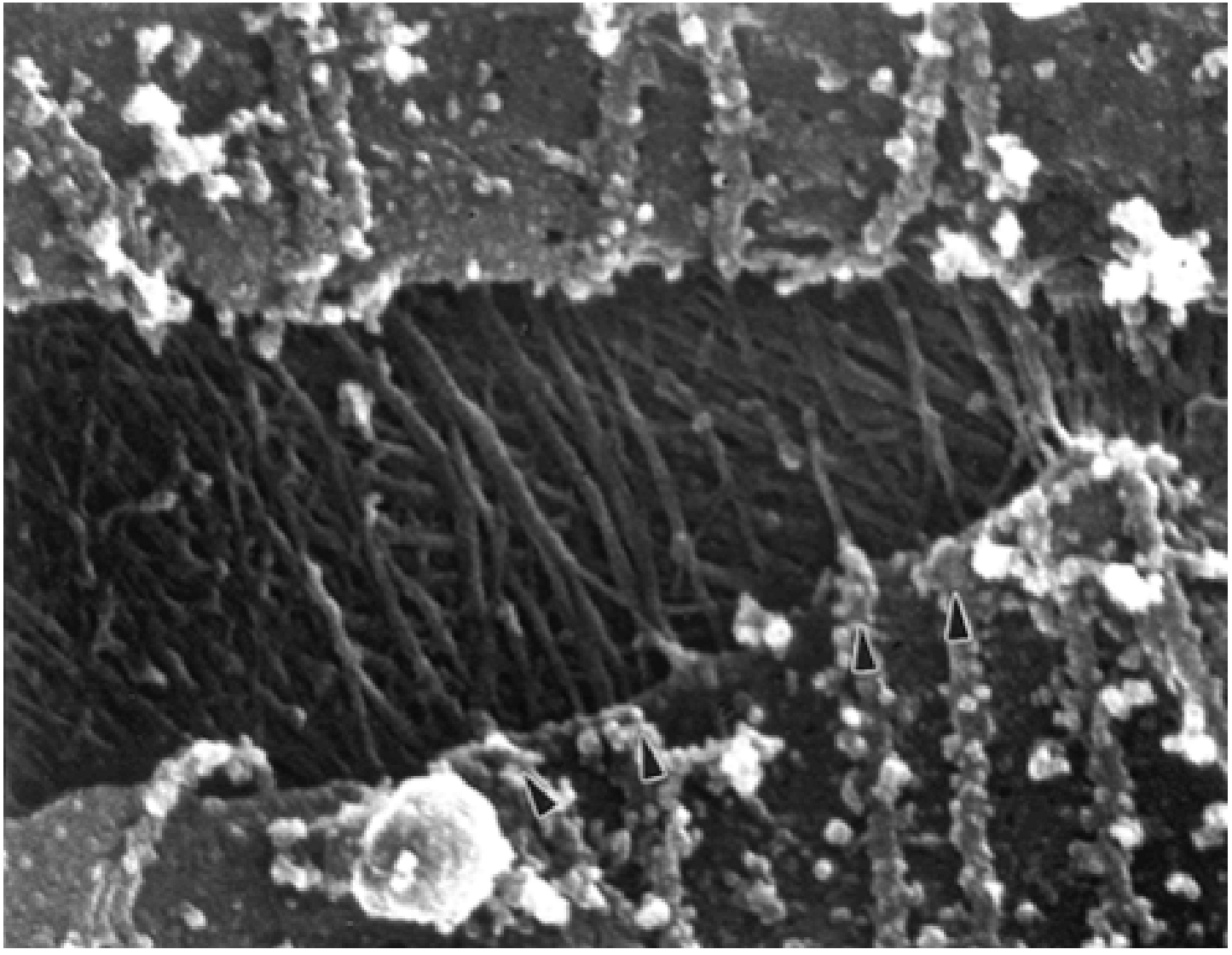


An experiment to test whether microfibrils can re-establish parallel order when the existing microfibril template is disordered and cortical microtubules are disorganized. After DCB treatment for 4 h, **(a)** cortical microtubules remain transversely oriented in *mor1-1* root cells, whereas **(b)** the 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

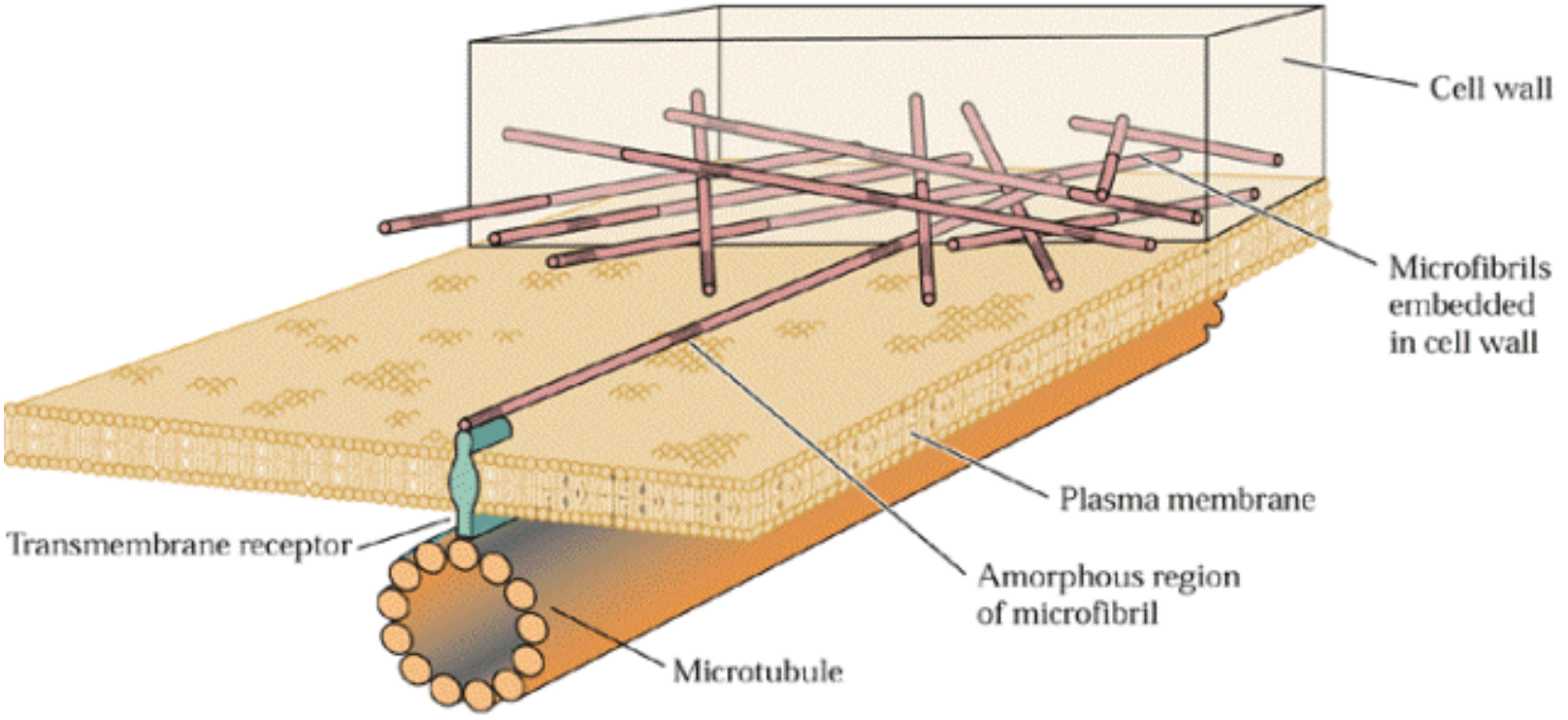
2-6-dichlorobenzonitril

Asociace mezi kortikálními mt a stěnou

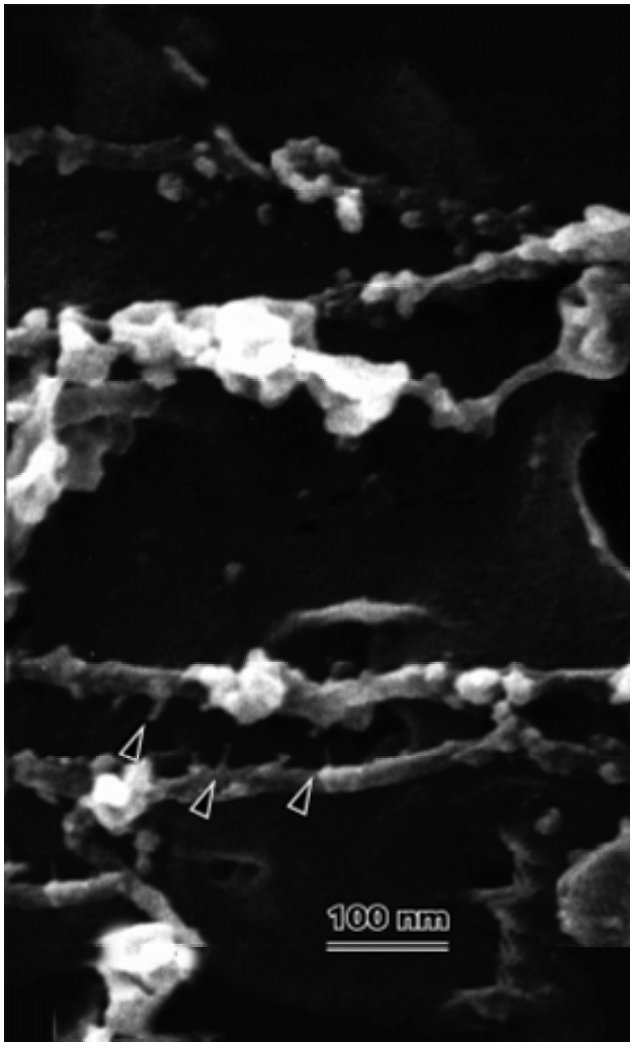


onion root

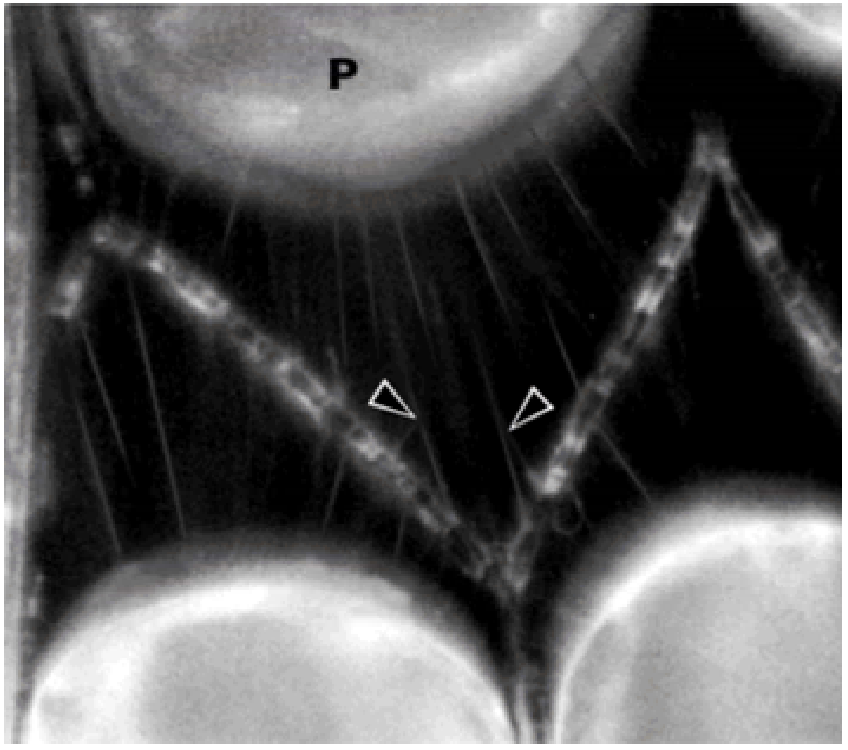
Asociace mezi kortikálními MT a stěnou



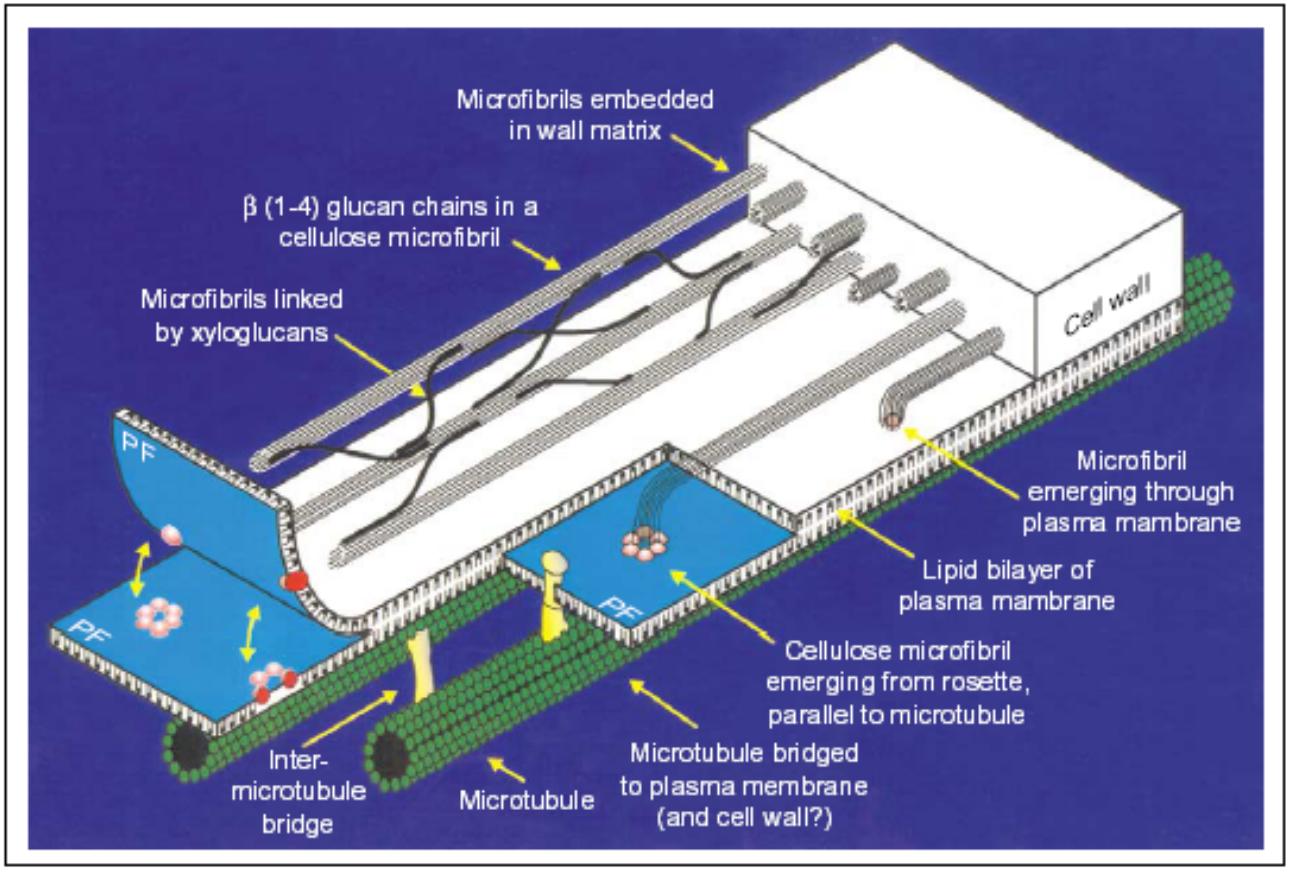
Interakce MT a buněčné stěny



cibule

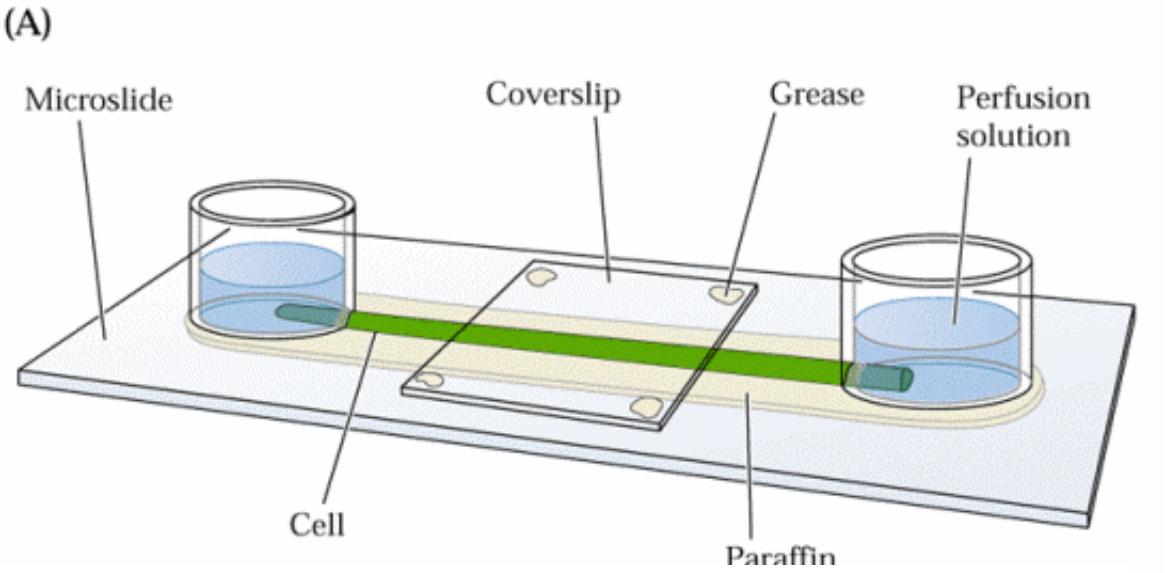


Hechtovy provazce

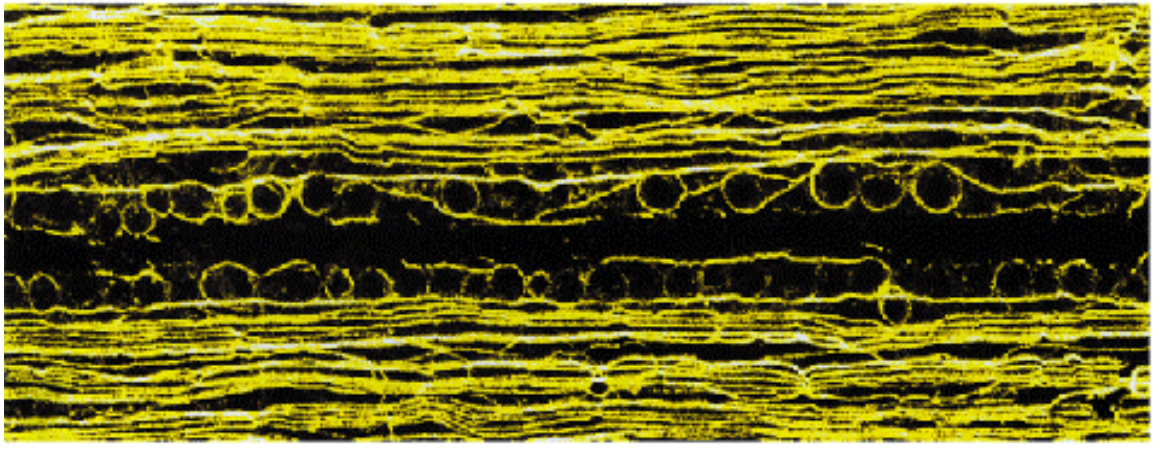


Relationships between cellulose-synthesizing complexes (rosette type), wall microfibrils, plasma membrane and microtubules. Diagram provided by Brian Gunning.

