Funkční diverzita mikrotubulů: její funkce a regulace

Karel Souček

9-10-2008

Bi6051 Molekulární fyziologie živočichů

Mikrotubuly

- struktura
- dynamika
- posttranslační modifikace

Cytoskelet

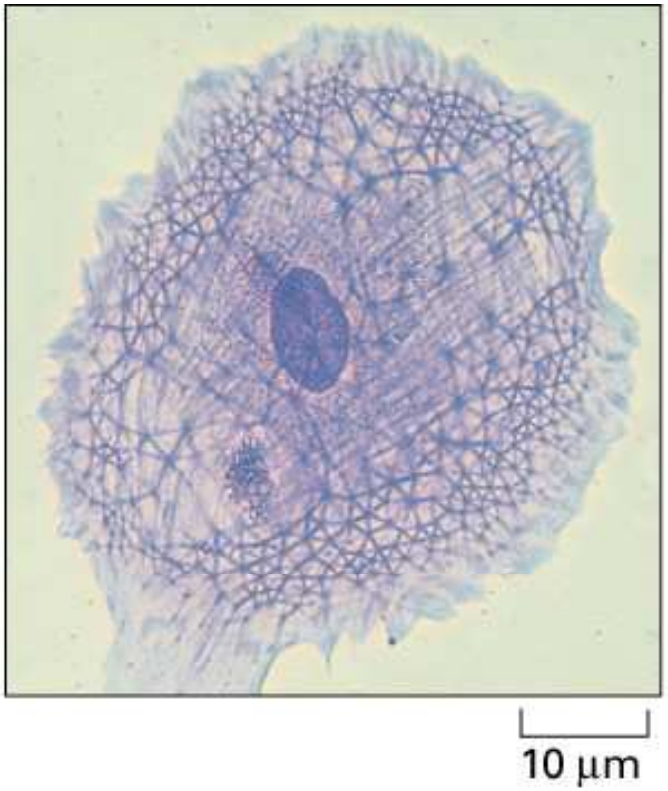
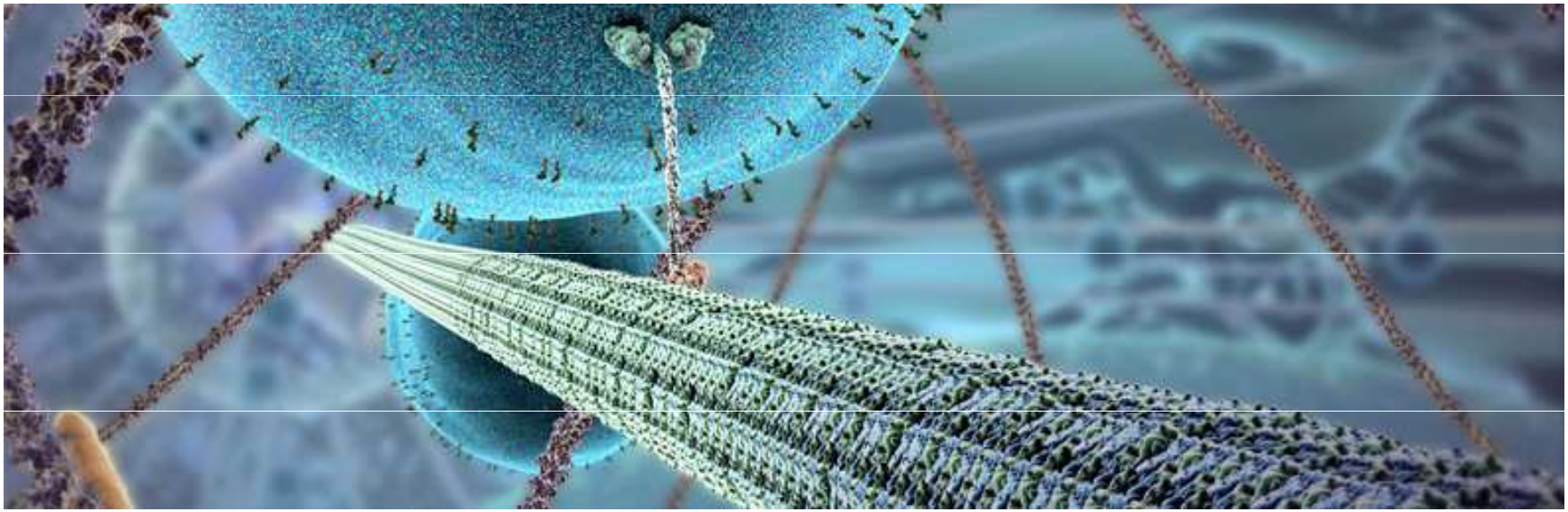
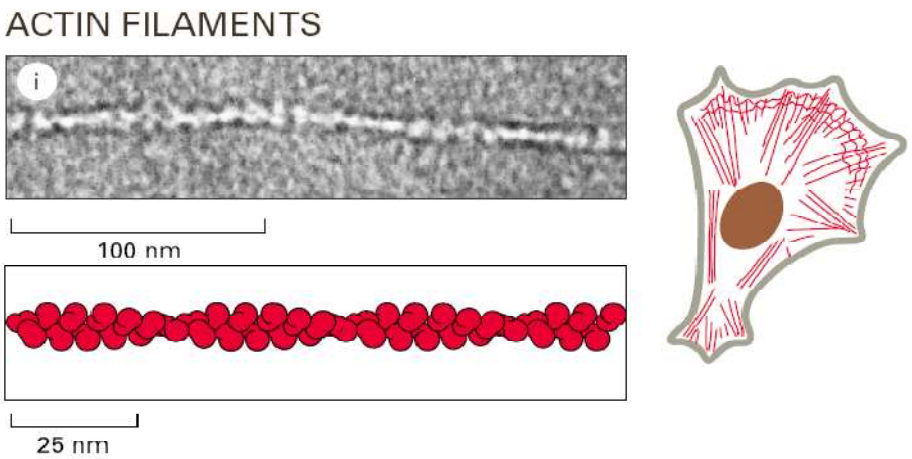


Figure 16–1. Molecular Biology of the Cell, 4th Edition.

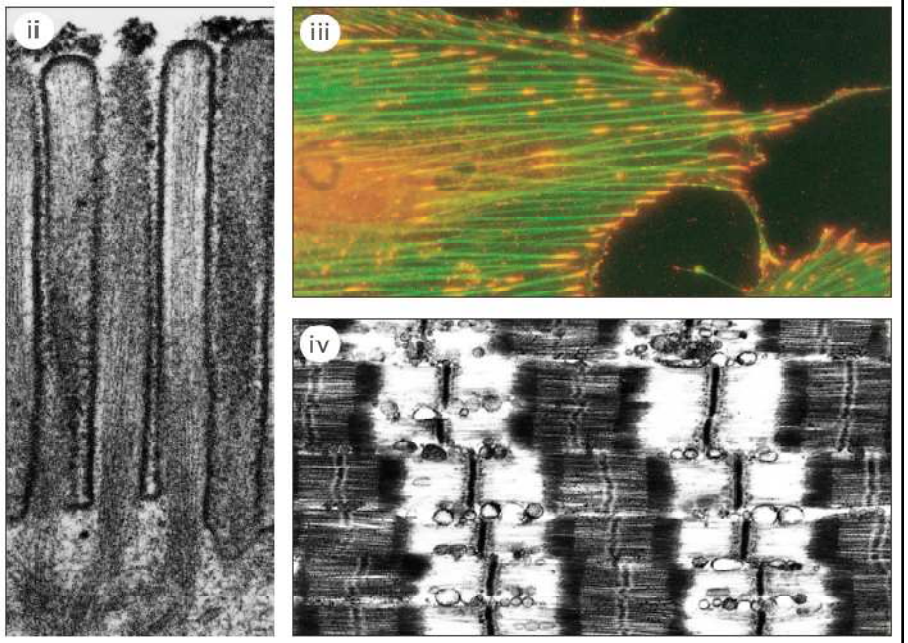
Cellular Visions: *The Inner Life of a Cell*



Aktinová filamenta

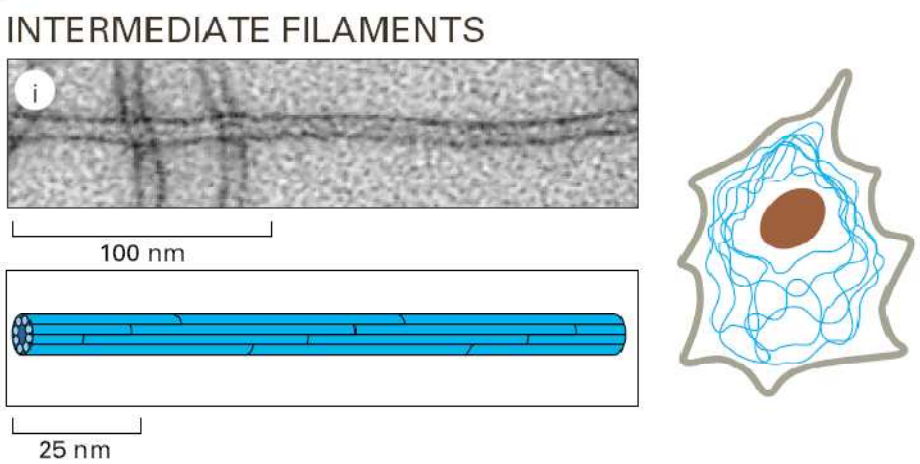


Actin filaments (also known as *microfilaments*) are two-stranded helical polymers of the protein actin. They appear as flexible structures, with a diameter of 5–9 nm, and they are organized into a variety of linear bundles, two-dimensional networks, and three-dimensional gels. Although actin filaments are dispersed throughout the cell, they are most highly concentrated in the *cortex*, just beneath the plasma membrane.

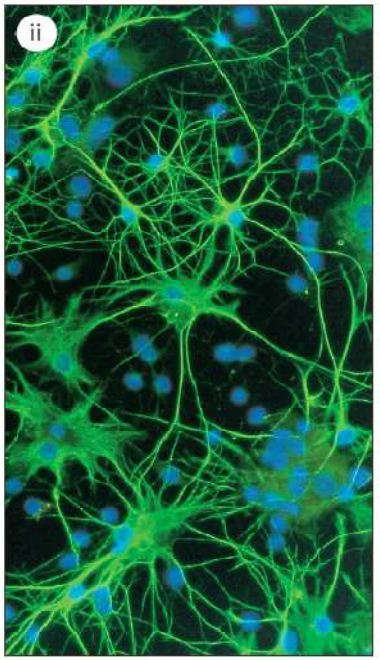


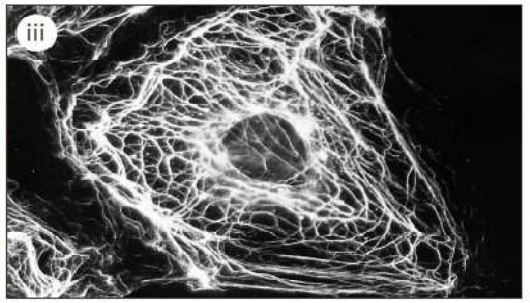
Micrographs courtesy of Roger Craig (i and iv); P.T. Matsudaira and D.R. Burgess (ii); Keith Burridge (iii).

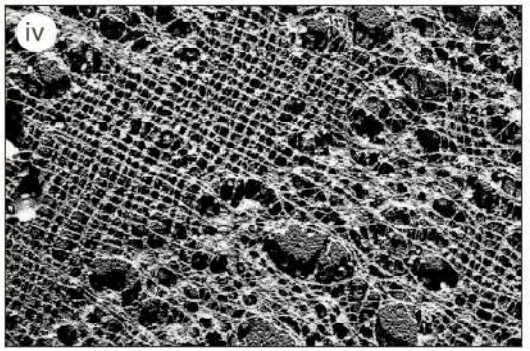
Intermediární (střední) filamenta



Intermediate filaments are ropelike fibers with a diameter of around 10 nm; they are made of intermediate filament proteins, which constitute a large and heterogeneous family. One type of intermediate filament forms a meshwork called the nuclear lamina just beneath the inner nuclear membrane. Other types extend across the cytoplasm, giving cells mechanical strength. In an epithelial tissue, they span the cytoplasm from one cell-cell junction to another, thereby strengthening the entire epithelium.



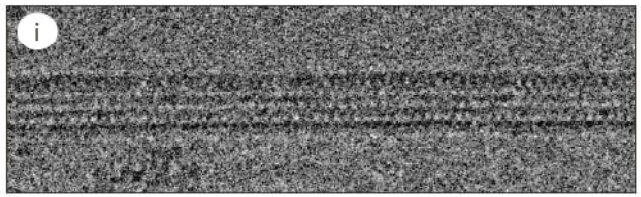


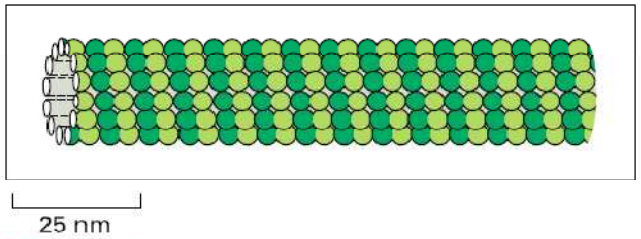


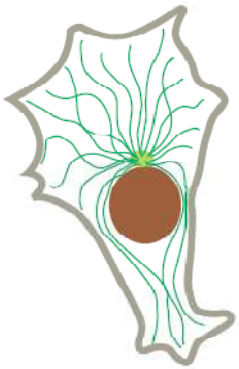
Micrographs courtesy of Roy Quinlan (i); Nancy L. Kedersha (ii); Mary Osborn (iii); Ueli Aebi (iv).

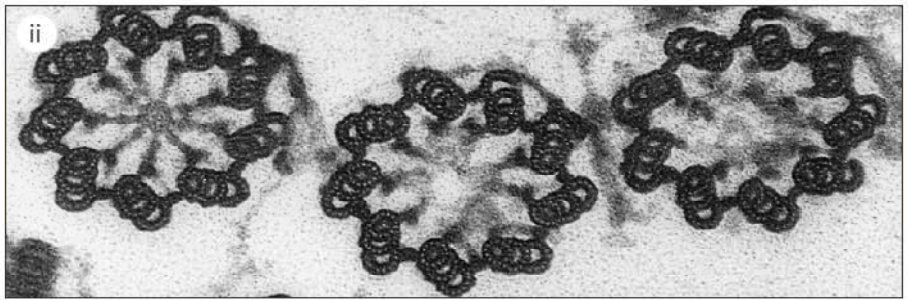
Mikrotubuly

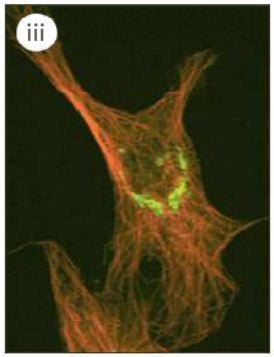
MICROTUBULES

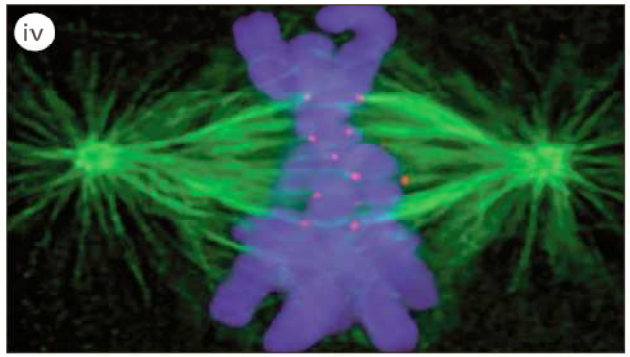










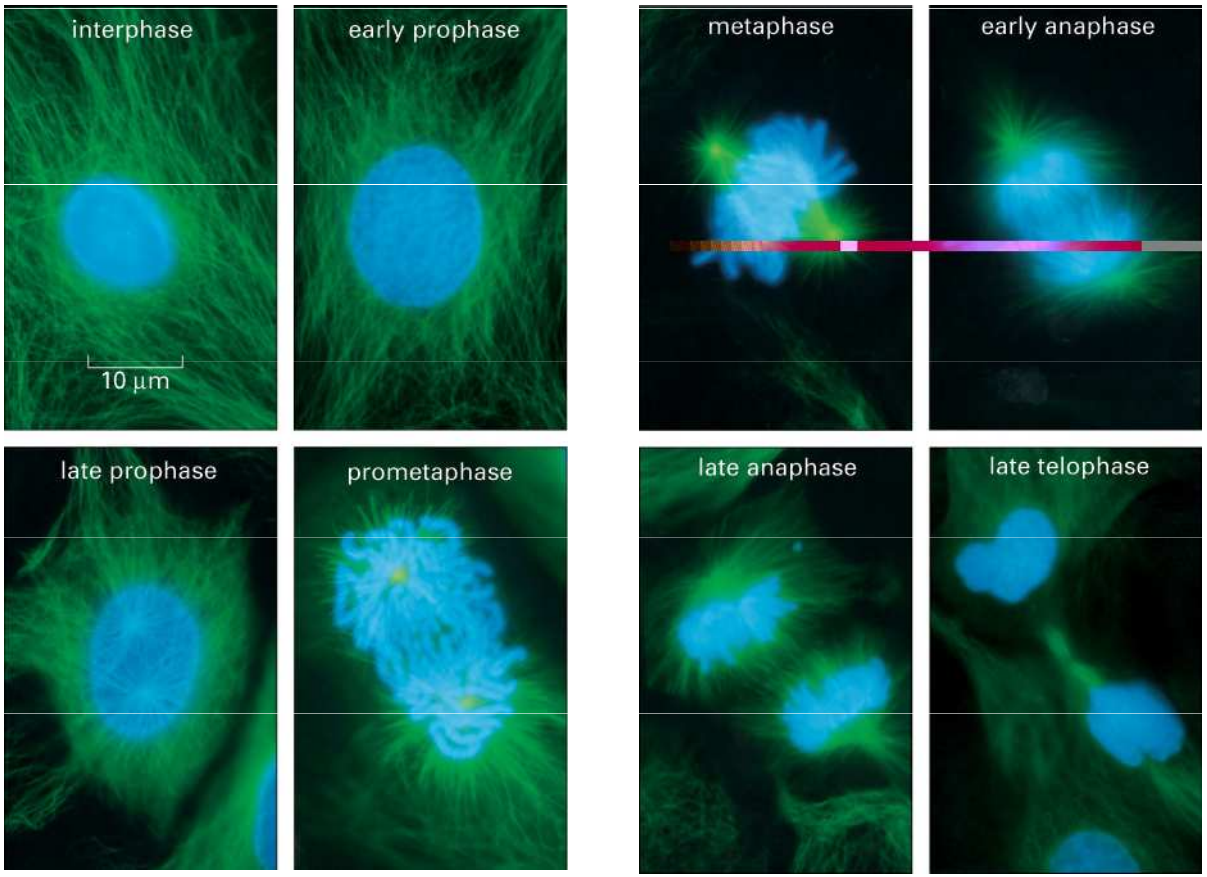


Microtubules are long, hollow cylinders made of the protein tubulin. With an outer diameter of 25 nm, they are much more rigid than actin filaments. Microtubules are long and straight and typically have one end attached to a single microtubule-organizing center (MTOC) called a *centrosome*, as shown here.

Micrographs courtesy of Richard Wade (i); D.T. Woodrow and R.W. Linck (ii); David Shima (iii); A. Desai (iv).

Microtubuly – klíčové složky cytoskeletu

- vývoj
- udržování tvaru
- transport organel
- buněčná signalizace
- buněčné dělění a mitóza



Molecular Biology of the Cell (4th Ed.)

