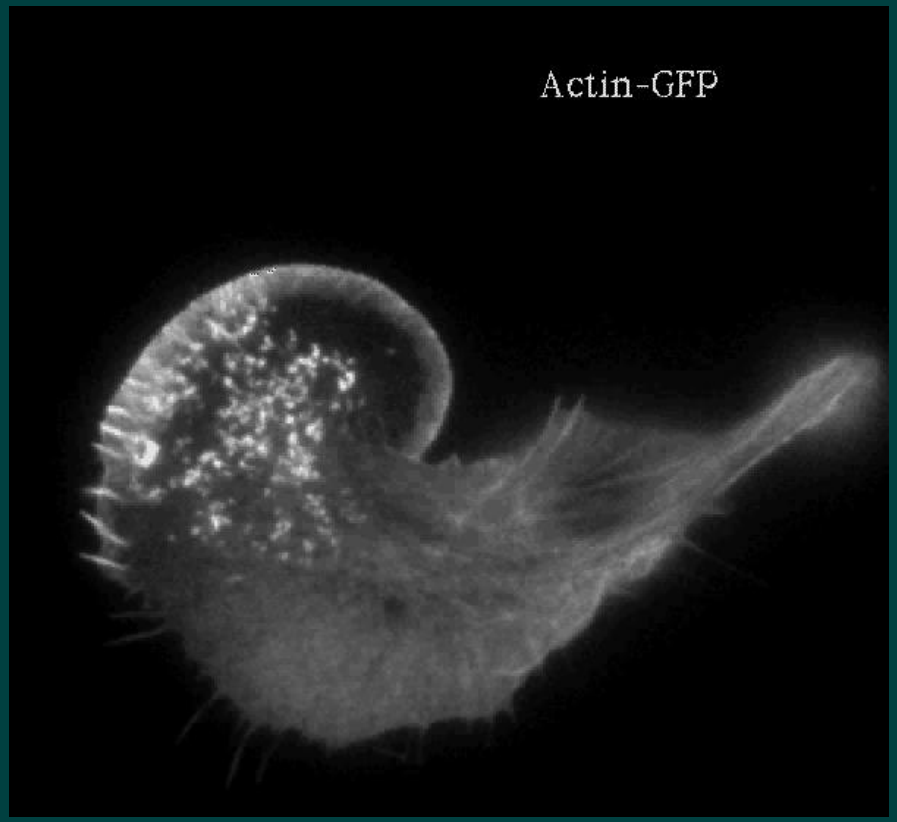
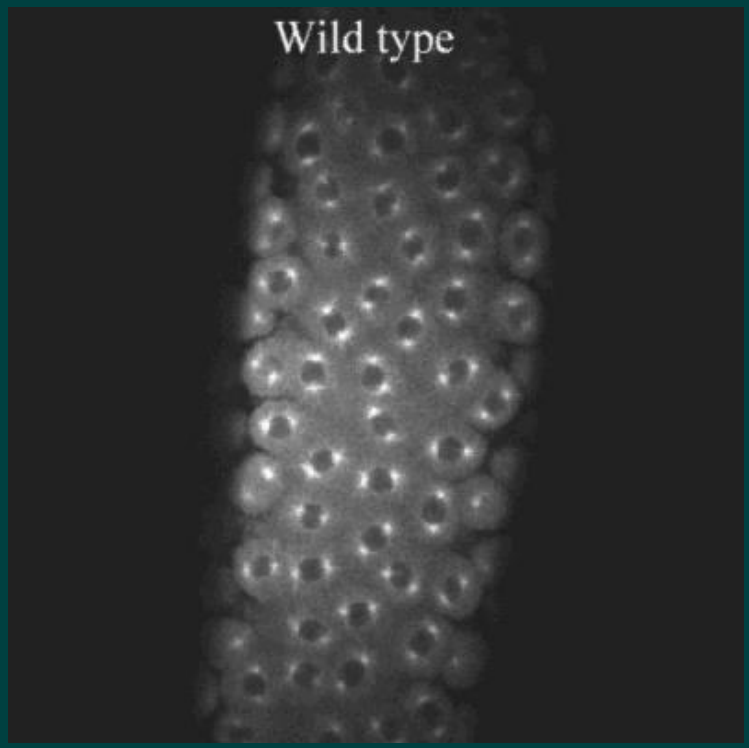
dynamics (assembly/disassembly) of microfilaments and microtubules

actin microfilaments



Crawling B16 cell
Vic Small

microtubules



Mitosis in Drosophila embryo
Sharp et al.,
Mol. Biol. Cell, 2000

assembly conditions

Actin filaments:

100 mN KCl
or/and 2mM Mg^{2+} or Ca^{2+}

ATP

Microtubules:

Mg^{2+}, no Ca^{2+}

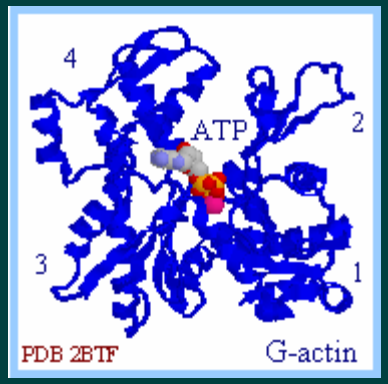
GTP

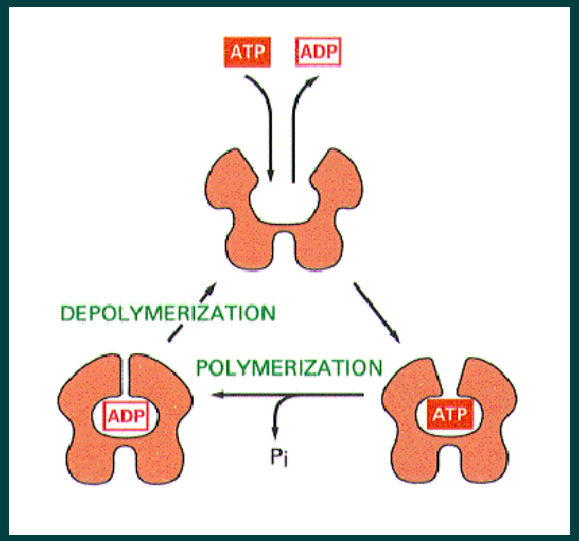
37^{o} C

Glycerol, or polycations, or MAPs

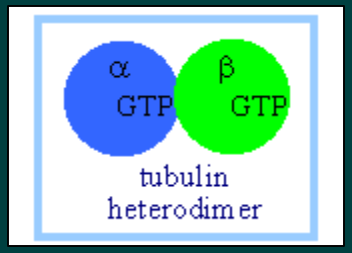
nucleotides in actin and tubulin assembly

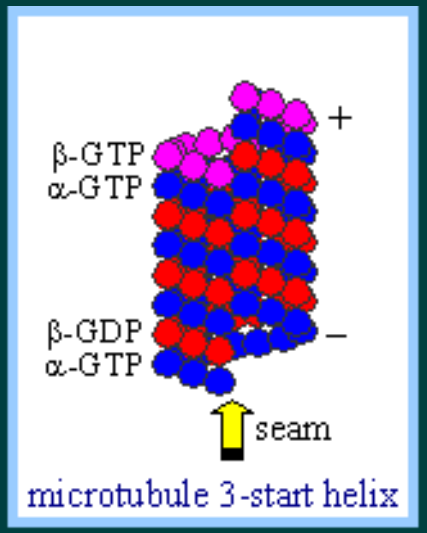
actin

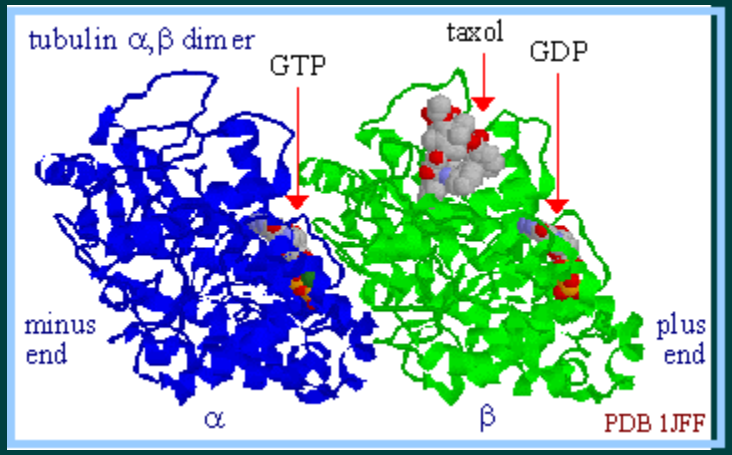




tubulin







https://www.rpi.edu/dept/bcbp/molbiochem/MBWeb/mb2/part1/

Assembly poisons

Actin:

cytochalasin, latrunculin
(block assembly)

phalloidin, jasplakinolide
(block disassembly)

Microtubules:

colchicin, colcemid, vinblastin,
nocodazole (block assembly)

taxol (block disassembly)

Equilibrium view of filament assembly

$$P_n + P_1 \overset{K_d}{\rightleftarrows} P_{n+1},$$

$$K_d = \frac{[P_n][P_1]}{[P_{n+1}]}$$

$$K_d = \frac{[P_1][P_1]}{[P_2]} = \frac{[P_1]^2}{[P_2]} \Rightarrow [P_2] = \frac{[P_1]^2}{K_d}$$

$$K_d = \frac{[P_2][P_1]}{[P_3]} = \frac{[P_1]}{[P_3]} \cdot \frac{[P_1]^2}{K_d} \Rightarrow [P_3] = \frac{[P_1]^3}{K_d^2}$$

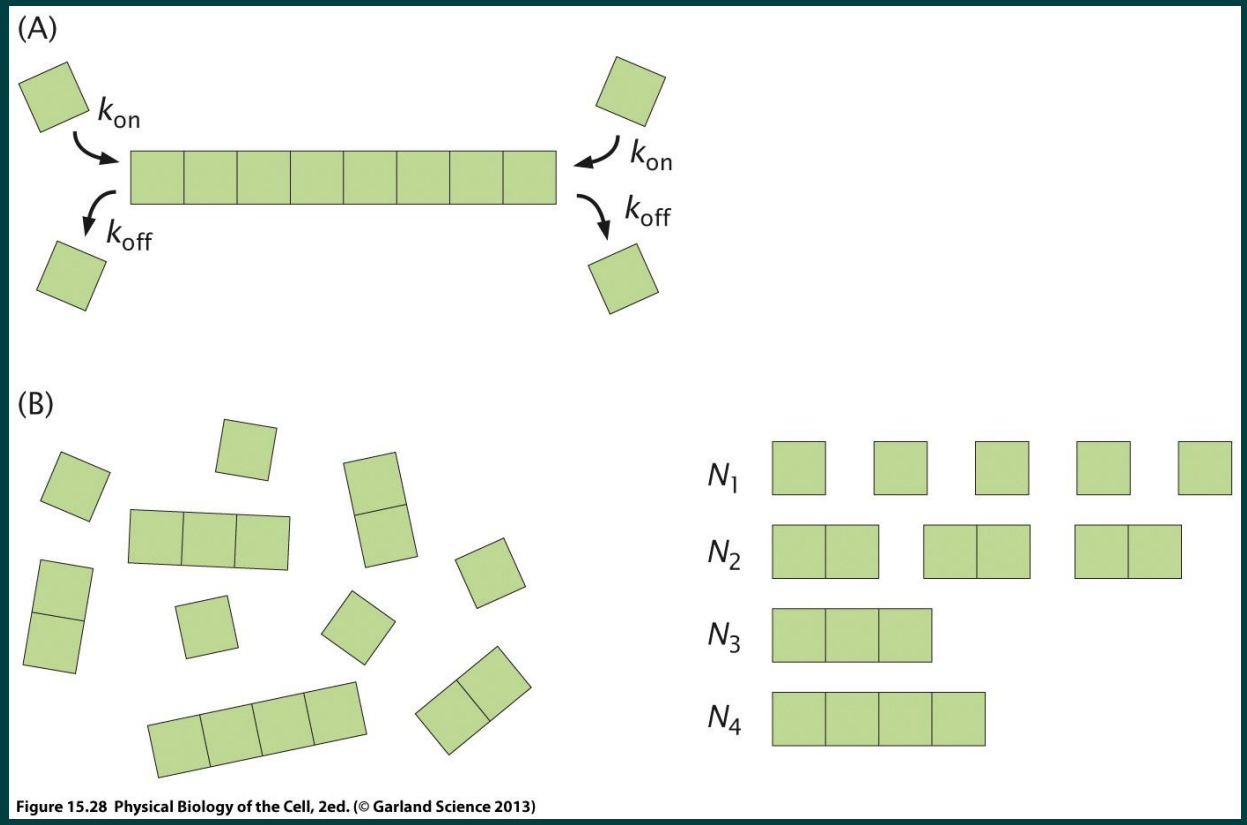


Figure 15.28 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

$$[P_n] = \frac{[P_1]^n}{K_d^{n-1}} = K_d \left(\frac{[P_1]}{K_d}\right)^n$$

$$[P_n] = K_d e^{n \ln([P_1]/K_d)} = K_d e^{-\alpha n}$$

short filaments prevail, length very sensitive to monomer concentration

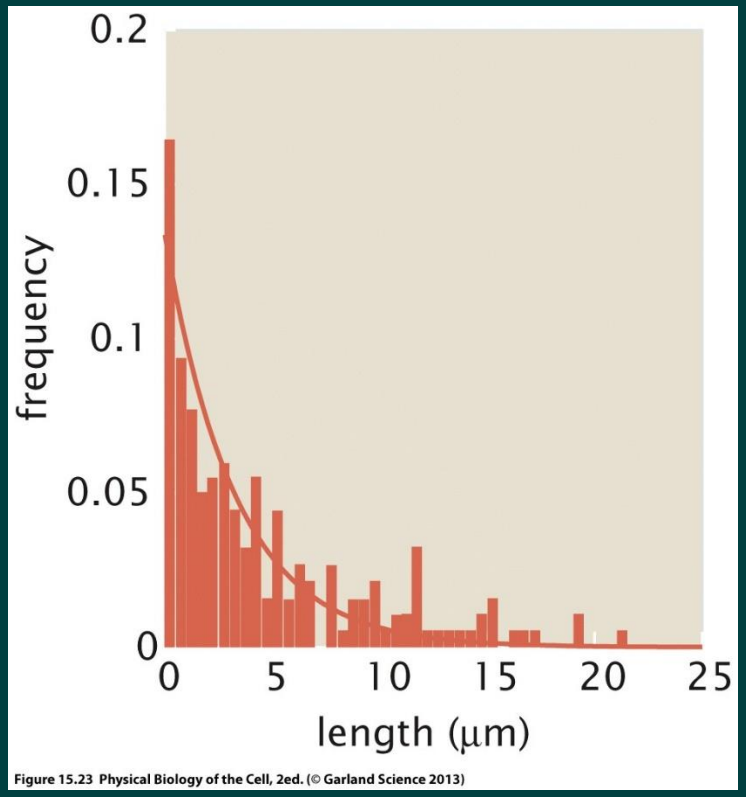


Figure 15.23 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

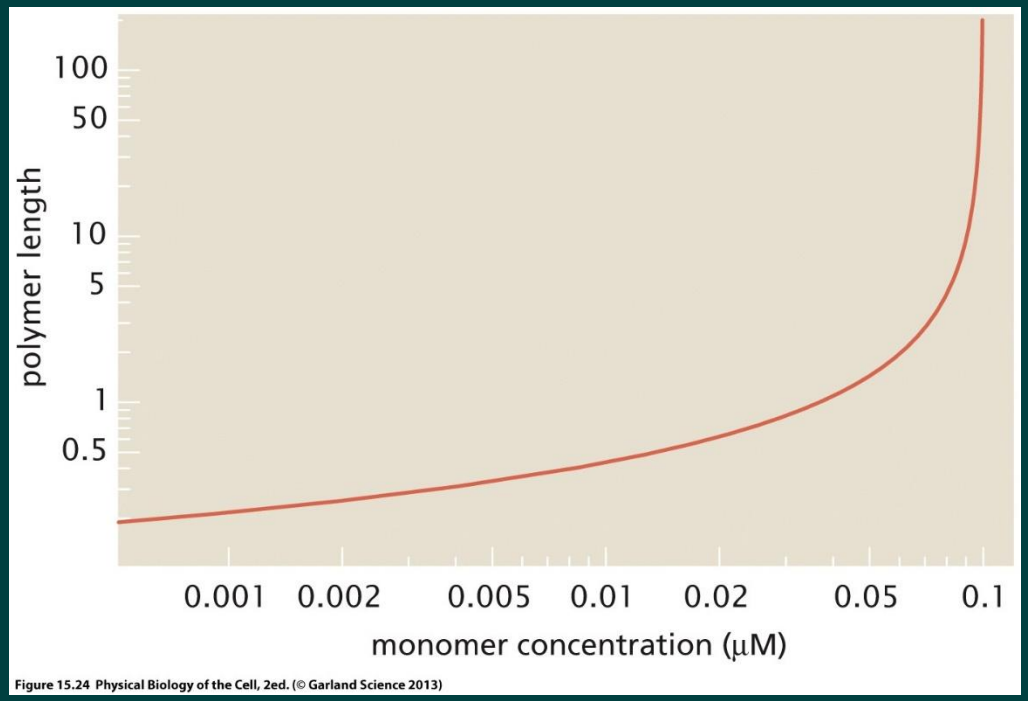


Figure 15.24 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

In reality, formation of dimers and short oligomers is not the same as filament elongation,