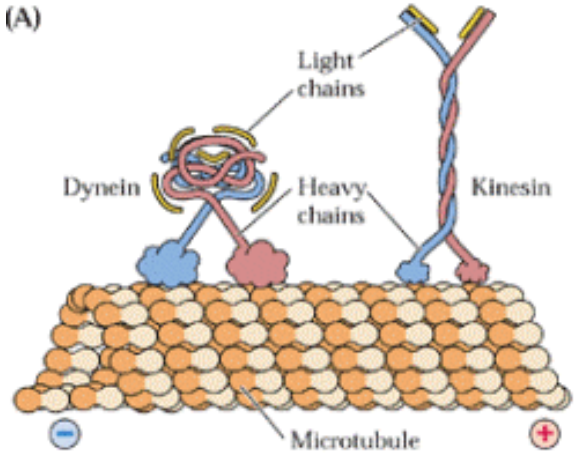
Chara: jak se to měřilo

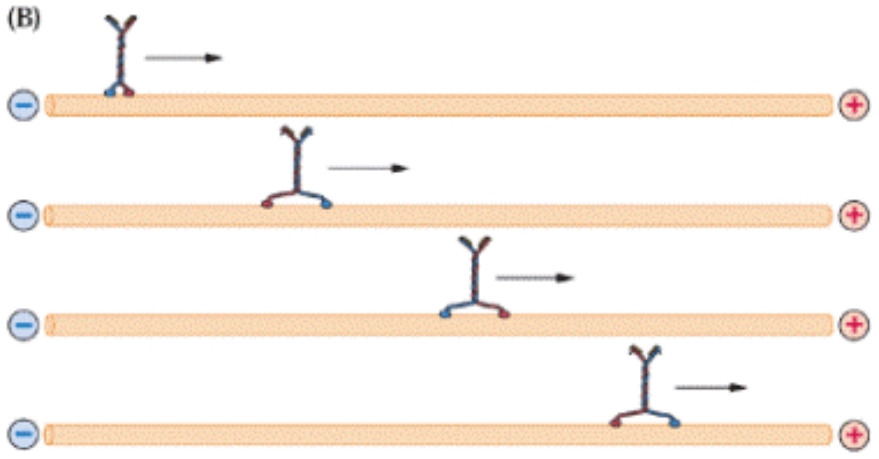


(D)

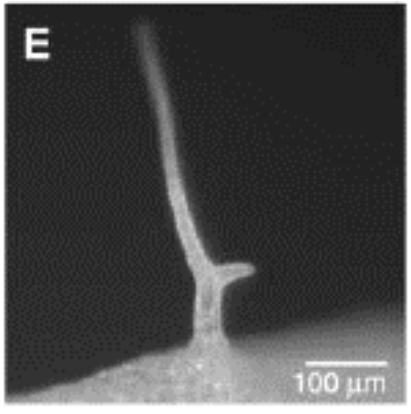


(aktin)

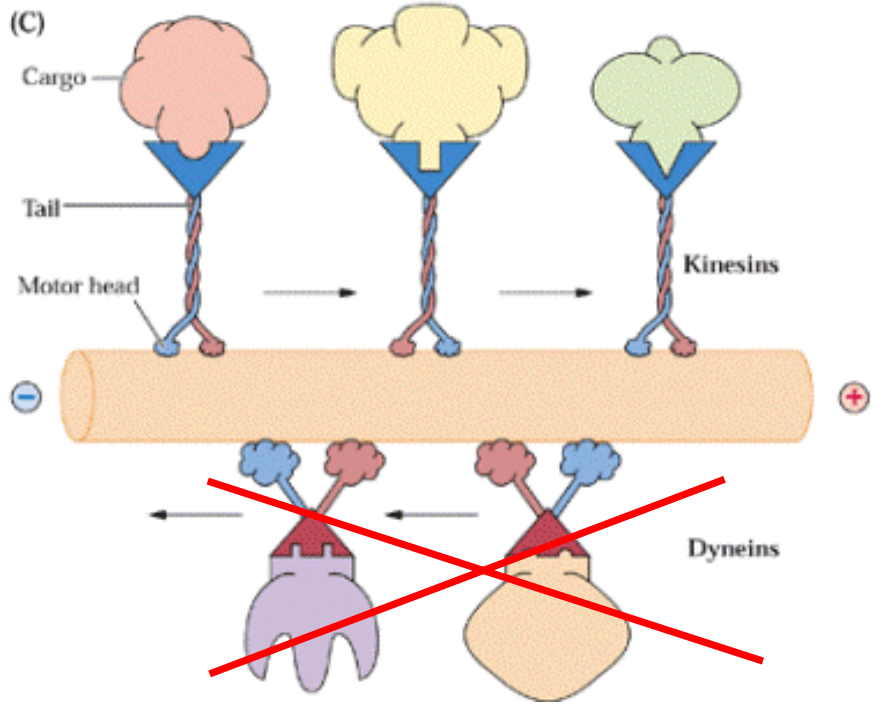




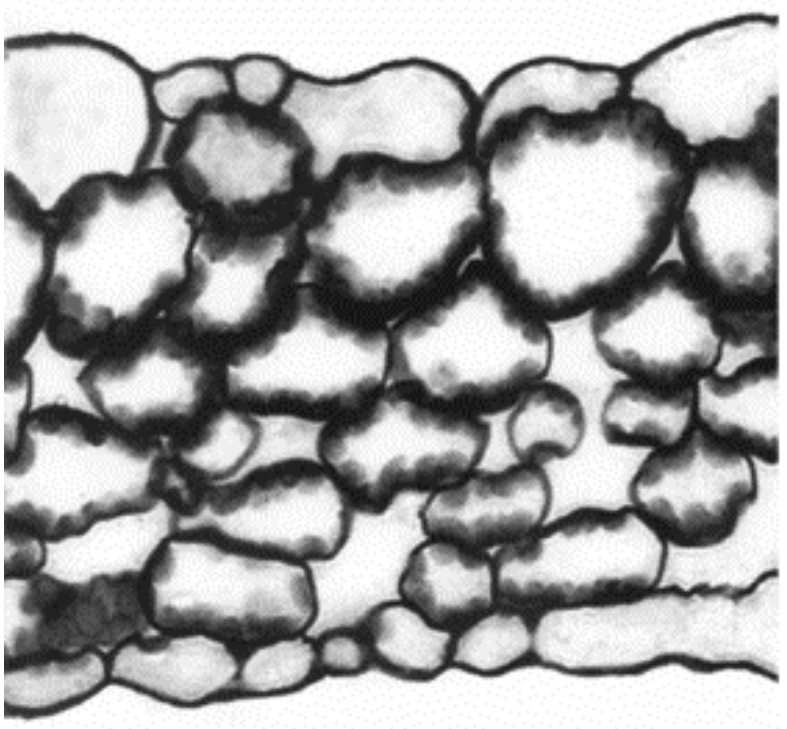
ZWICHEL kóduje kinesin!



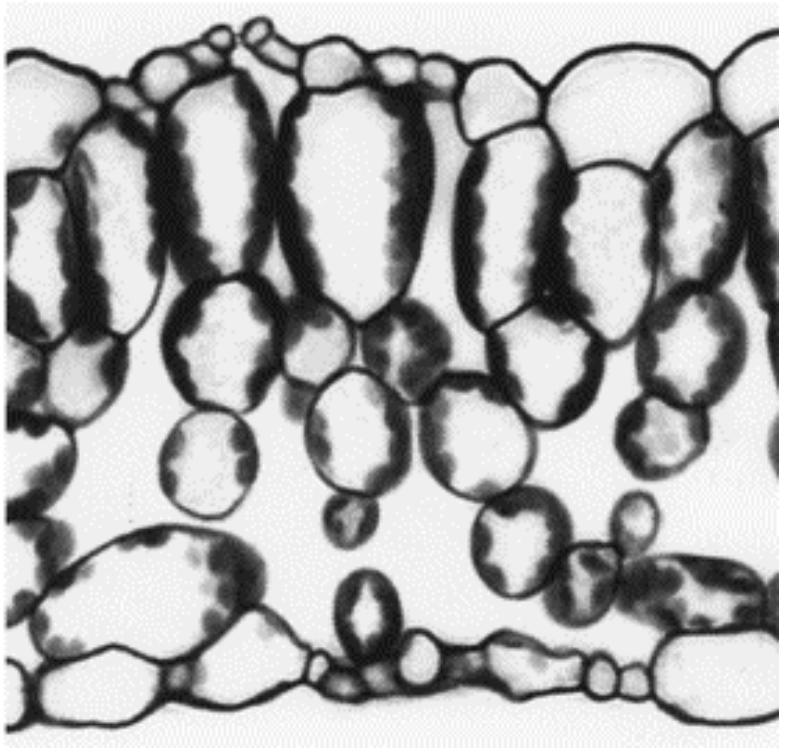
(jeden z mnoha!)



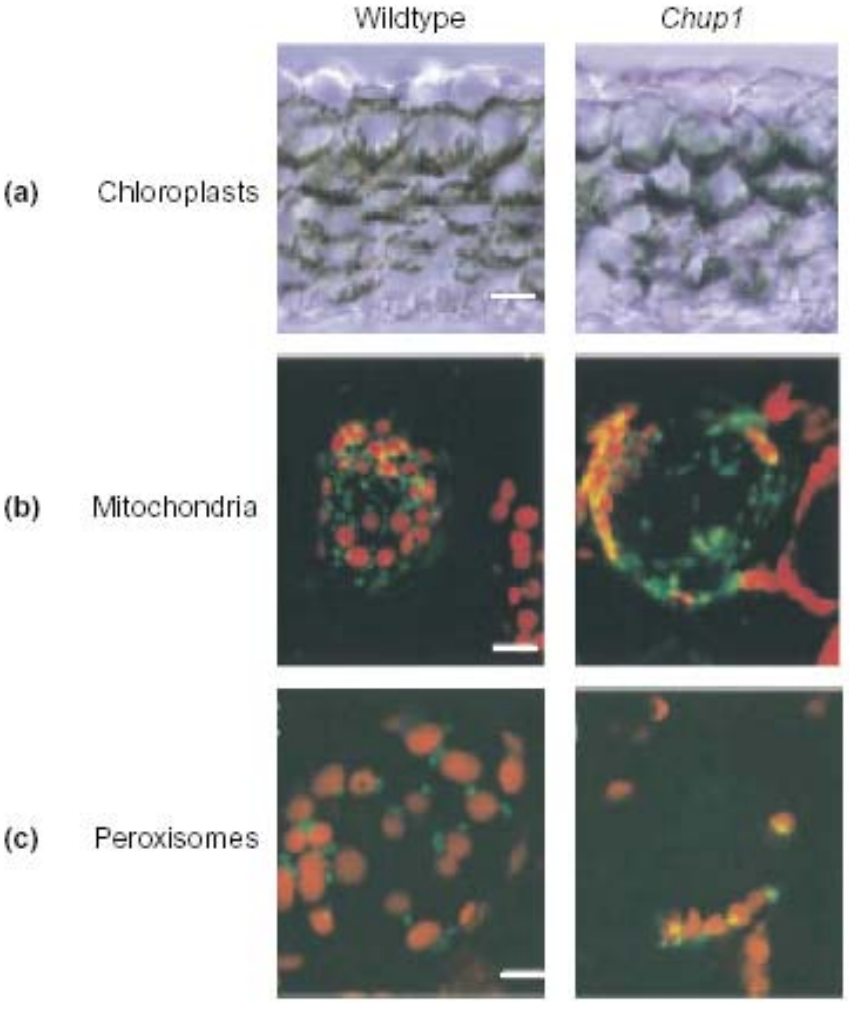
(A)



(B)

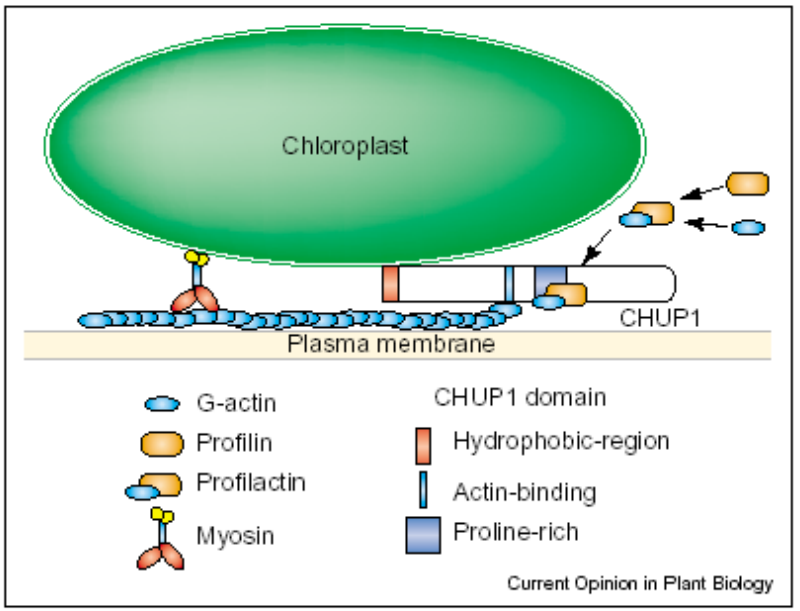


Mutant *chup1* (chloroplast unusual positioning)



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.

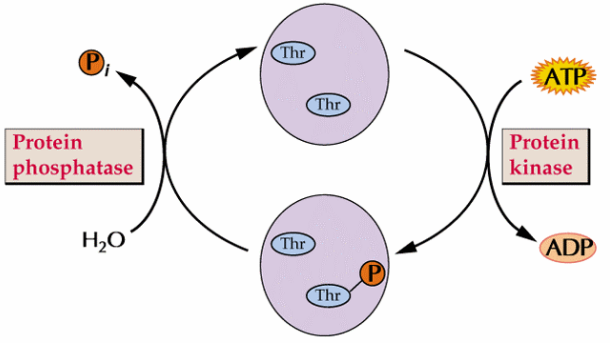
CHUP1: actin binding!

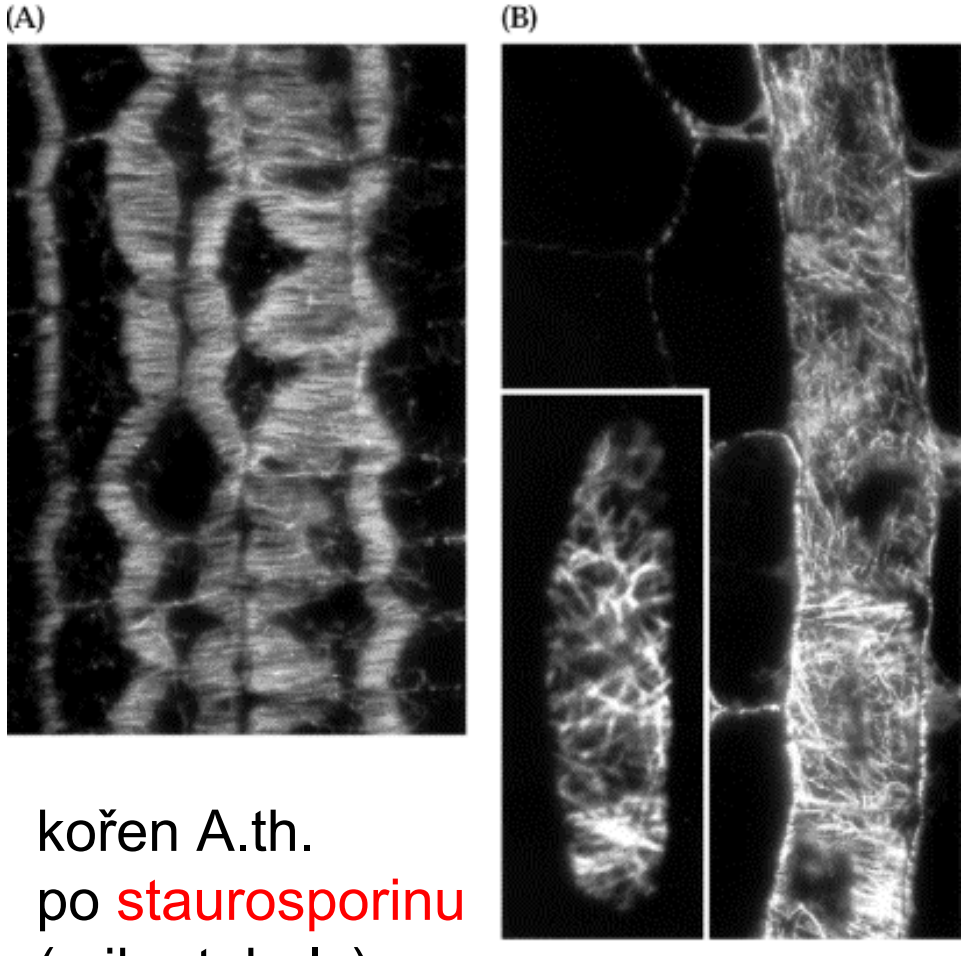


Working model of chloroplast positioning. The amino-terminal hydrophobic region of CHUP1 can target to the chloroplast outer membrane. The proline-rich motif of CHUP1 may serve in actin polymerization to recruit profilactin. CHUP1 binds polymerized F-actin through its actin-binding domain. These functions 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

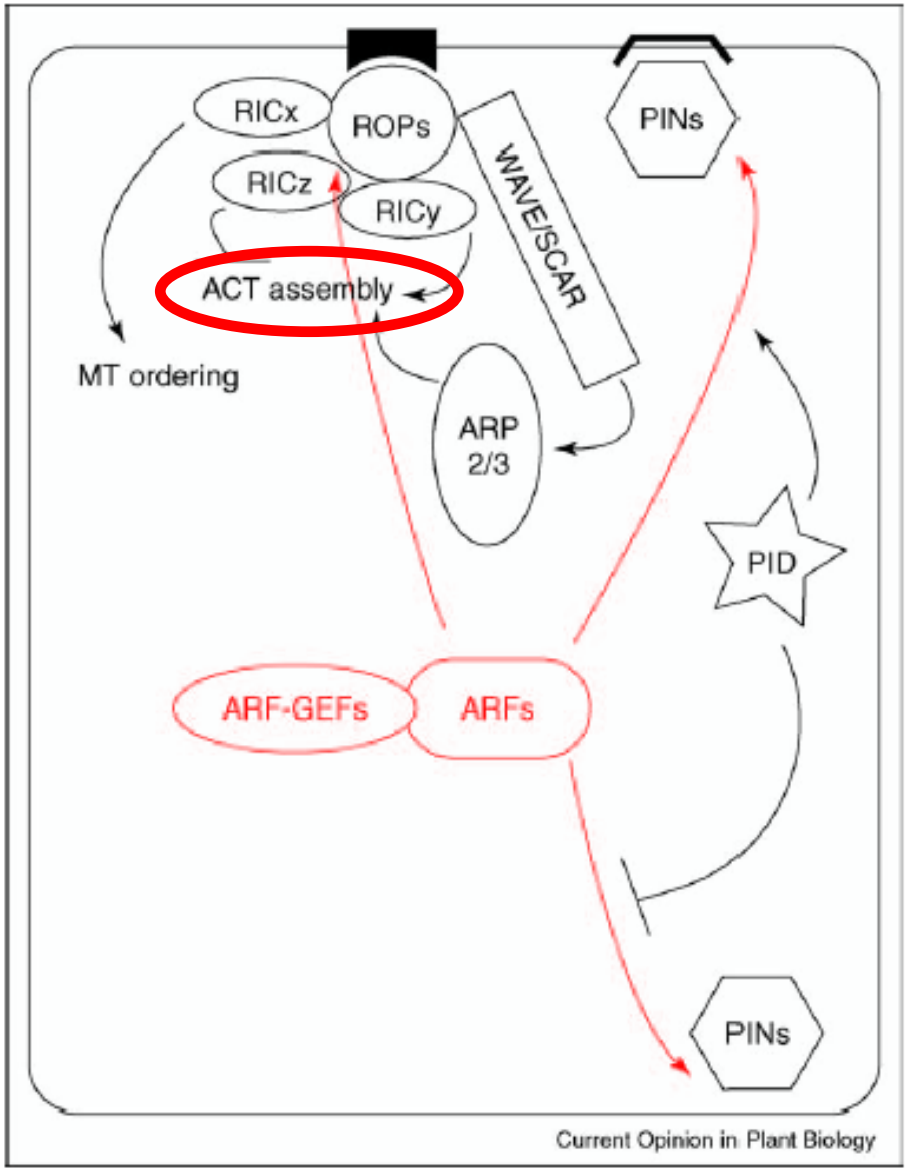
- Jako všude:
  proteinkinázy
  a fosfatázy





kořen A.th.
po staurosporinu
(mikrotubuly)

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.