Mechanické vlastnosti aktinu, tubulinu a intermediárních filament

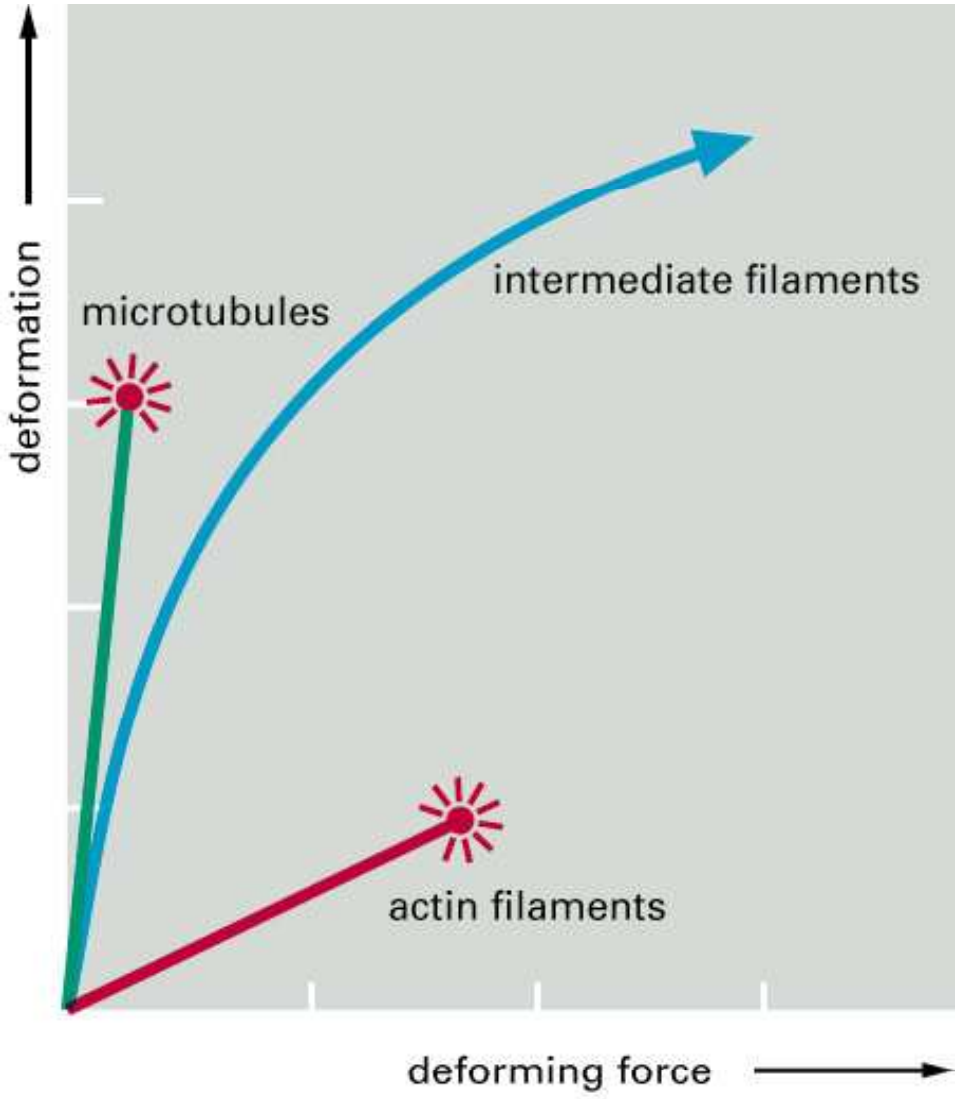
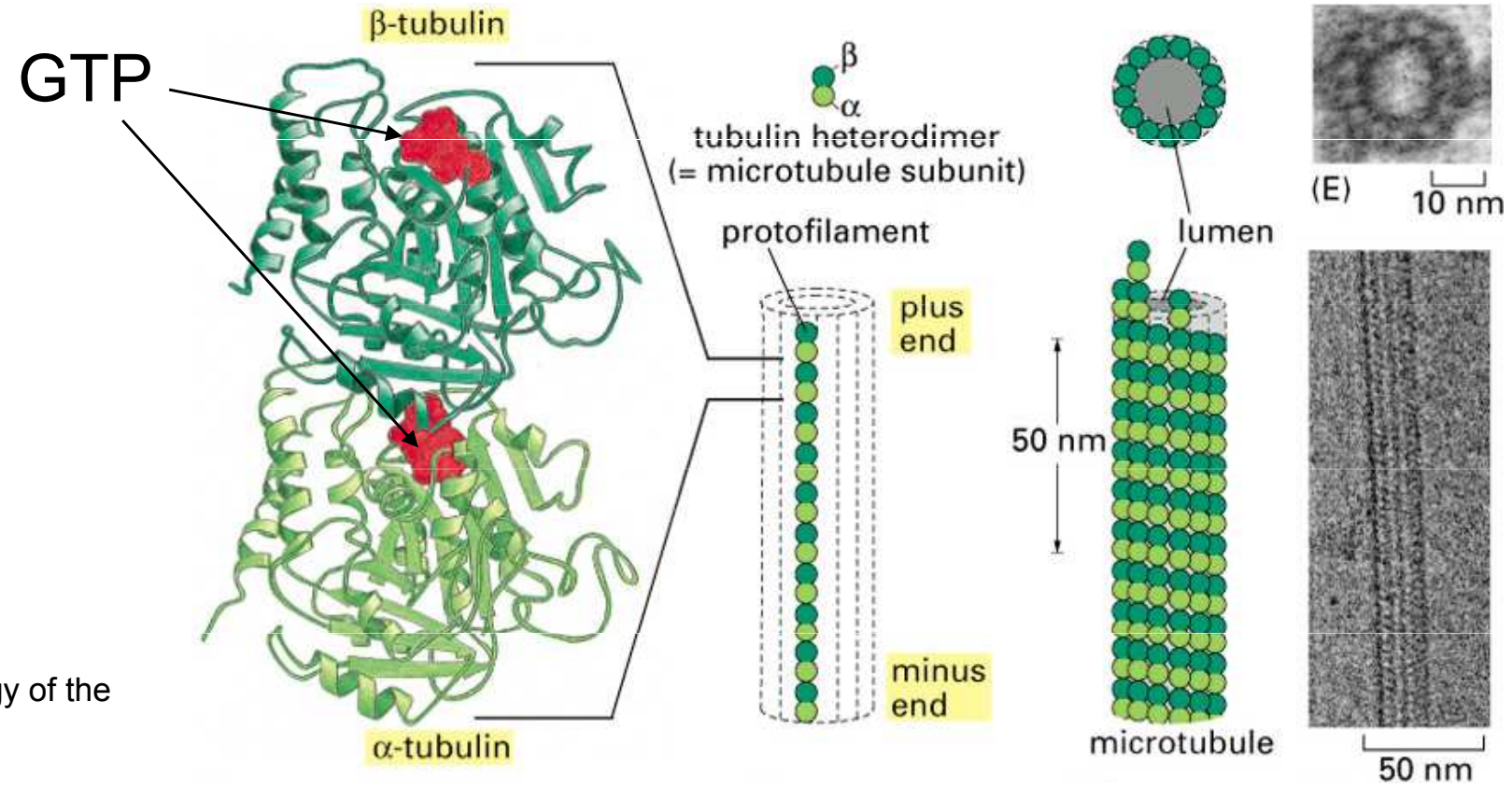


Figure 16–17. Molecular Biology of the Cell, 4th Edition.

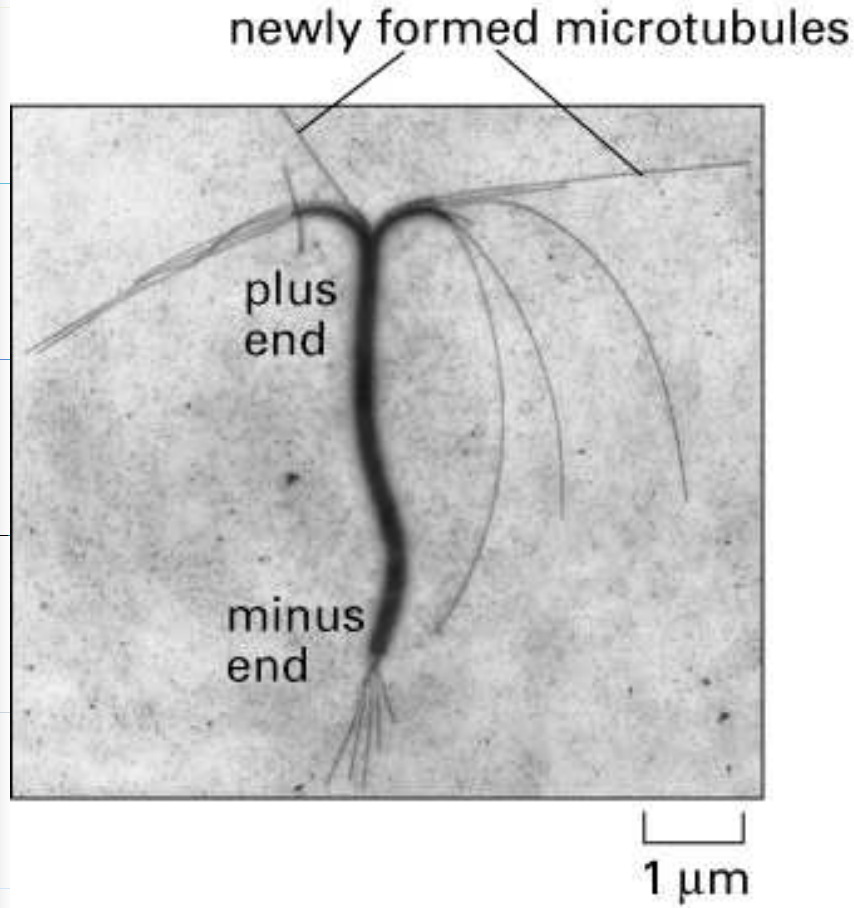
α-tubulin a β-tubulin heterodimer
- vysoce dynamický polymer

Funkční diversita:
-Vazba proteinů asociujících s mikrotubuly (MAP);
-exprese různých isoforem (6 forem α-tubulinu a 7 forem β-tubulinu);
-posttranslační modifikace.



Molecular Biology of the Cell (4th Ed.)

Růst mikrotubulu na + konci



- mikrotubuly rostou rychleji na „plus“ konci

Figure 16–8. Molecular Biology of the Cell, 4th Edition.

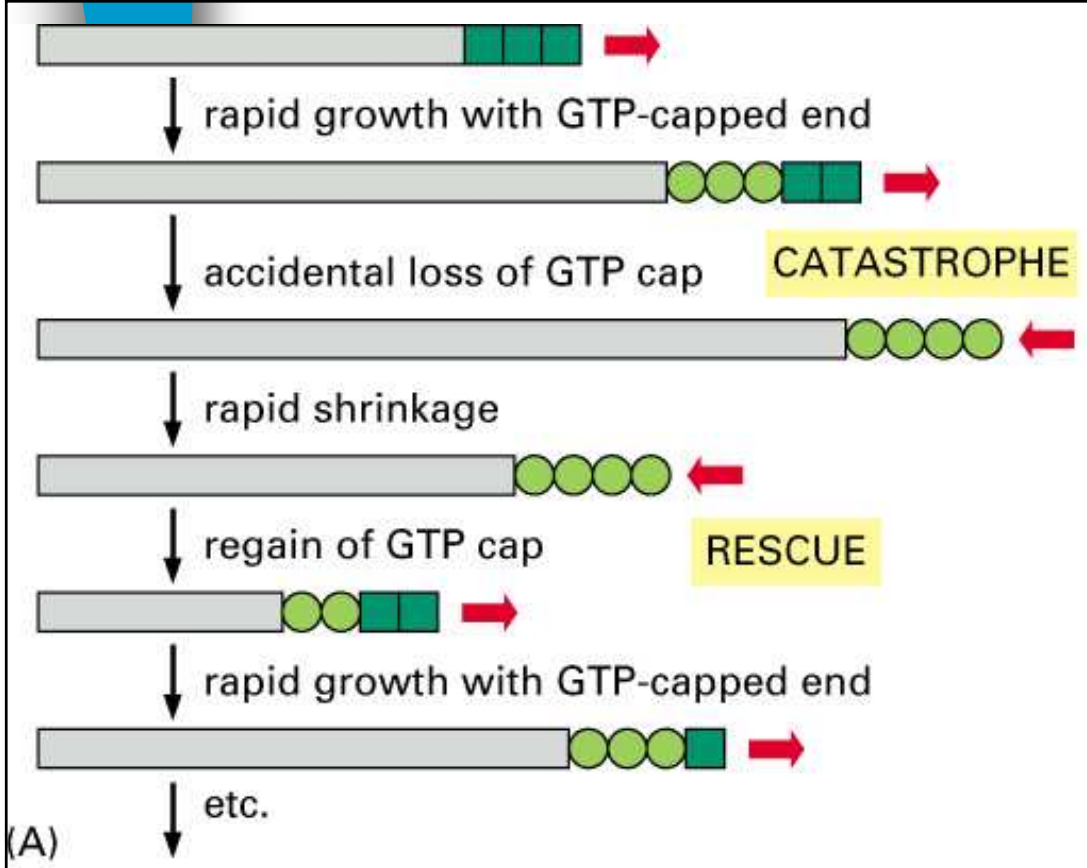
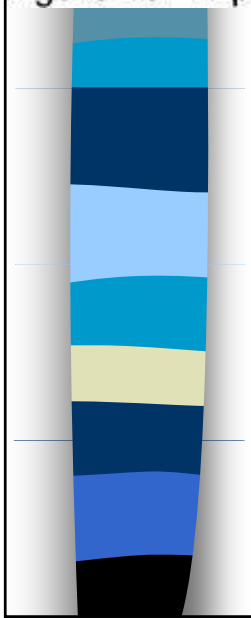


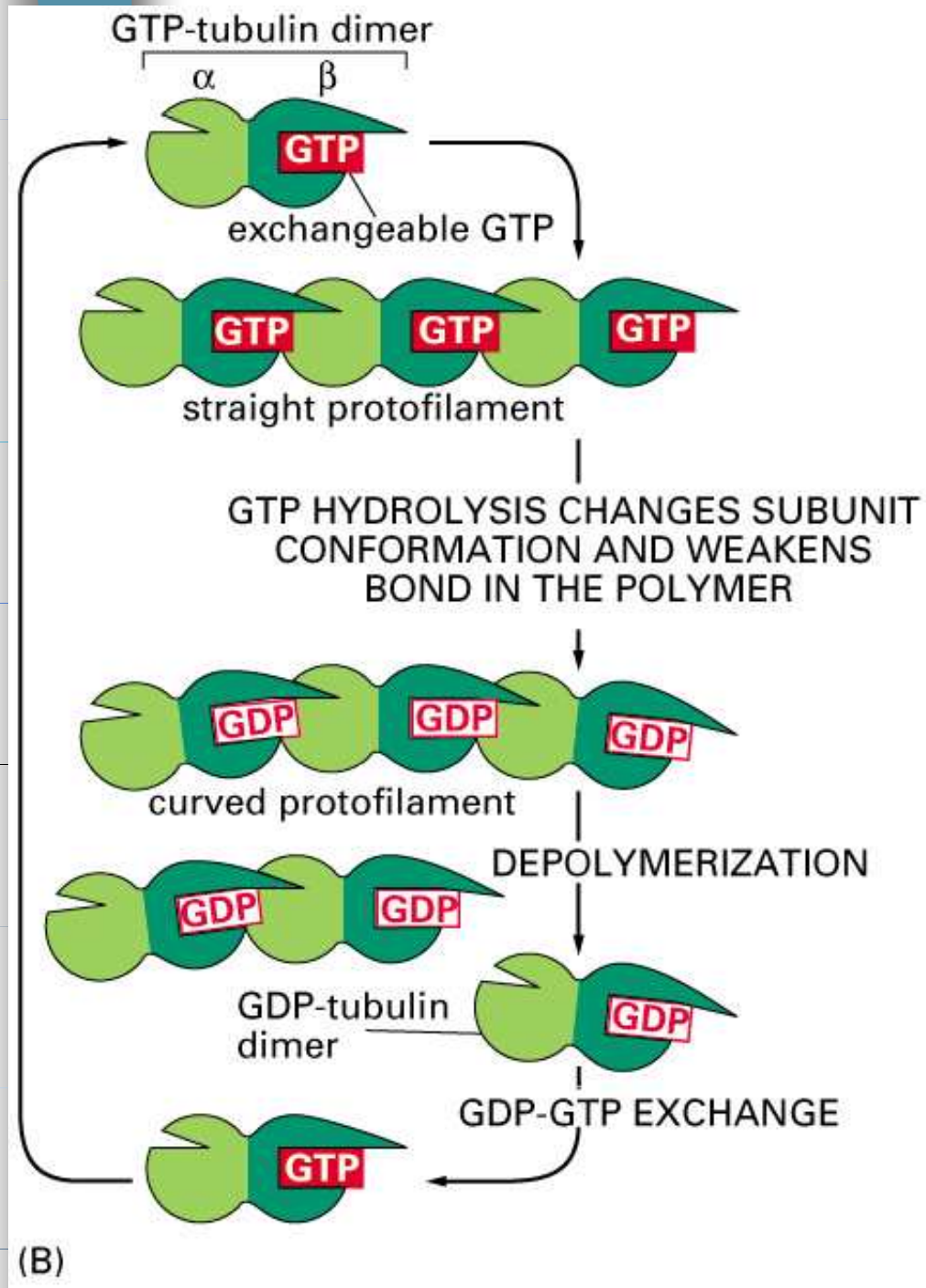
Figure 16–11 part 1 of 3. Molecular Biology of the Cell, 4th Edition.



Dynamická nestabilita

- Rostoucí MT má na konci β podjednotku s GTP
- pokud dojde k hydrolýze dochází ke smršťování

Hydrolýza GTP



- Zeslabení vazby v polymeru
- depolymerizace

Figure 16–11 part 2 of 3. Molecular Biology of the Cell, 4th Edition.