hence:

nucleation, elongation, steady state

polymerization – condensation assembly mechanism (Oosawa and Kasai, 1962):

critical monomer concentration
at steady state $= K_- / K_+$

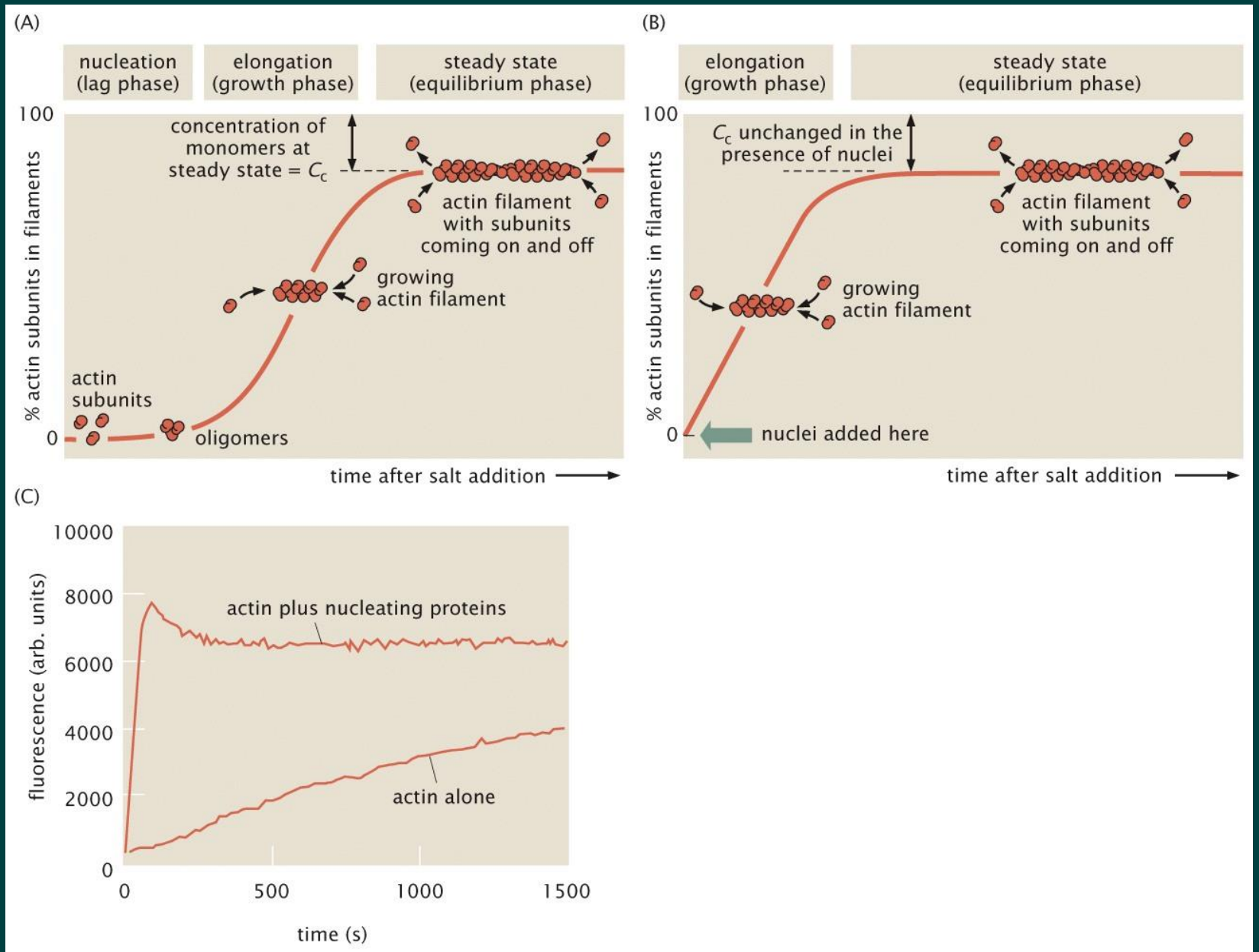
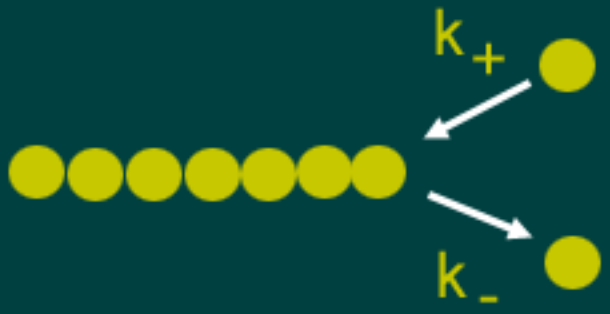


Figure 15.19 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

at the steady state



$$dP/dt = k_+ C\ N - k_-\ N$$

$$dP/dt = 0 \qquad C_c = k_-/k_+$$

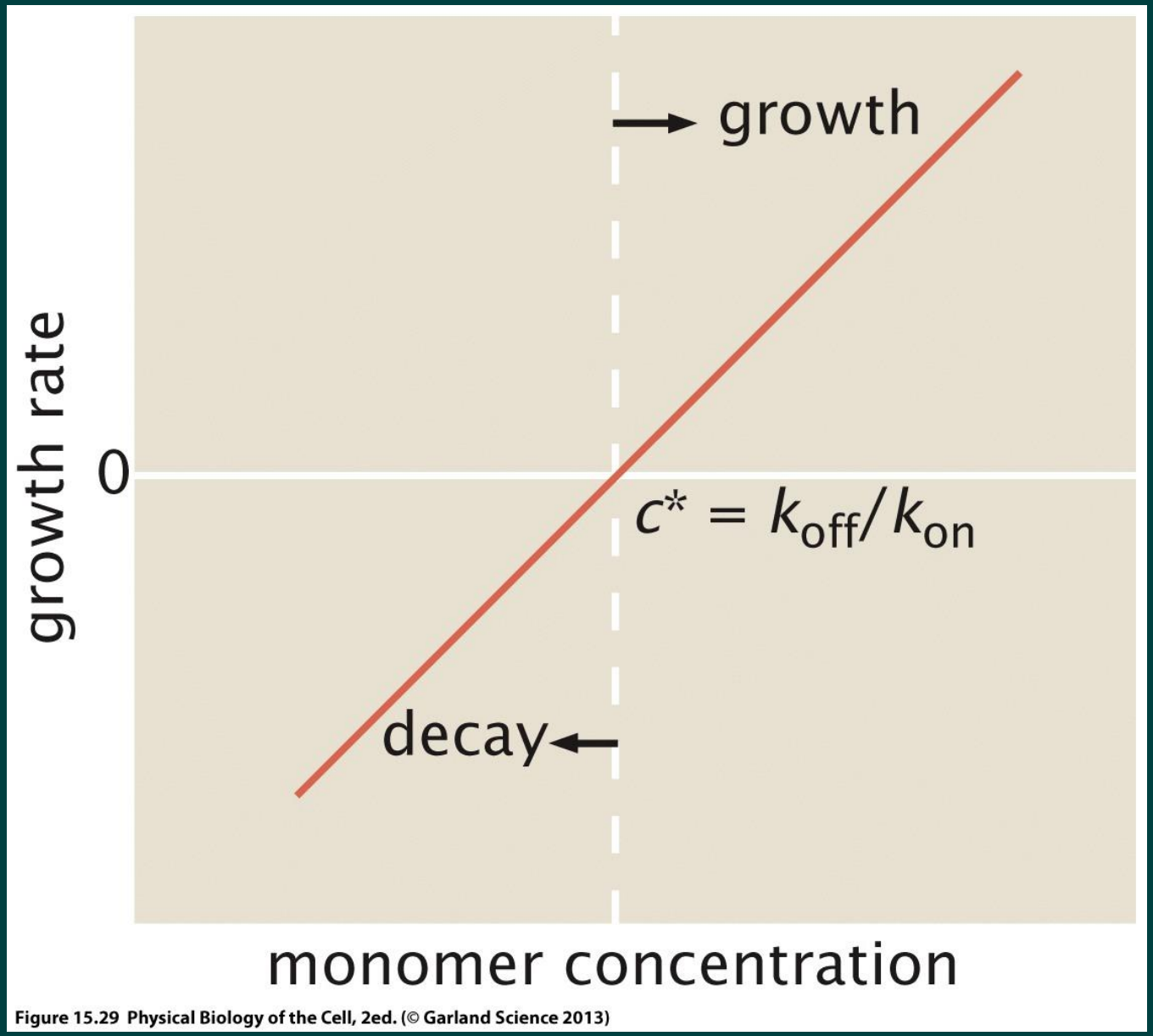


Figure 15.29 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

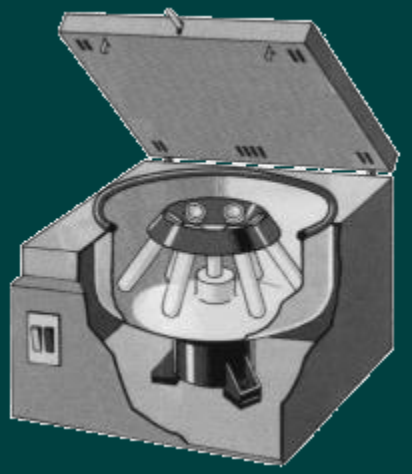
how to measure polymerization

e.g., centrifugation to separate polymer from the subunits

sedimentation velocity $v_{drift} = mg_c/(\text{friction coefficient})$

for a sphere at low Reinolds number,

friction coefficient = $6\pi\eta R$



$$v_{drift} = \frac{4/3\pi R^3 (\rho - \rho_{solvent})\, g_c}{6\,\pi\eta R} = 2\, R^2 (\rho - \rho_{solvent})\, g_c /9\eta$$

sedimentation velocity strongly depends on the size –
- polymer can be separated from subunits (need 10^3-10^4 g)

Opposite end assembly and disassembly of microtubules at steady state in vitro (assayed in the bulk using centrifugation and GTP-labeled tubulin)

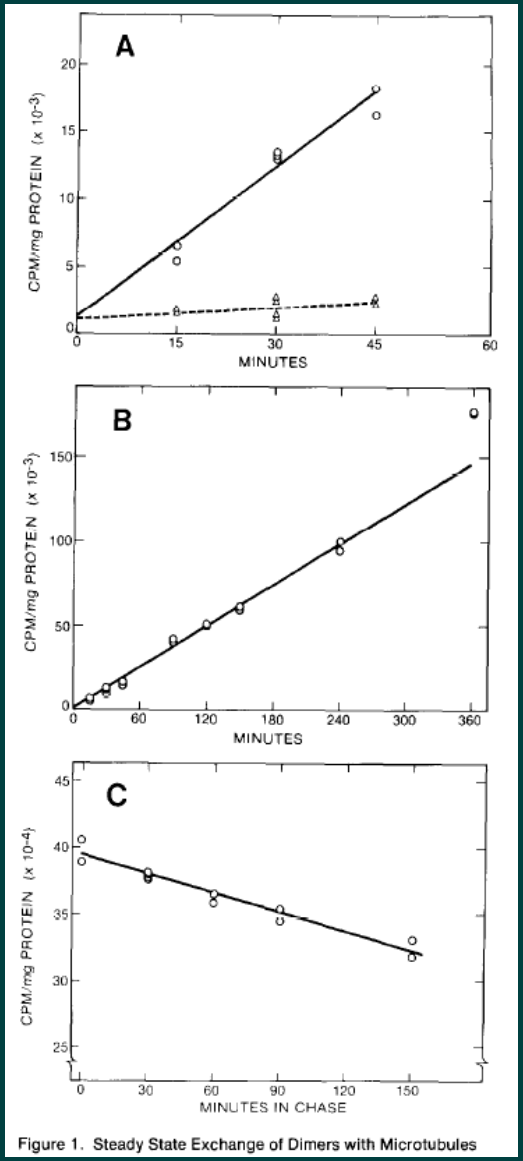


Figure 1. Steady State Exchange of Dimers with Microtubules

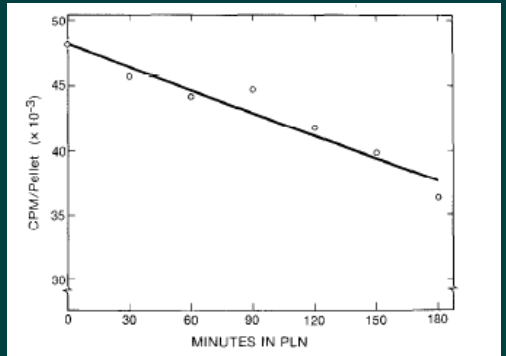


Figure 2. Podophyllotoxin Effect on Steady State Loss of Subunits from Microtubules

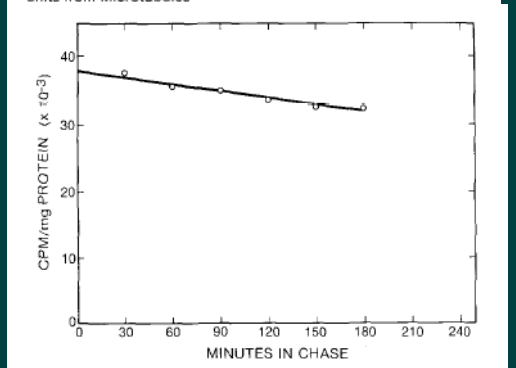


Figure 3. Loss of Tubulin from the Assembling End of the Microtubule

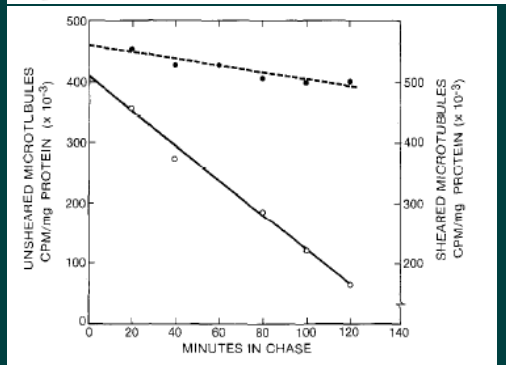


Figure 4. Effect of Polymer Length on Steady State Exchange of Subunits on Microtubules