Příklad: diferenciace epidermálních buněk

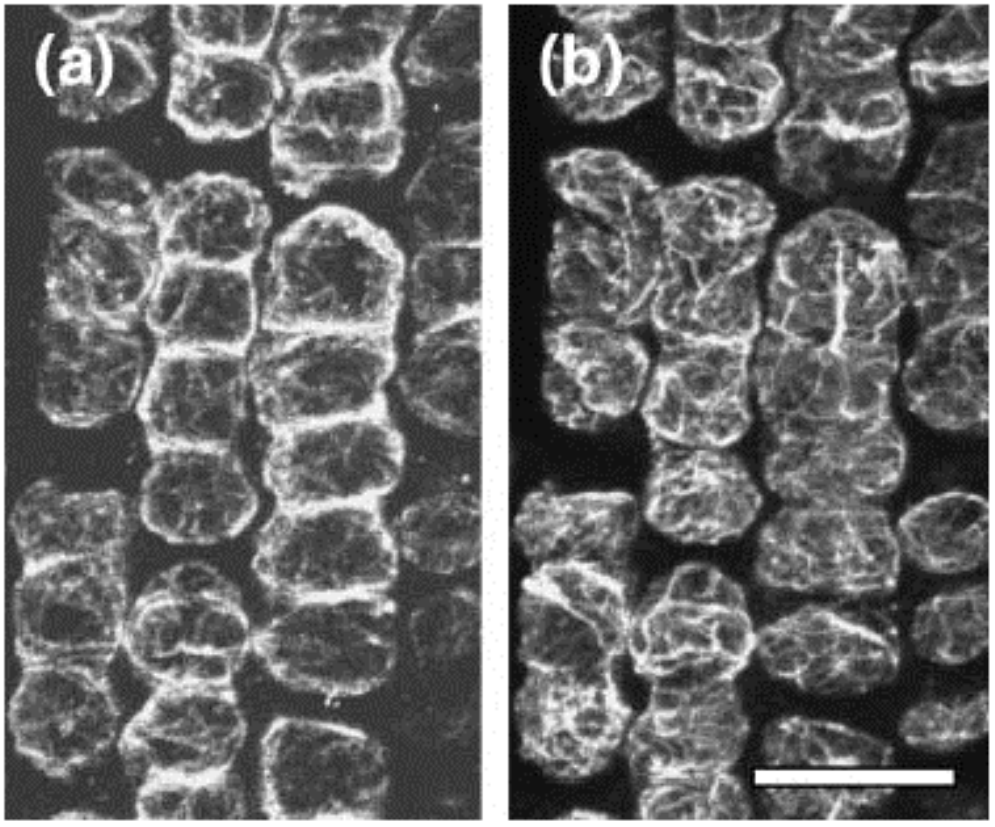
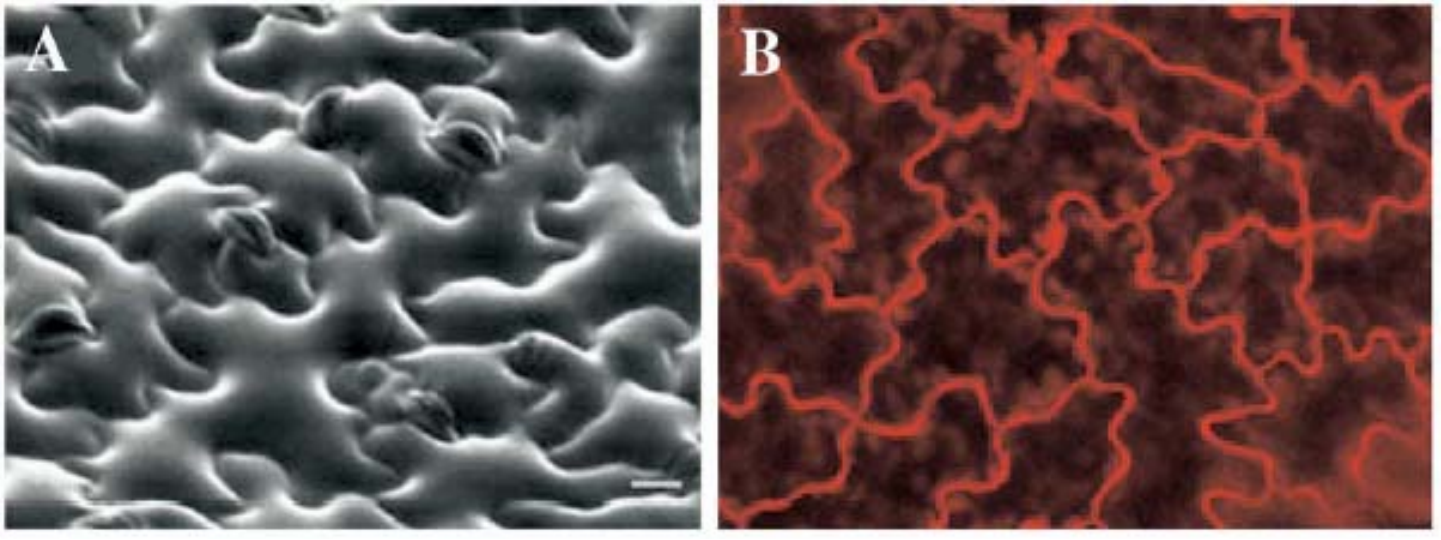


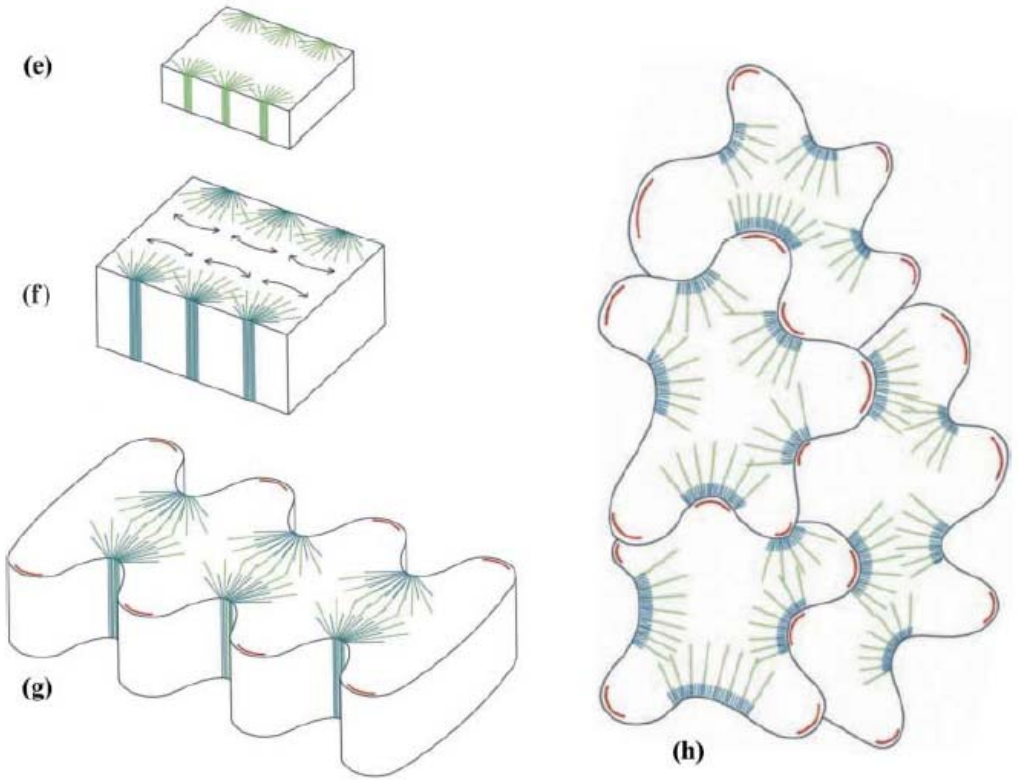
Fig. 1 Confocal-laser-scanning microscope (CLSM) images of lobed mesophyll cells of *Zea mays* after immunostaining of microtubules (MTs) (a) and visualization of actin filaments (AFs) with fluorescent phalloidin (b). Cortical MTs form ring-like bands (a), while AFs (b) exhibit diffuse arrangement. Bar,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

(Panteris a Galatis 2005)

Regulační aspekty: ví se hlavně o aktinu

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





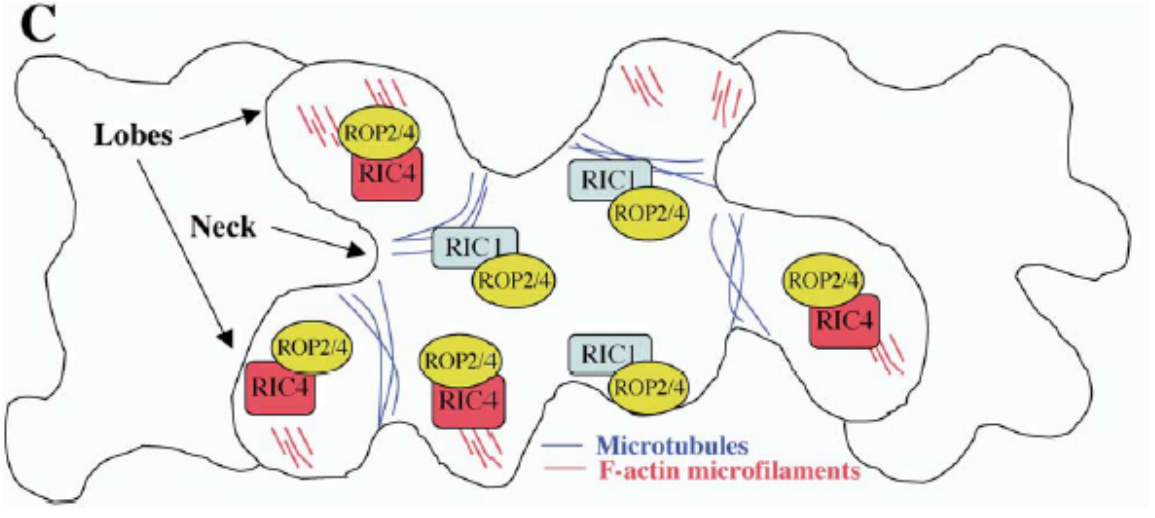
mikrotubuly
celulóz. mikrofibrily
aktin

(Panteris a Galatis 2005)

Fig. 3 Drawing presenting the successive stages of mesophyll (a–d) and pavement (e–h) cell morphogenesis. Microtubules (MTs) are shown in green, cellulose microfibrils (CMF) in blue and actin filament (AF) patches in red. In (a–d) cell wall thickenings, with CMFs parallel to MTs, are not shown. (a) Young mesophyll cell; the cortical MTs form interconnecting ring-like bundles. (b) As mesophyll cells grow, lobes and constrictions are created. New MT bundles also form. (c) Further mesophyll cell growth leads to formation of additional lobes. (d) Microtubule rings form at the base of growing mesophyll cell lobes, while AF patches (red lines) assemble at their domes. New axes of cell growth (arrows) are established. Apart from the AF patches, AFs exhibit diffuse pattern of organization and are not depicted. (e) Cortical MTs form bundles that line the anticlinal walls and radial MT arrays at the junctions of the external periclinal with the anticlinal walls in a young pavement cell. (f) Later morphogenetic stage of a pavement cell. Local cell wall thickenings with CMFs parallel to the underlying MTs are deposited. Arch-like tangential expansion (arrows) is imposed on the external periclinal cell face. (g) Lobes and constrictions are initiated in this morphogenetic stage of pavement cells, while AF patches (red lines) assemble at the domes of growing cell lobes. (h) Paradermal view of an epidermal area during cell lobe formation and expansion. Note that cortical MT arrays, wall thickenings with CMFs and AF patches are alternate between neighboring pavement cell resulting in a 'jigsaw-puzzle' top view.

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

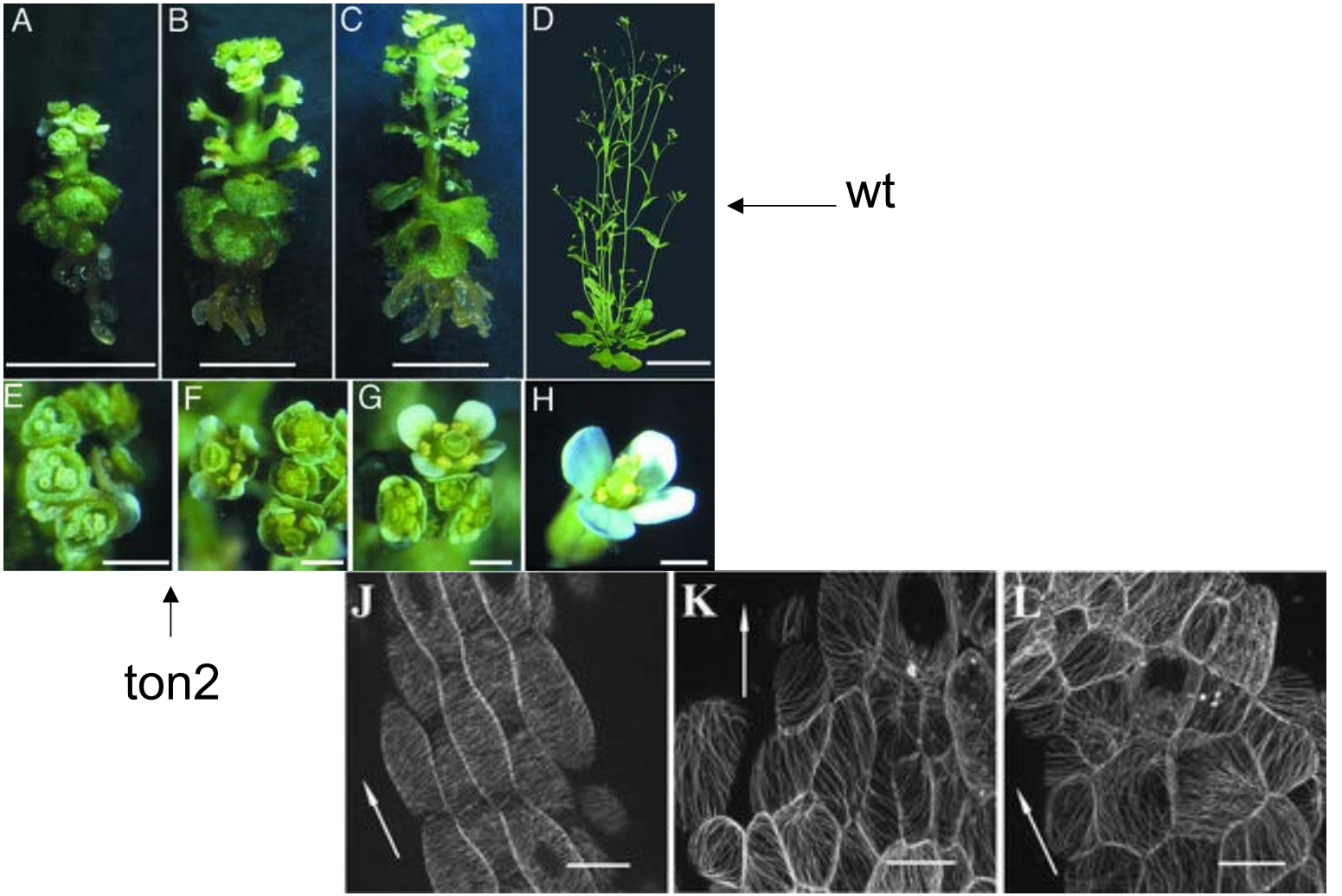


(C) Schematic representation of the model proposed by Fu et al. to explain the role of ROP GTPases and their effectors (RICs) in leaf morphogenesis. ROP2/4 GTPases, via activation of RIC4, promote actin microfilament formation in regions of growing lobes. At the same time, the ROPs, via RIC1 binding, 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.