Růst a smršťování MT

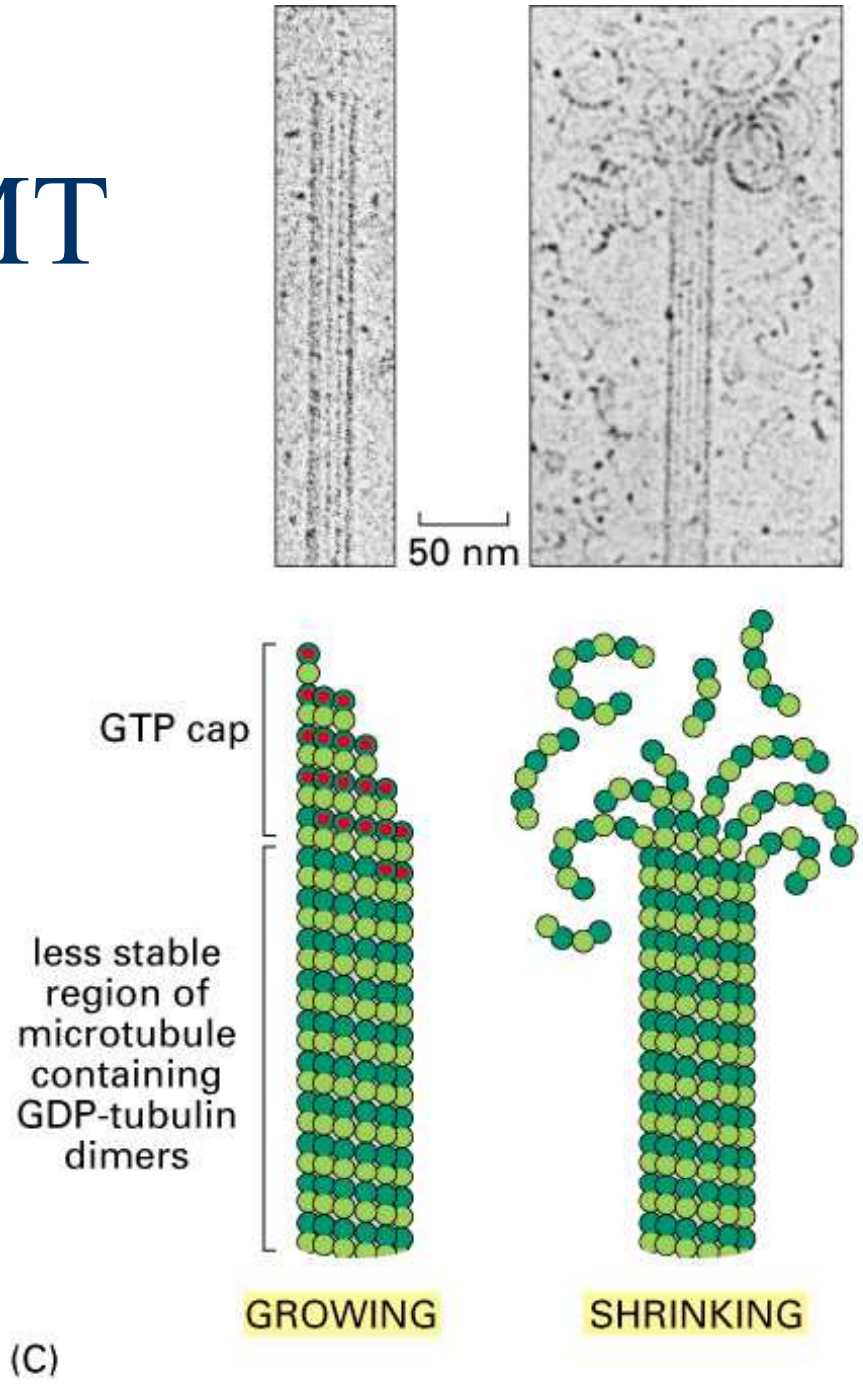
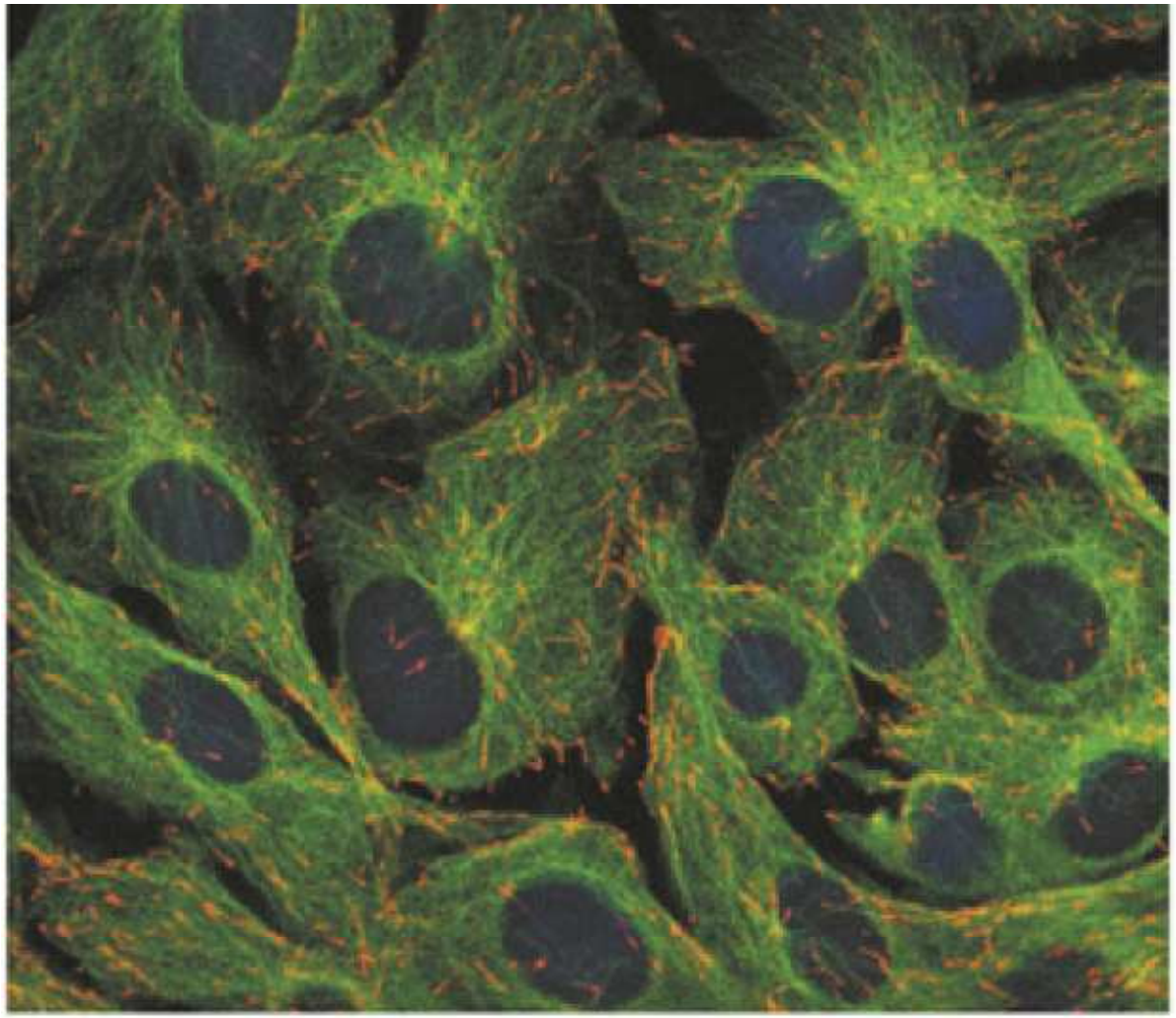


Figure 16–11 part 3 of 3. Molecular Biology of the Cell, 4th Edition.

Dynamická nestabilita

Rat Vascular Smooth Muscle Cells
EB1 a α-Tubulin značení



Rychlé změny v přestavbě cytoskeletu během vývoje embrya Drosophily a Caenorhabditis

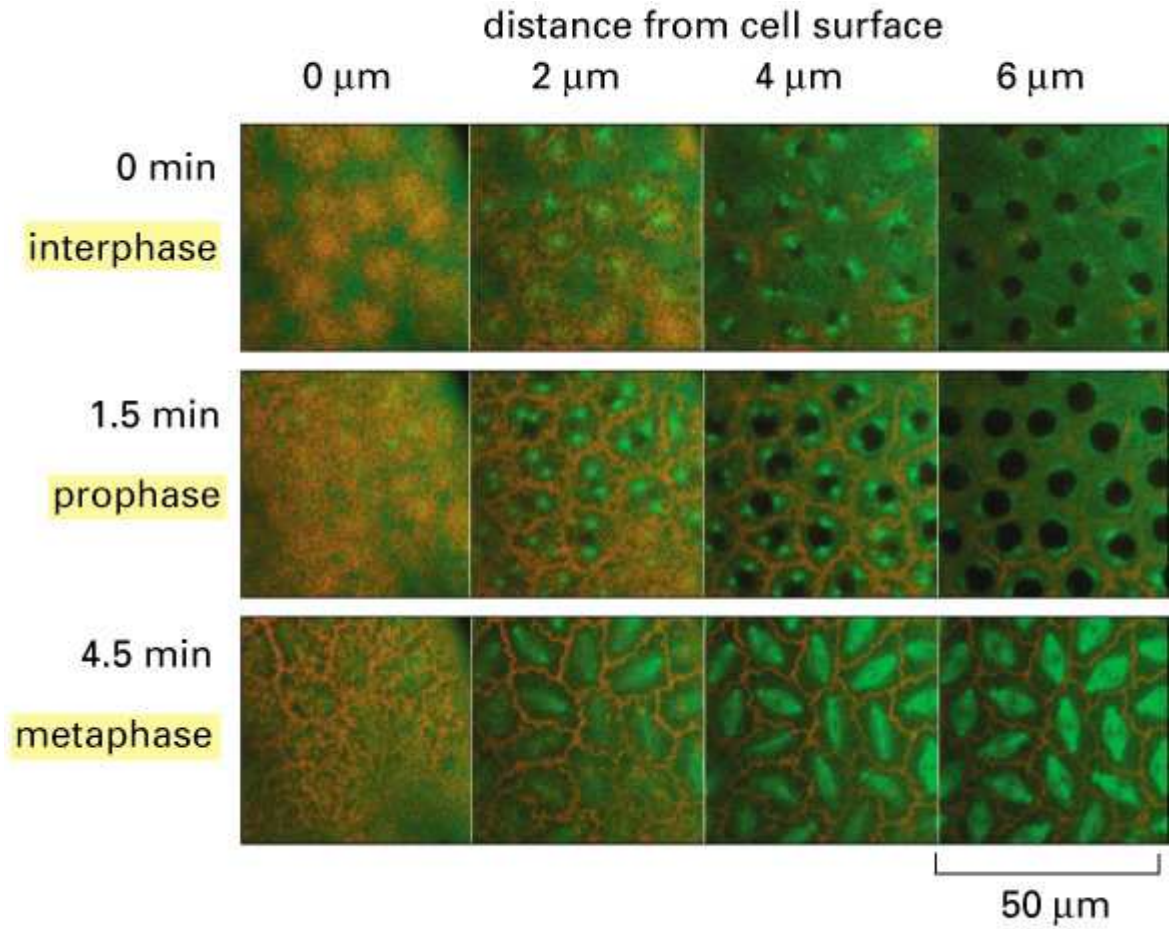


Figure 16–13. Molecular Biology of the Cell, 4th Edition.

Cell, Vol. 96, 517–527, February 19, 1999, Copyright ©1999 by Cell Press

CLIP-170 Highlights Growing Microtubule Ends In Vivo

Franck Perez,* Georgios S. Diamantopoulos, Romaine Stalder, and Thomas E. Kreis†
Department of Cell Biology
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University of Geneva
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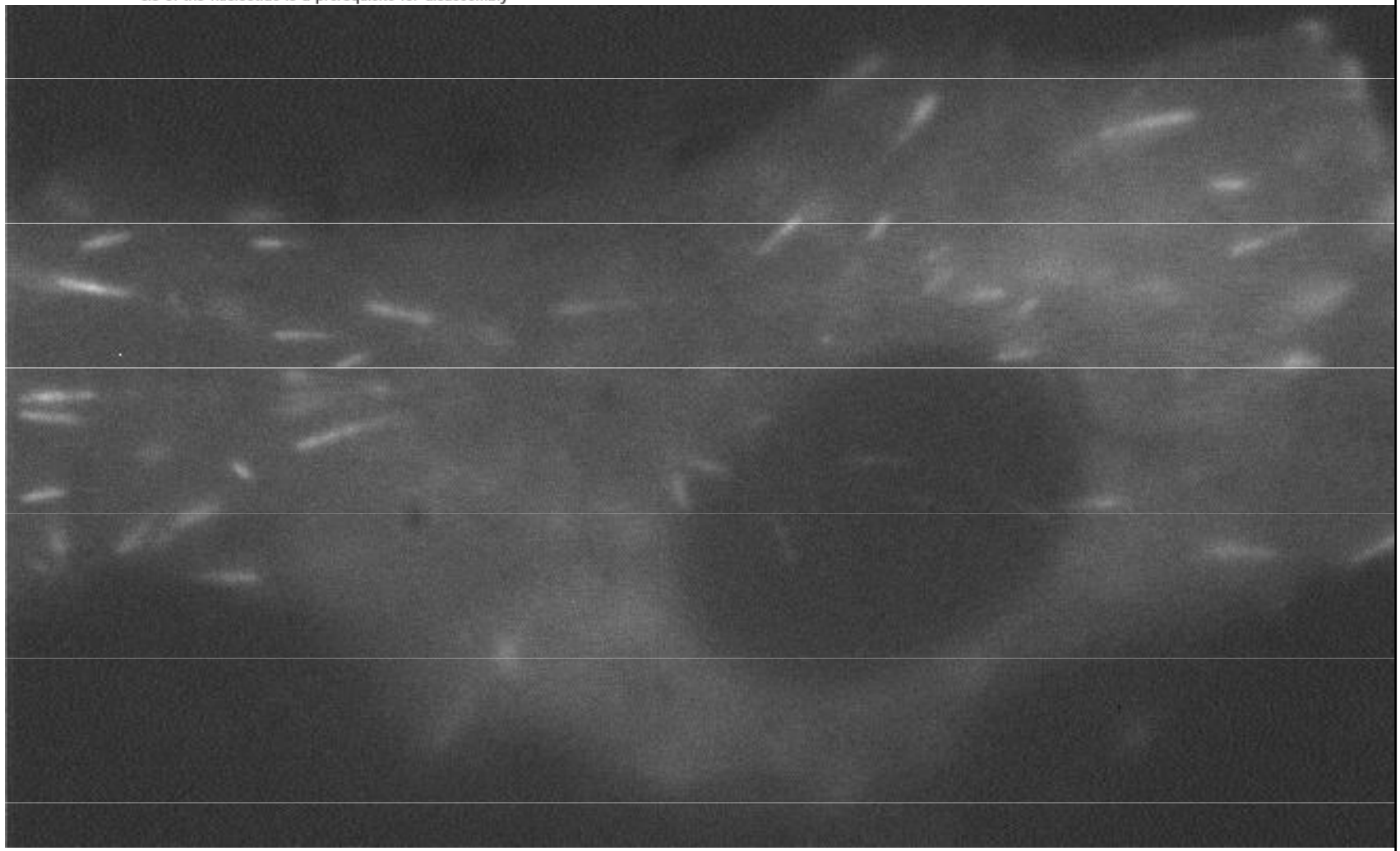
Switzerland

bound GTP polymerizes at the plus ends, and, as microtubules grow, the GTP bound to the β subunits is hydrolyzed so that normally only a small segment of GTP-tubulin should remain (discussed by Caplow, 1992; Desai and Mitchison, 1997). This GTP cap is thought to be necessary for elongation of the polymer and to prevent microtubules from depolymerization, while hydrolysis of the nucleotide is a prerequisite for disassembly



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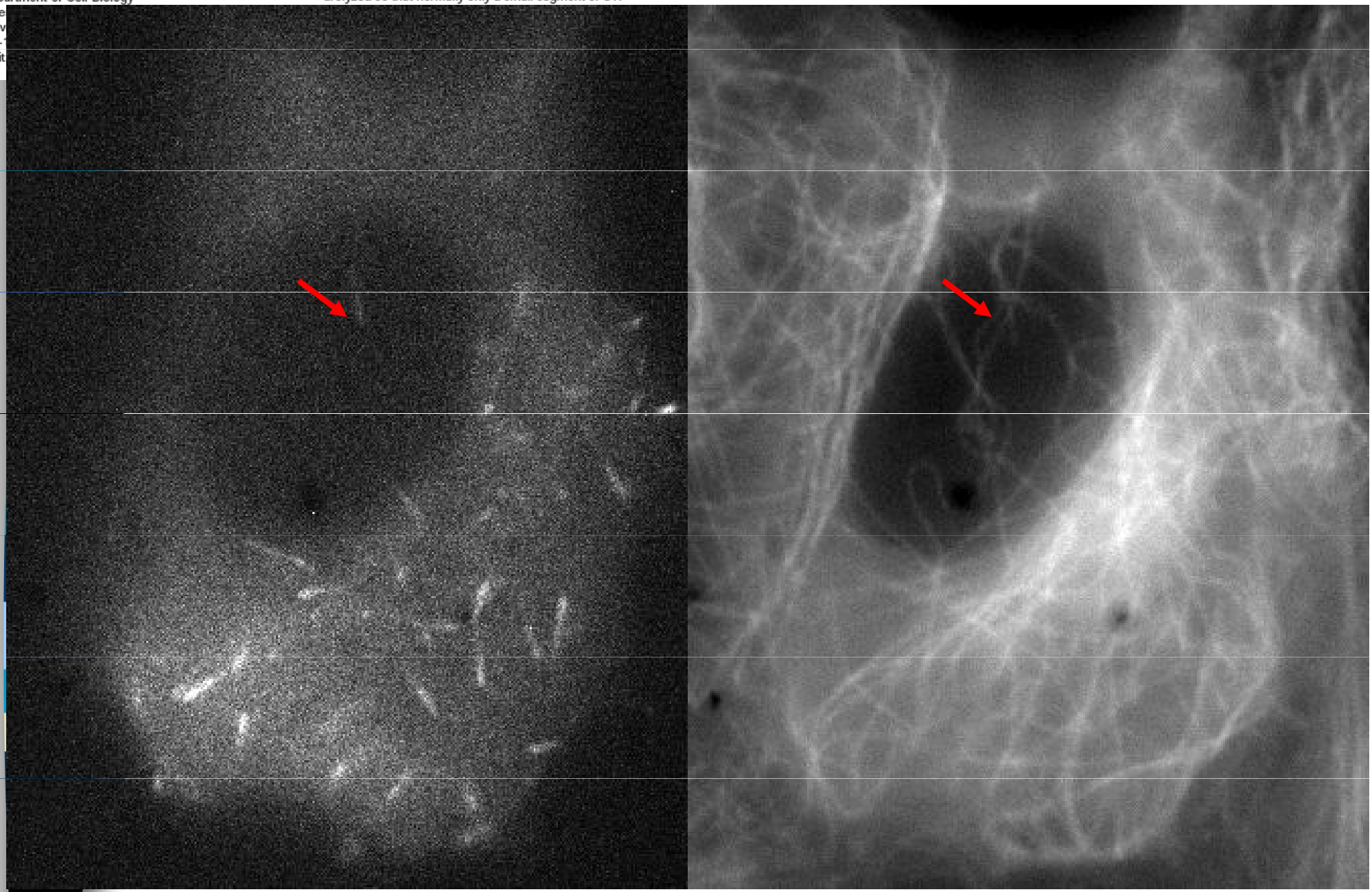
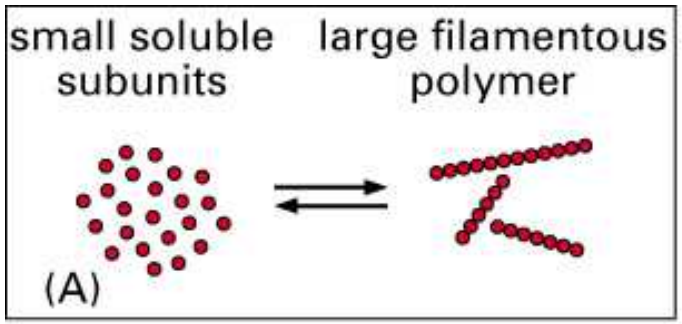


Cytoskelet a změna tvaru



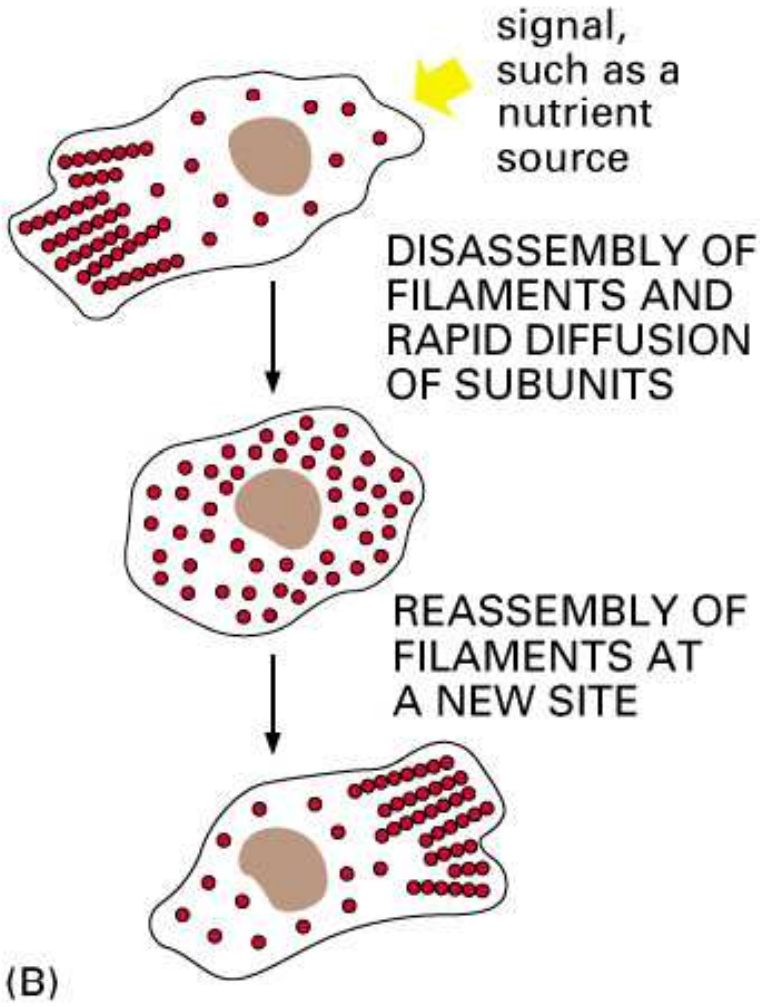
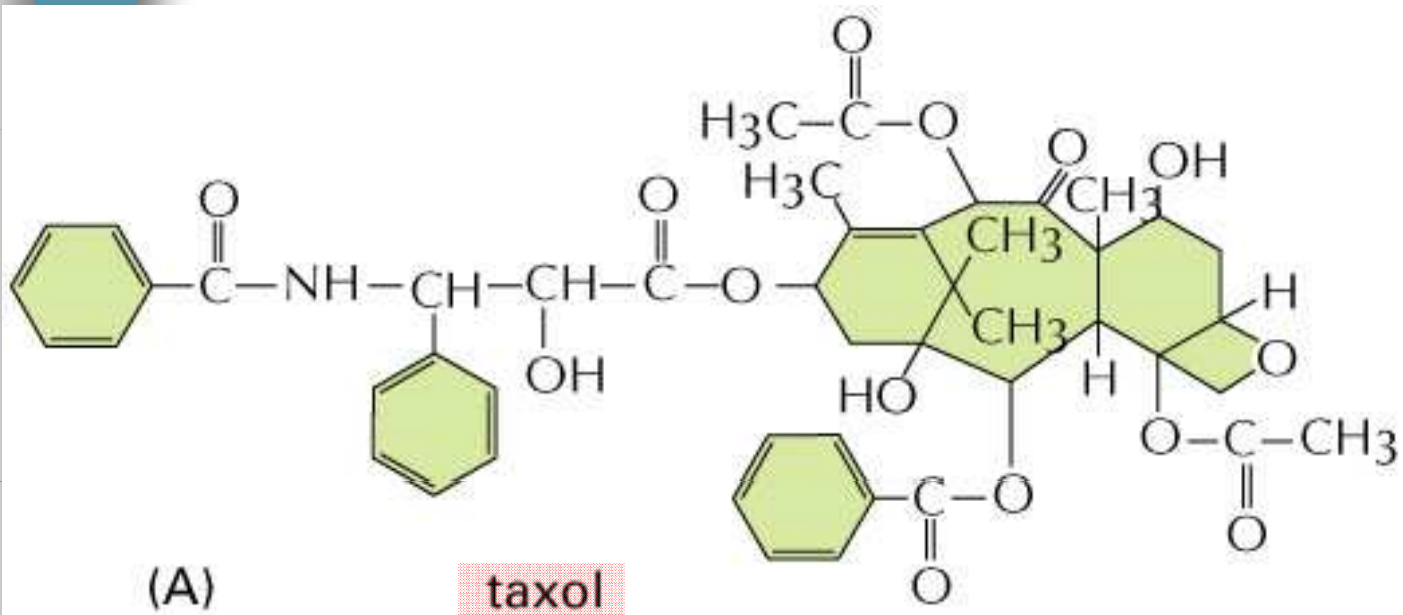
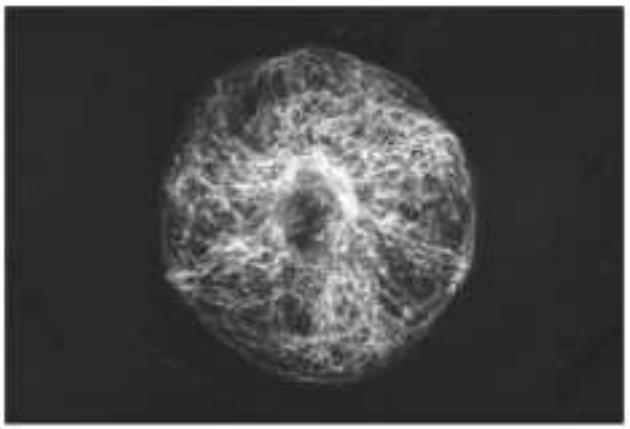
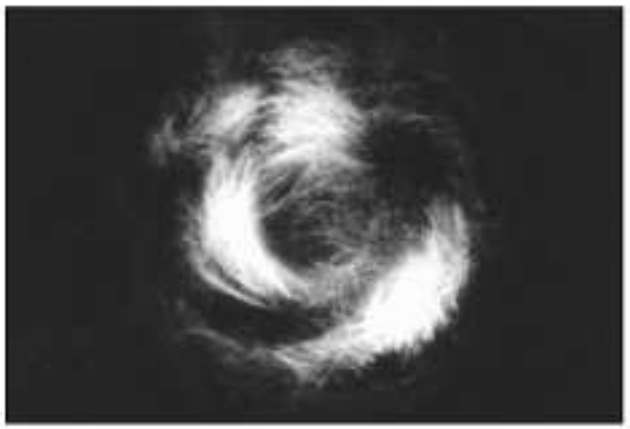