Margolis and Wilson, Cell, 1978

expectation from equilibrium assembly-disassembly

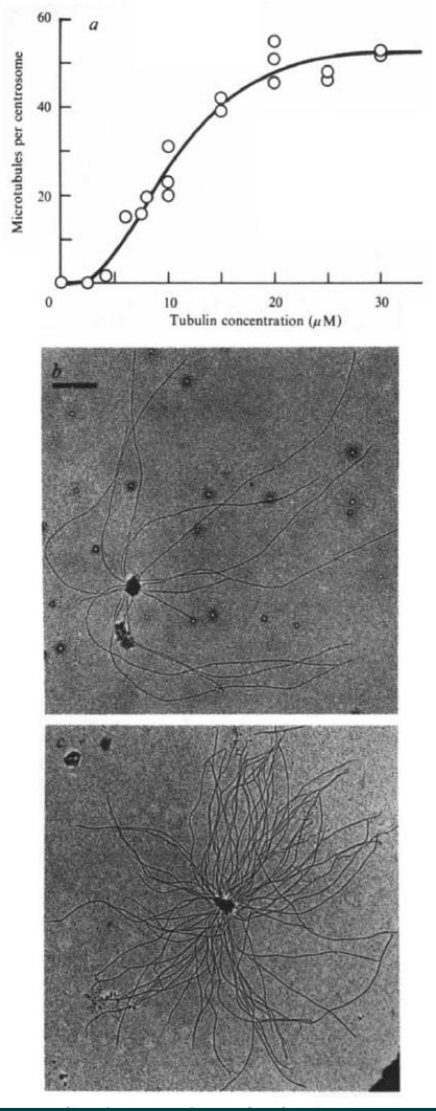
microtubule length variance at equilibrium:

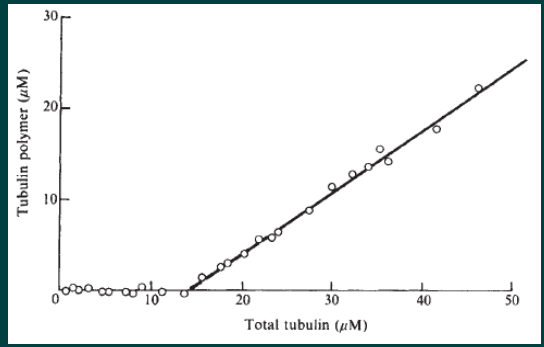
$\langle L^2 \rangle = 2a^2 k_{off} t$ (a – length increment per dimer)

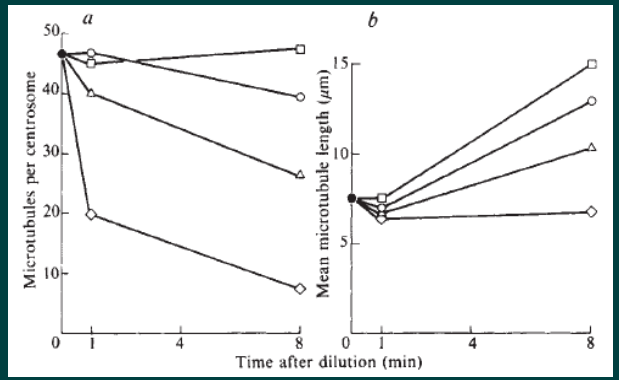
subunit incorporation is not linear with time

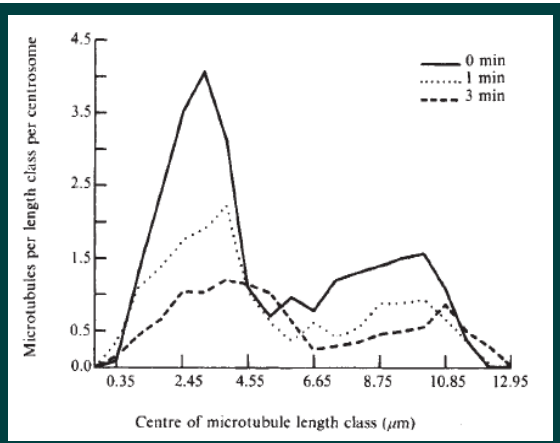
Polymer could be observed by electron microscopy

Discovery of dynamic instability









Mitchison and Kirschner, 1984

with different rate constants at the two ends

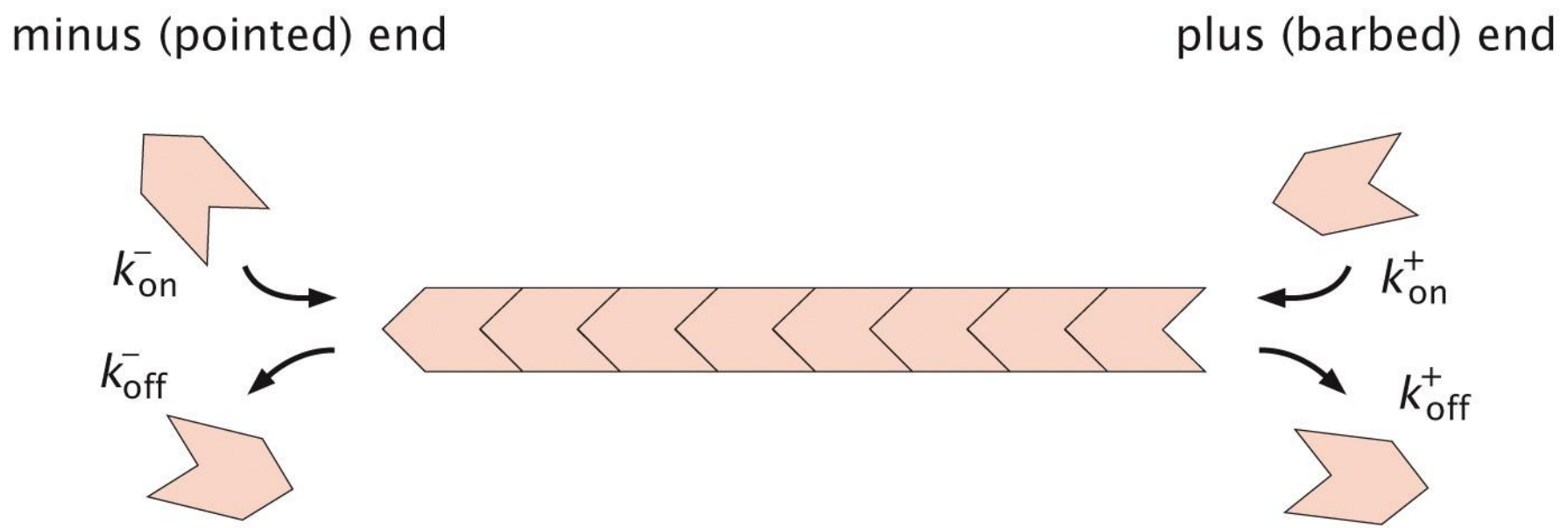


Figure 15.21b Physical Biology of the Cell, 2ed. (© Garland Science 2013)

critical concentration should be the same at both ends

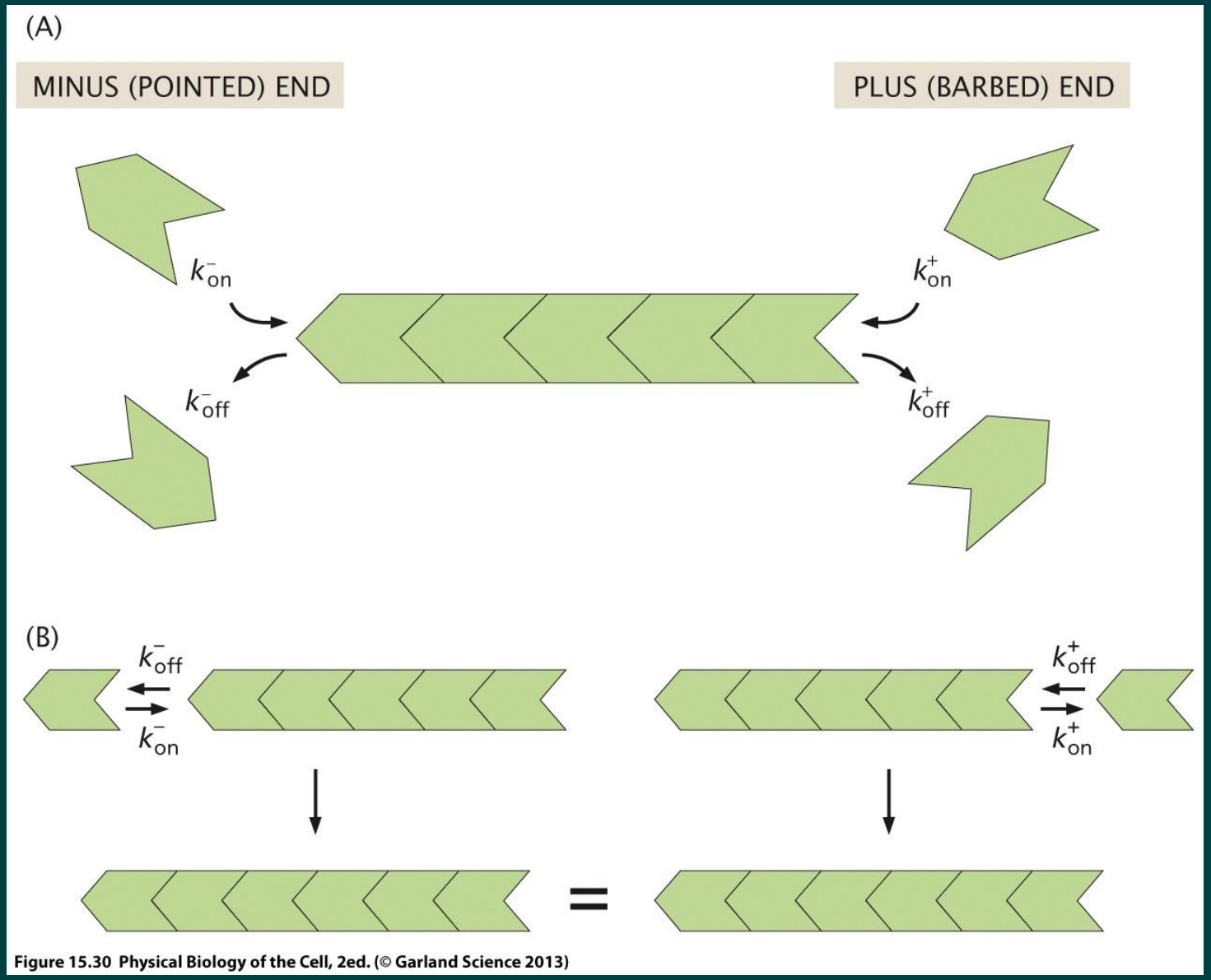


Figure 15.30 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

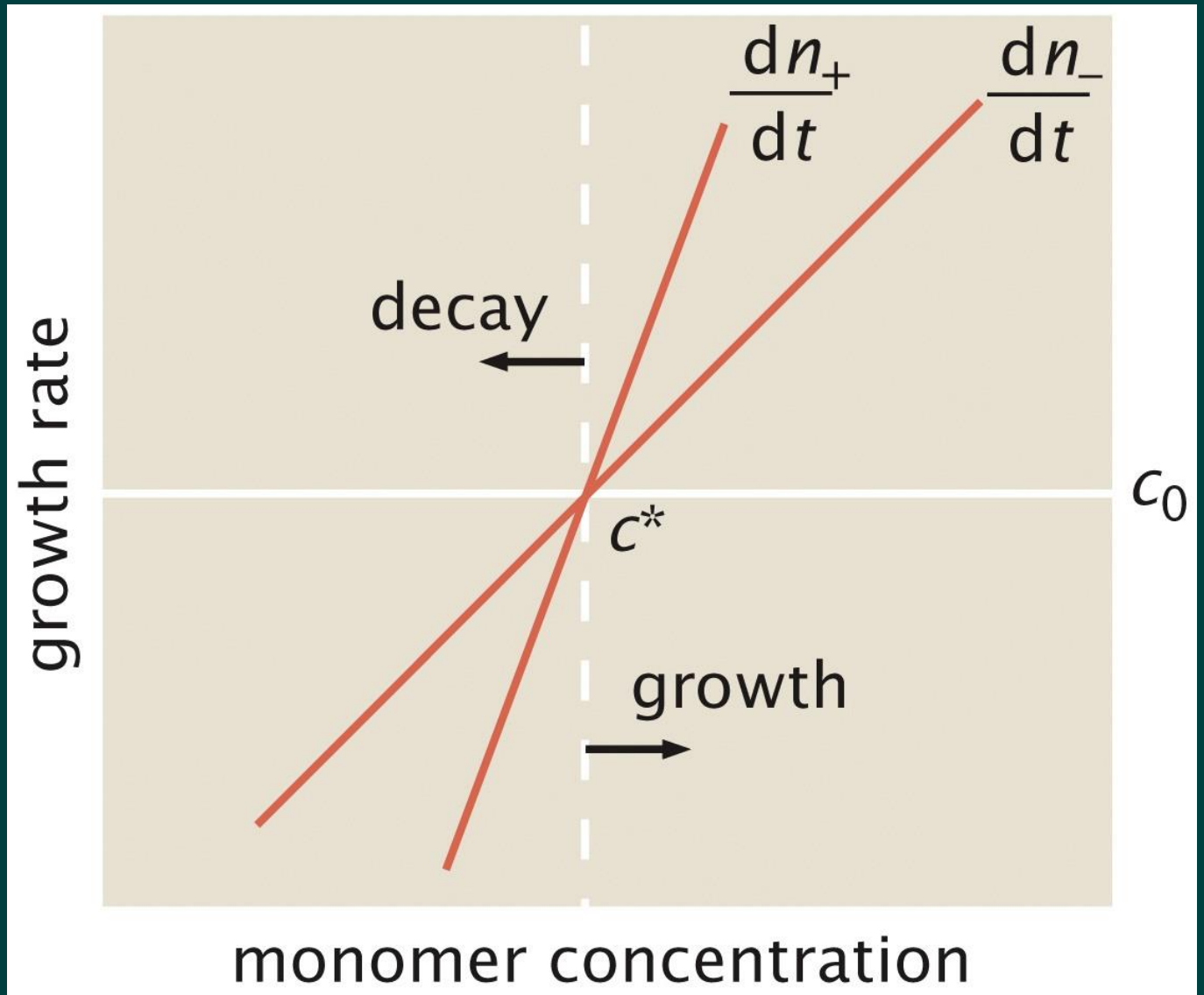
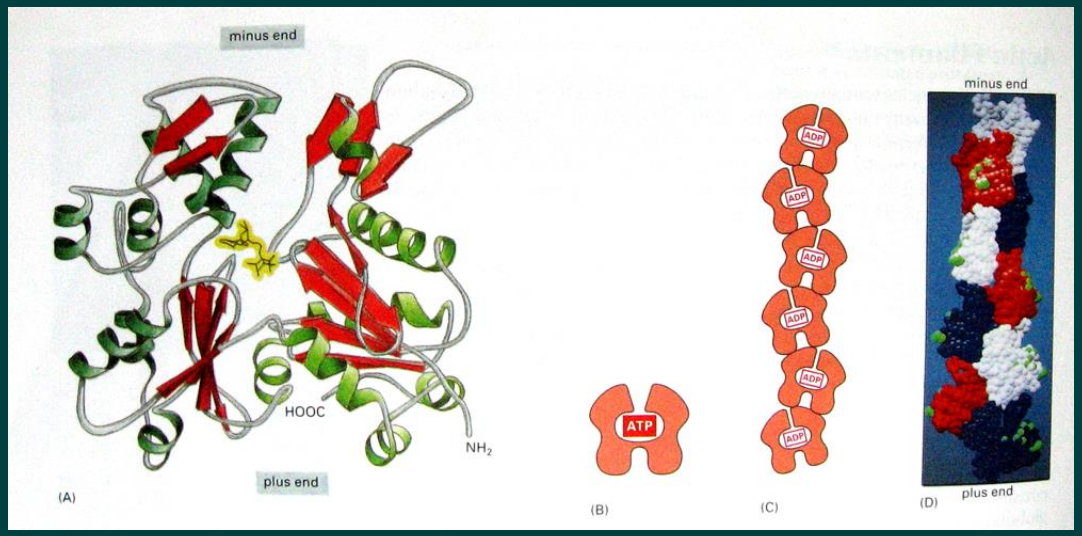
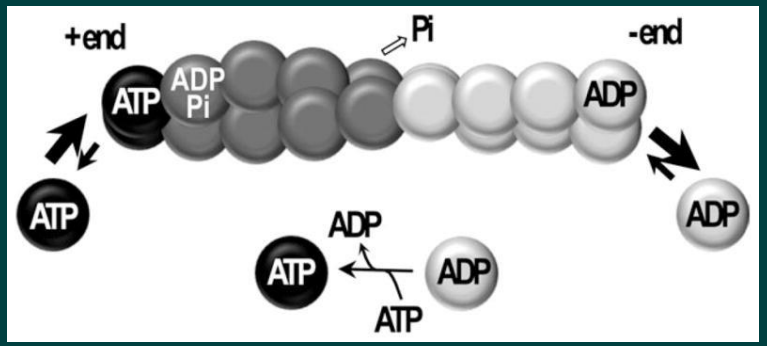
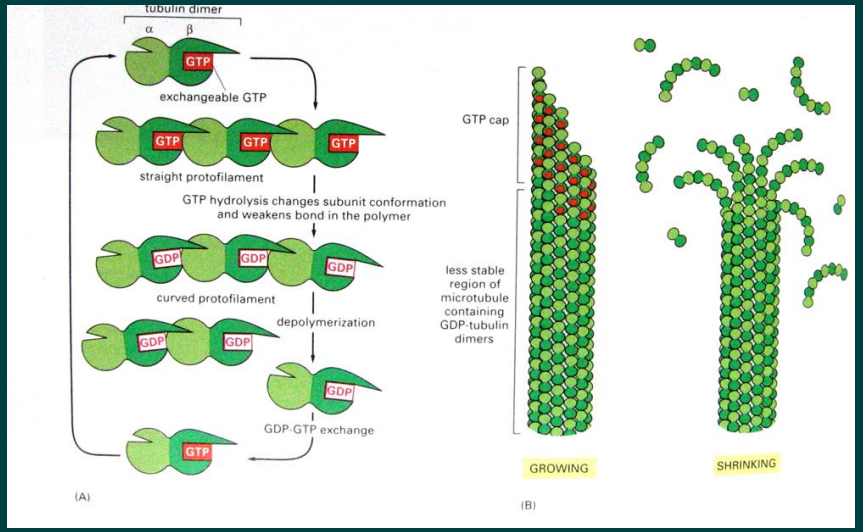