ROP GTPázy = Rho of plants (Rho GTPázy)

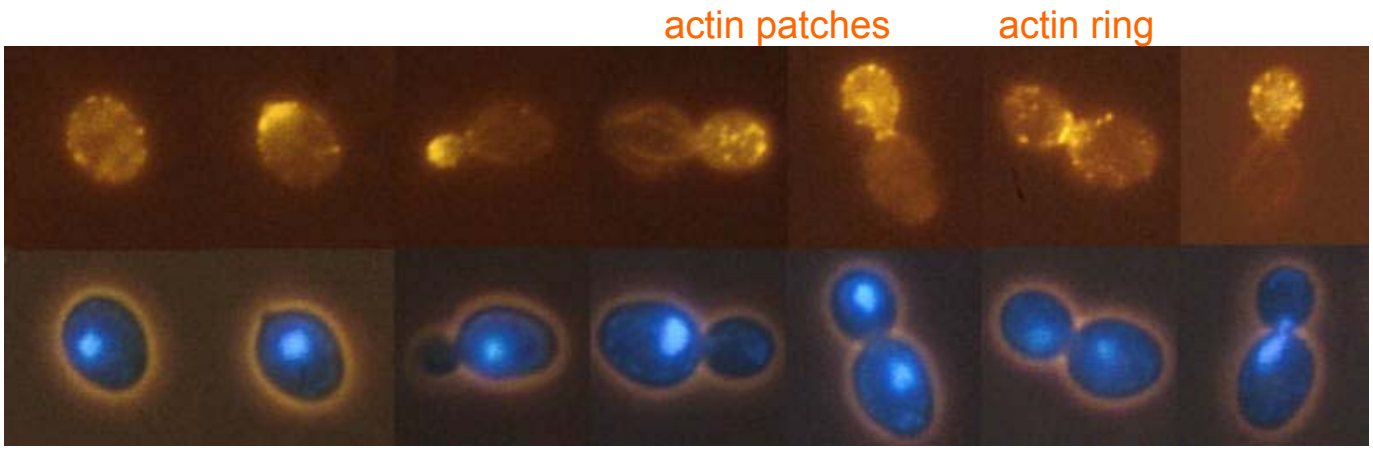
CRIB doména = Cdc42/Rac interactive binding domain

TON2 kóduje protein fosfatázu A



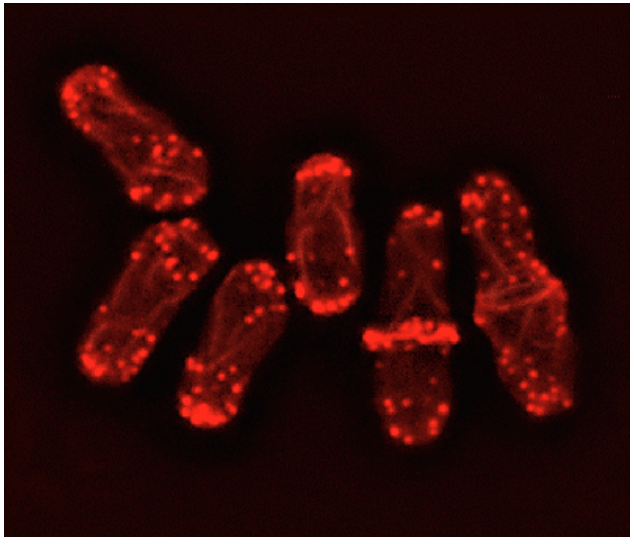
Cytoskelet kvasinek

Aktinový cytoskelet během buněčného cyklu

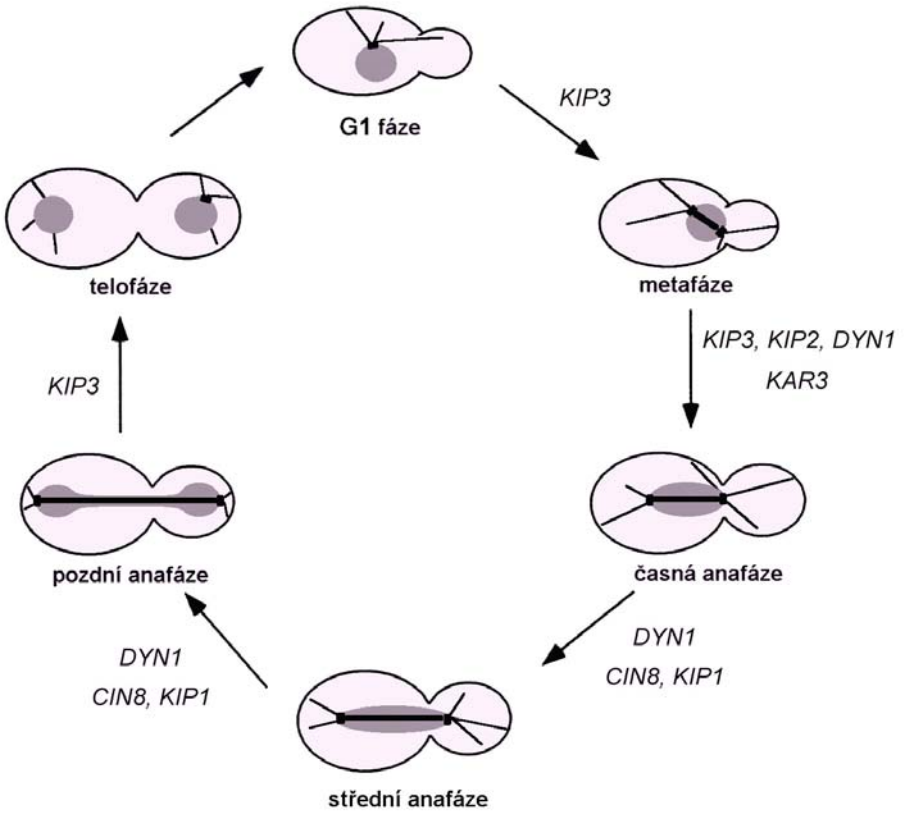


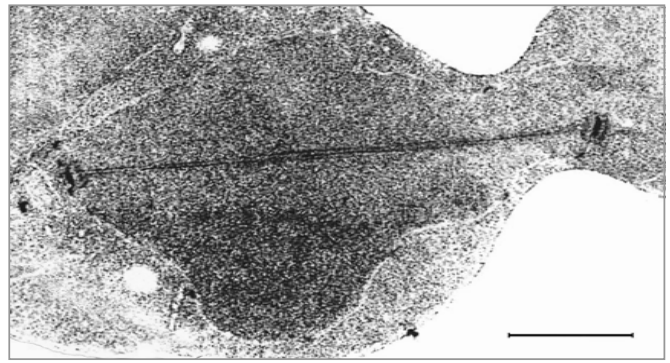
Saccharomyces cerevisiae

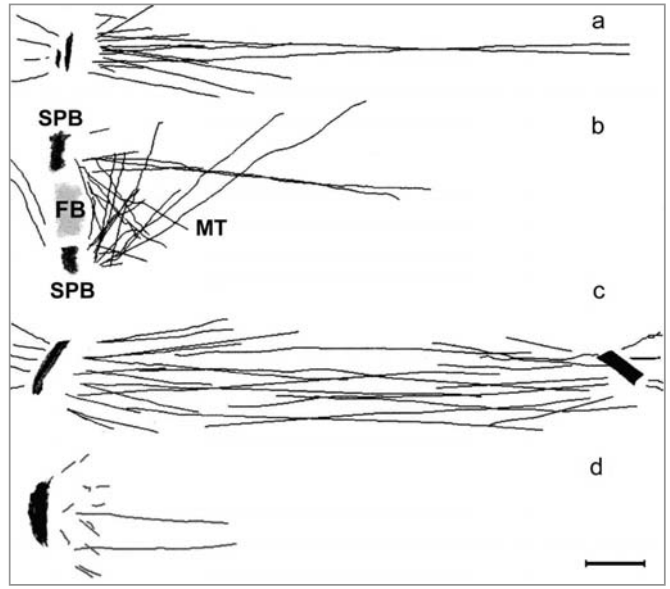
Schizosaccharomyces pombe



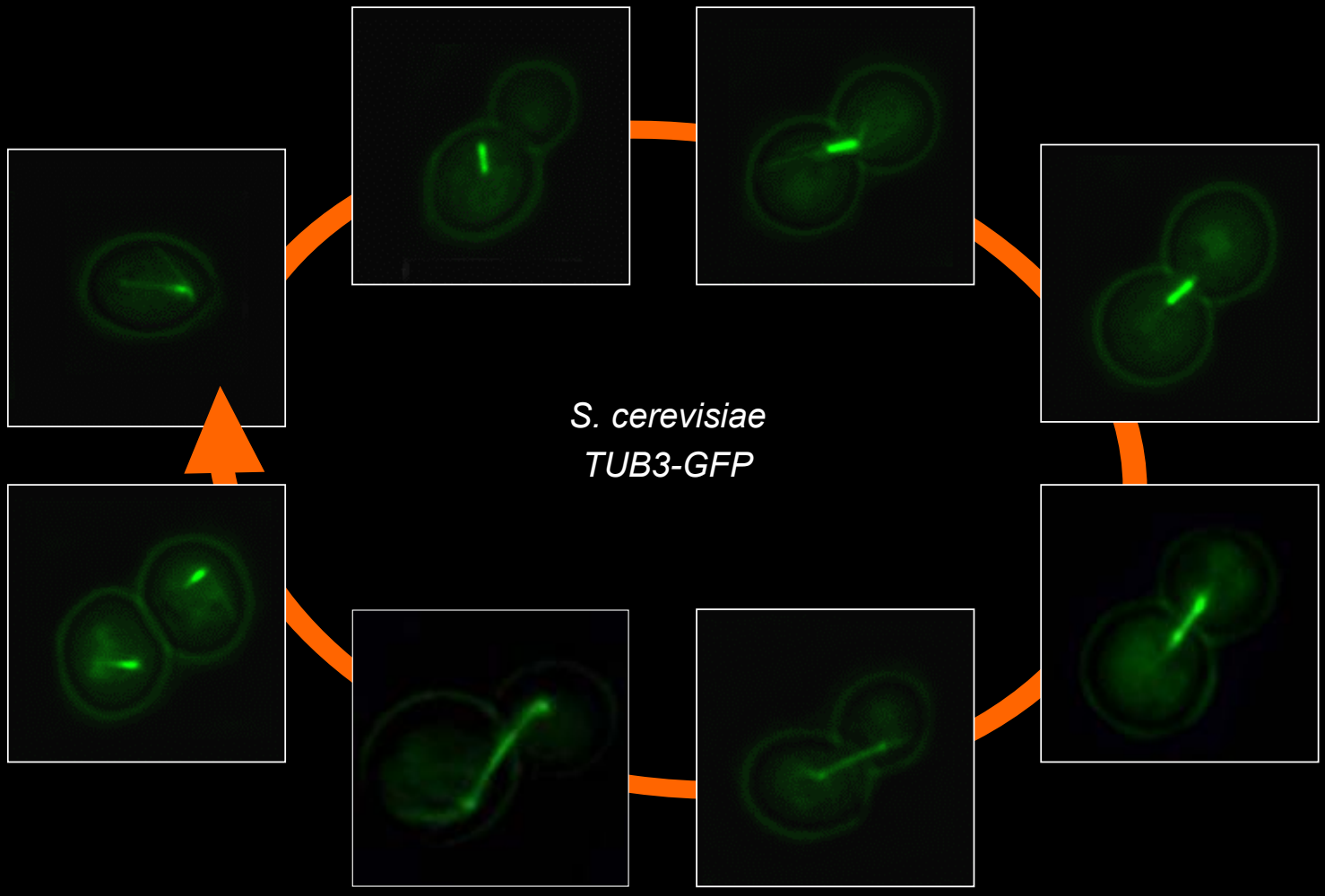
Mitotické vřeténko během buněčného cyklu



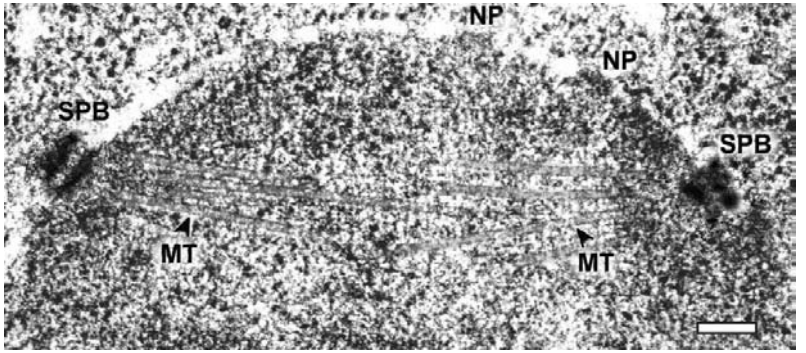


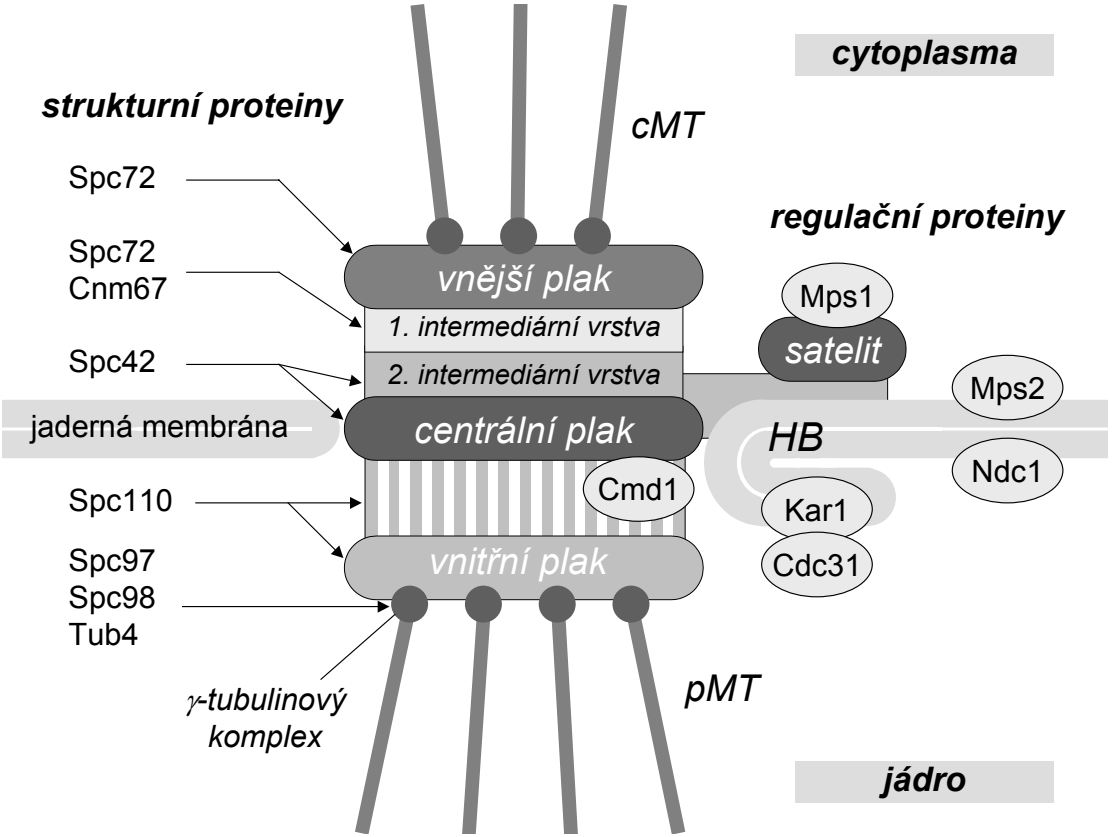


Tubulinový cytoskelet během buněčného cyklu

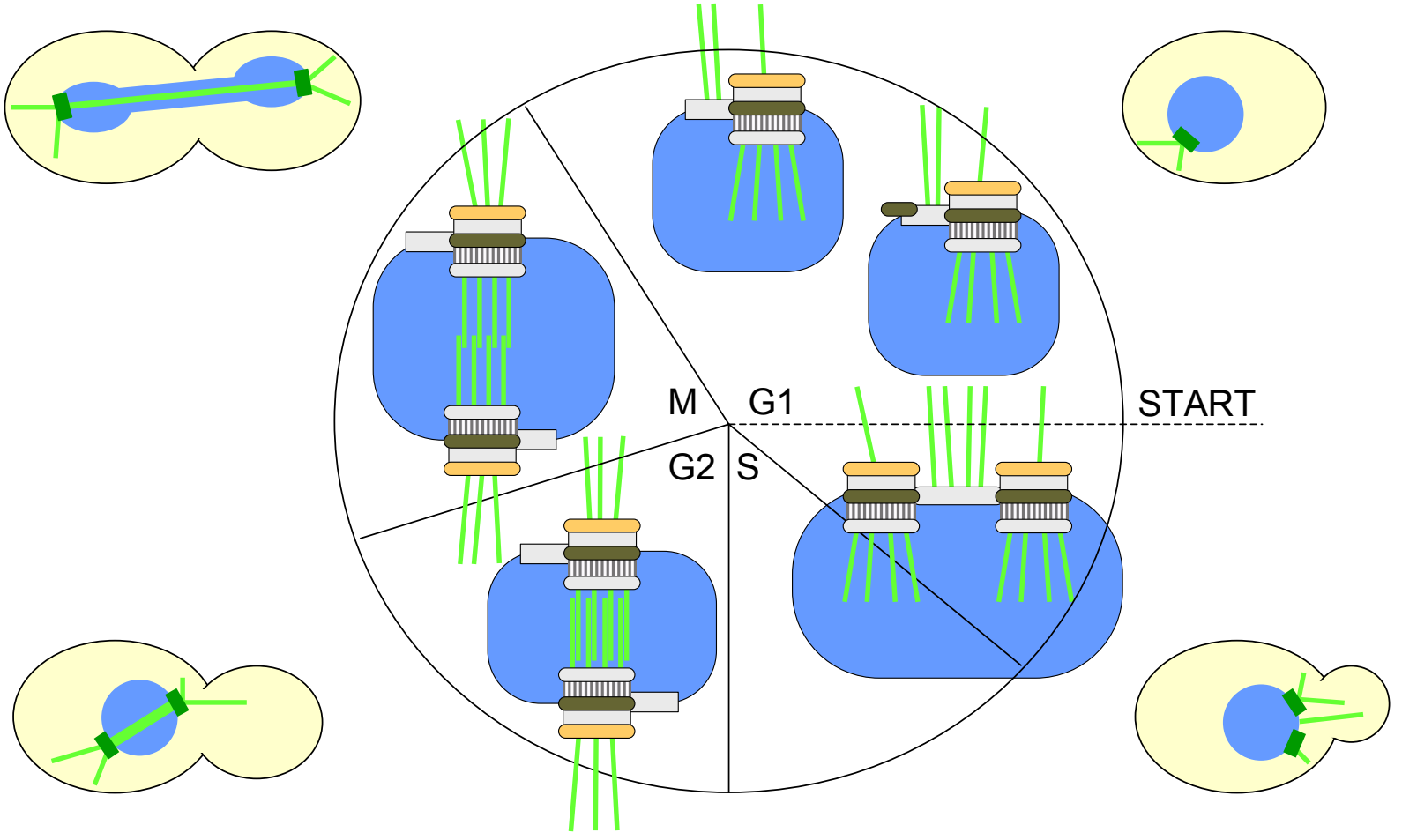


Spindle pole body (SPB)