Figure 16–2. Molecular Biology of the Cell, 4th Edition.

- Formování filament z podjednotek malých proteinů
- rychlá reorganizace cytoskeletu v odpovědi na vnější signál







(B)

15 µm

(C)

(D)

Figure 16–21. Molecular Biology of the Cell, 4th Edition.

Microtubuly – cíle protinádorové léčby

Table 1 | **Antimitotic drugs, their diverse binding sites on tubulin and their stages of clinical development**

Binding domain	Related drugs or analogues	Therapeutic uses	Stage of clinical development	References
Vinca domain	Vinblastine (Velban)	Hodgkin's disease, testicular germ-cell cancer	In clinical use; 22 combination trials in progress	75–77,131
	Vincristine (Oncovin)	Leukaemia, lymphomas	In clinical use; 108 combination trials in progress	132–134
	Vinorelbine (Navelbine)	Solid tumours, lymphomas, lung cancer	In clinical use; 29 Phase I–III clinical trials in progress (single and combination)	135–137
	Vinflunine	Bladder, non-small-cell lung cancer, breast cancer	Phase III	131,138
	Cryptophycin 52	Solid tumours	Phase III finished	139,140
	Halichondrins (such as E7389)	–	Phase I	58,141–143
	Dolastatins (such as TZT-1027)	Potential vascular-targeting agent	Phase I; Phase II completed	144
	Hemiasterlins (such as HTI-286)	–	Phase I	145,146
Colchicine domain	Colchicine	Non-neoplastic diseases (gout, familial Mediterranean fever)	Appears to have failed trials, presumably because of toxicity	89–90
	Combretastatins (AVE8062A, CA-1-P, CA-4-P, *N*-acetylcolchicinol-*O*-phosphate, ZD6126)	Potential vascular-targeting agent	Phase I, II	91,147
	2-Methoxyestradiol	–	Phase I	148,149
	Methoxybenzene-sulphonamide (such as ABT-751, E7010)	Solid tumours	Phase I, II	150
Taxane site	Paclitaxel (Taxol), TL00139 and other analogues of paclitaxel	Ovarian, breast and lung tumours, Kaposi's sarcoma; trials with numerous other tumours	In clinical use; 207 Phase I–III trials in the United States; TL00139 is in Phase I trials	82, 151–153
	Docetaxel (Taxotere)	Prostate, brain and lung tumours	8 trials in the United States (Phases I–III)	154,155
	Epothilones (such as BMS-247550, epothilones B and D)	Paclitaxel-resistant tumours	Phases I–III	156–159
	Discodermolide	–	Phase I	160–164
Other microtubule binding sites	Estramustine	Prostate	Phases I–III, in numerous combinations with taxanes, epothilones and *Vinca* alkaloids	122, 165–168

Information on clinical trials was obtained from the National Institutes of Health Clinical Trials web site (www.clinicaltrials.gov), the European Organisation for Research and Treatment of Cancer web site (www.eortc.be) and the Proceedings of the American Association for Cancer Research meeting in 2003 (www.aacr.org). CA-4-P, combretastatin-A-4 3-*O*-phosphate; CA-1-P, combrestatin A-1-phosphate.

Inhibice polymerizace

Inhibice polymerizace

Stablizace polymerizace

Jordan M.A. and Wilson L.
Nature Rev. Cancer,
Vol 4, 2004, 253-265

Pirotenin – specifický pro α-tubulin (váže kovalentně Lys)

Usui T. et.al.
Chem. Biol., Vol 11, 2004, 799-806.

Shrnutí - I

- Mikrotubuly jsou klíčové komponenty cytoskeletu
- Hydrolýza GTP je důležitá pro dynamiku MT
- Mikrotubuly jsou cílem protinádorové terapie

Regulace tvorby filament

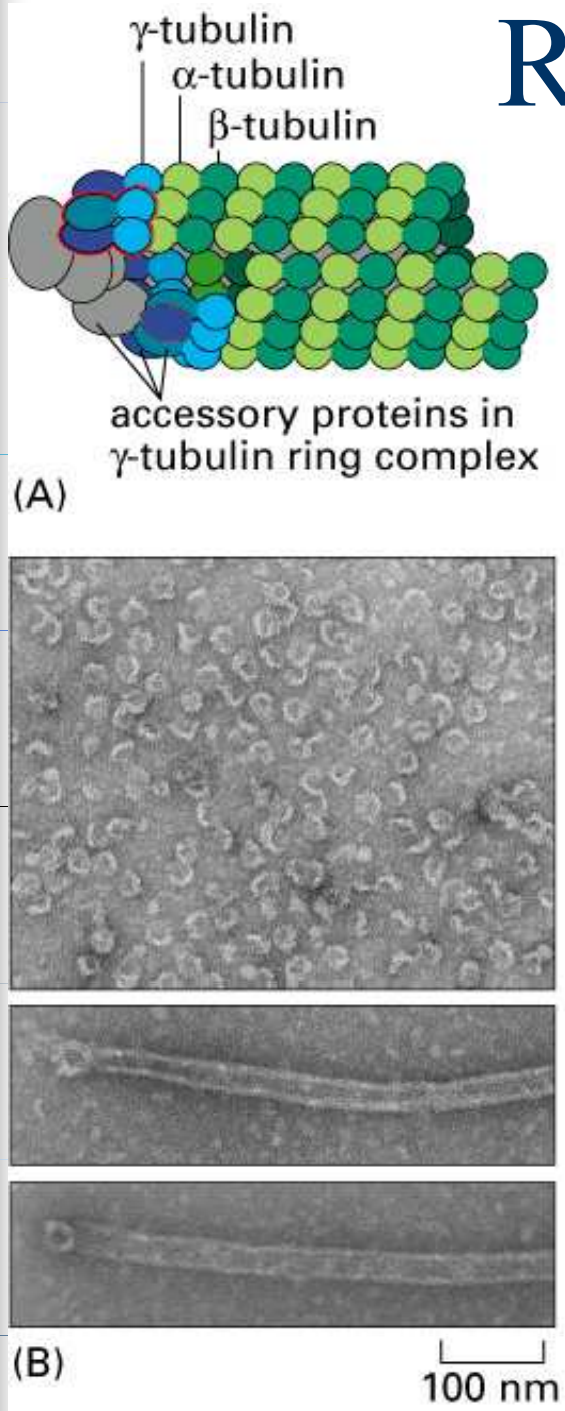


Figure 16–22. Molecular Biology of the Cell, 4th Edition.

- γ-tubulinový prstenec tvoří počátek – nukleační místo MT
- centrosom – organizační místo MT

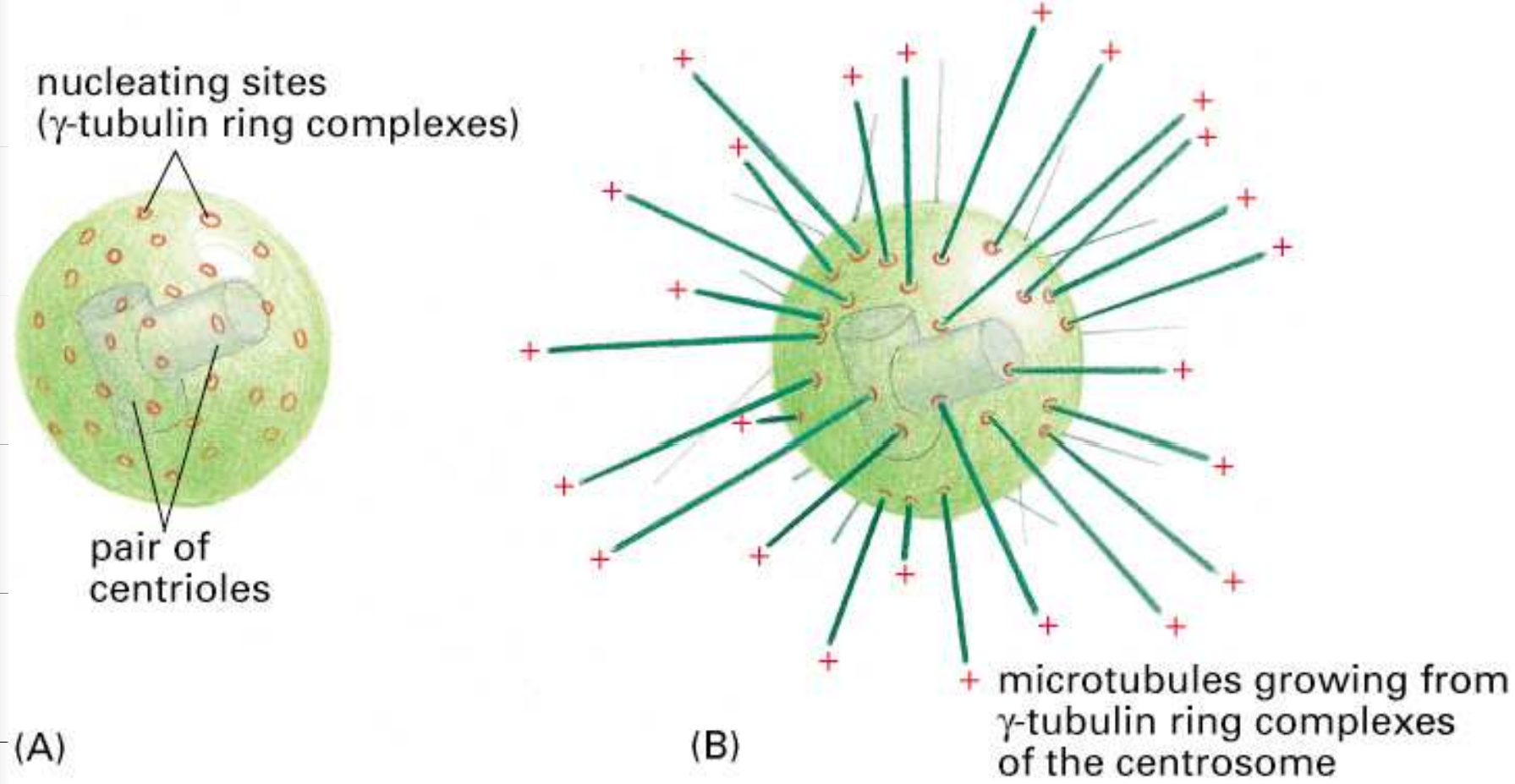
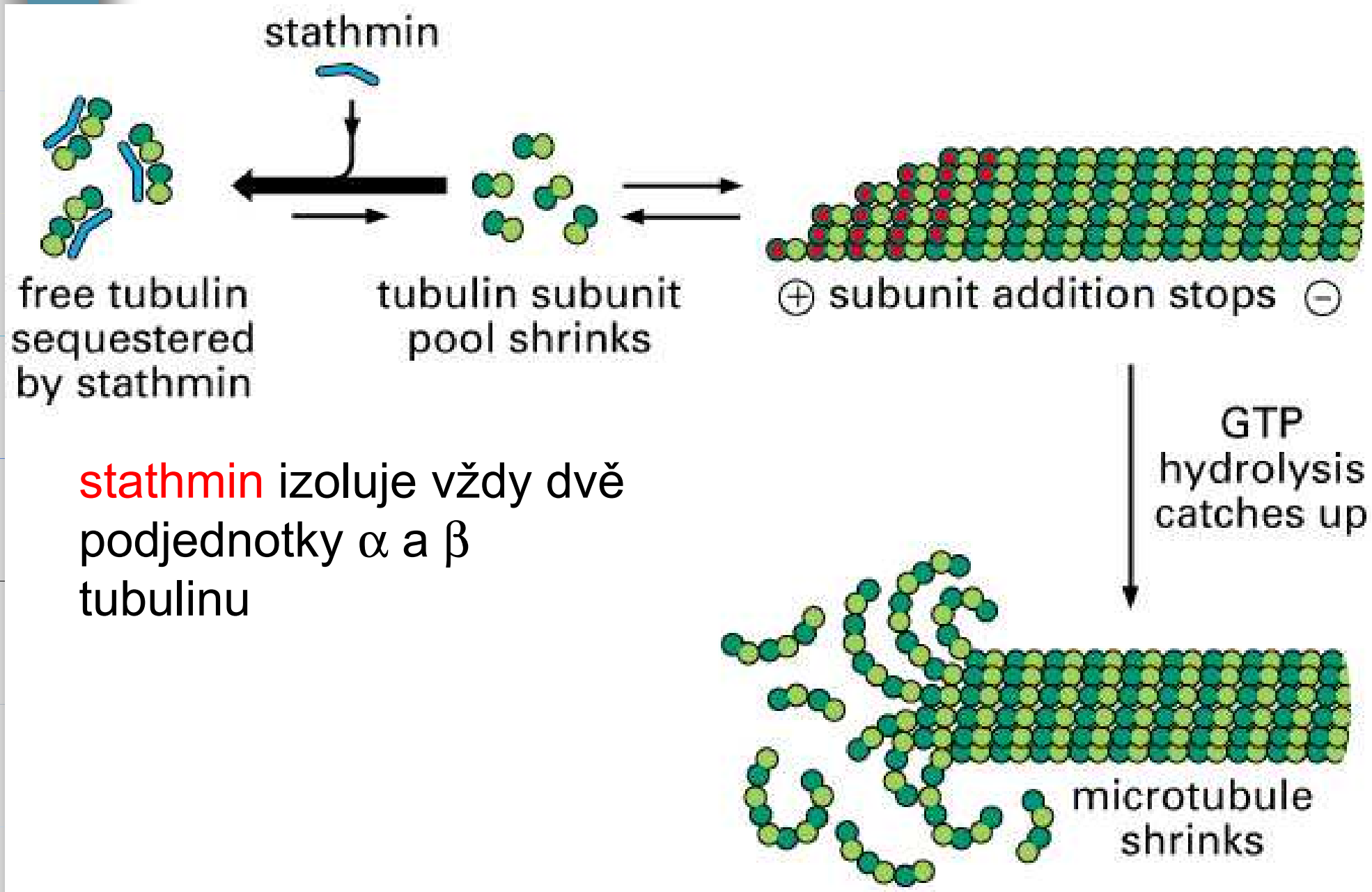


Figure 16–23. Molecular Biology of the Cell, 4th Edition.

- Centrioly jsou součástí centrosomu – válcovitě uspořádané krátké MT



stathmin izoluje vždy dvě podjednotky α a β tubulinu

Figure 16–31. Molecular Biology of the Cell, 4th Edition.

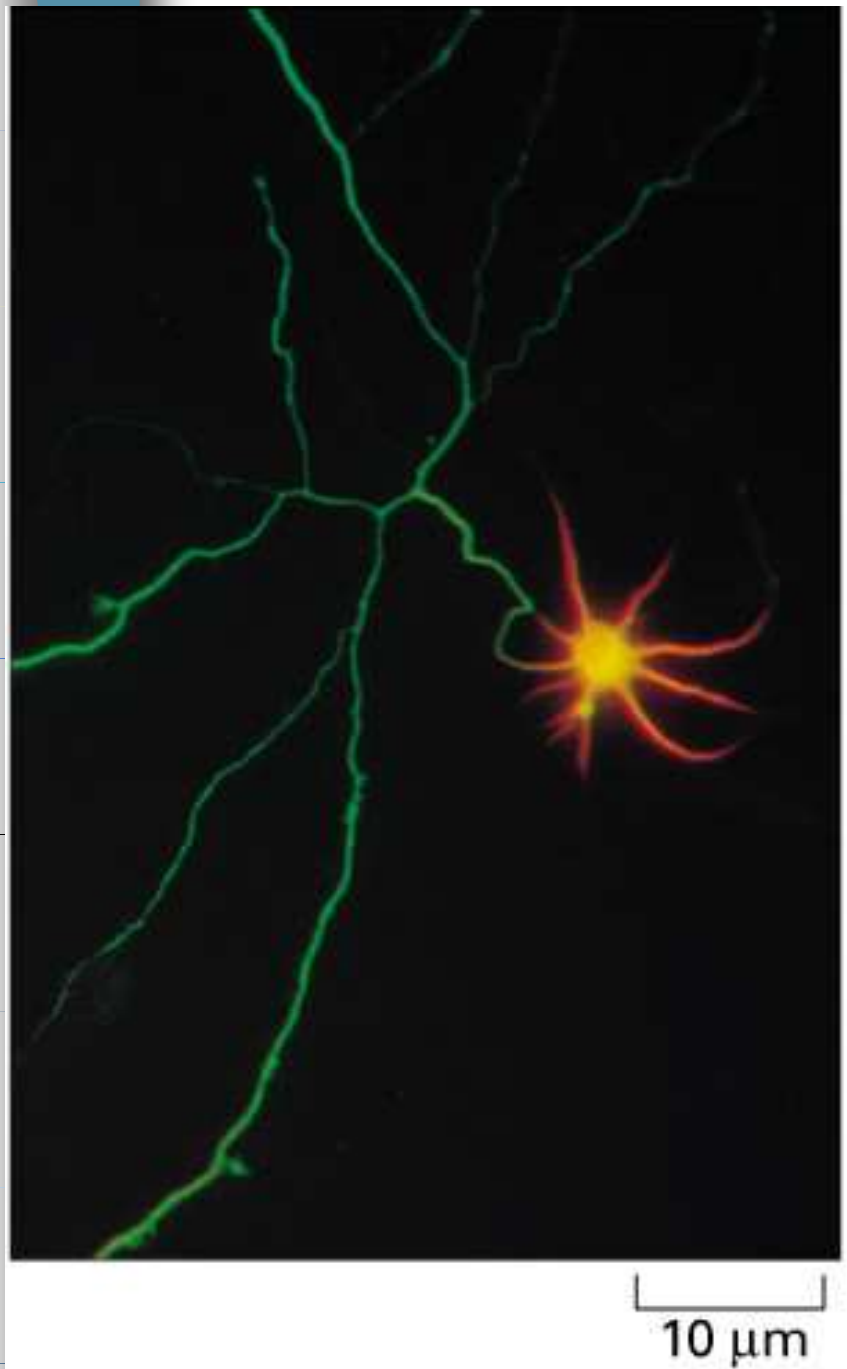


Figure 16–32. Molecular Biology of the Cel

MAP mohou regulovat stabilitu MT

- **MAP2** = prostornější MT
-**tau** = kompaktní MT
-**catastrophin** = destabilizuje MT