Figure 15.31 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

Role of nucleotide in assembly







four kinds of ends: NTP +end
NDP +end
NTP -end
NDP –end

therefore – four critical concentrations

with nucleotide hydrolysis

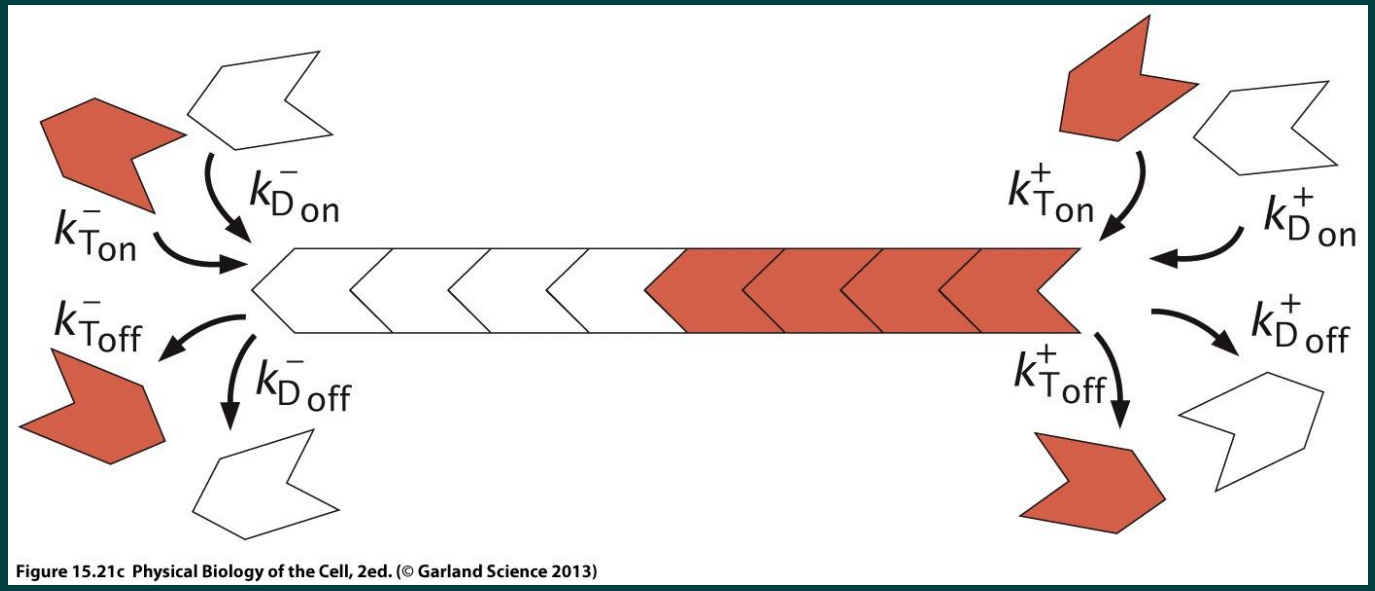


Figure 15.21c Physical Biology of the Cell, 2ed. (© Garland Science 2013)

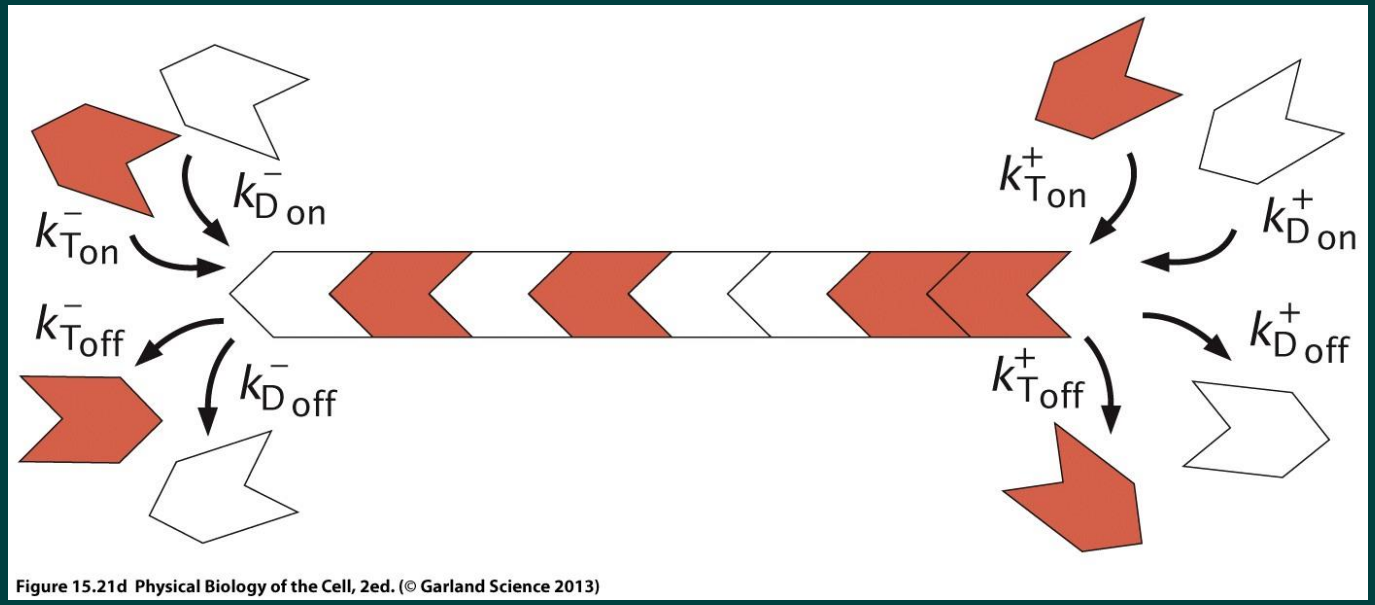


Figure 15.21d Physical Biology of the Cell, 2ed. (© Garland Science 2013)

critical concentration differ for nucleotide state and for two ends

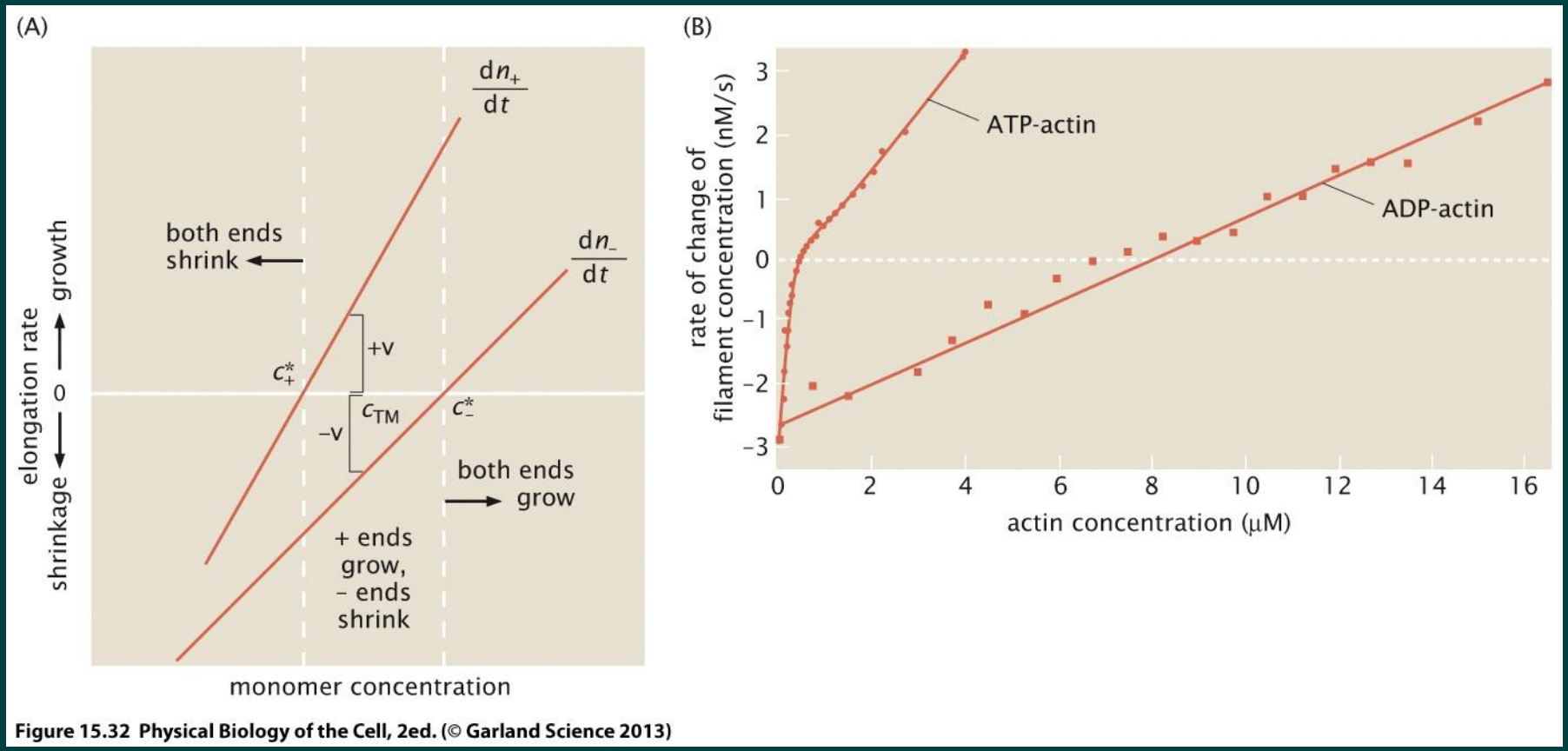


Figure 15.32 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

theoretically possible at steady state:

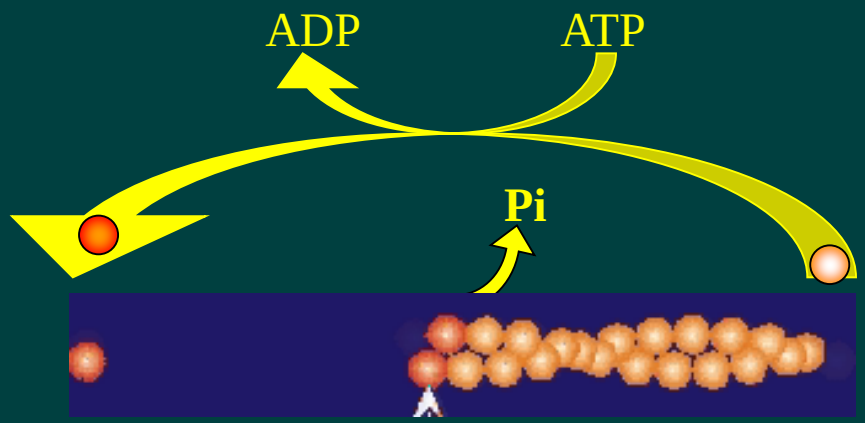
net addition of NTP-subunits at +end
net loss of NDP-subunits at –end

This was termed treadmilling

also possible:

assembly and disassembly alternate at the same end
depending on the nucleotide state