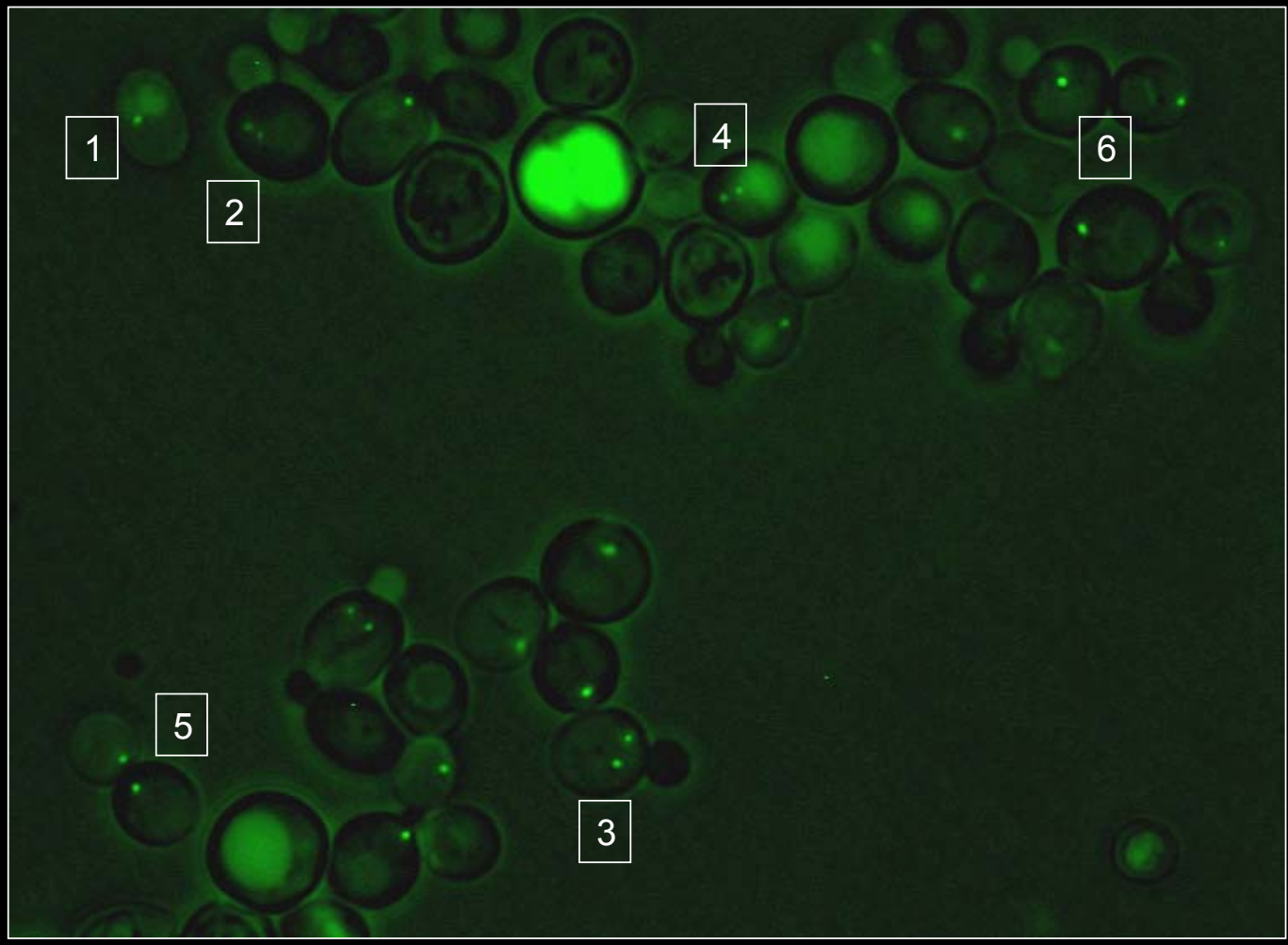




SPB během buněčného cyklu



SPB během buněčného cyklu



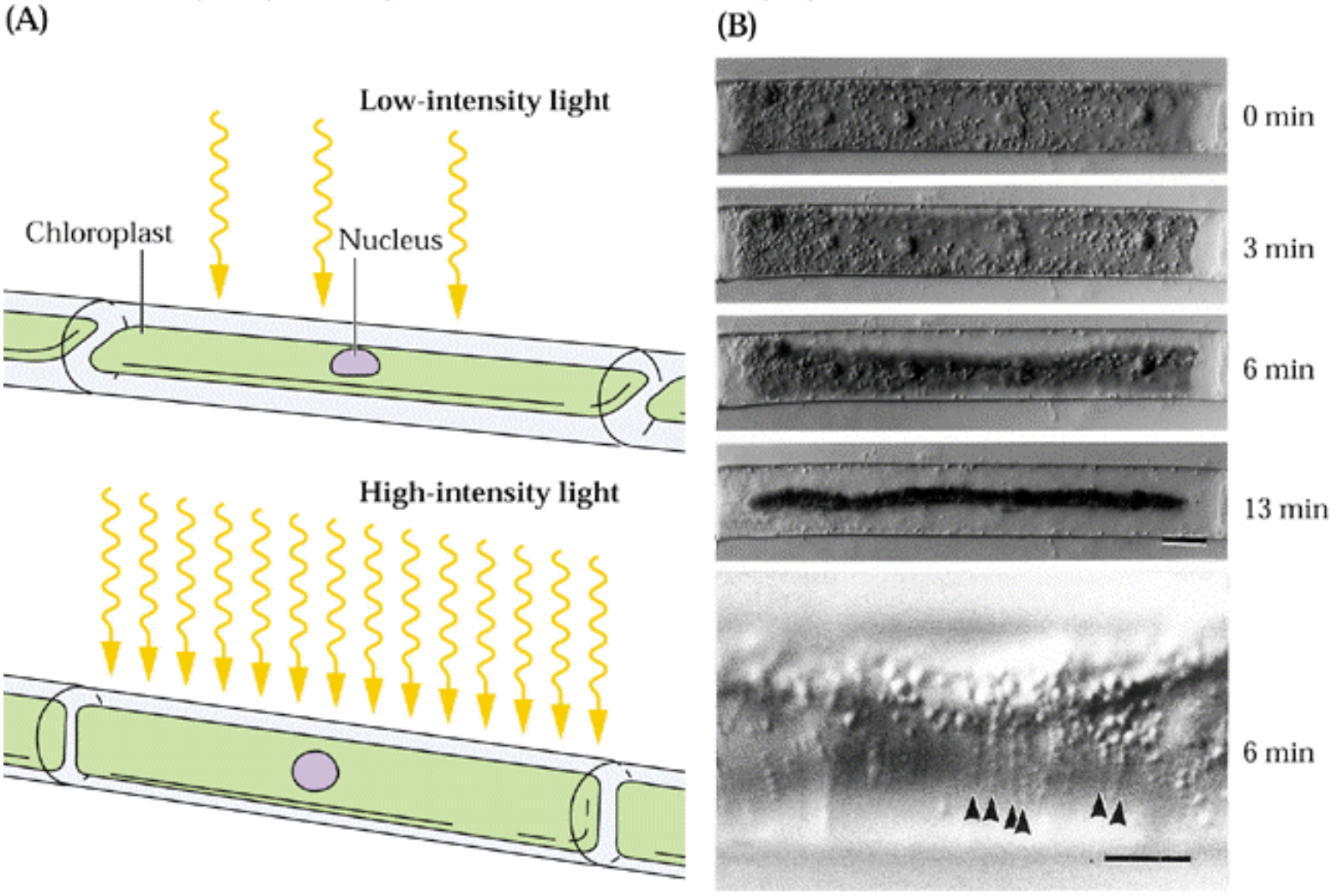
Molekulové motory

Molekulové motory

- 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 a mitotického vřeténka, membr. váčků, organizace mikrotubulů ...
  - kinesiny (typicky k +konci) a dyneiny (k -konci)

Pohyby organel: plastidy jdou za světlem



Mougeotia

MOLEKULOVÉ MOTORY

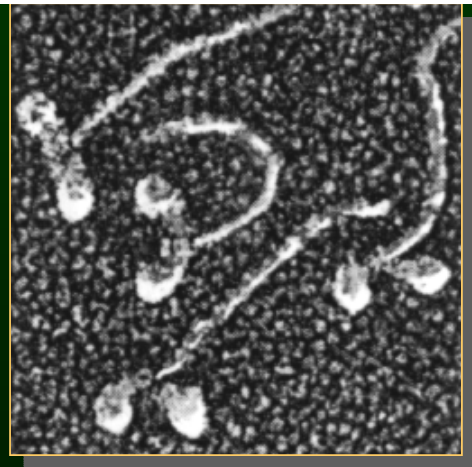
DEFINICE

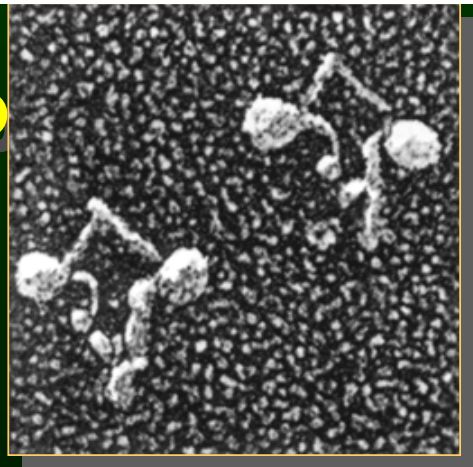
Proteiny, které jsou schopné posouvat se na úkor energie ATP po cytoskeletálních vláknech vždy jedním určitým směrem. Hydrolýza ATP vede k cyklickým konformačním změnám jejich molekul, což umožňuje jejich pohyb.

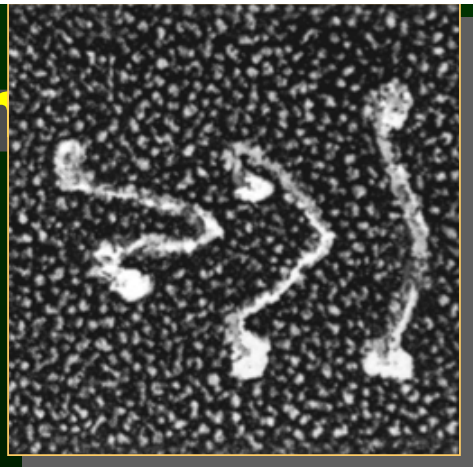
1965 - axonemální dynein (Gibbons)
1985 - kinezin (Brady, Vale , Scholey)
1987 - cytoplazmatický dynein (Lye, Scholey, McIntosh)

FUNKCE MOLEKULOVÝCH MOTORŮ

- transport váčků
  (endocytóza, exocytóza, přenos signálů, atd.)
- transport molekul nebo komplexů (mRNA)
- pohyb organel
- proudění cytoplazmy
- segregace chromosomů v mitóze a meióze
- splývání jader (karyogamie)
- buněčná polarita, morfogeneze
- vliv na dynamiku mikrotubulů
- komunikace
- svalový stah



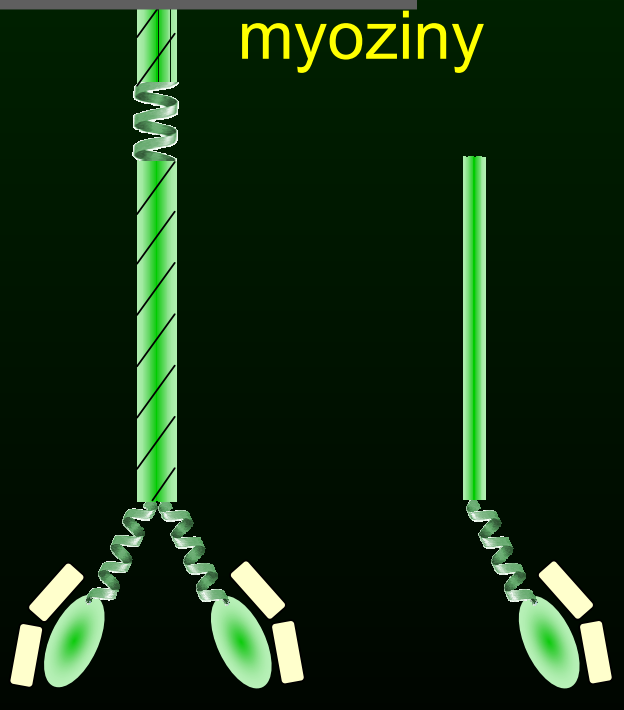


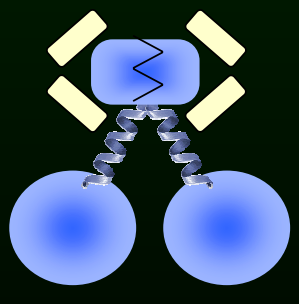


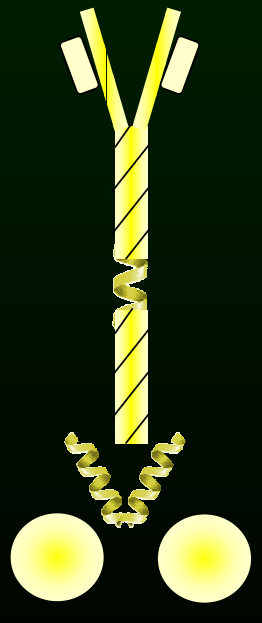
myoziny

dyneiny

kineziny







mikrofilamenta | IF | mikrotubuly

Nadrodiny molekulových motorů

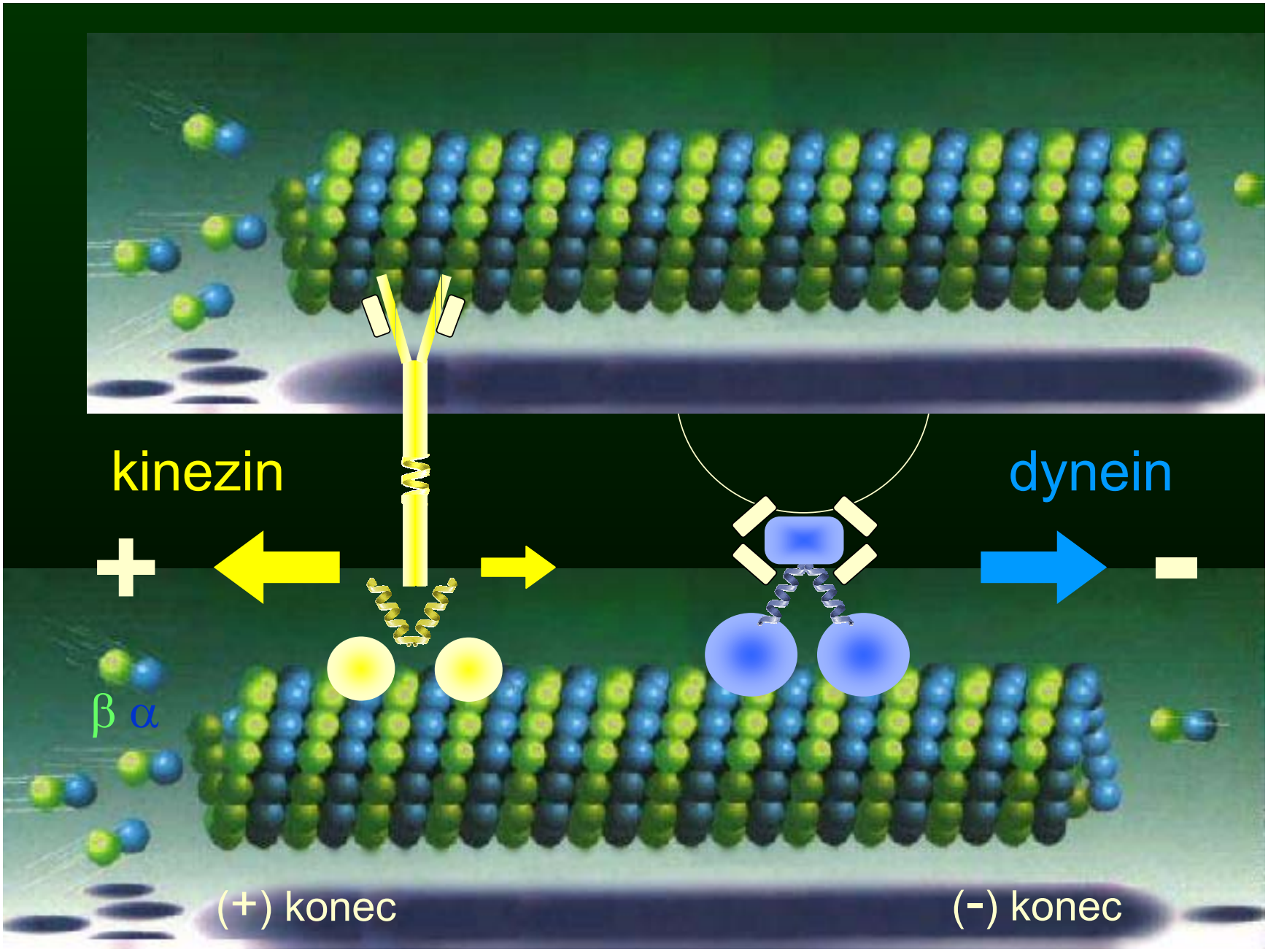
	S. cer.	*A. th.*	počet tříd
myoziny	5	17	15
kineziny	6	61	10 + nezař.
dyneiny	1	0	

- funkční redundance

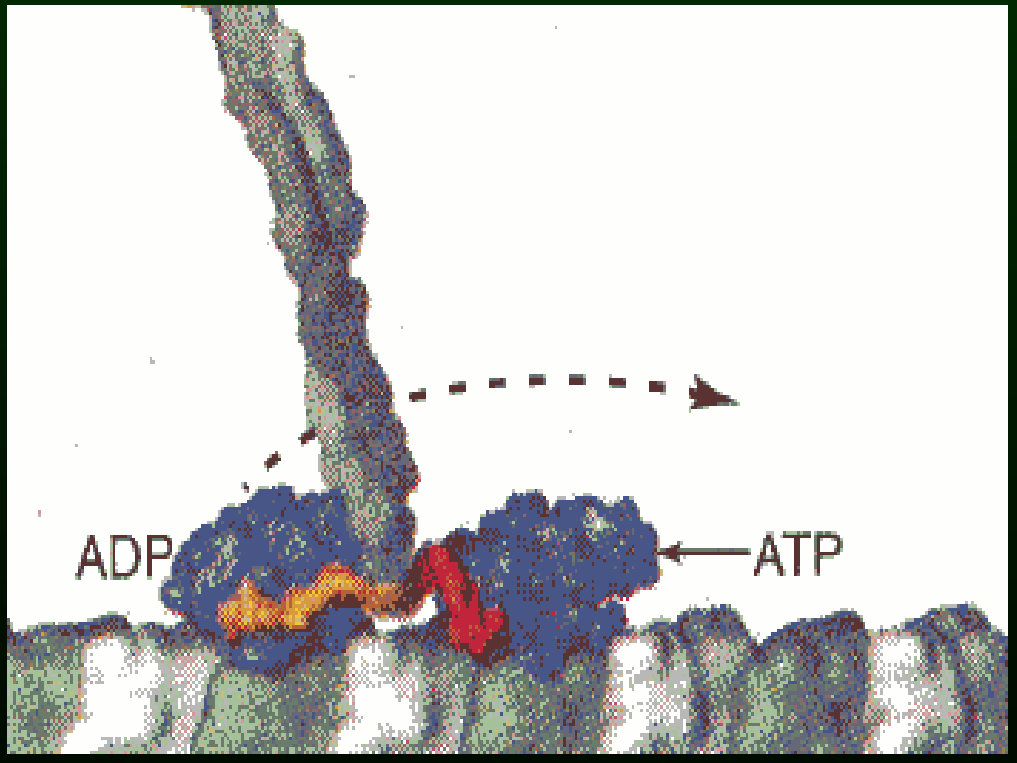
Kineziny

Arabidopsis má 61 kinezinů

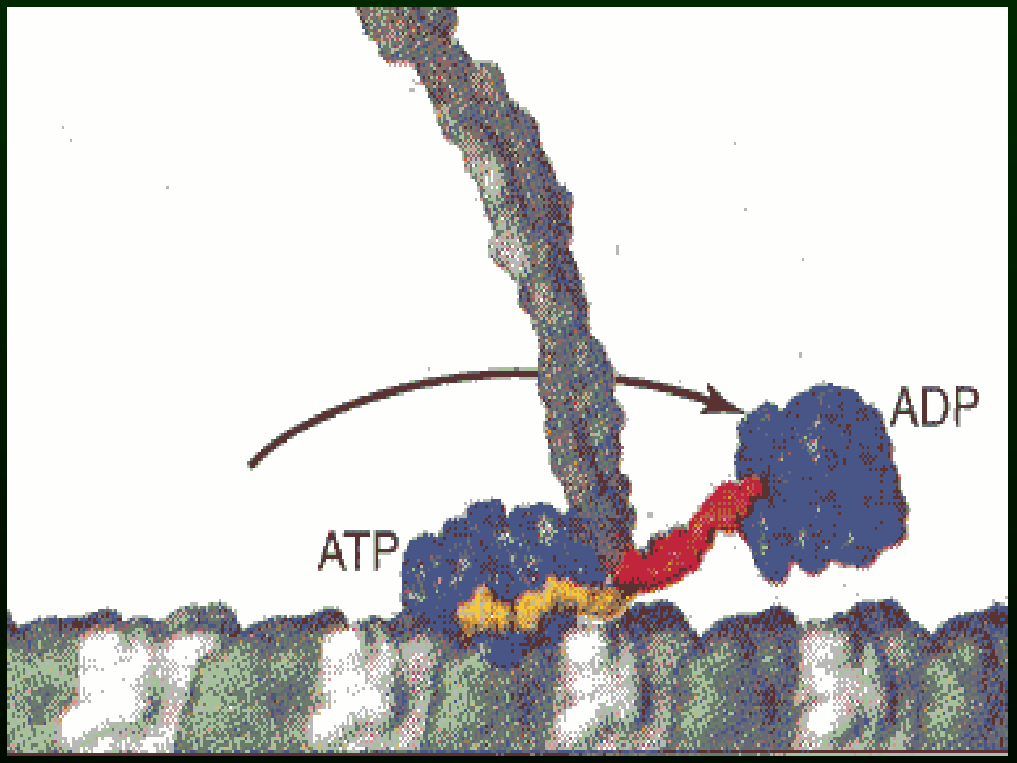
- pohyb po mikrotubulech oběma směry (zajišťován různými proteiny), směr určuje „krček“
- většina (živočišné a 40 u A.th.) + end-directed
  - často role v cytokinezi (PAKPs – phragmoplast-associated kinesin proteins)
- zbytek (21) – end-directed
  - ATK1 – funkce vřeténka
  - ZWI – regulace calmodulinem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