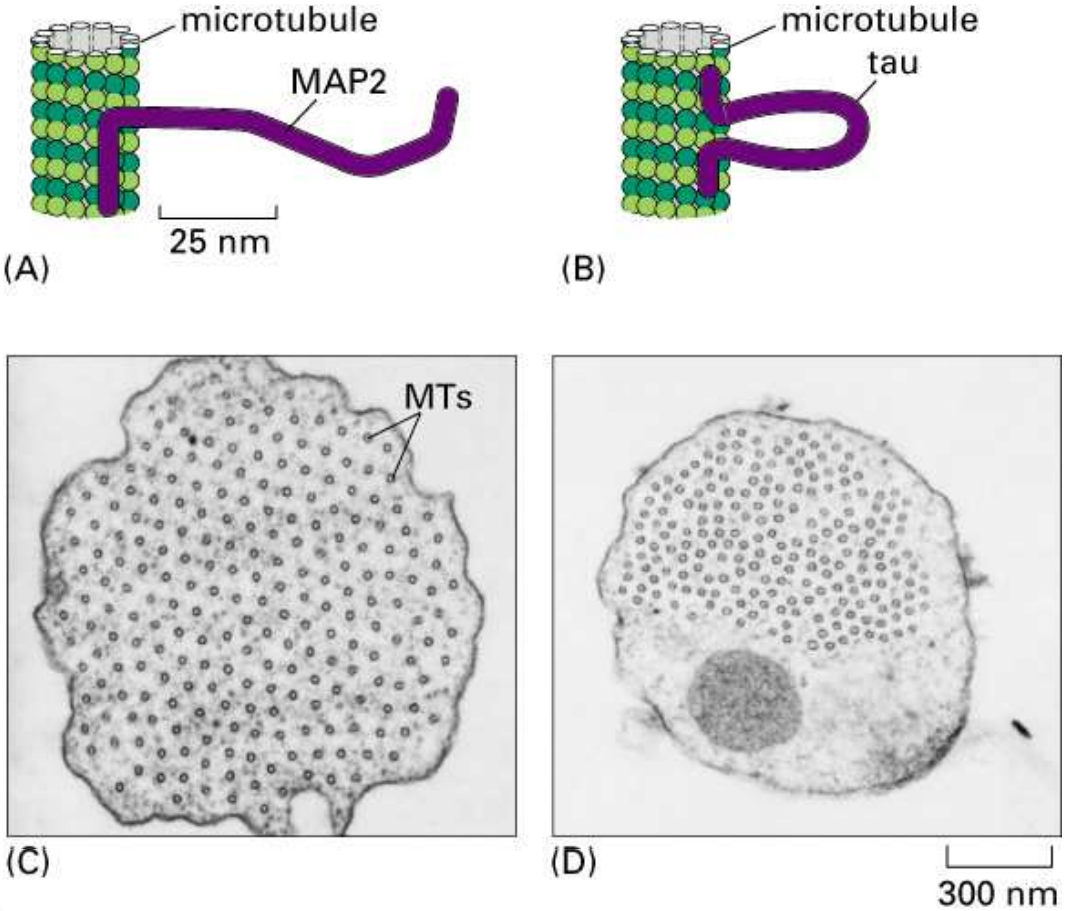


Figure 16–33. Molecular Biology of the Cell, 4th Edition.

Regulace stability MT

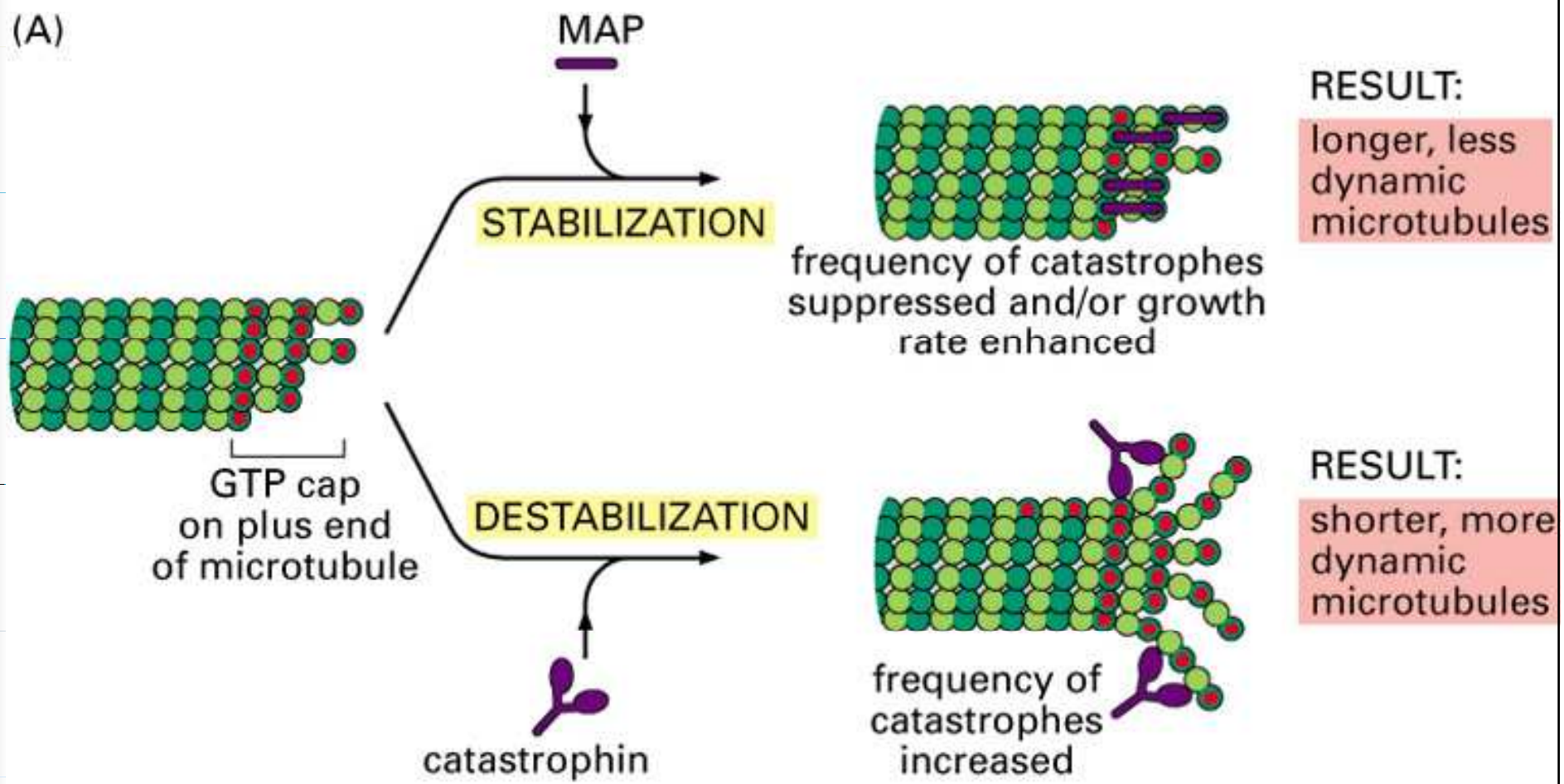
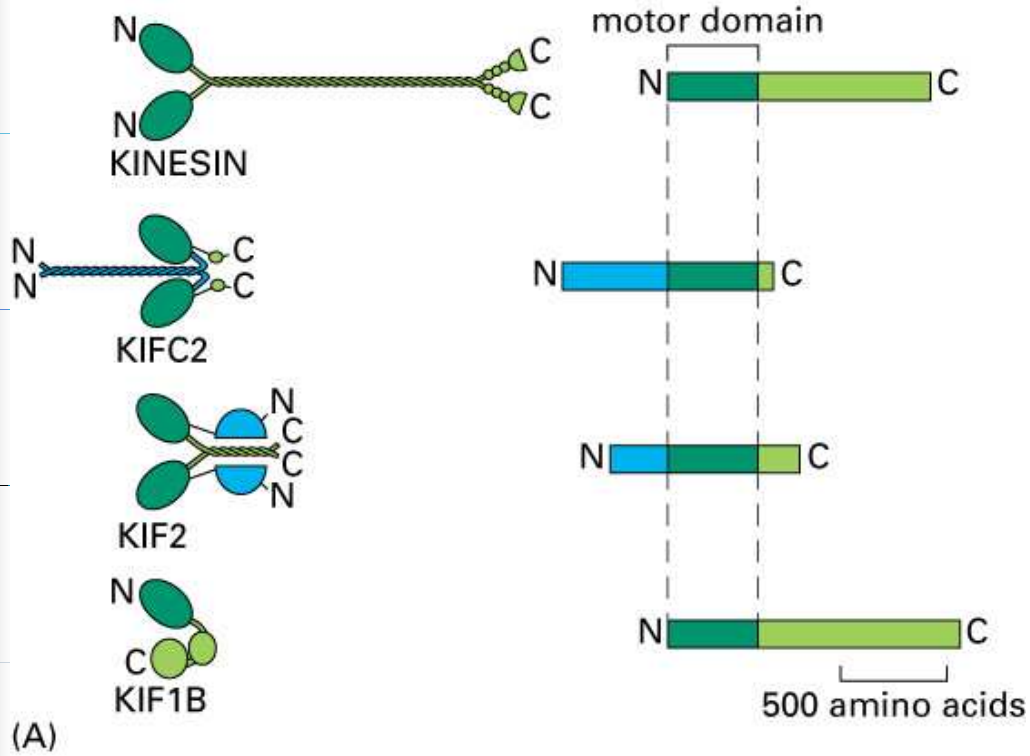


Figure 16–36 part 1 of 2. Molecular Biology of the Cell, 4th Edition.

Molekulové motory spojené s mikrotubuly - Kinesiny (➔+) a dyneiny (➔ -)



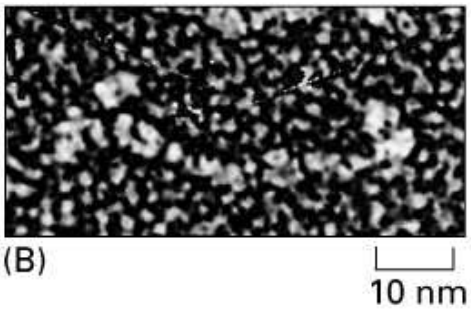


Figure 16–55. Molecular Biology of the Cell, 4th Edition.

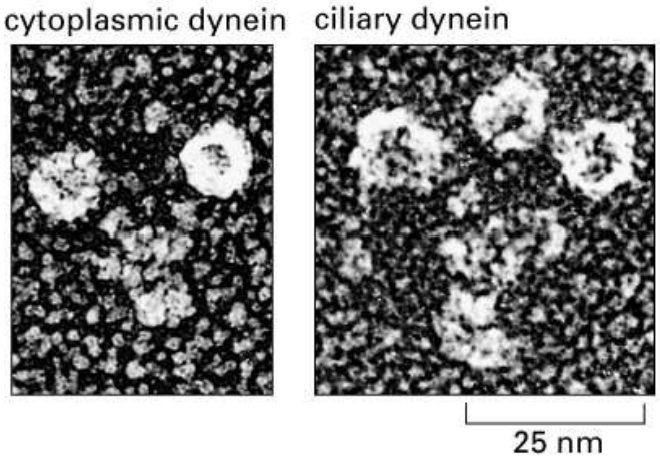


Figure 16–56. Molecular Biology of the Cell, 4th Edition.

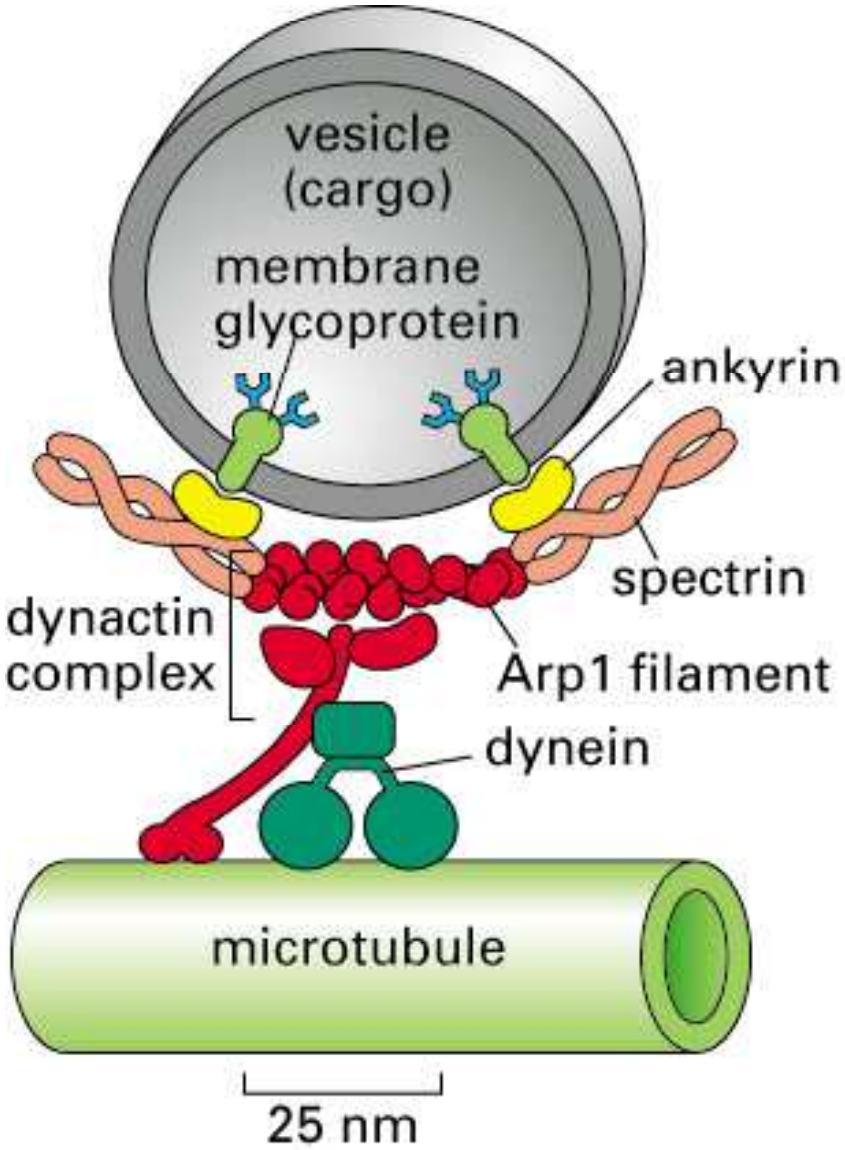


Figure 16–63. Molecular Biology of the Cell, 4th Edition.

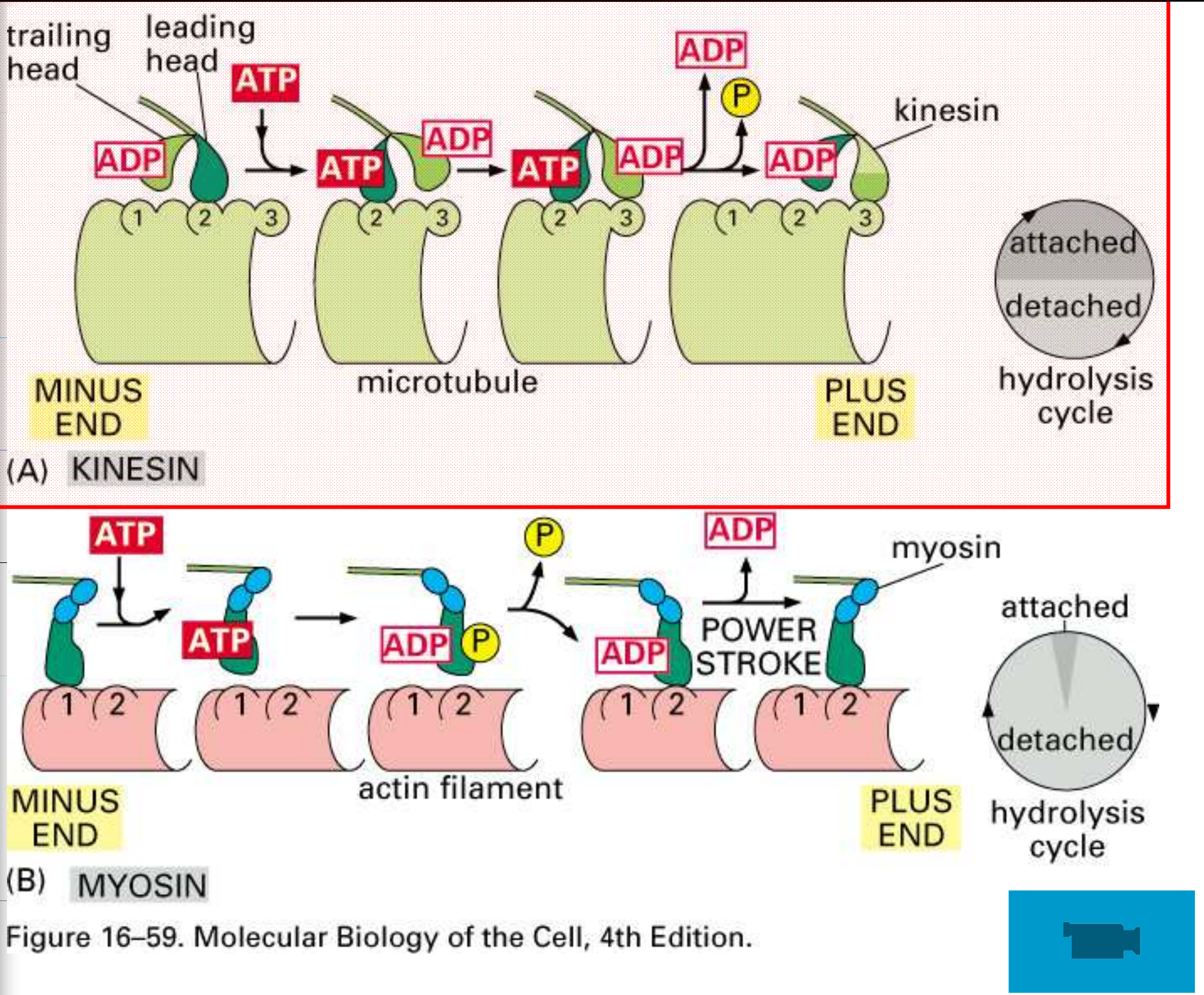
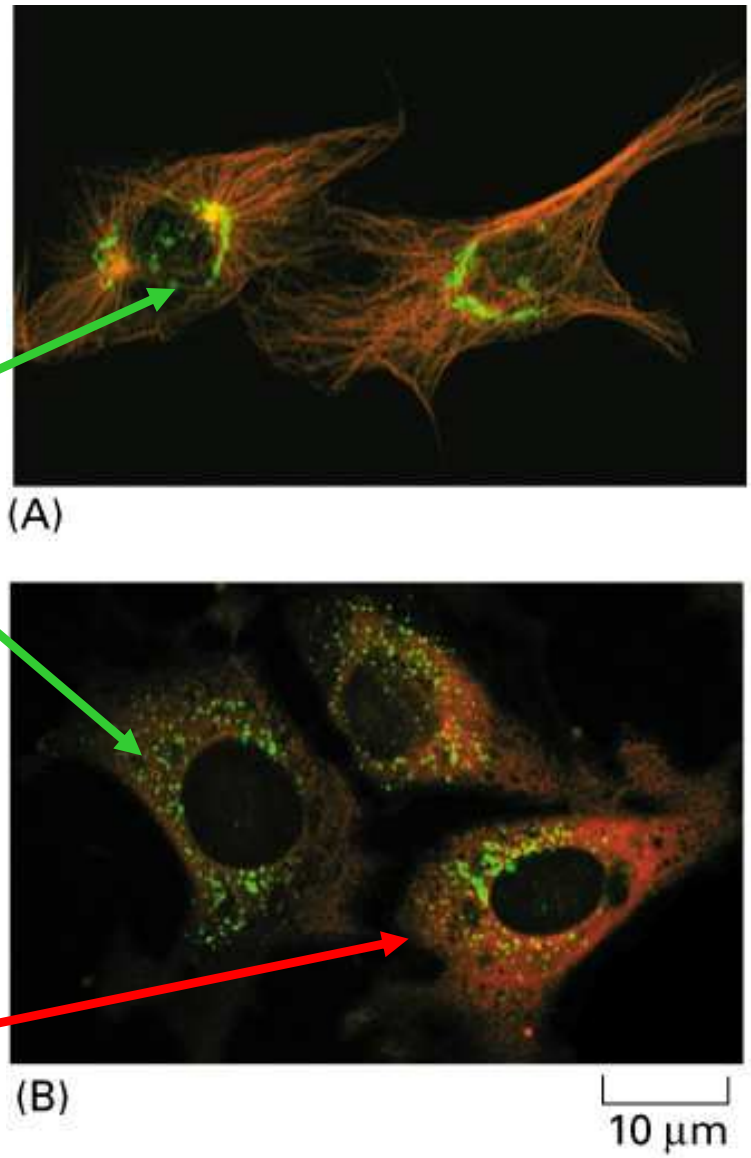


Figure 16–59. Molecular Biology of the Cell, 4th Edition.