This was termed dynamic instability



Actin Intrinsic Treadmilling

catastrophe happens when the end monomers (cap) hydrolyze their nucleotides

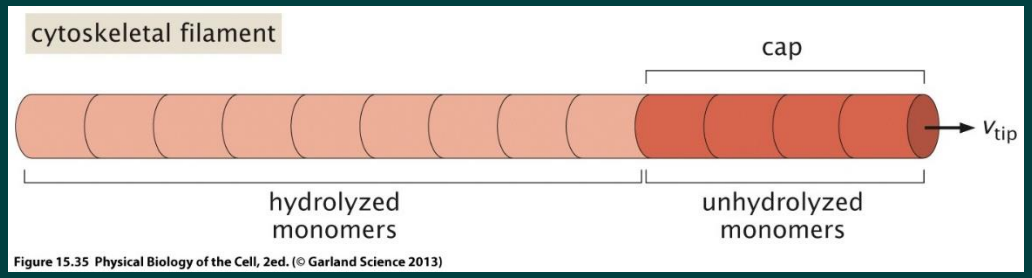


Figure 15.35 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

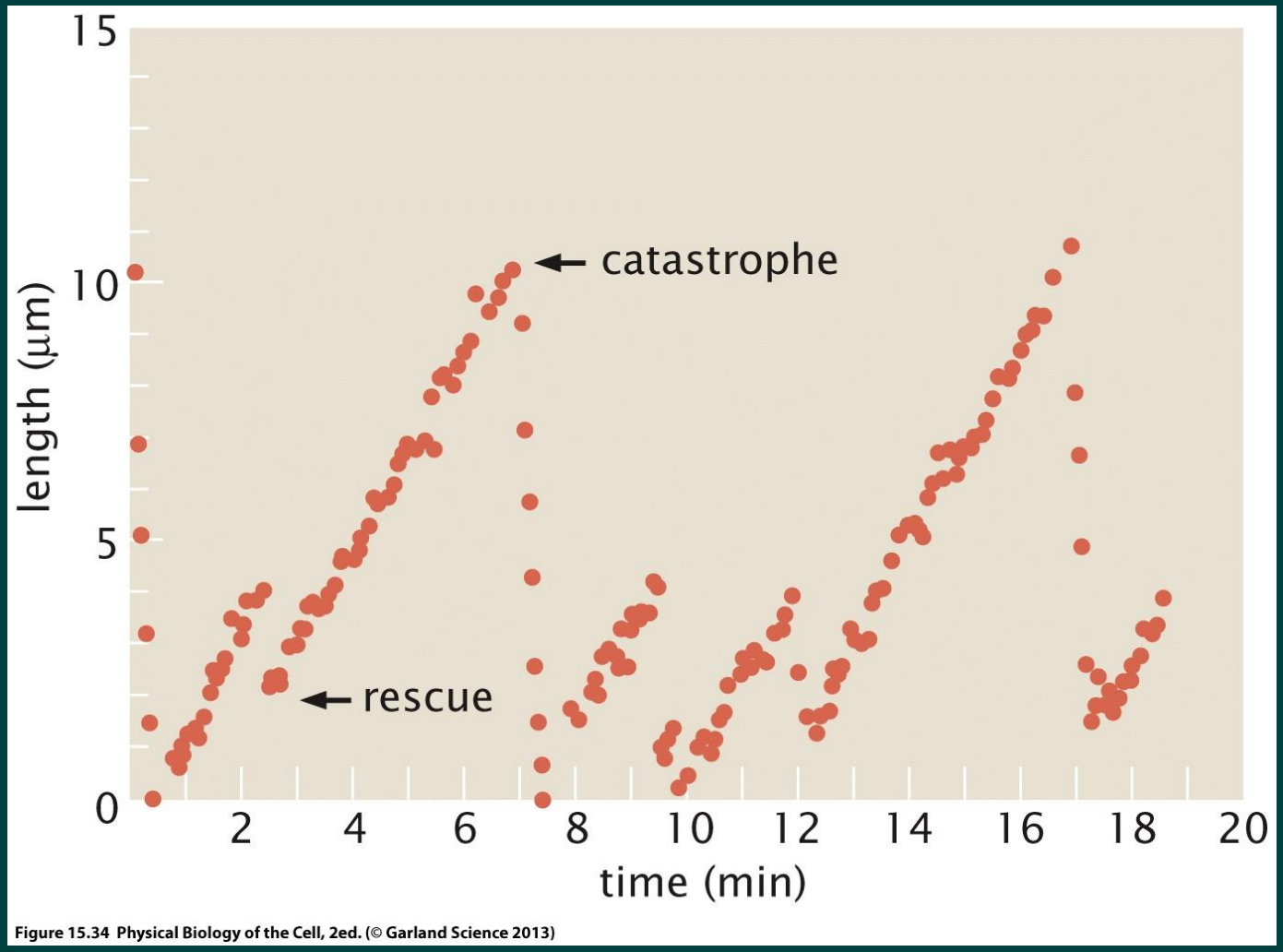
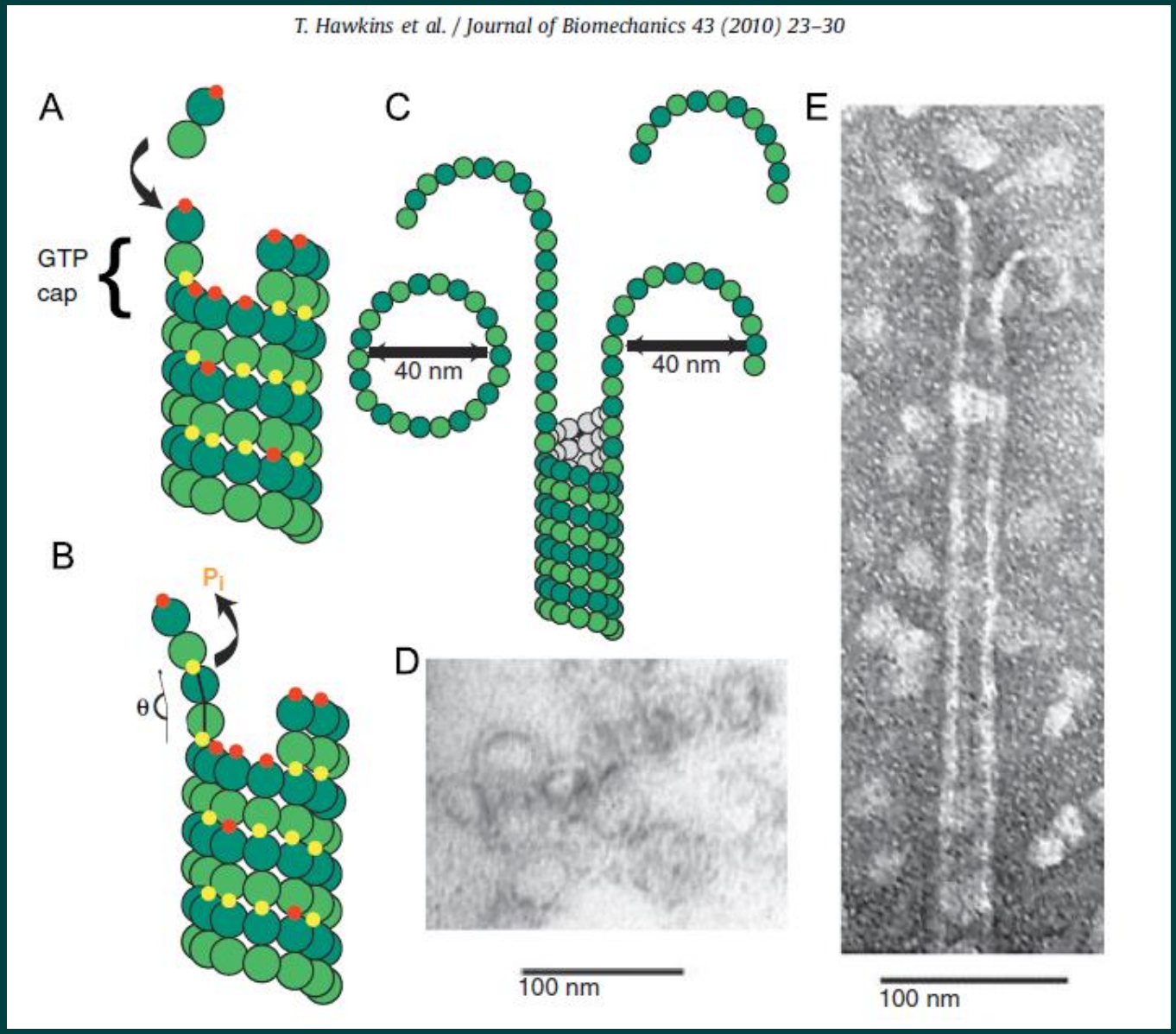
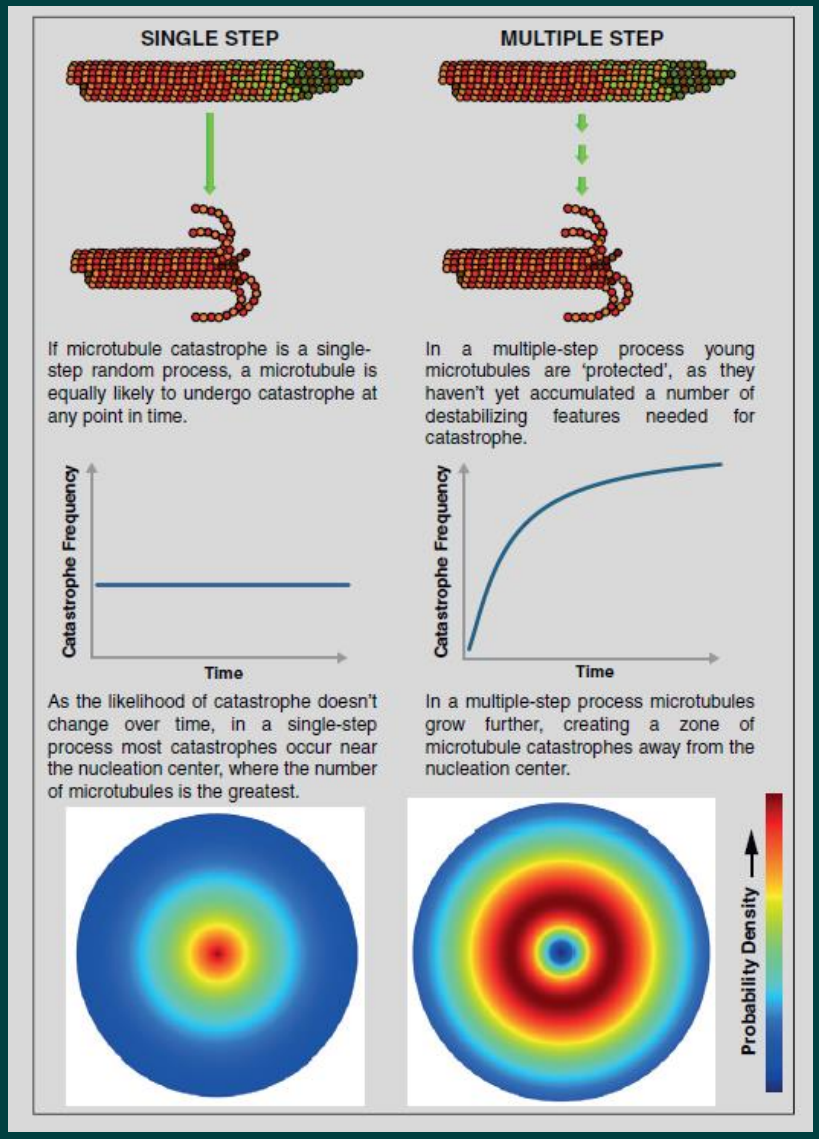


Figure 15.34 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

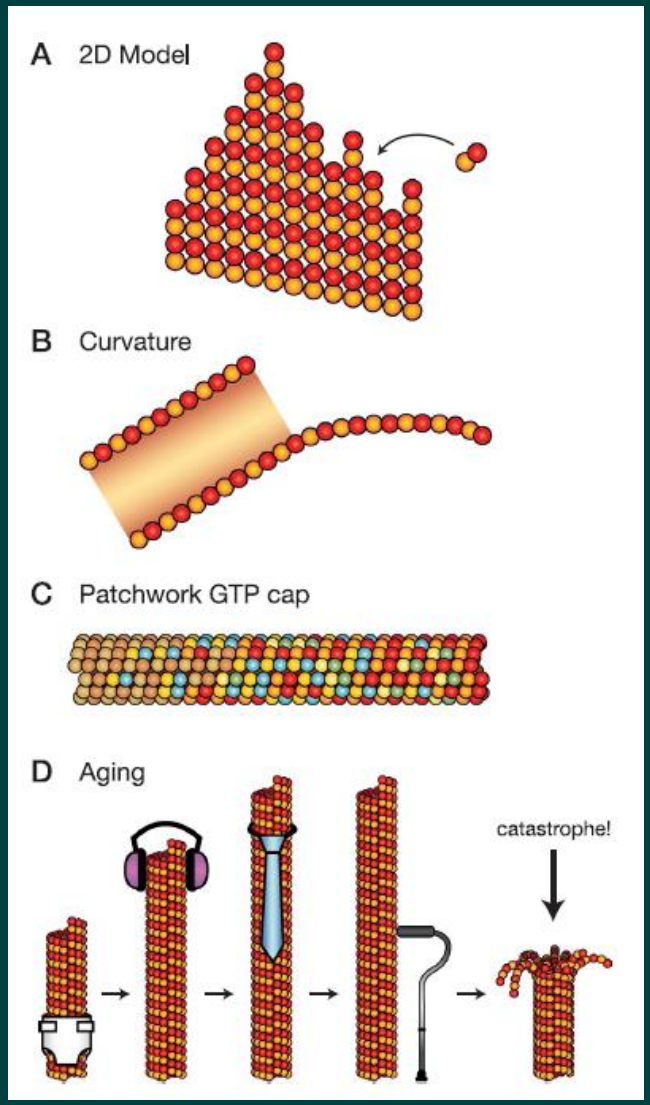
in microtubules, catastrophe may be related to the change in protofilament curvature



catastrophe frequency may be age-dependent



Gardner et al, 2013



Brouhard, 2015

How to measure polymerization (depolymerization)?

challenges:

separate polymer from subunits

distinguish newly assembled polymer

distinguish one filament end from the other

distinguish polymer from subunits

in bulk:

centrifugation

light scattering

viscosity

fluorescence (pyrene-modified actin)

at a single filament level:

direct observation by light or electron microscopy

distinguish new assembly and one end from the other: selective labeling

at the individual filament level:

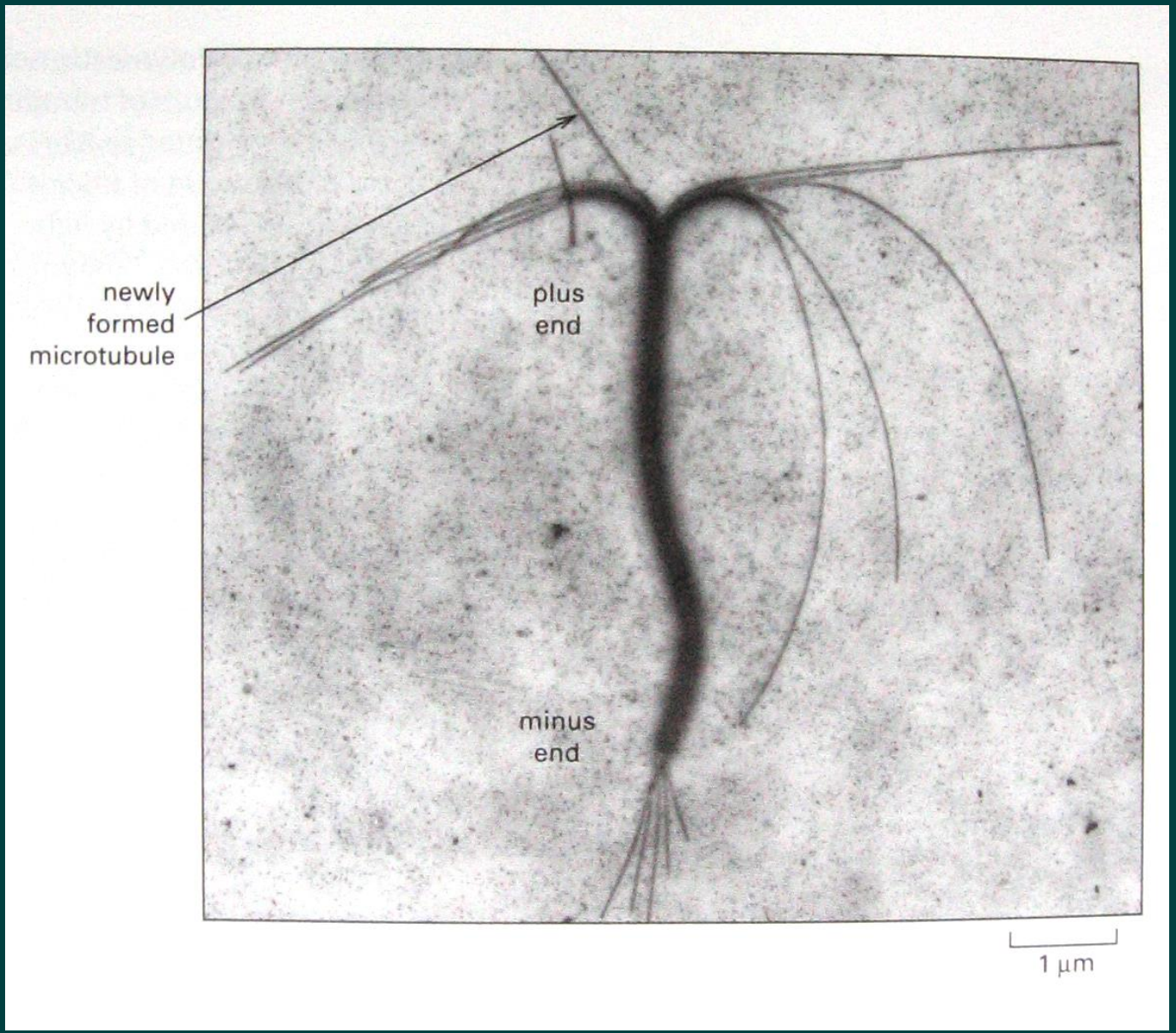
electron microscopy (fixed)

immunofluorescence microscopy (fixed)

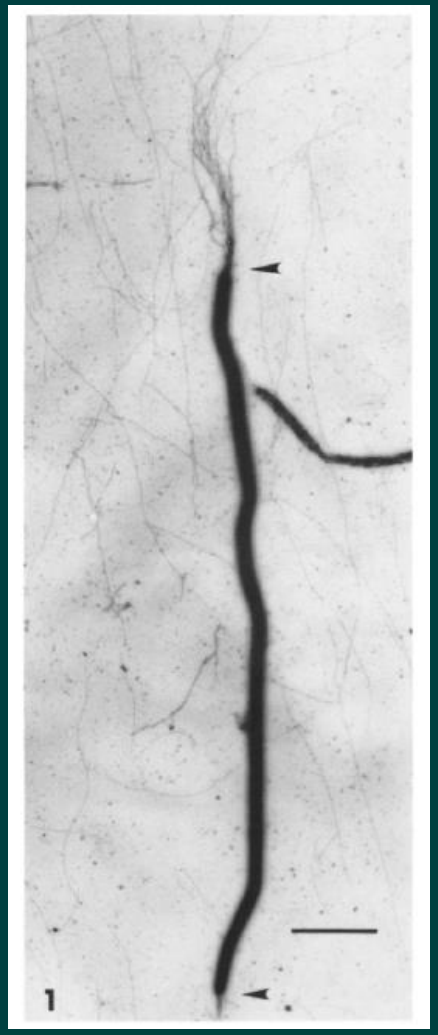
video-enhanced microscopy (live)