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

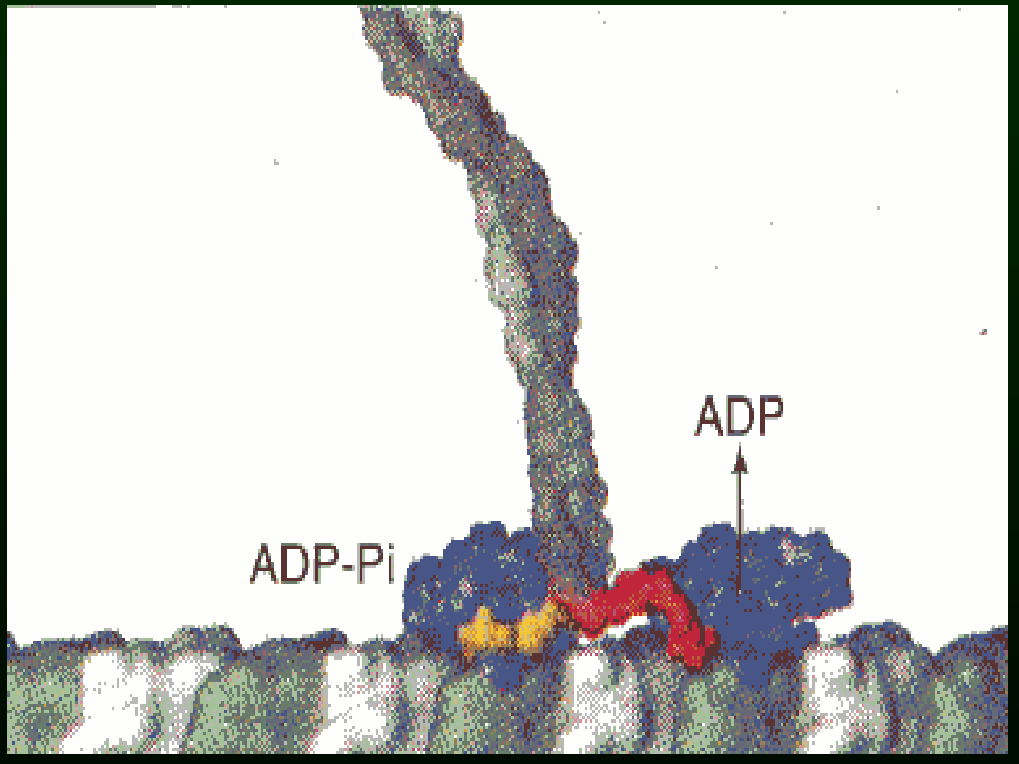
mechanismus pohybu kinezinu



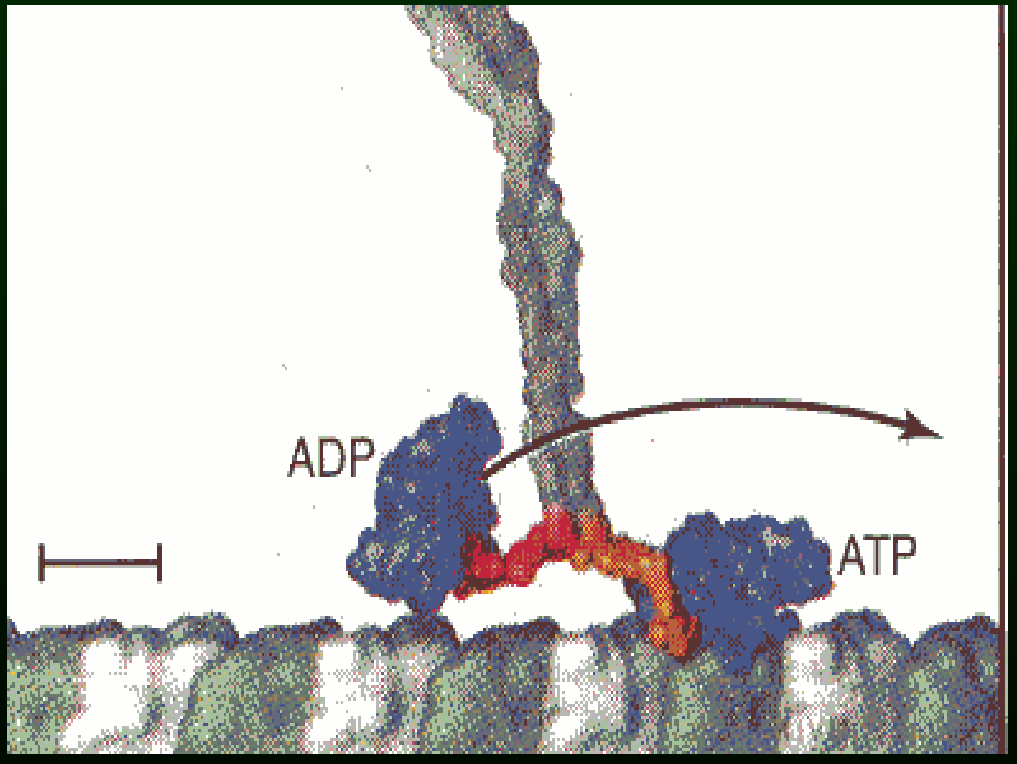
mechanismus pohybu kinezinu



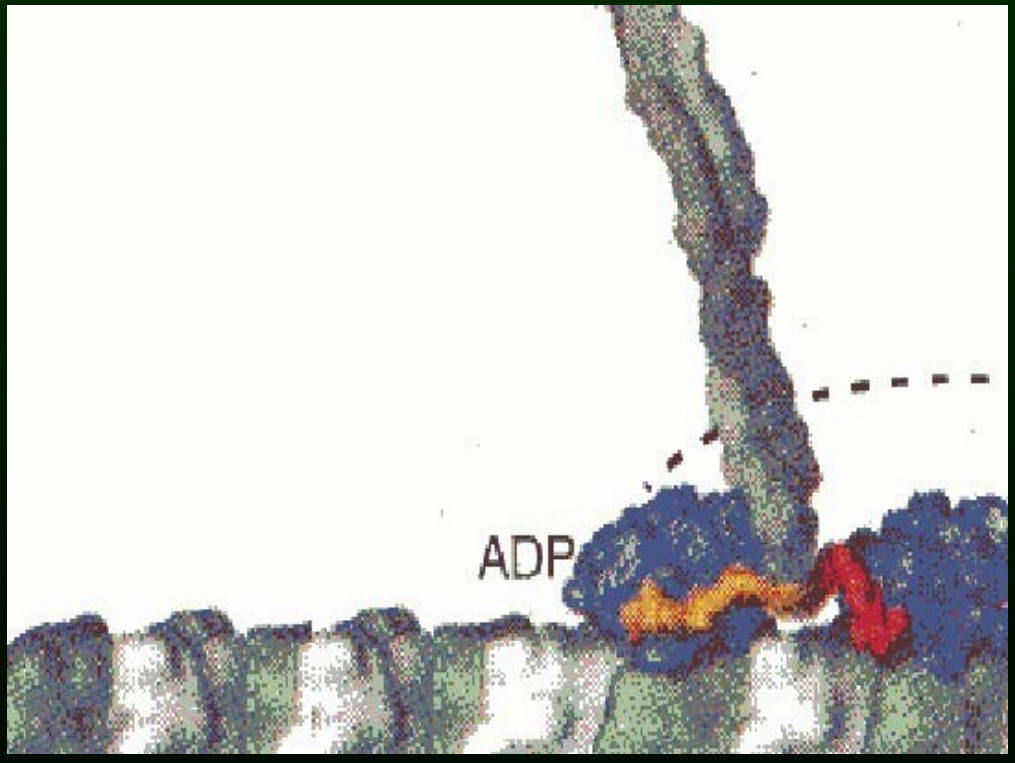
mechanismus pohybu kinezinu



mechanismus pohybu kinezinu

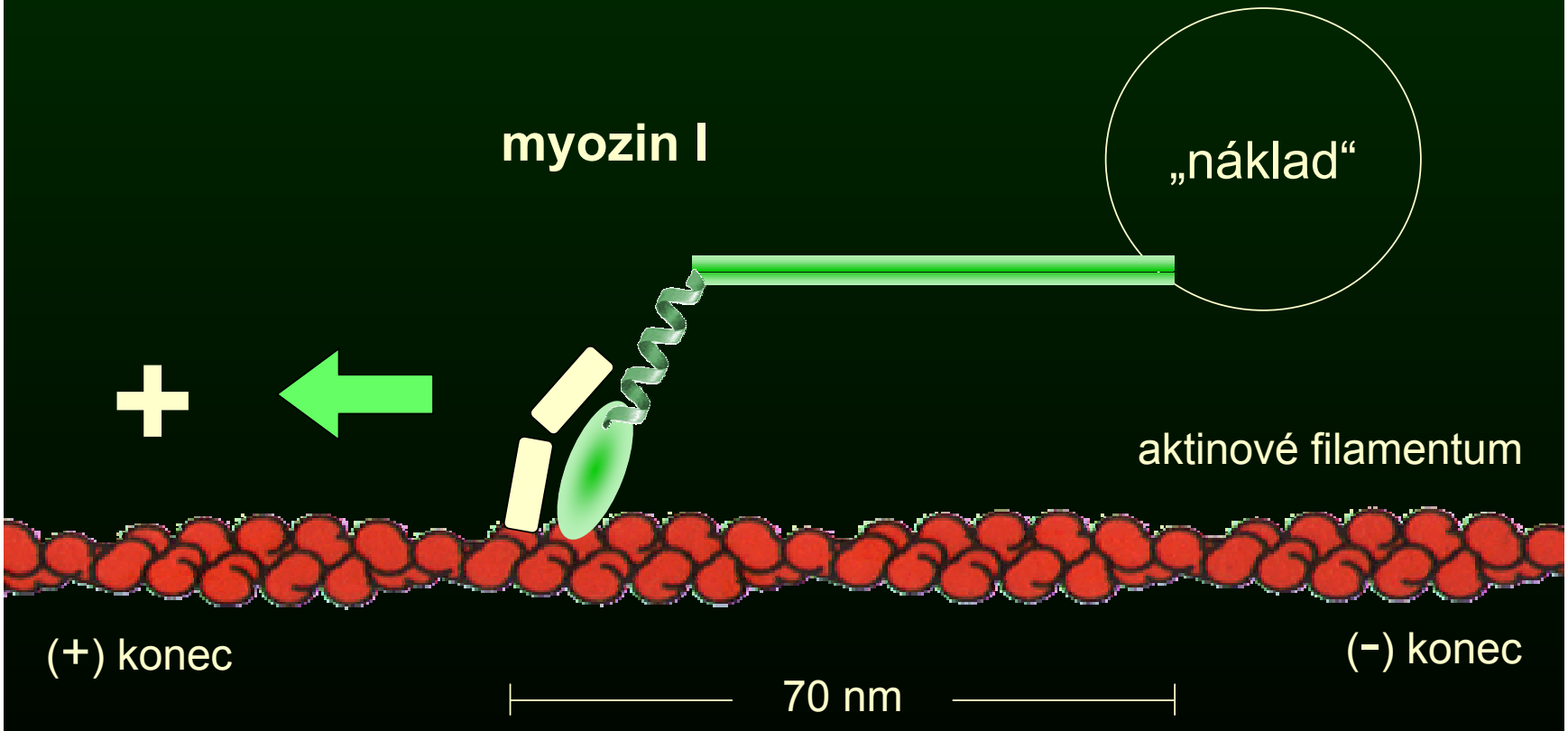


mechanismus pohybu kinezinu

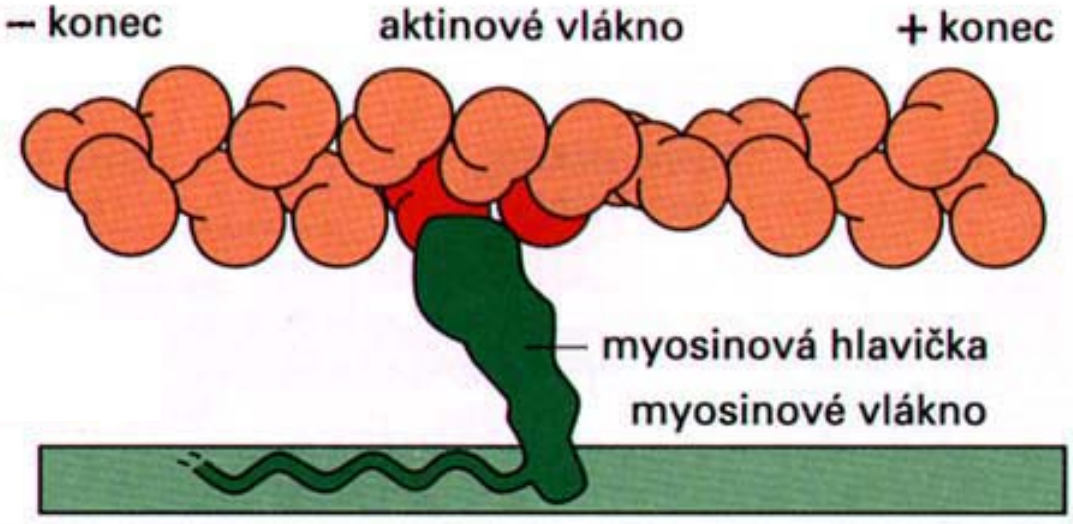


ANIMACE 1

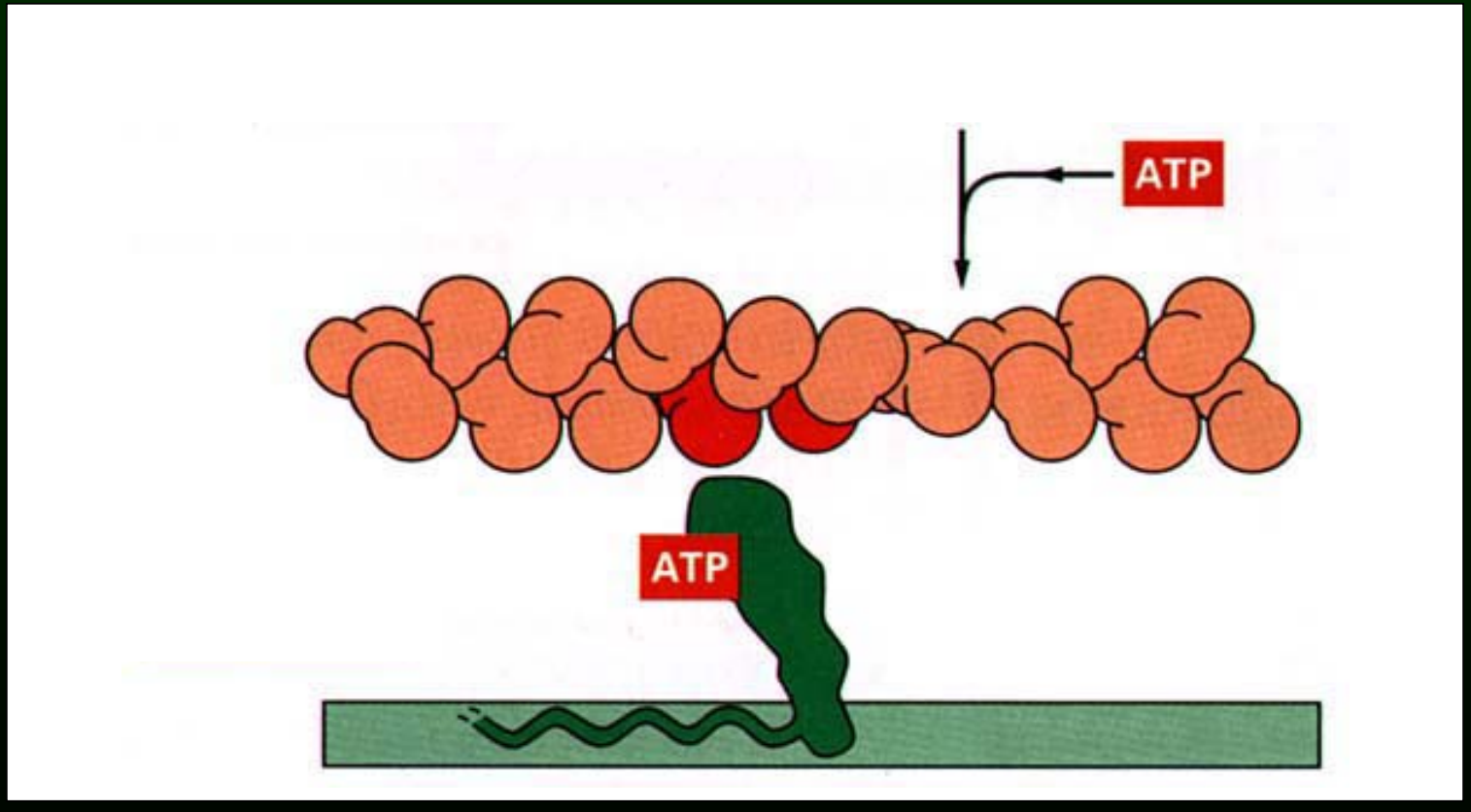
Myoziny



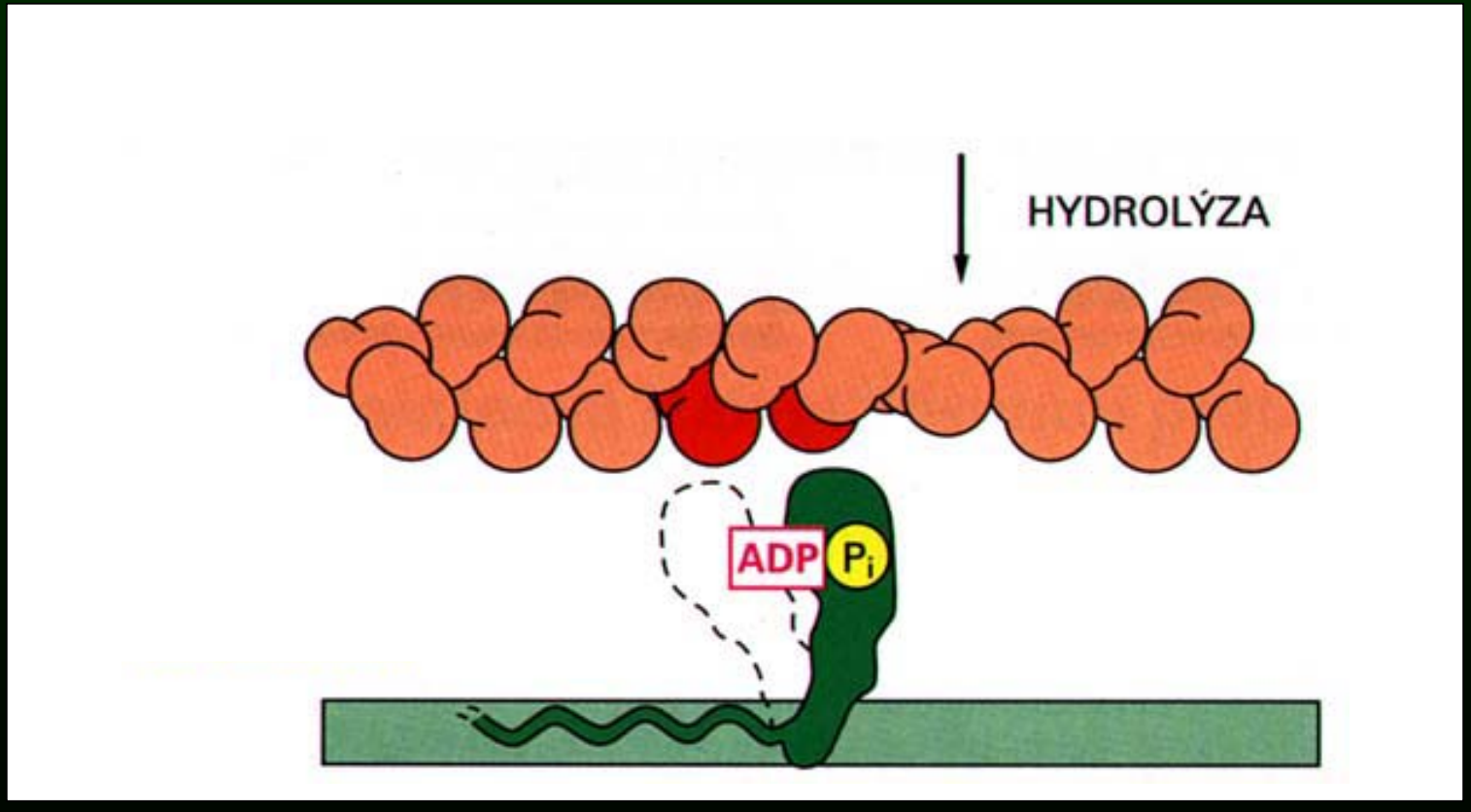
mechanismus pohybu myozinu



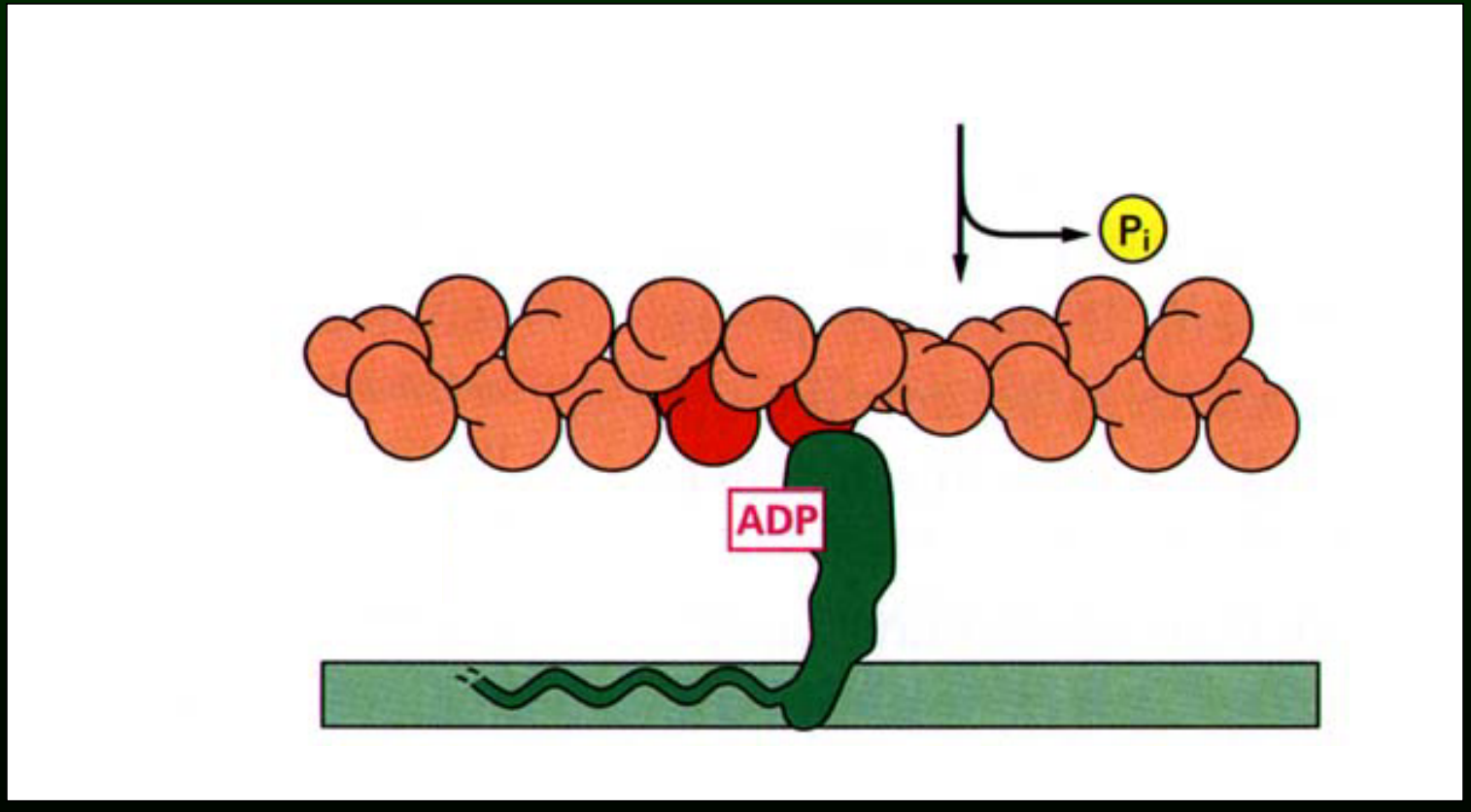
mechanismus pohybu myozinu



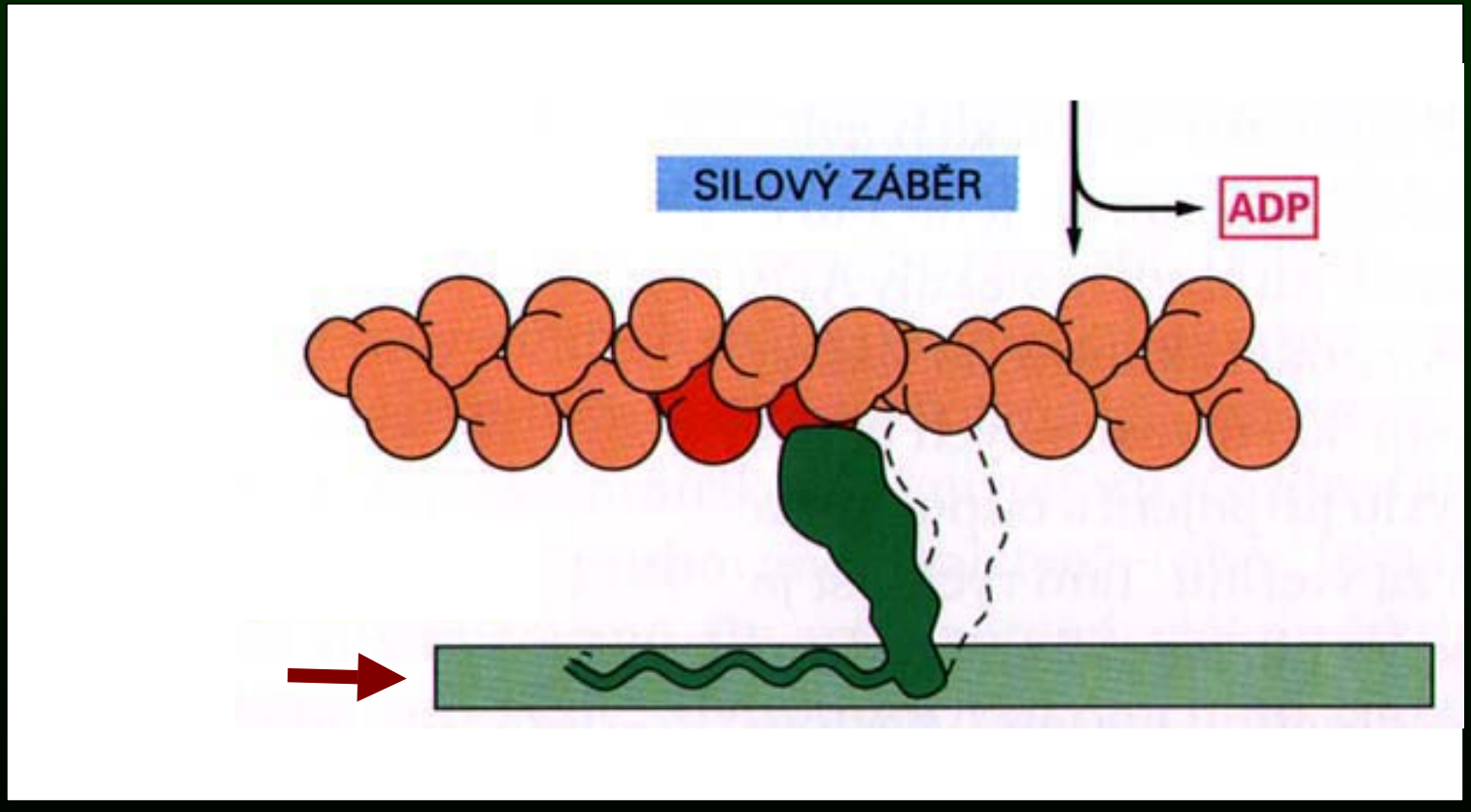
mechanismus pohybu myozinu



mechanismus pohybu myozinu

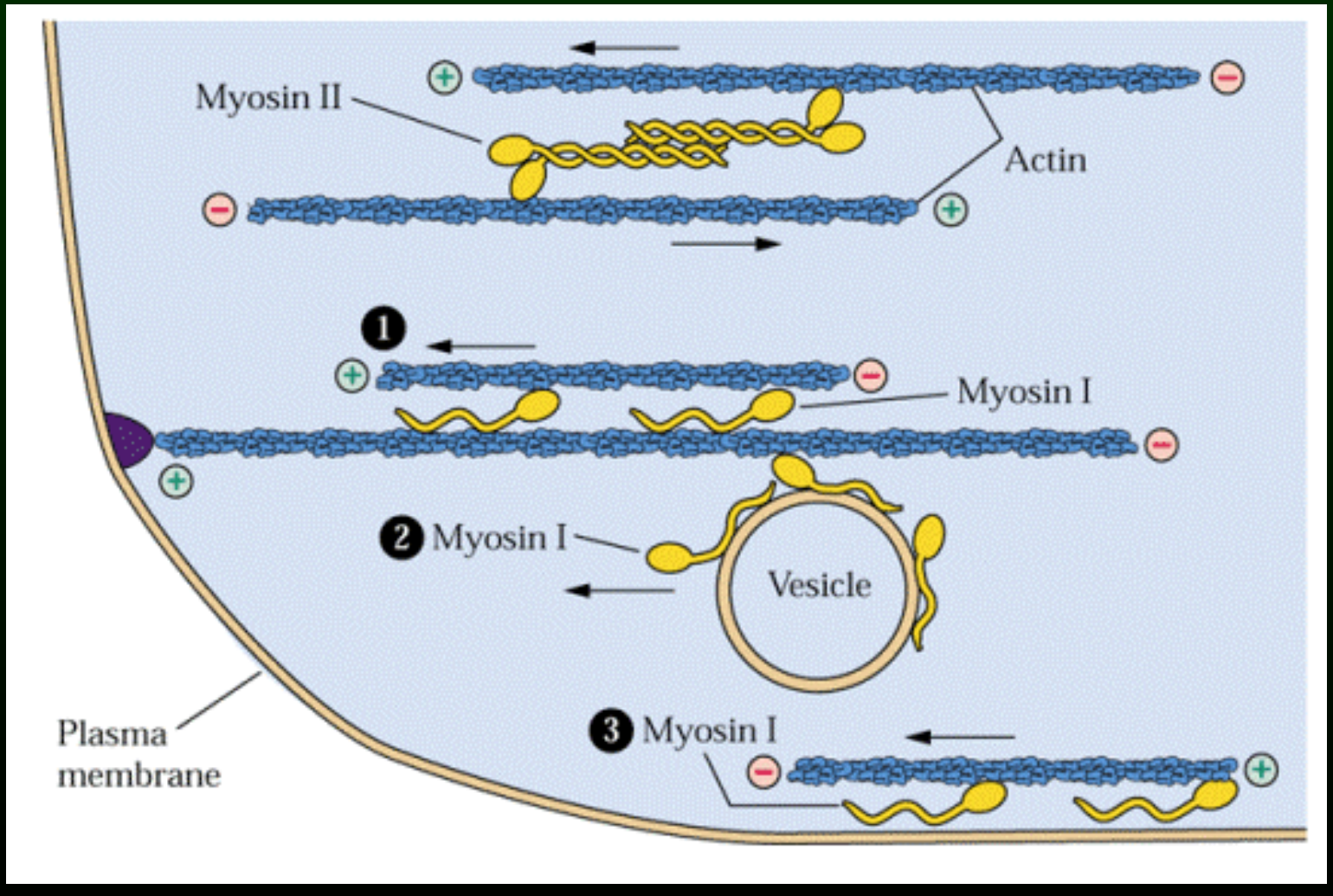