Polymerizované MT jsou klíčové pro lokalizaci Golgiho aparátu

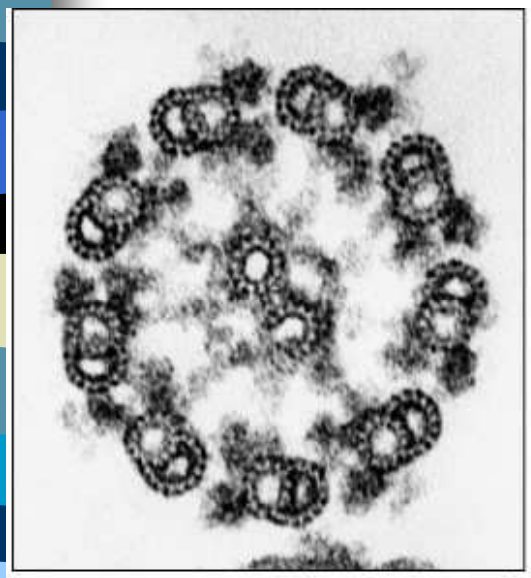


Nocodazole – depolymerizace MT

Figure 16–62. Molecular Biology of the Cell, 4th Edition.

Uspořádání mikrotubulů v řasince nebo bičíku

(9+2)



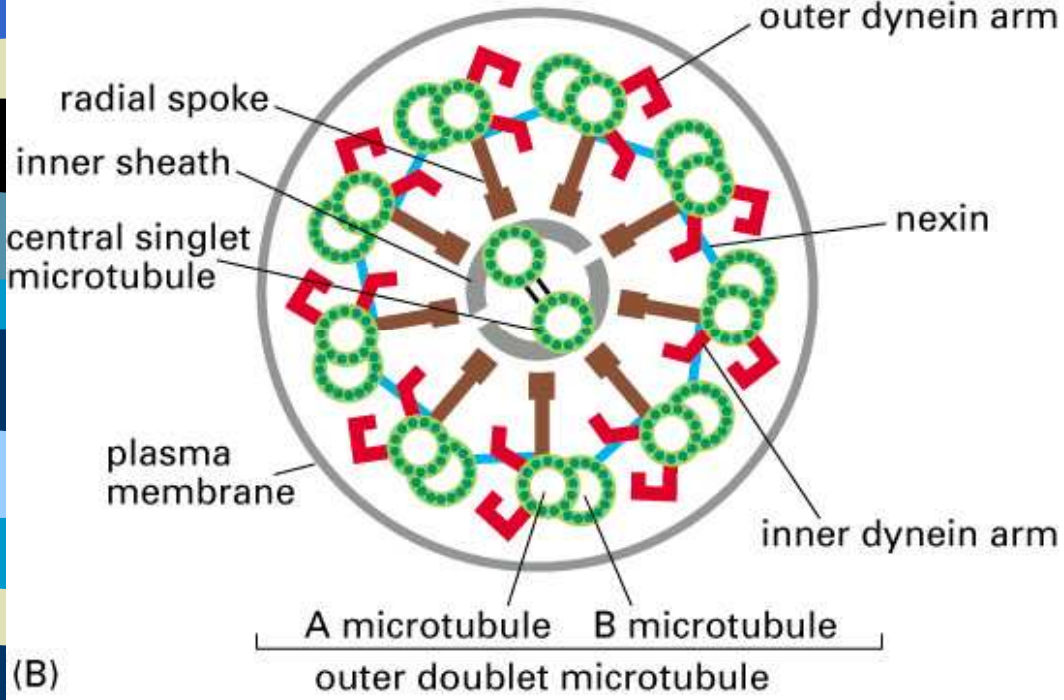
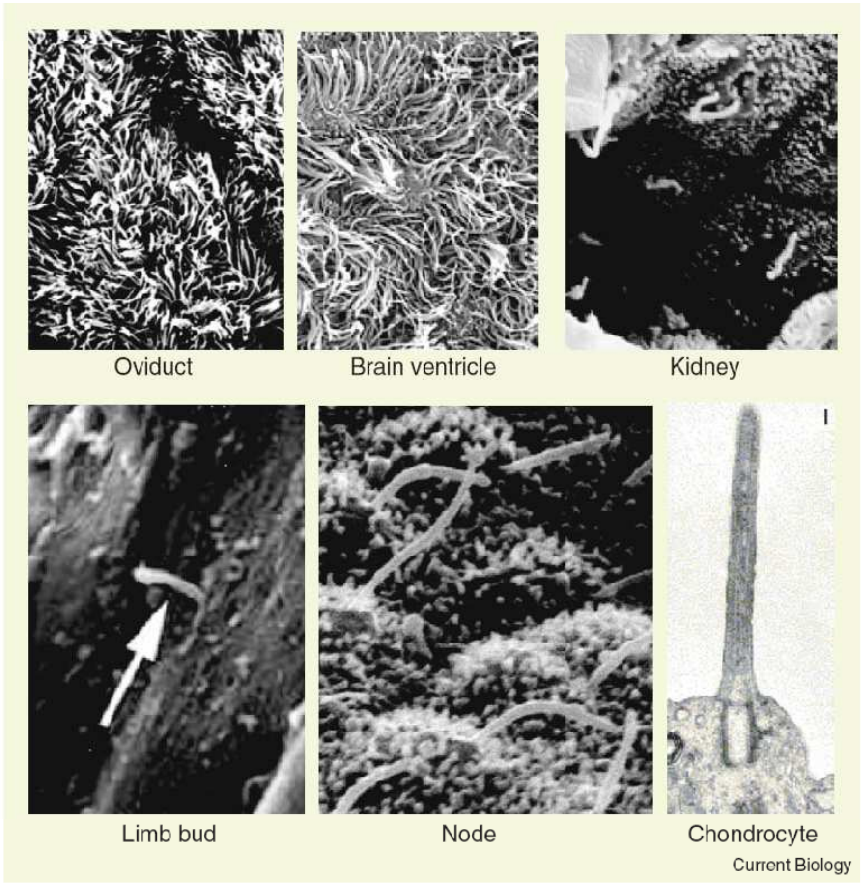
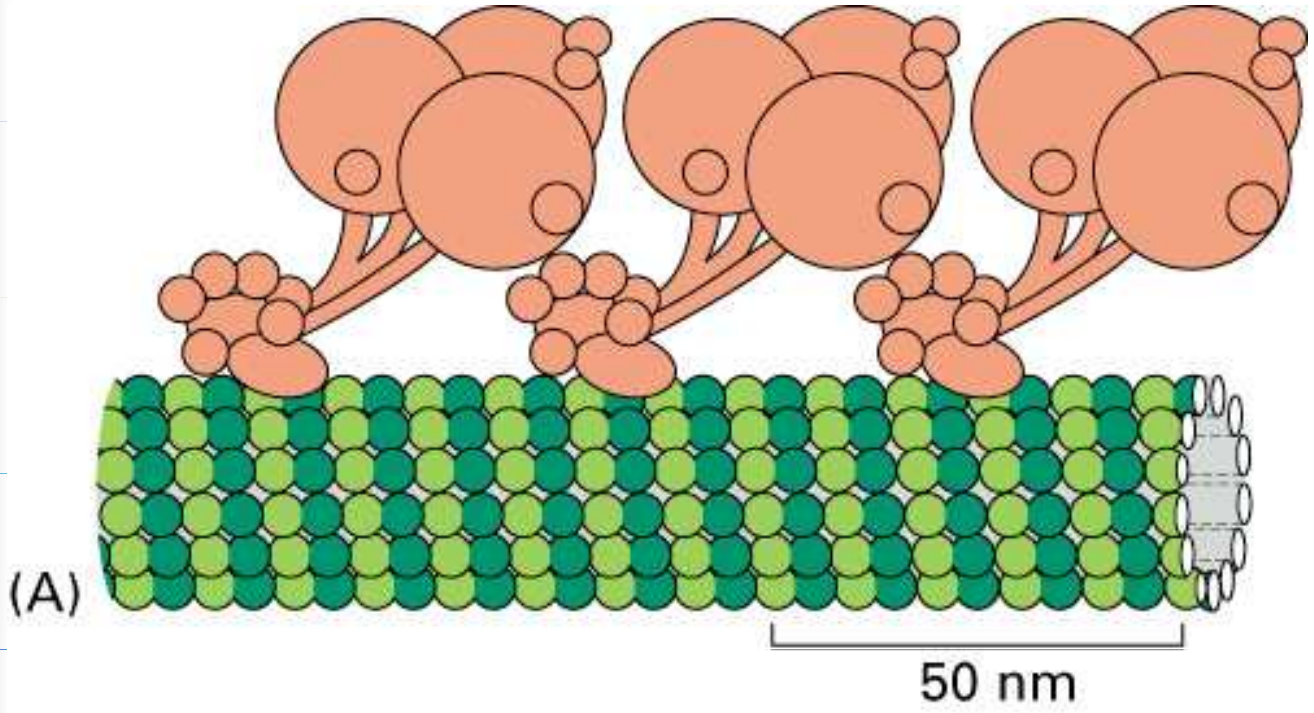
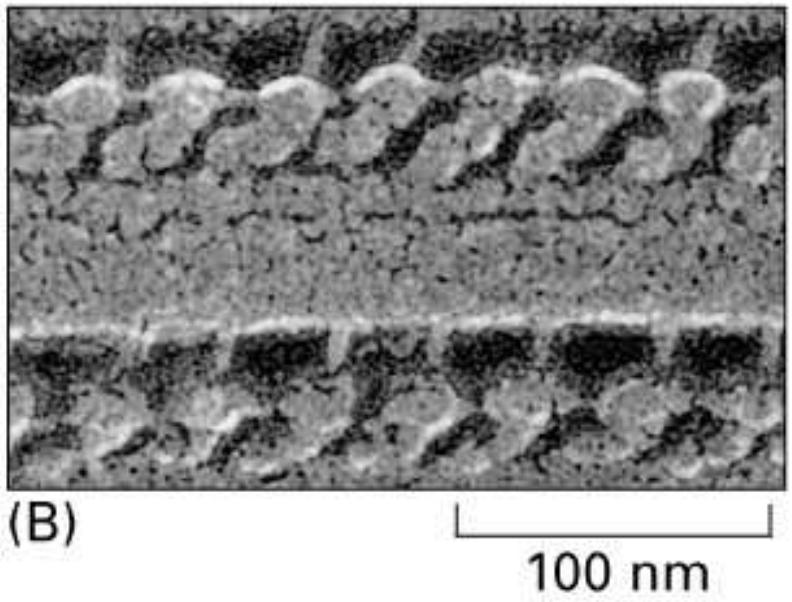


Figure 16–77. Molecular Biology of the Cell, 4th Edition.







Řasinkový dynein

Figure 16–78. Molecular Biology of the Cell, 4th Edition.

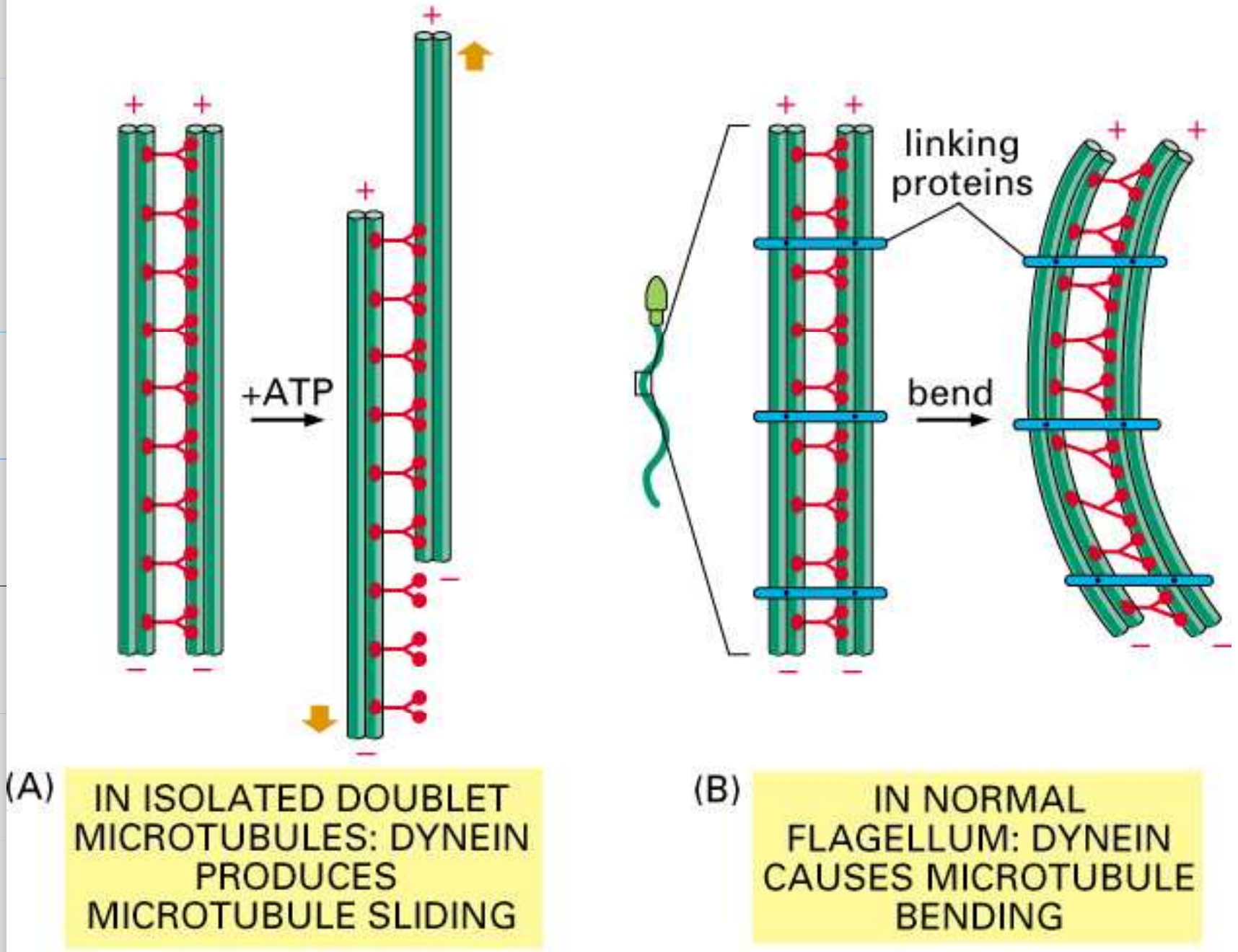


Figure 16–79. Molecular Biology of the Cell, 4th Edition.

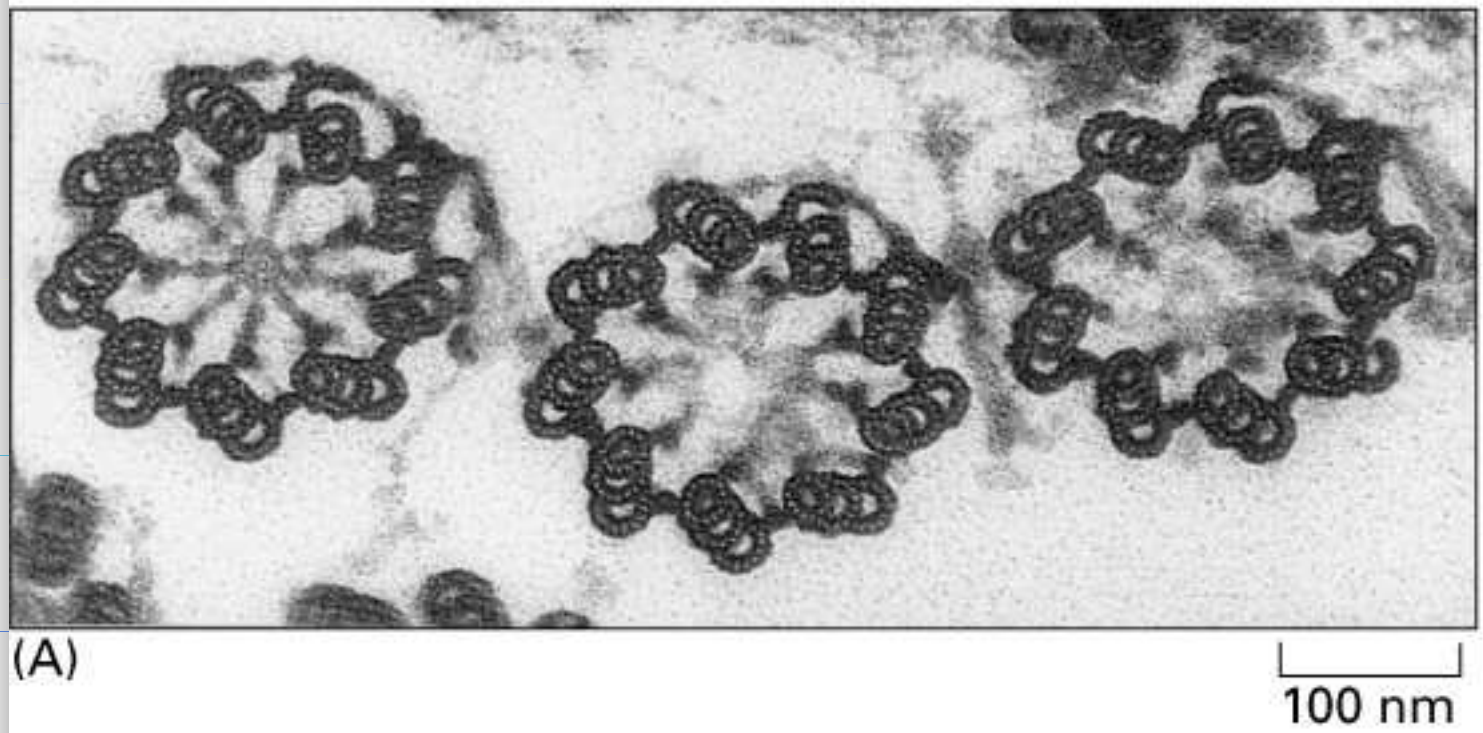


Figure 16–80. Molecular Biology of the Cell, 4th Edition.