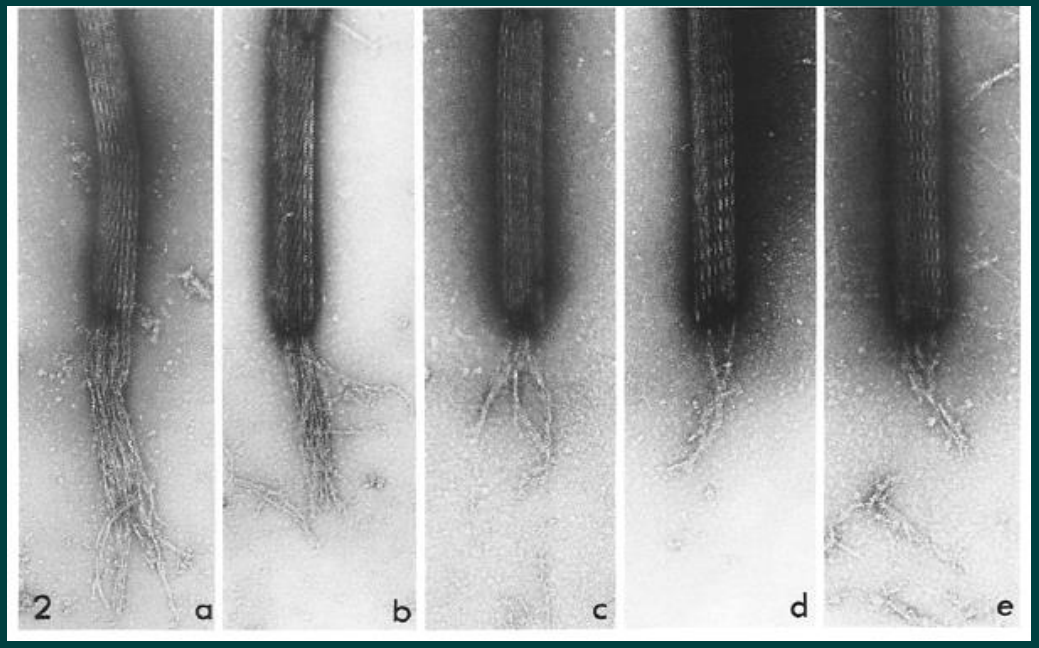
fluorescence microscopy with labeled proteins of interest (live!!)

EM-visualization of polarized assembly of microtubules



Actin disassembly at –end at a steady state
Visualization with Limulus acrosomal actin bundle





Coluccio and Tilney, JCB, 1983

rate constants measured with EM

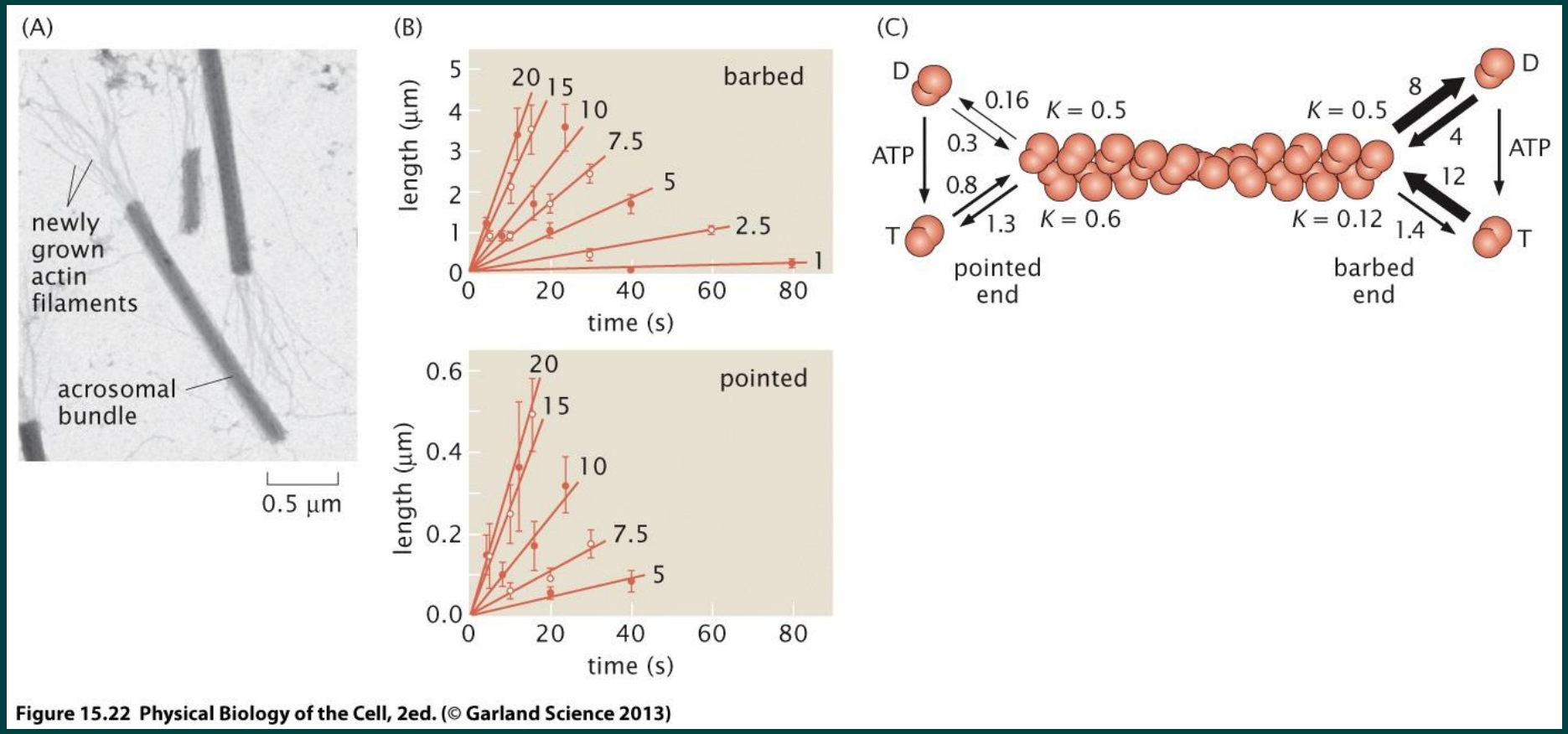
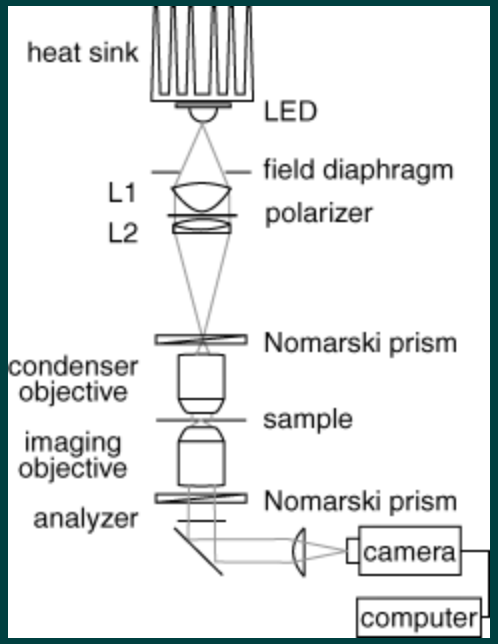
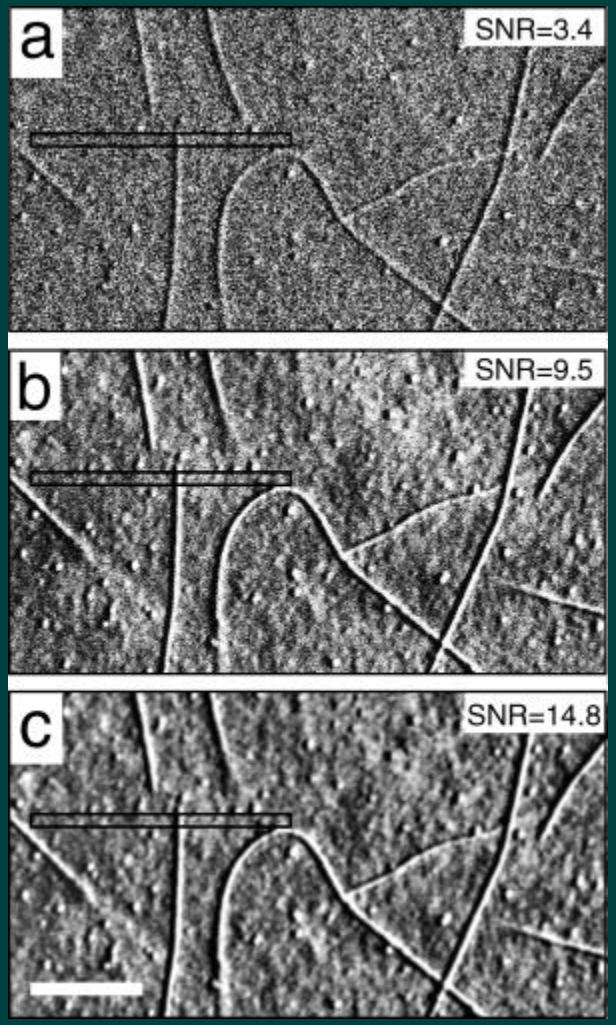
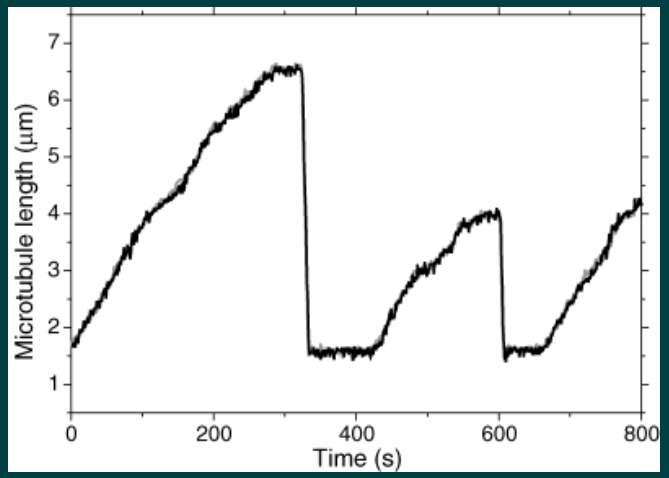


Figure 15.22 Physical Biology of the Cell, 2ed. (© Garland Science 2013)