mechanismus pohybu myozinu

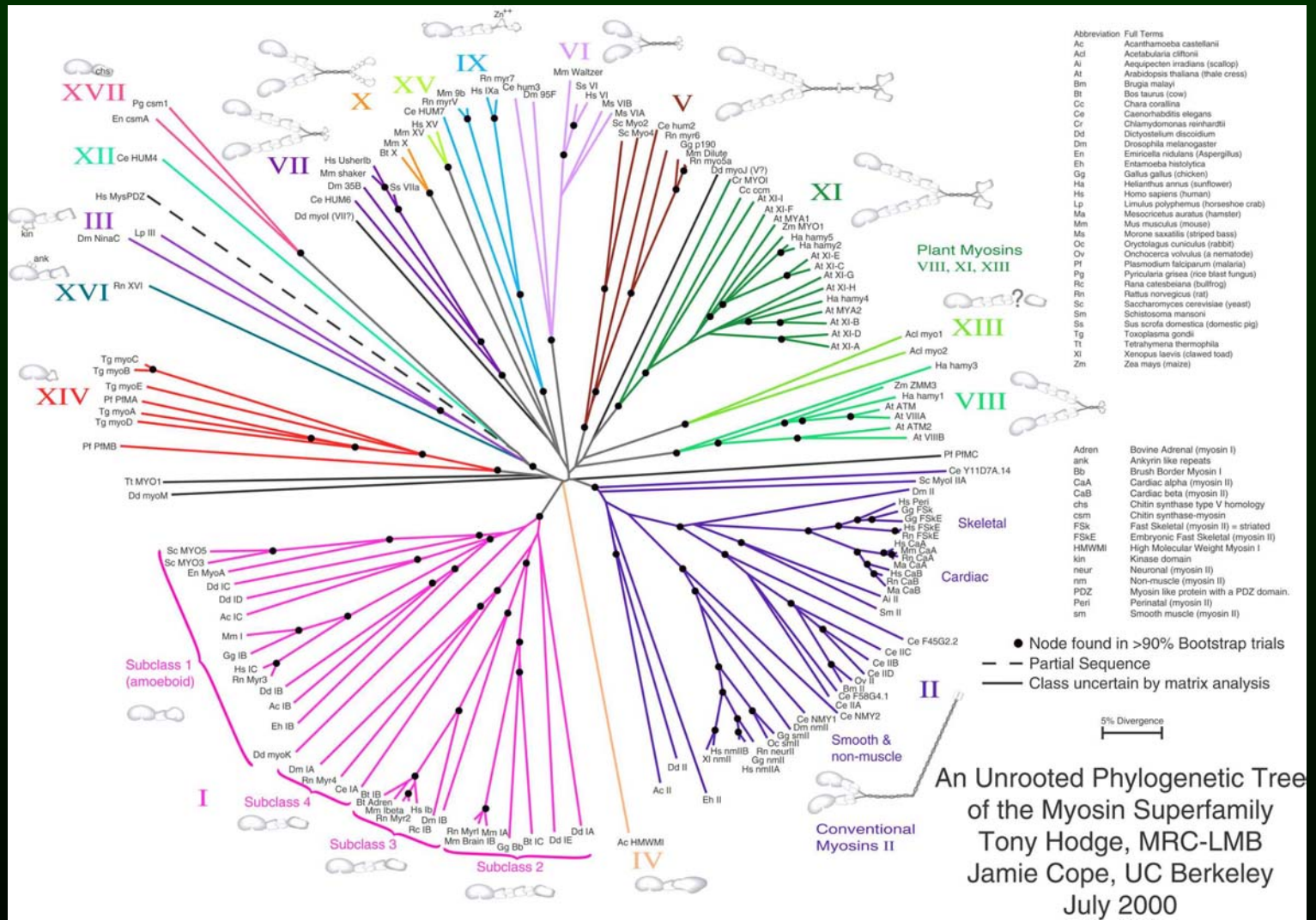


ANIMACE 2

funkce myozinů



Myozniny



některé třídy specifické jen pro určité skupiny organismů

Arabidopsis má 17 myosinů (class VIII, XI, XIII)

Regulace molekulových motorů

regulace přes Ca^{2+} a calmodulin

- ⇨ IQ motivy myozinů
- ⇨ KCBP kinezin
- ⇨ injekce Ca^{2+} urychluje pohyb chromosomů

fosforylace

Interakce motorů operujících na mikrotubulech a mikrofinamentech

interakce obou skupin motorů → koordinace buněčného transportu (MT – long-distance, MF – short-distance)

- ⇨ SMY1 suprimuje mutaci v MYO2 (fyzická interakce jejich ocasových domén) – *S. cer.*
- ⇨ KCBP je z části kinezin, z části myozin - *A. th.*
- ⇨ přímá interakce MyoVa s KhcU - savci