Organizace mikrotubulů ve fibroblastu a neuronu

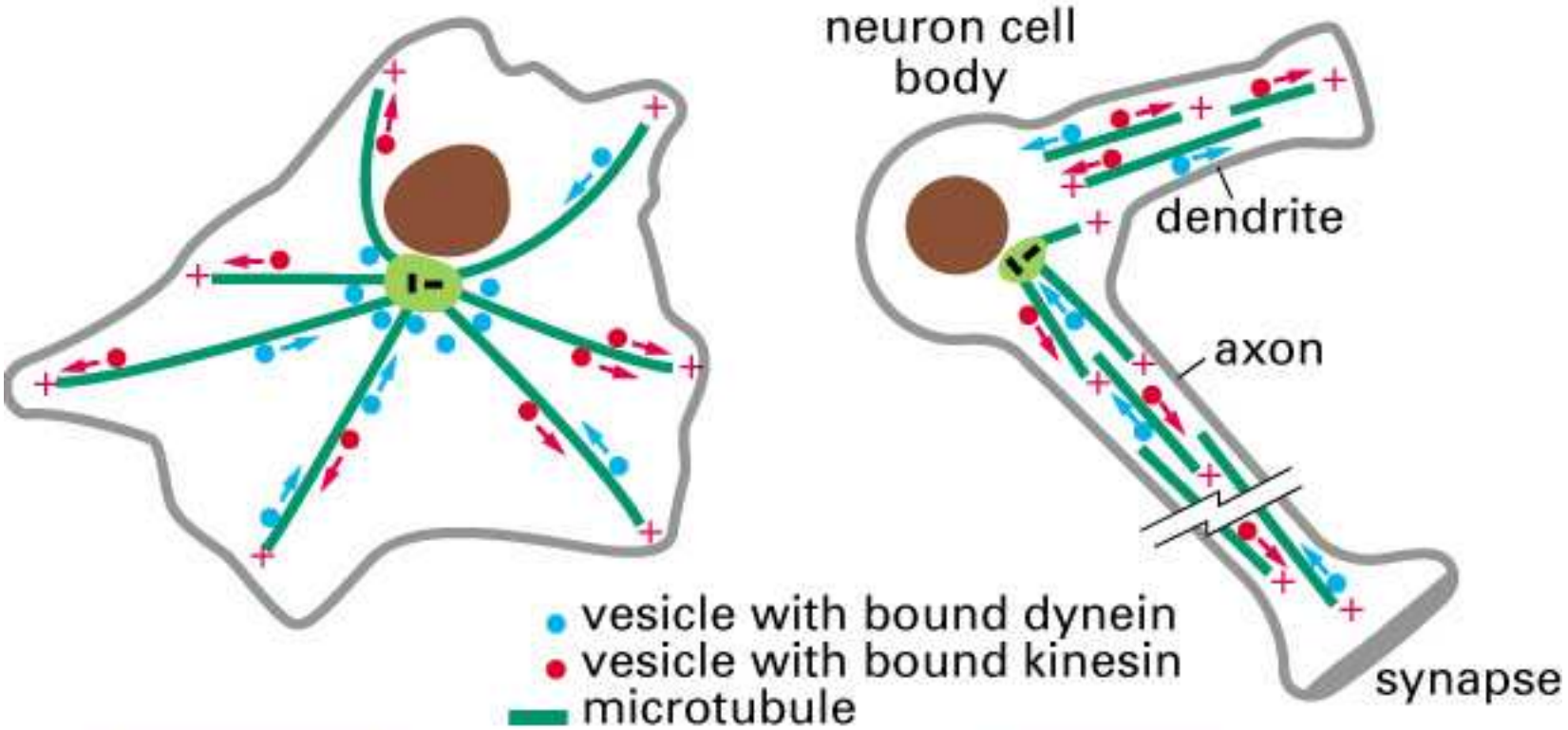
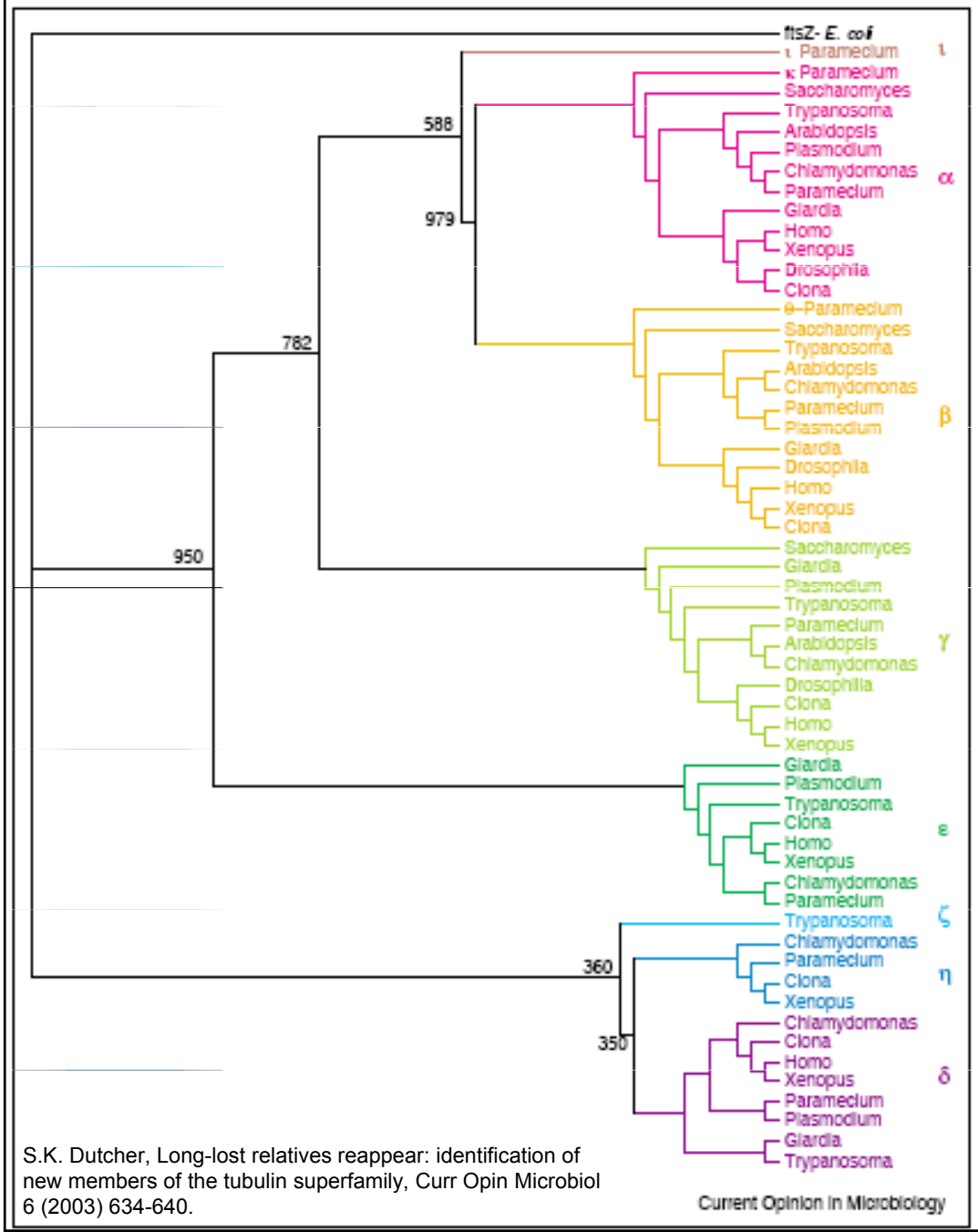


Figure 16–98. Molecular Biology of the Cell, 4th Edition.

Shrnutí - II

- dynamika MT může být regulována pomocí MAP
- hydrolýza ATP je zdroj energie pro pohyb molekulových motorů asociovaných s MT - kinesinu a dyneinu
- dynein-MT komplex je zdrojem pohybu řasinek a bičíků
- organizace MT závisí na buněčném typu

Mikrotubuly – rozlehlá rodina proteinů



S.K. Dutcher, Long-lost relatives reappear: identification of new members of the tubulin superfamily, Curr Opin Microbiol 6 (2003) 634-640.

αβ - heterodimeric protein; basic building block of microtubules

γ - essential role in initiating microtubule assembly at MTOCs, such as spindle pole body, centrosome and basal body

δ - localized to the centrosome or basal body and other regions

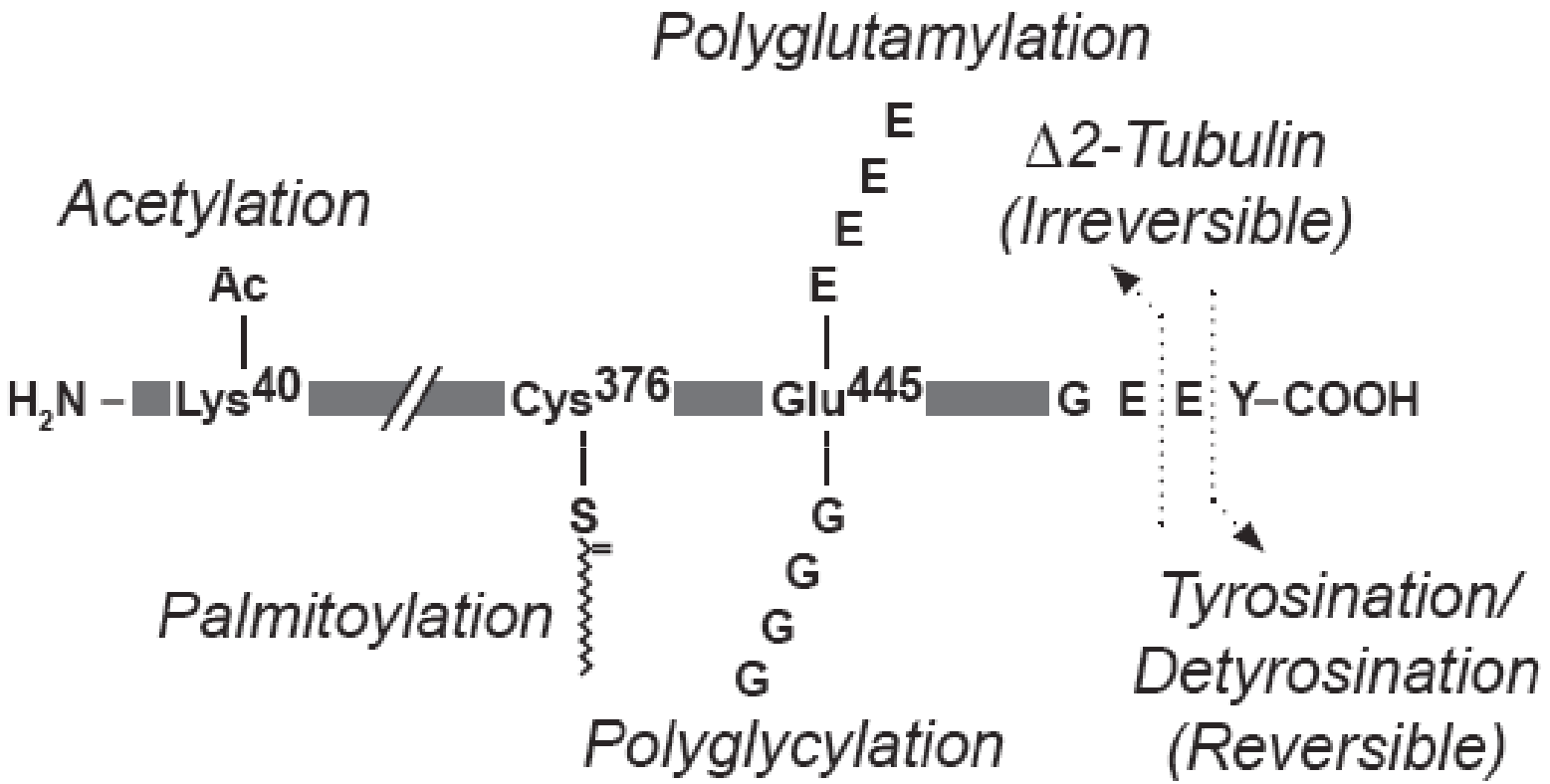
ε - localised to the centrosome in cell-cycle-dependent manner

ζ - localized to the basal body of *Trypanosoma brucei*

η - no localization data available

McKean P.G. et.al.
J. Cell Sci., 114, 2001, 2723-2733

Posttranslační modifikace tubulinu



Souček et al. Prostate 2006 podle
Westermann S., Weber K. Nature Rev. Mol. Cell. Biol., Vol 4, 2003, 938-947

Posttranslační modifikace tubulinu

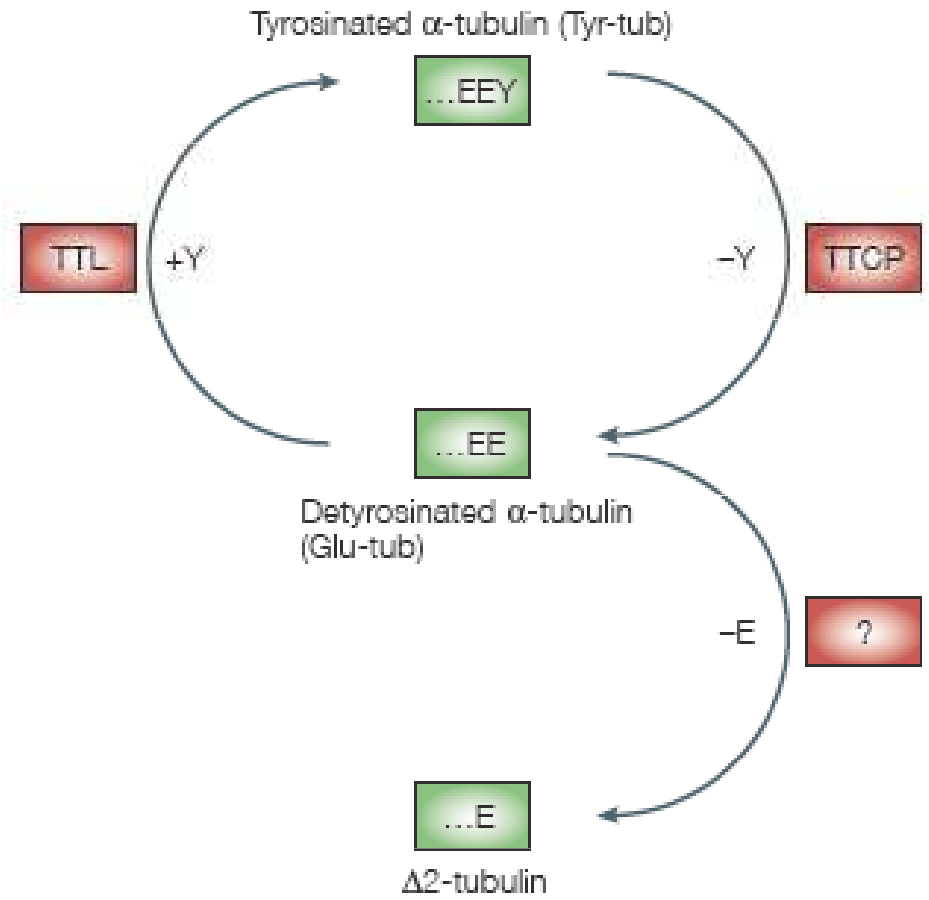
Table 1 | **Overview of the various tubulin modifications and their proposed functions**

α-tubulin	Modification	Comments	Enzymes	Proposed functions
H_2N Ac 40 GEEY	Acetylation/deacetylation	Only α-tubulin; marker for stable microtubules	HDAC6, SIRT2	Regulation of cell motility, binding of MAPs to microtubules
H_2N GEE	Tyrosination/detyrosination	Reversible; enzyme TTL cloned	TTL	Crosstalk to intermediate filaments; differentiation
H_2N GE	Generation of Δ2-tubulin	Only α-tubulin; marker for stable microtubules	?	Removing tubulin from tyrosination cycle; marking microtubules for polyglycylation?
H_2N 445 E E E E GEEY	Polyglutamylation	α- and β-tubulin; multiple glutamylation sites possible; up to 20 side-chain residues	Nek (Cf)	Centriole maturation and stability; flagellar and ciliary motility; regulation of interaction with MAPs
H_2N 445 G G G G GEEY	Polyglycylation	α- and β-tubulin; multiple glycylation sites possible; up to 30–40 side-chain residues	?	Essential in *Tetrahymena* for: axonemal organization, ciliary motility, cytokinesis (severing of microtubules)
H_2N 376 GEEY	Palmitoylation	Demonstrated for budding yeast α-tubulin on residue 376	?	Positioning of astral microtubules in budding yeast; interaction with cell cortex?
H_2N P GEEY	Phosphorylation	Better established for β-tubulin on Ser441/444	?	Neuronal differentiation?

Ac, acetate; Cf, *Crithidia fasciculata*; E, glutamic acid; G, glycine; Nek, NIMA (never in mitosis gene A)-related kinase; HDAC6, histone deacetylase 6; MAP, microtubule-associated protein; P, phosphate; SIRT, Sir2 homologue; TTL, tubulin tyrosine ligase; Y, tyrosine.

Westermann S., Weber K.
Nat[illegible] Mol. Cell. Biol., Vol 4, 2003, 938-947

The tyrosination cycle of α-tubulin

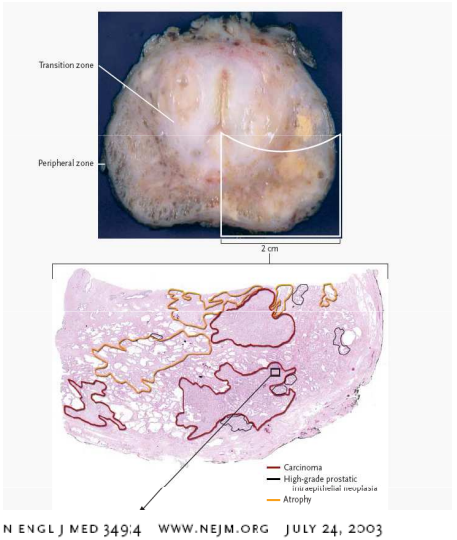


Westermann S., Weber K.
Nat… Mol. Cell. Biol., Vol 4, 2003, 938-947

Profil posttranslačních modifikací α-tubulinu jako možný „fingerprint“ nádorů prostaty.

Souček, K. *et al.*, 2006

Rakovina prostaty



Histologické změny

Muži starší 50ti let: 30%
Muži starší 80ti let: 70%

Klinický výskyt

Nejčastěji diagnostikovaný novotvar u mužů.
Primární nádory: maligní, žlázového původu – adenokarcinomy.
Sekundární nádory: kostní metastáze.

Prognóza

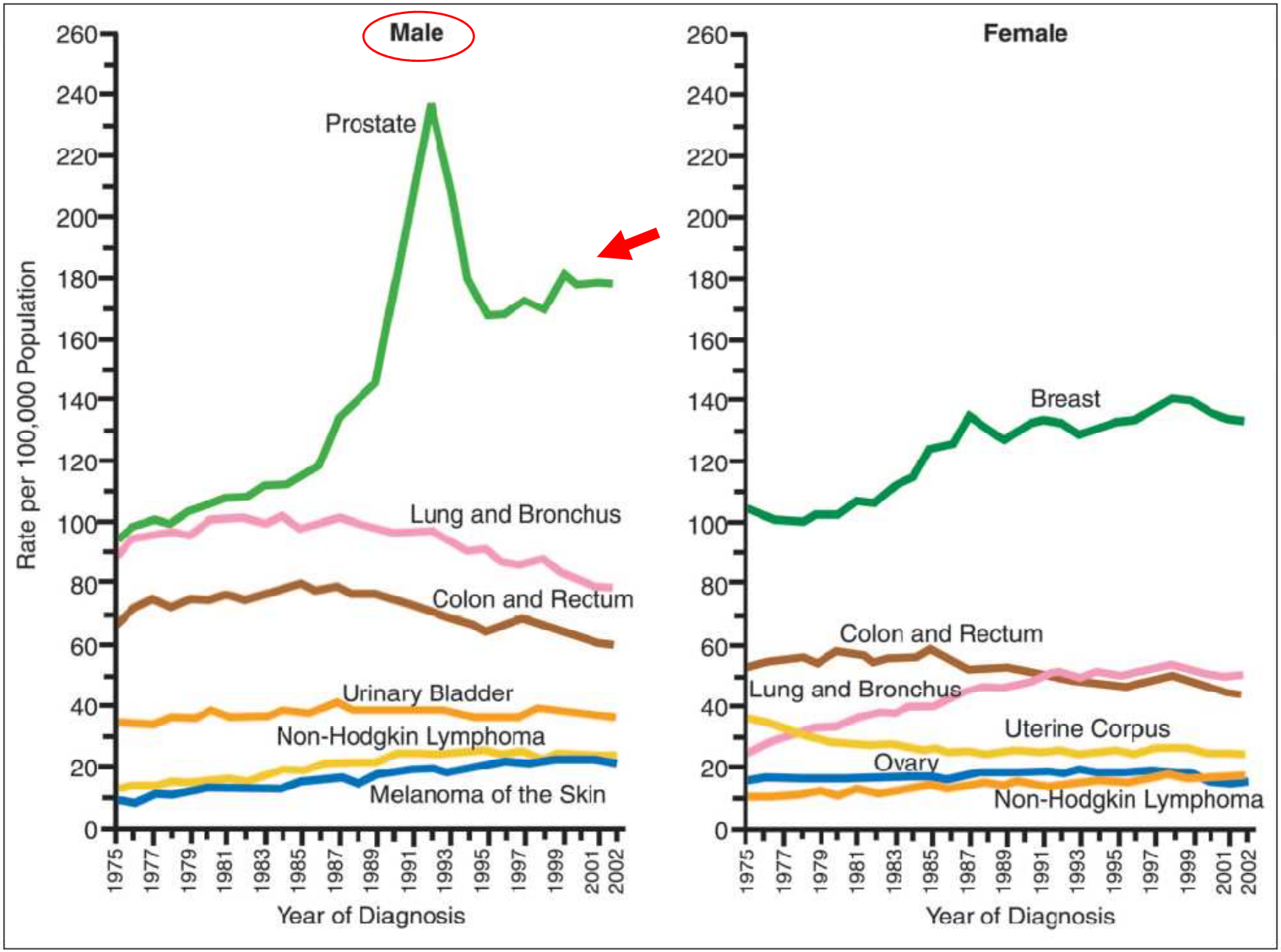
Závisí na fázi ve které je nádor zachycen a léčen. U stádií 1-3 >60% pacientů přežívá >5let. Ve stadiu 4 (metastáze) má pouze 30% naději na dožití >5let.