individual microtubules visualized with video-enhanced DIC

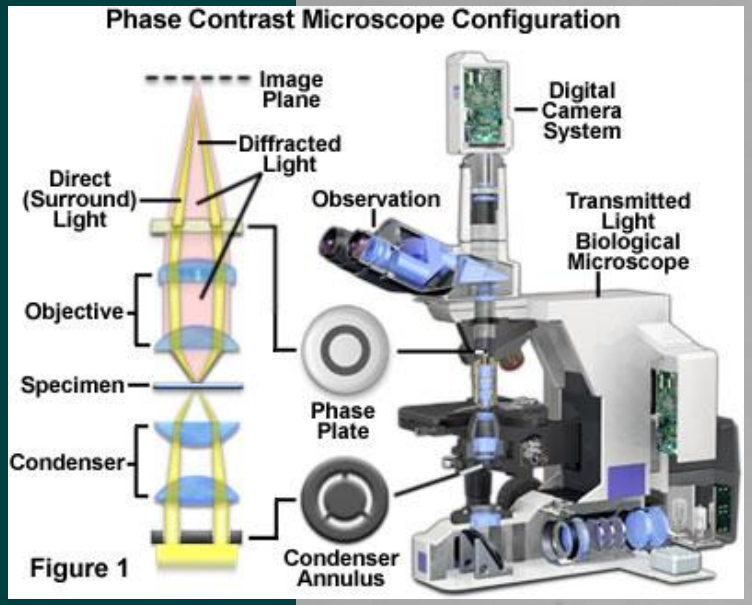






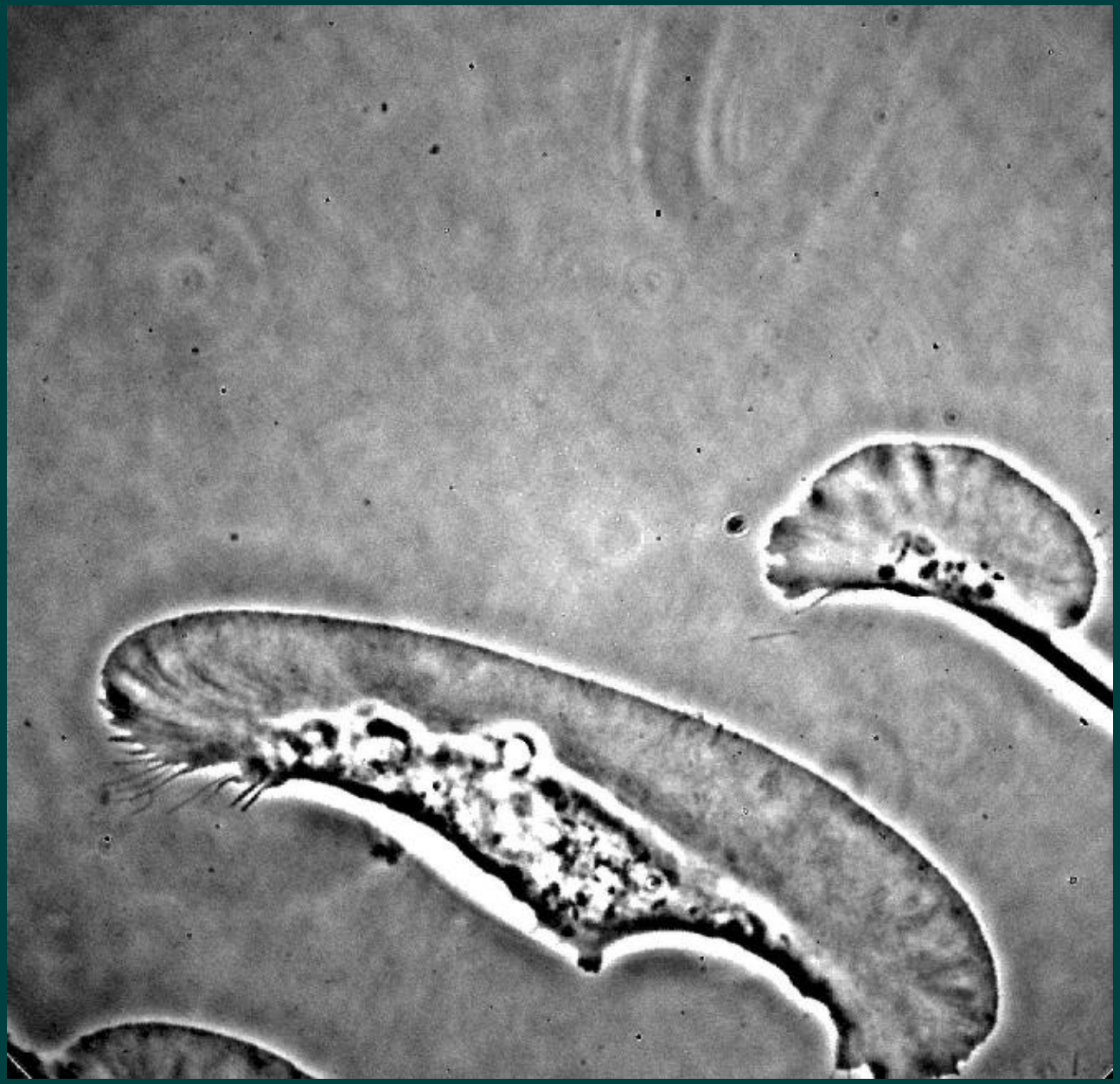
Bormuth et al., 2007

Video-enhanced phase contrast

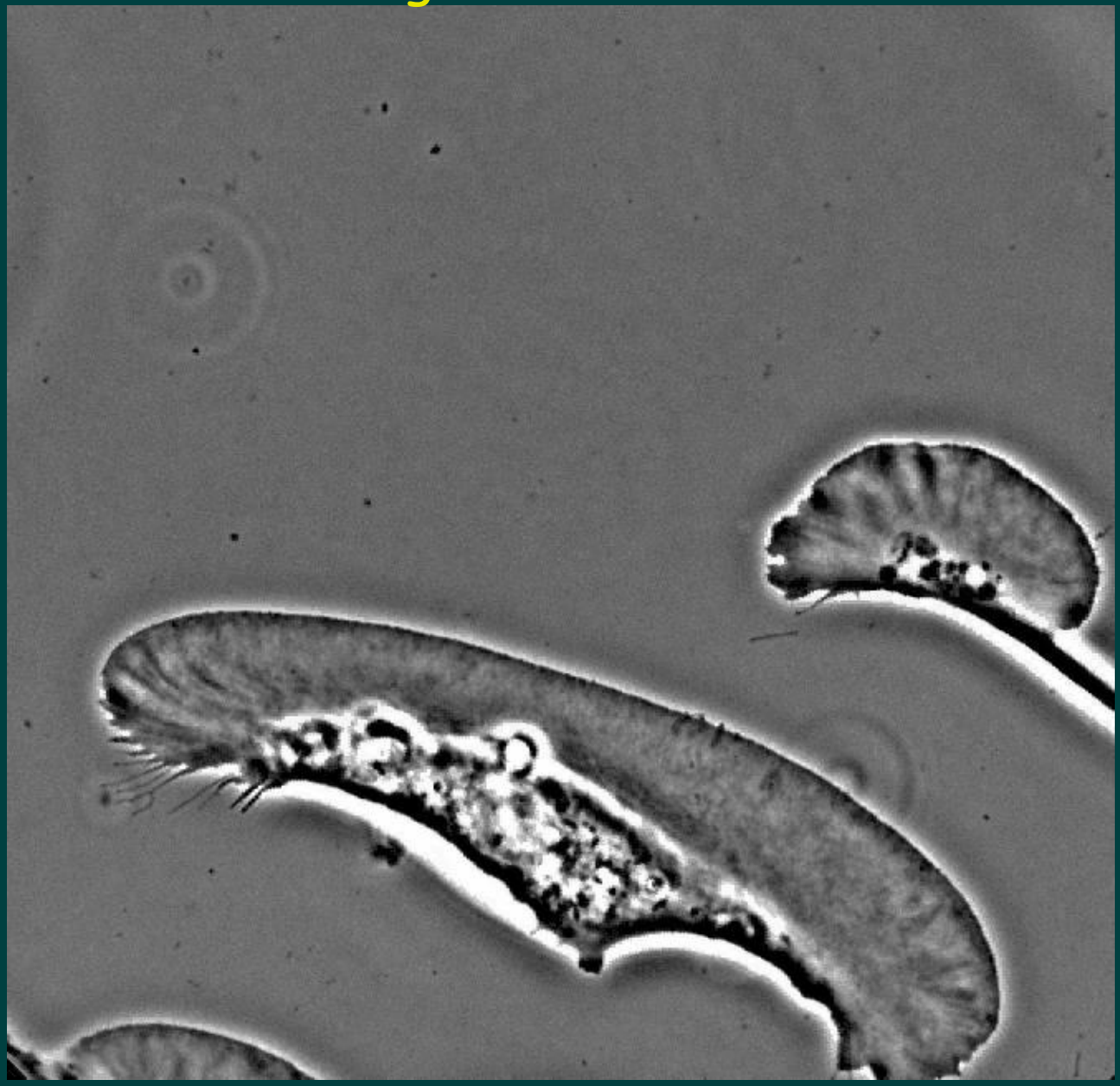


raw image

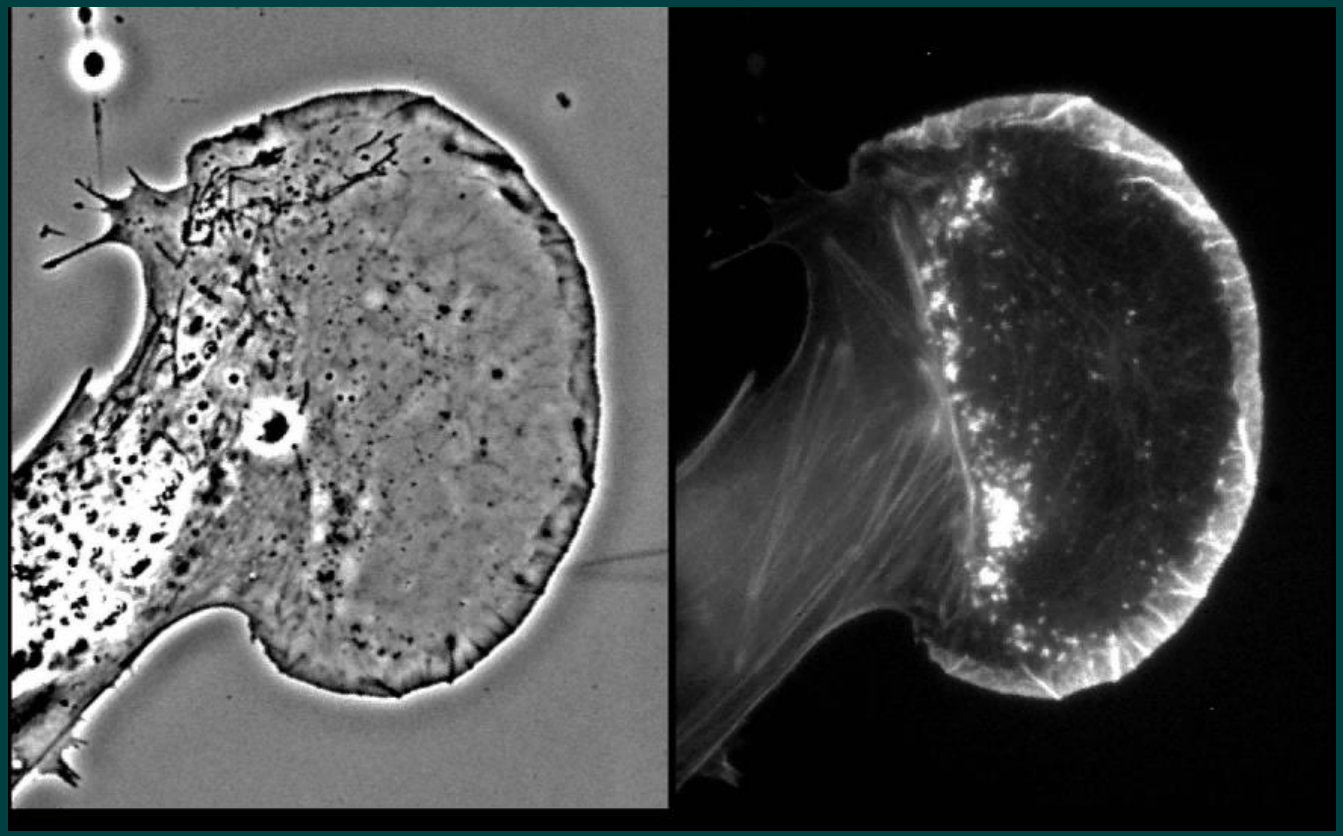
contrasted

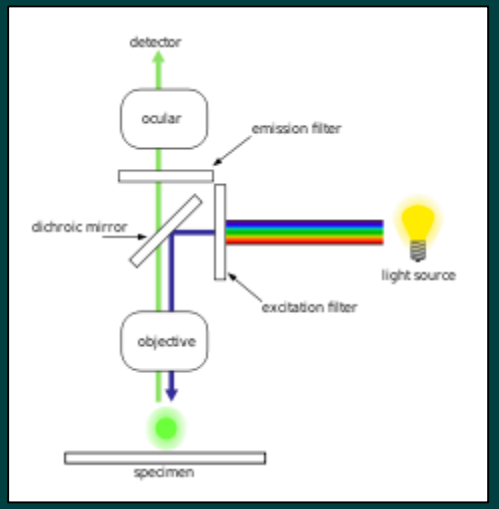


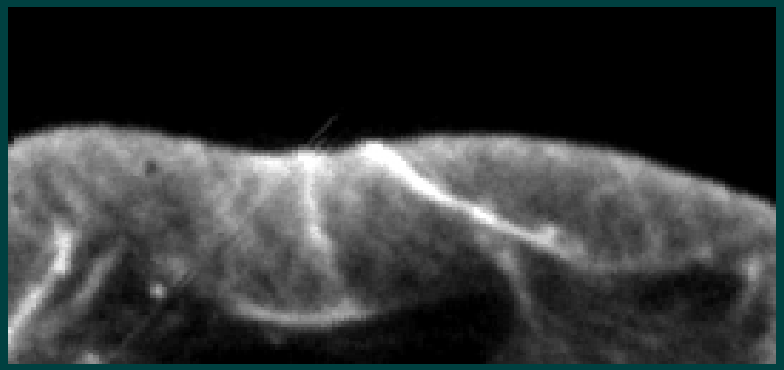
background-subtracted



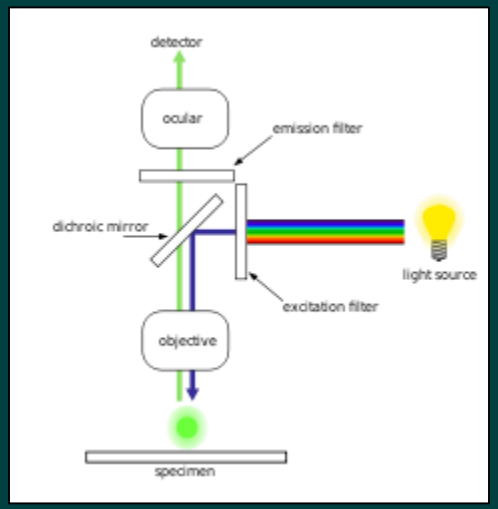
Phase contrast vs fluorescence



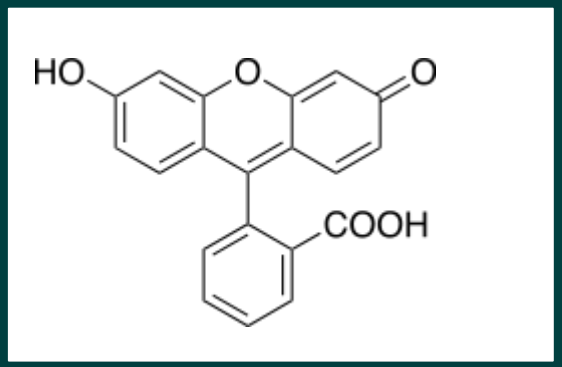




fluorescence microscopy

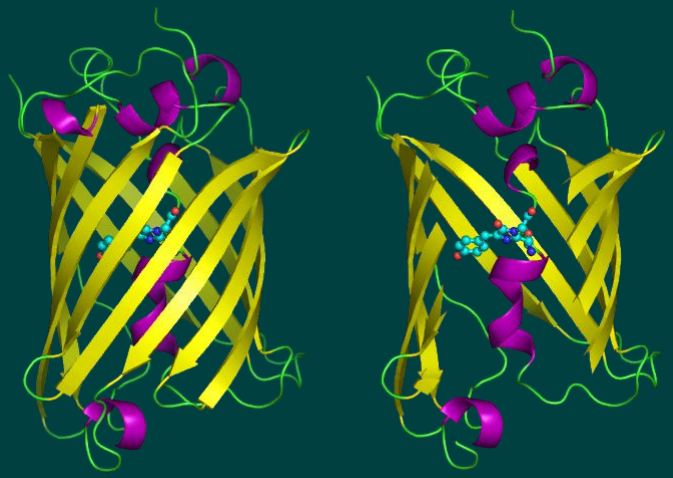


chemical dyes attached either to antibodies or directly to the protein of interest



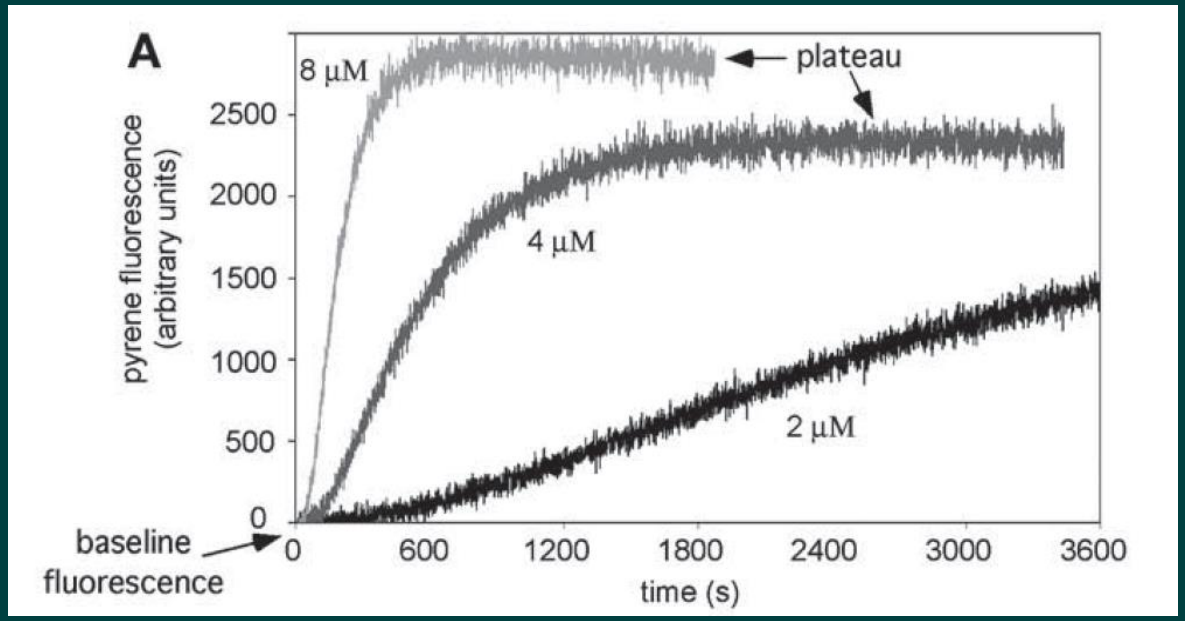
e.g., fluorescein

green fluorescent protein (GFP) and its variants (2008 Nobel prize)

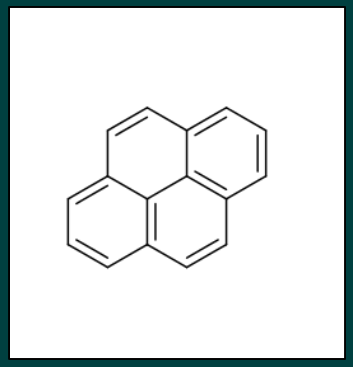


chromophore is formed by three amino acids: Ser65–Tyr66–Gly67

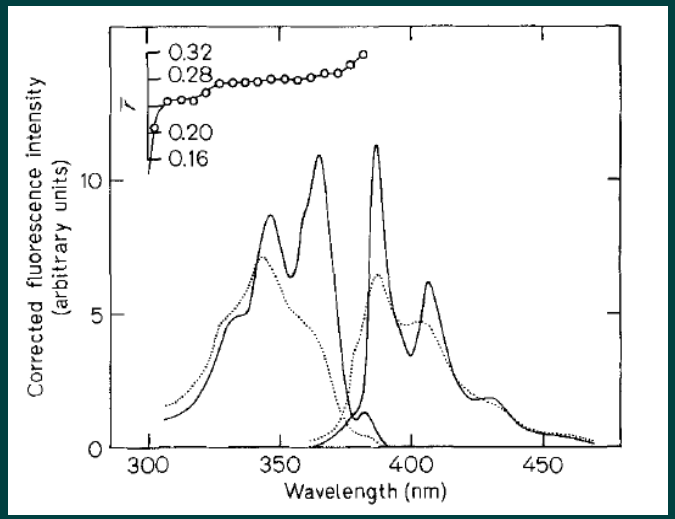
polymerization kinetics by fluorescence of pyrene-labeled actin



Zuchero, Meth. in Mol. Biol.