Cytoplasmic streaming

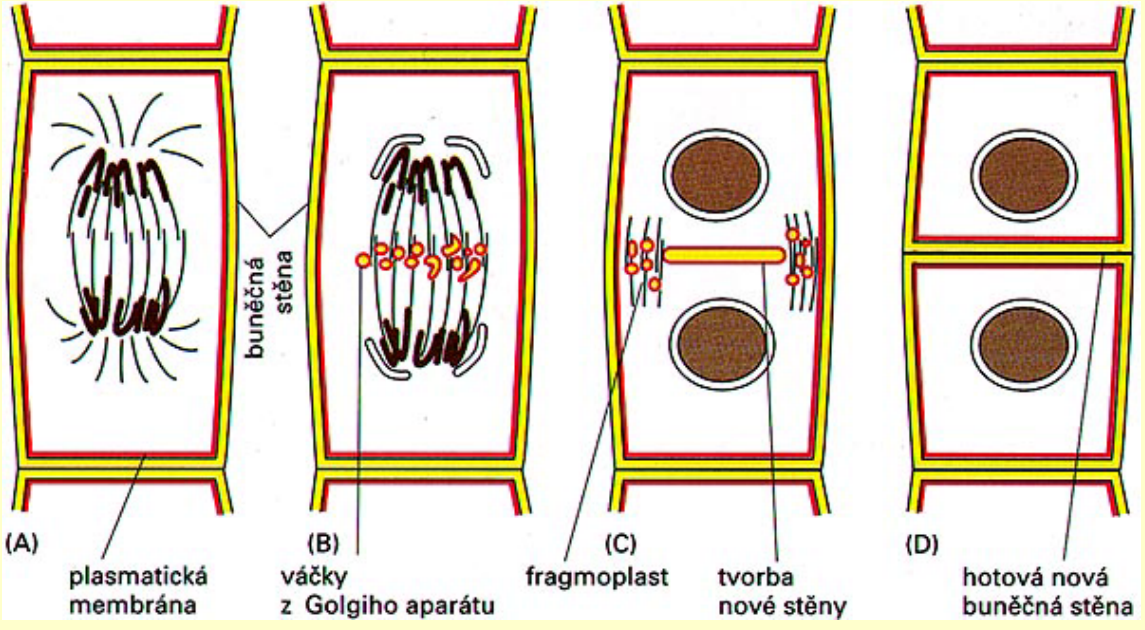
Pohyb organel, váčků a různého materiálu podél aktinového cytoskeletu (~ 70 μm/s), které provádějí **motorové proteiny operující na aktinových filamentech.**

- ⇨ kolokalizace mikrofilament a “proudů” cytoplasmy
- ⇨ depolymerizace mikrofilament inhibuje *streaming*
- ⇨ depolymerizace mikrotubulů neinhibuje *streaming*
- ⇨ kosedimentace myozinu s váčky
- ⇨ imunolokalizace myozinů:
  - myozin I kolem jádra, mitochondrií, chloroplastů
  - myozin II kolem mitochondrií, chloroplastů
  - myozin V kolem malých organel (váčky)

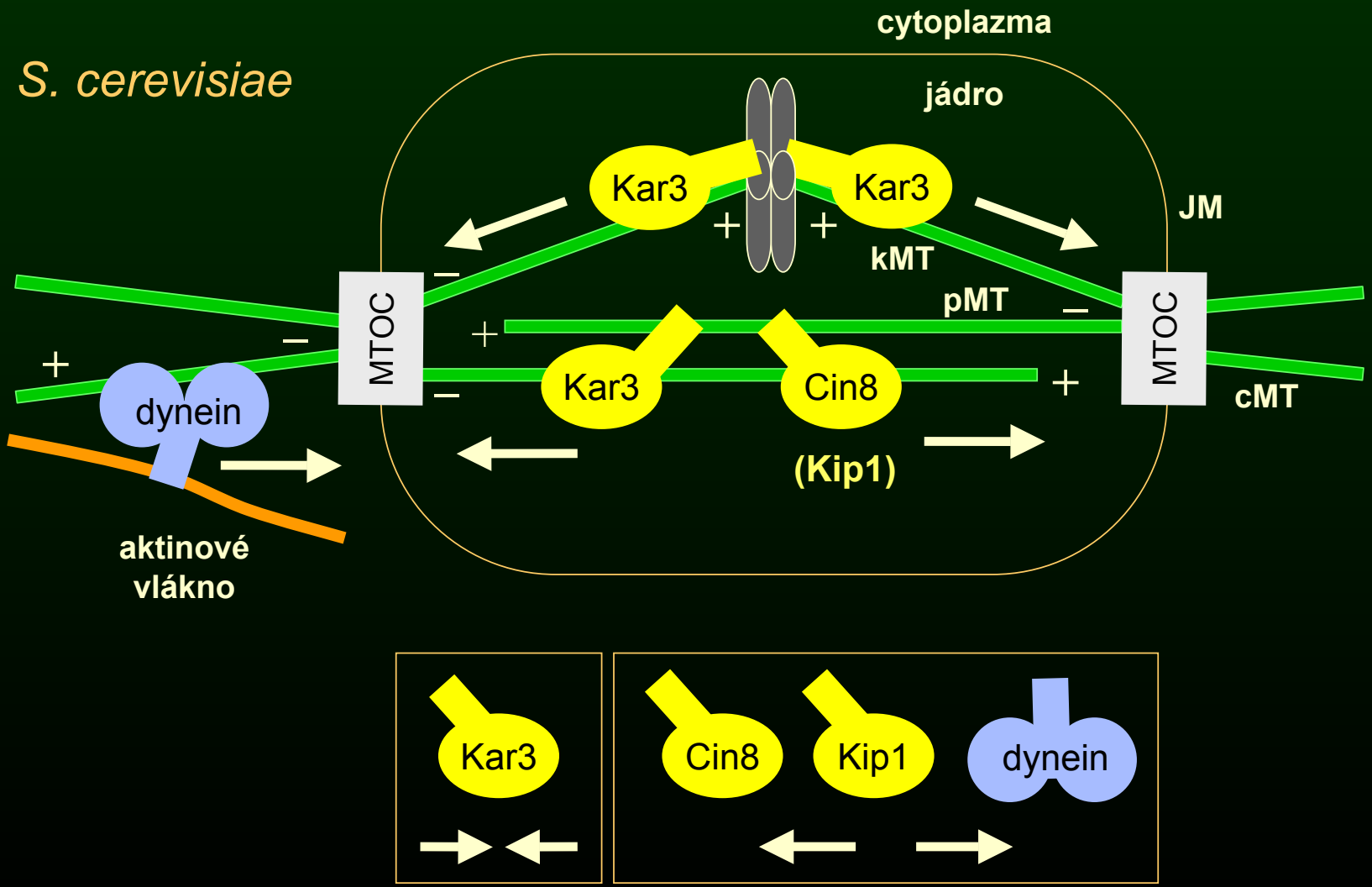
Buněčné dělení

- kortikální mikrotubuly ⇨ preprofázový prstenec
- vytváření dělicího vřeténka
- segregace chromosomů
- tvorba plazmatické destičky

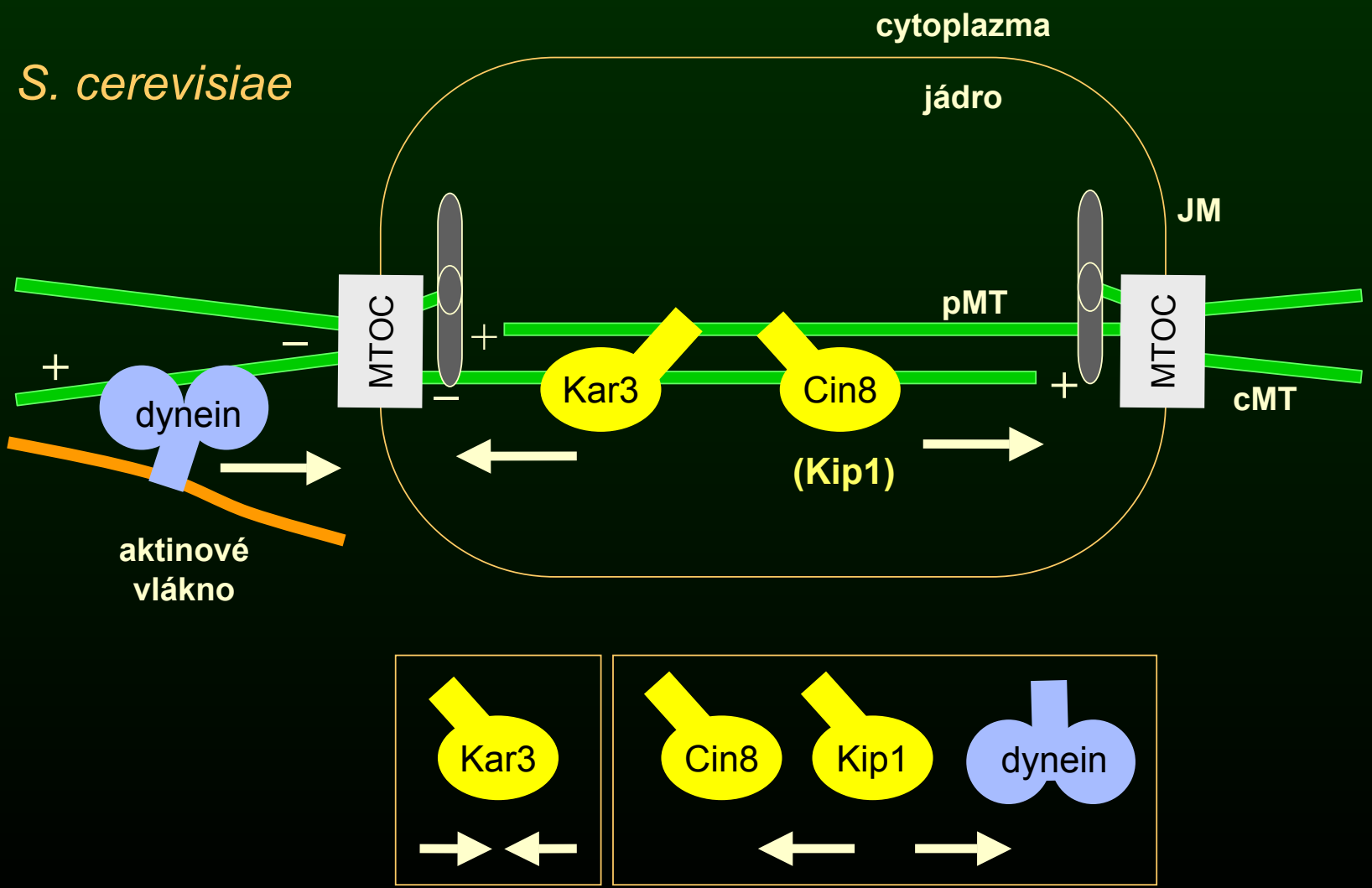
ANIMACE 3



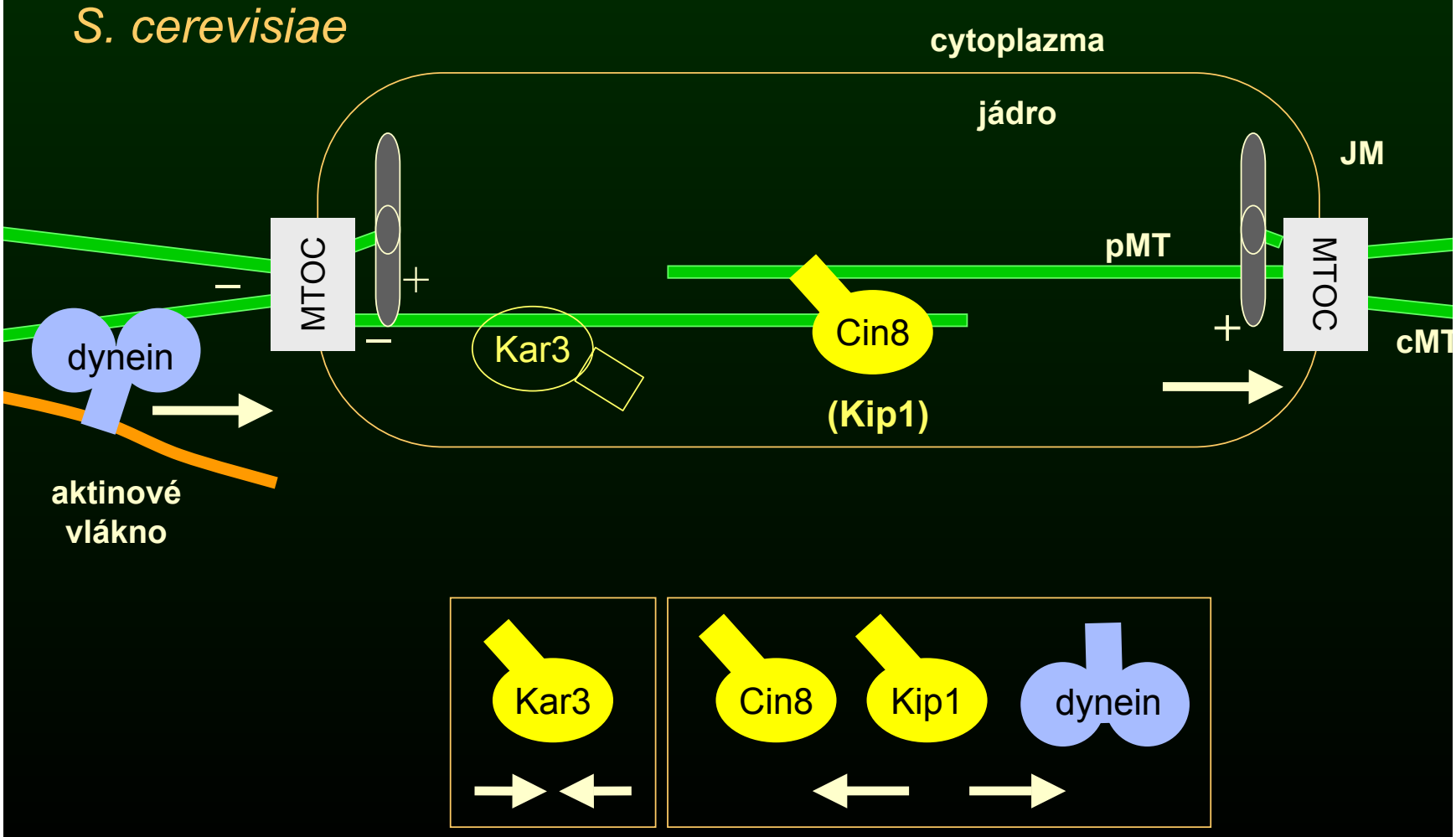
segregace chromosomů



segregace chromosomů



segregace chromosomů



segregace chromosomů

rostliny

motorové proteiny operující na mikrotubulech

- ⇨ zvýšená hladina exprese kinezinů během mitózy (KatB/C, KCBP, TKRP125)
- ⇨ lokalizace některých kinezinů podél mikrotubulů vřeténka (N- i C-koncové)
- ⇨ injekce KCBP-protilátky zablokuje mitózu v metafázi

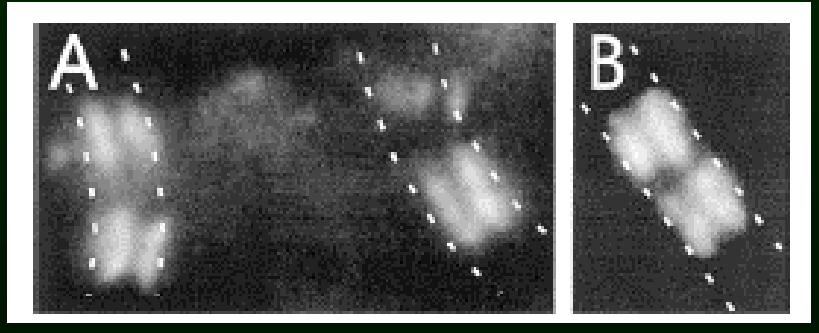
tvorba plazmatické destičky

motorové proteiny operující na mikrotubulech

- ⇨ asociace váčků s mikrotubuly fragmoplastu
- ⇨ depolymerizace mikrotubulů inhibuje tvorbu destičky
- ⇨ depolymerizace mikrofilament neinhibuje tvorbu destičky (vede pouze k abnormální pozici destičky)
- ⇨ lokalizace některých motorů u fragmoplastu; N-koncové kineziny (TKRP125) i C-koncové (KatA a KCBP)
- ⇨ injekce KCBP-protilátky po anafázi blokuje tvorbu destičky

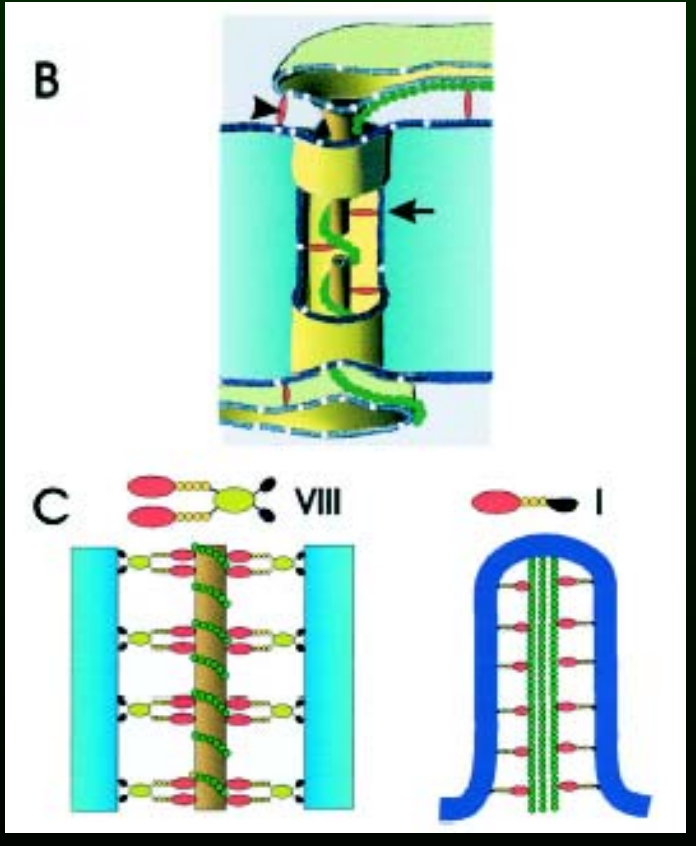
buněčná komunikace

MyoVIII funguje v plasmodesmech

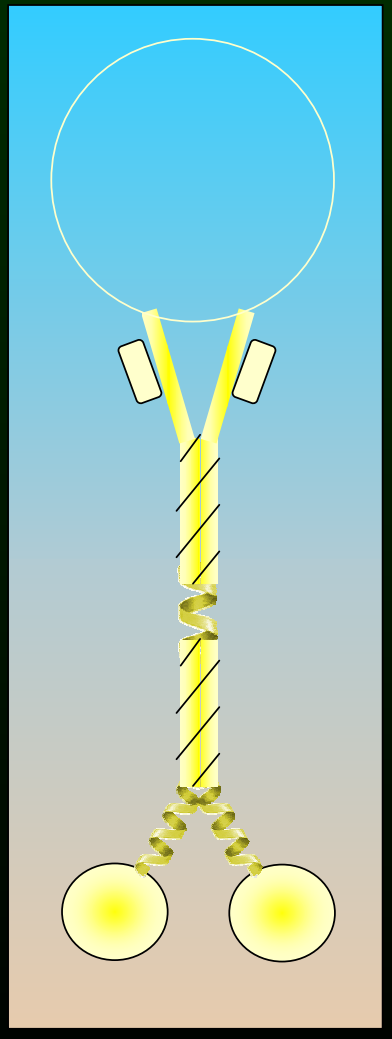


mikrotubuly a mikrofilamenta jsou nezbytné pro regulovaný transport makromolekul přes plazmodesmy

⇨ pravděpodobná účast molekulových motorů



„mikrovilus naruby“? Baluška et al. 2001



náklad

tělo

motorová doména