Léčba

Chirurgická, radioterapie, anti-androgenní terapie, chemoterapie.

Úmrtnost

Třetí nejčastější příčina úmrtí na nádorové onemocnění.



Cancer Statistics, 2006

CA
A Cancer Journal for Clinicians

Stages 1 and 2
(Cancer that is only in the prostate gland, PSA)

Between 65 - 98% with stage 1 and 2 prostate cancer will live for more than five years after they are diagnosed.

Stage 3
(Cancer cells have spread outside the covering (capsule) of the prostate gland to tissues around the prostate but not to the lymph nodes.)

About 60% diagnosed with stage 3 prostate cancer will live for more than five years after diagnosis.

Stage 4
(Cancer cells have spread (metastasized) to lymph nodes (near or far from the prostate gland) or to organs and tissues far away from the prostate such as the bone, liver, or lungs.)

About 20 -30% have cancer spread to another part of their body when they are diagnosed with prostate cancer.

About 30% men with advanced prostate cancer will live for more than five years after diagnosis.

On average, men in this situation can expect their cancer to respond to treatment for about 12 to 18 months. Average survival after that is about another two years.

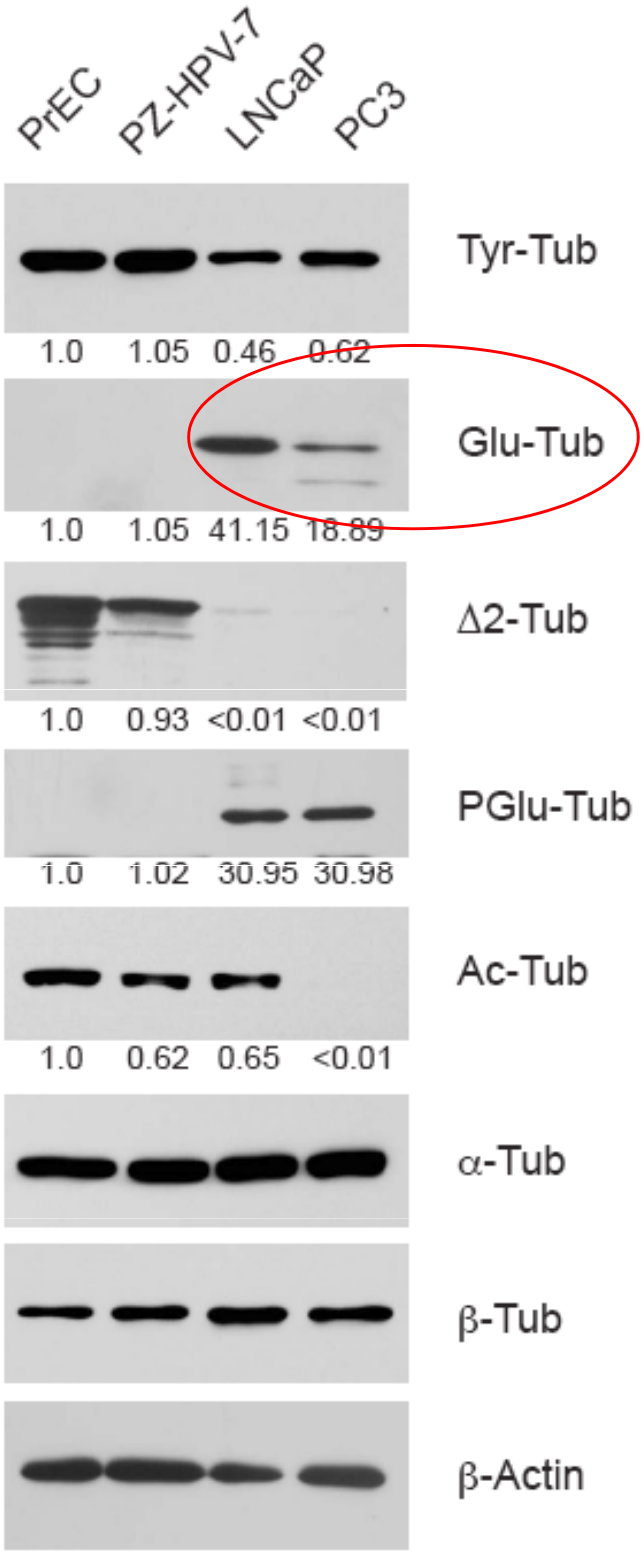
www.cancerhelp.org.uk, www.phoenix5.org/staging.html

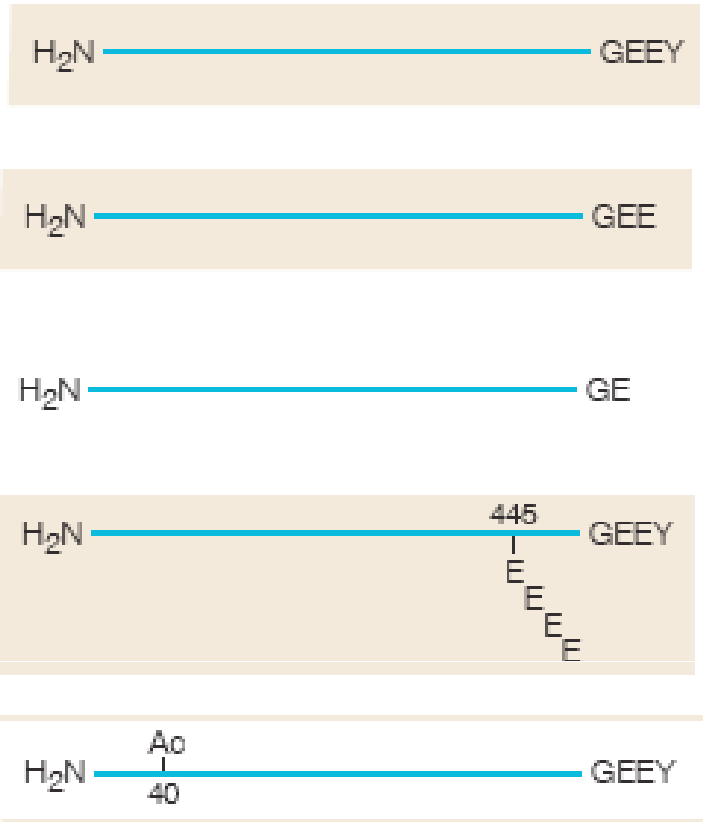
Future Diagnostic tools

- Gene Chip Analysis (prostate specific genes)
- Proteomic (tissue, serum, urine)

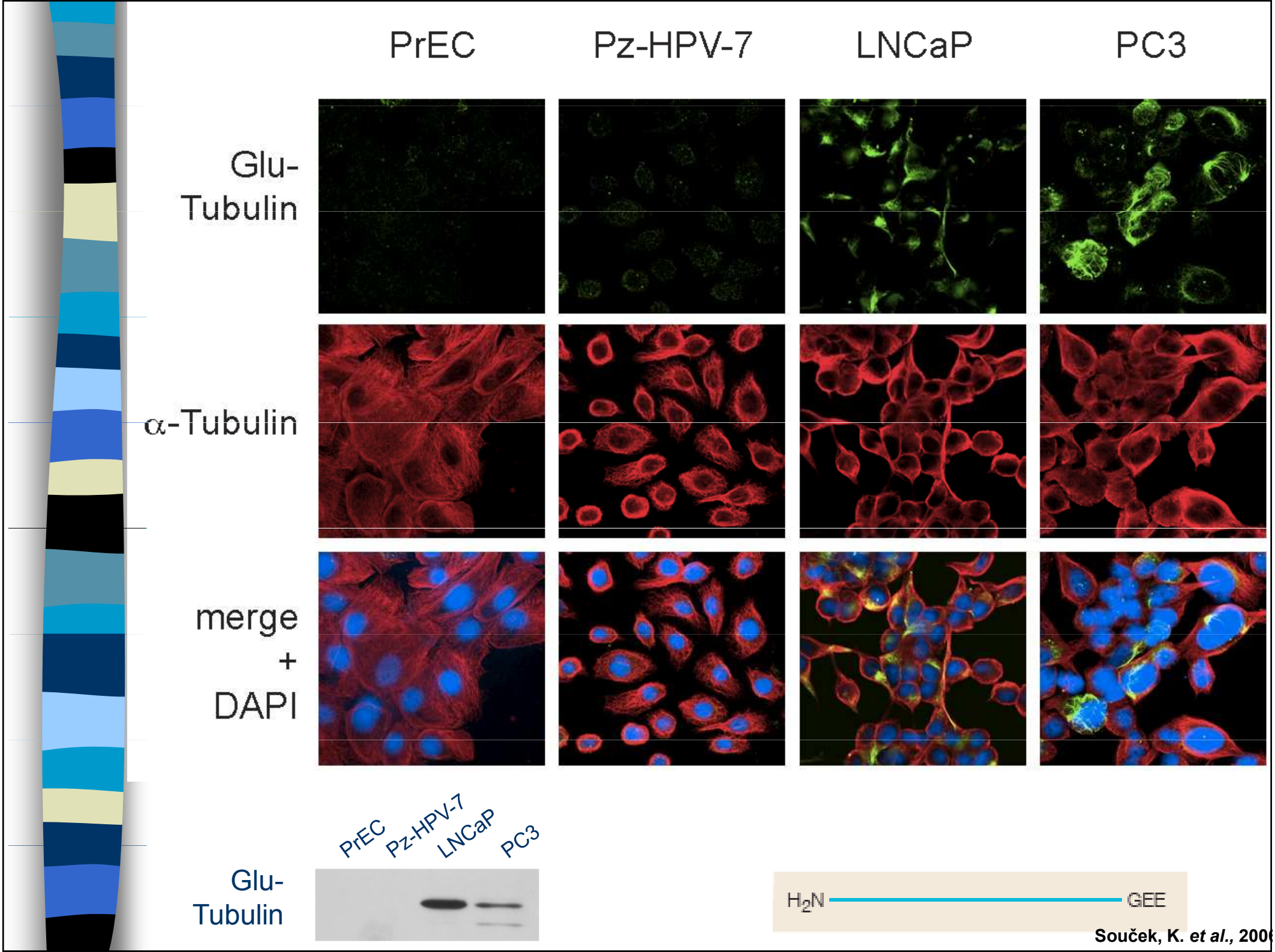
➔specific ~ “fingerprint”
~ “signature”

Post-translational modification of α-Tubulin in prostate epithelial cells





Souček, K. *et al.*, 2006



Souček, K. *et al.*, 200

Tubulin Tyrosine Ligase - potential tumor supressor?

Journal of Cell Science 111, 171-181 (1998)
Printed in Great Britain © The Company of Biologists Limited 1998
JCS9695

Suppression of tubulin tyrosine ligase during tumor growth

Laurence Lafanechère[1,*], Céline Courtay-Cahen[2,*,†], Toshiaki Kawakami[3], Michèle Jacrot[4], Manfred Rüdiger[5], Jürgen Wehland[6], Didier Job[1] and Robert L. Margolis[2,‡]

[CANCER RESEARCH 61, 5024–5027, July 1, 2001]

Tubulin Detyrosination Is a Frequent Occurrence in Breast Cancers of Poor Prognosis[1]

Agnes Mialhe,[2] Laurence Lafanechère,[2] Isabelle Treilleux, Nadine Peloux, Charles Dumontet, Alain Brémond, Meng-Hong Panh, Raoul Payan, Jürgen Wehland, Robert-Louis Margolis, and Didier Job[3]

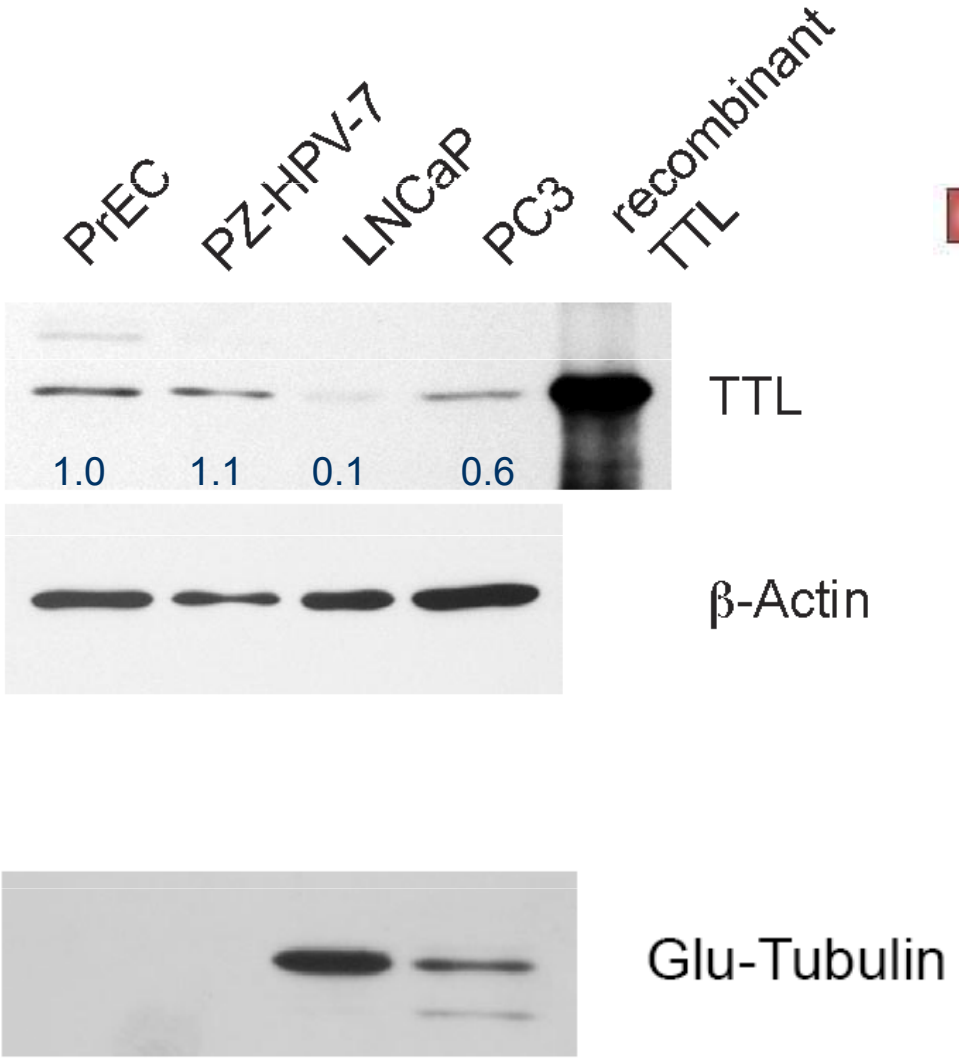
Int. J. Cancer: **112,** 365–375 (2004)

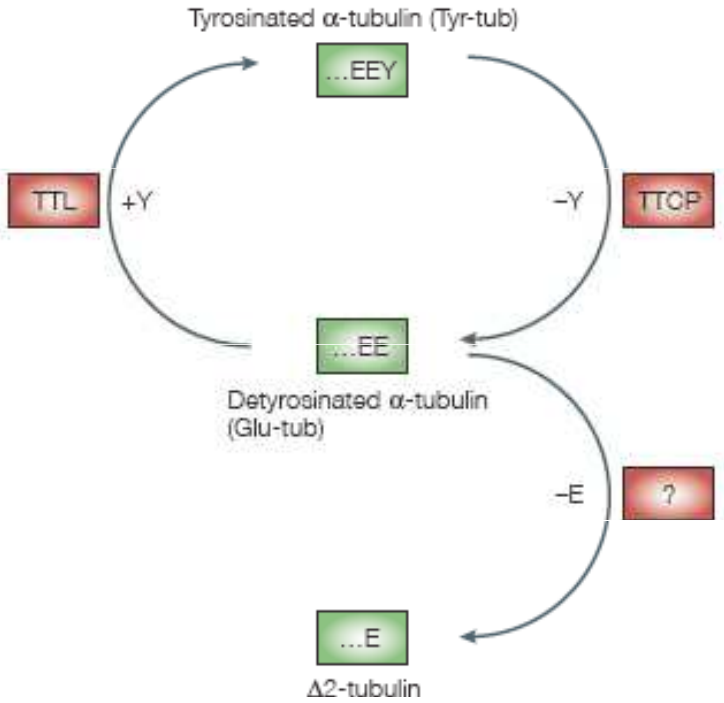
UICC Publication of the International Union Against Cancer

LOW EXPRESSION OF HUMAN TUBULIN TYROSINE LIGASE AND SUPPRESSED TUBULIN TYROSINATION/DETYROSINATION CYCLE ARE ASSOCIATED WITH IMPAIRED NEURONAL DIFFERENTIATION IN NEUROBLASTOMAS WITH POOR PROGNOSIS

Chiaki KATO[1,2], Kou MIYAZAKI[1], Atsuko NAKAGAWA[3], Miki OHIRA[1], Yohko NAKAMURA[1], Toshinori OZAKI[1], Toshio IMAI[2] and Akira NAKAGAWARA[1*]

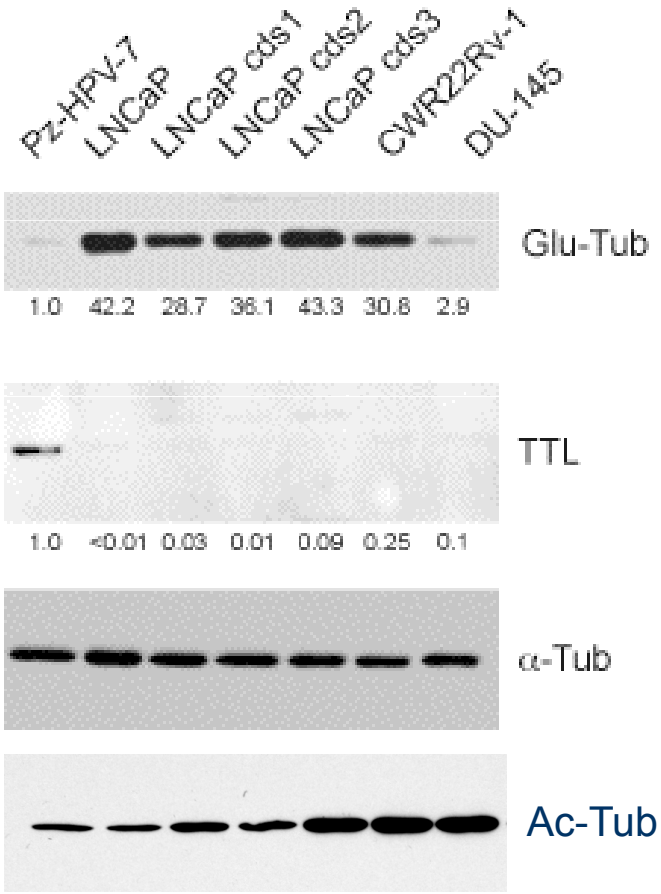
tubulin tyrosine ligase expression in prostate epithelial cells





Souček, K. *et al.*, 2006

Glu-Tubulin and Tubulin - Tyrosine Ligase expression in prostate epithelial cells - proof of the concept



Souček, K. *et al.*, 2006

SUMMARY

- Normal and prostate cancer cells display distinct molecular profiles of α-Tubulin posttranslational modifications
- Low expression of tubulin tyrosine ligase is characteristic also for prostate cancer cells
- Different profile of post-translation modifications α-Tubulin in various prostate epithelial cell lines show the possibility to distinguish the stages of cancer disease and has the potential to establish a novel tool to diagnose and treat prostate cancer.

Souček, K. *et al.*, 2006

Shrnutí - III

- Postranslační modifikace MT mohou ovlivňovat jejich funkci
- detyrosinace α-tubulinu je evolučně konzervovaný proces – klíčovým enzymem je tubulin tyrosin ligáza (TTL)
- detyrosinace α-tubulinu koreluje s rozvojem některých nádorových onemocnění

Shrnutí přednášky

- struktura
- dynamika
- posttranslační modifikace

Na konci dnešní přednášky by jste měli:

1. znát základní strukturu mikrotubulů (MT);
2. umět popsat způsob jakým řízena dynamika a stabilita MT;
3. znát základní postranslační modifikace MT.