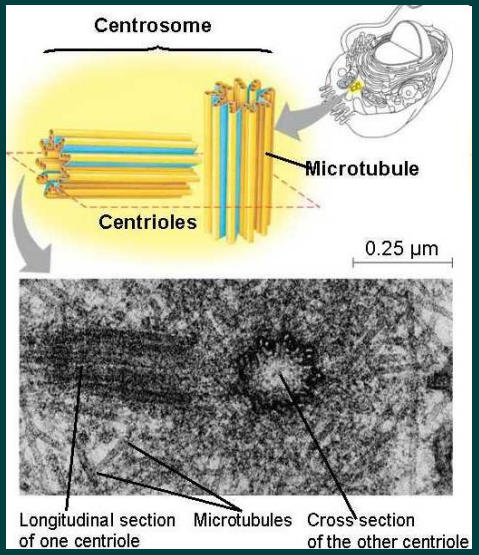
pyrene: fluorescence sensitive to environment

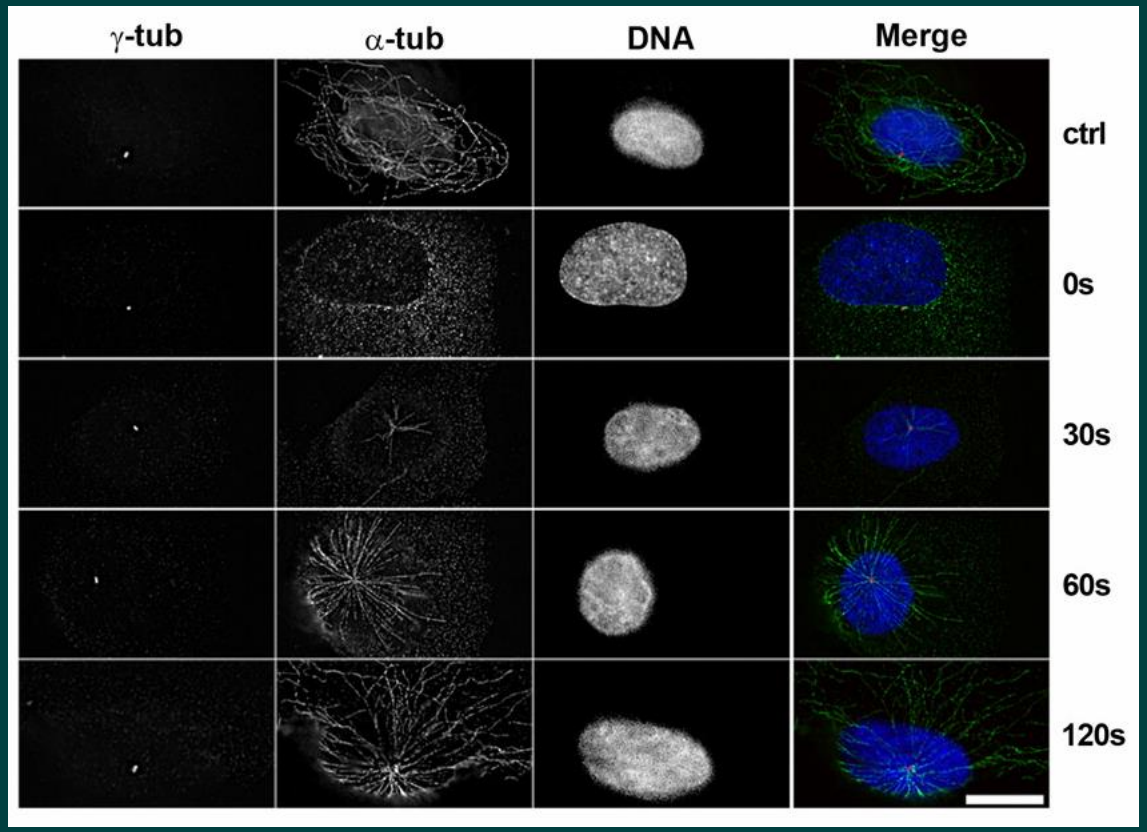


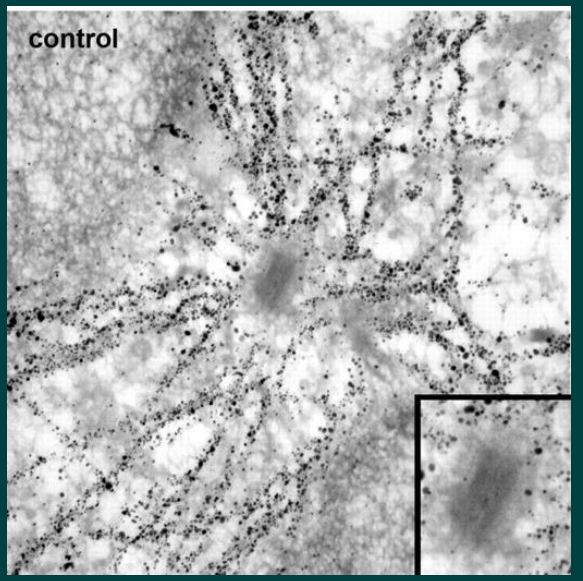
Kouyama and Mihashi, 1981

Cytoskeletal polarity and sites of assembly in the cell

After disassembly, microtubules grow from the centrosome

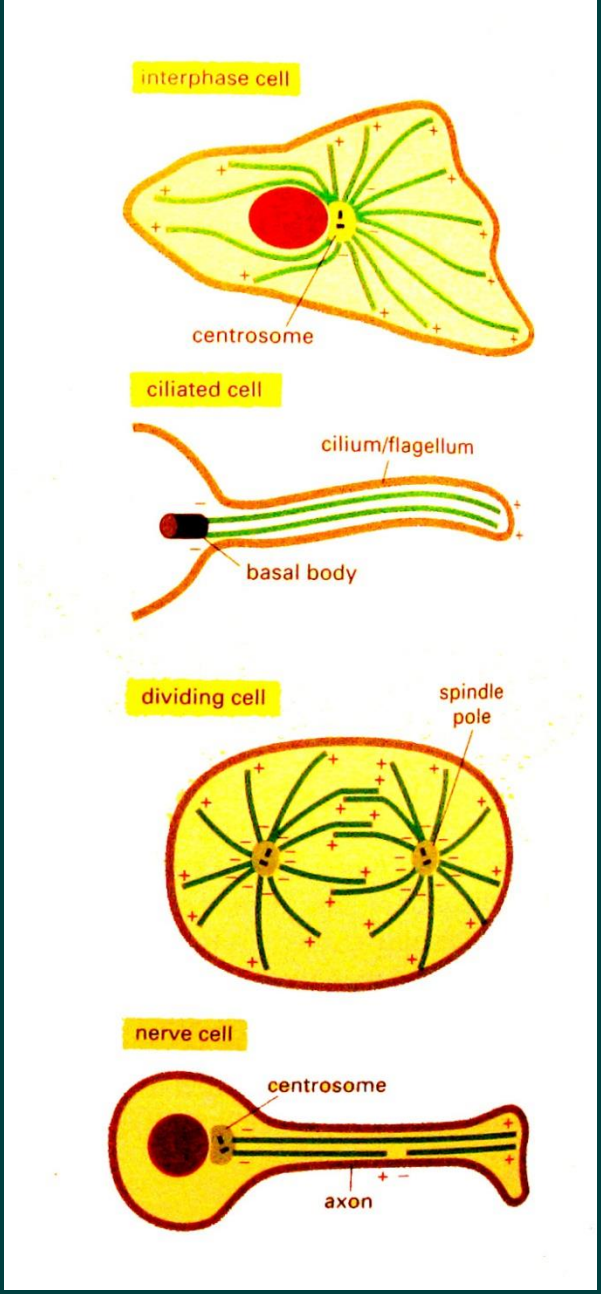




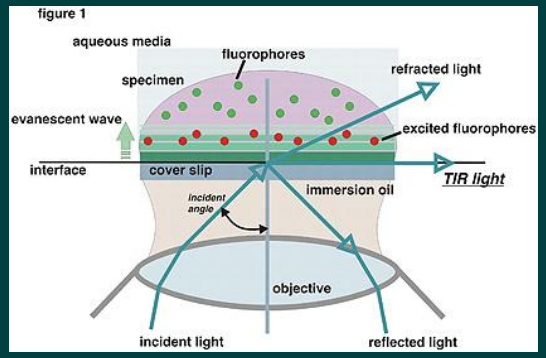


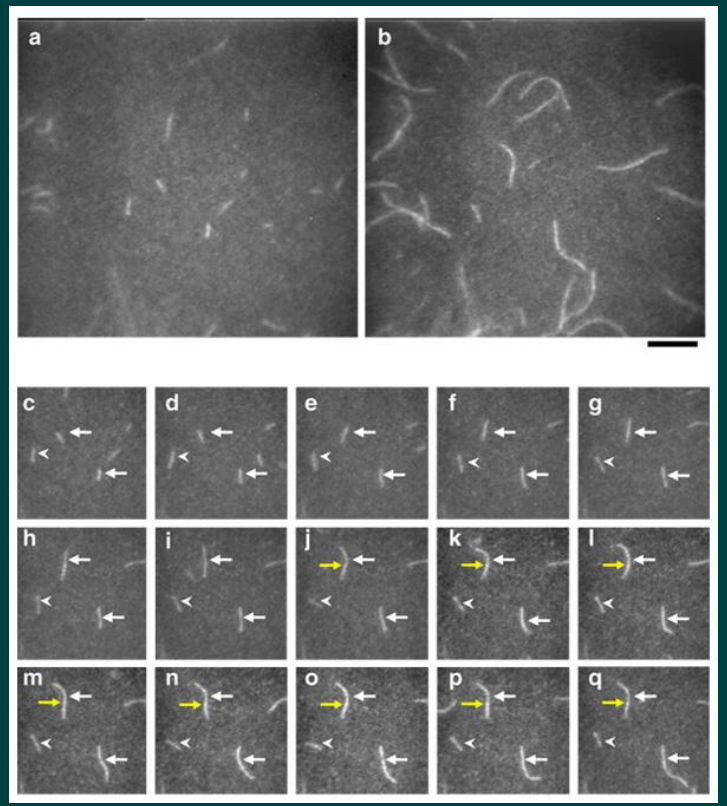
Didier et al., 2008

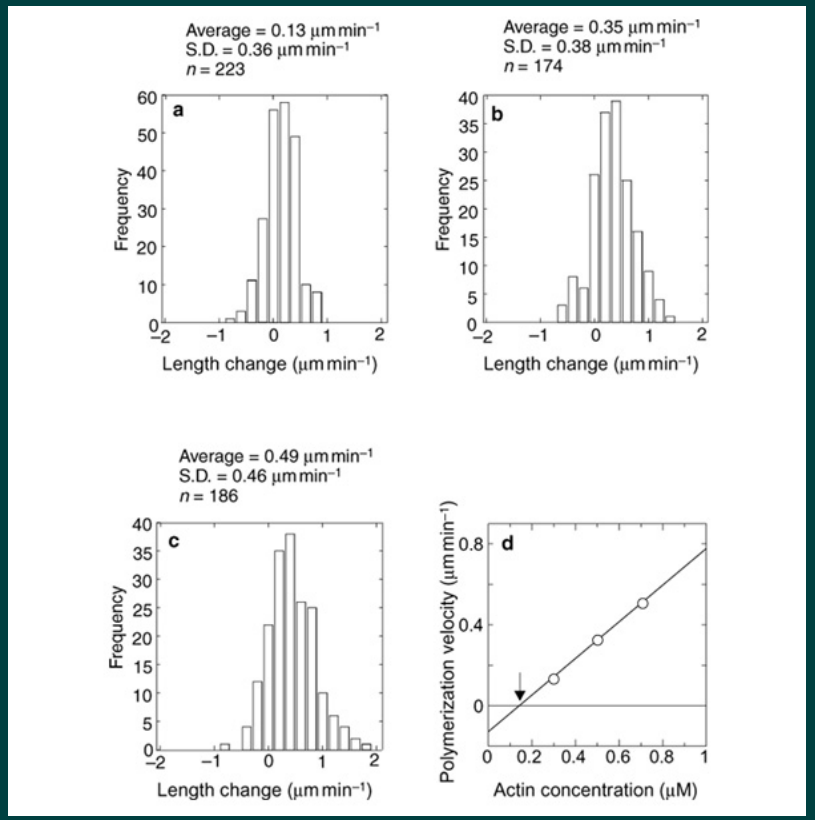
Microtubule polarity in different cells



Observation of assembly of individual actin filaments with TIRFM (total internal reflection fluorescence microscopy)







Fujiwara et al., NCB, 2002

Table 1 **Polymerization and depolymerization rate constants.**

Conditions		k^+ (M^{-1} s^{-1})	k^- (s^{-1})	C_0 (μM)
		$\times 10^7$		
Steady-state phase*	Ca	18	25	—
	Mg	45	29	—
Polymerization phase†	Ca	0.61	0.85	0.14
	Mg	1.0	0.64	0.064
Solution + 0.5%	Ca	0.75	0.94	0.13
methylcellulose‡	Mg	1.2	0.80	0.067
Solution§	Ca	0.50	0.57	0.11
	Mg	0.95	0.90	0.095

x10^7

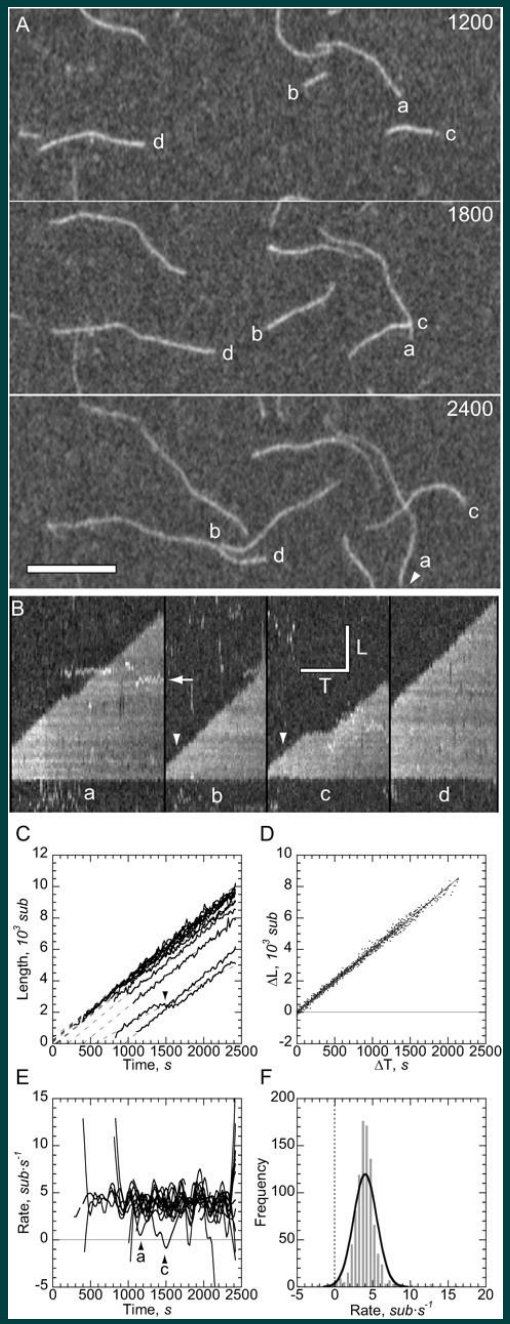
Conditions: 30 mM potassium chloride, 2 mM magnesium chloride, 4 mM ATP, 20 mM MOPS at pH 7.0, 10 mM DTT, with*†‡ or without§ 0.5% (w/v) methylcellulose.

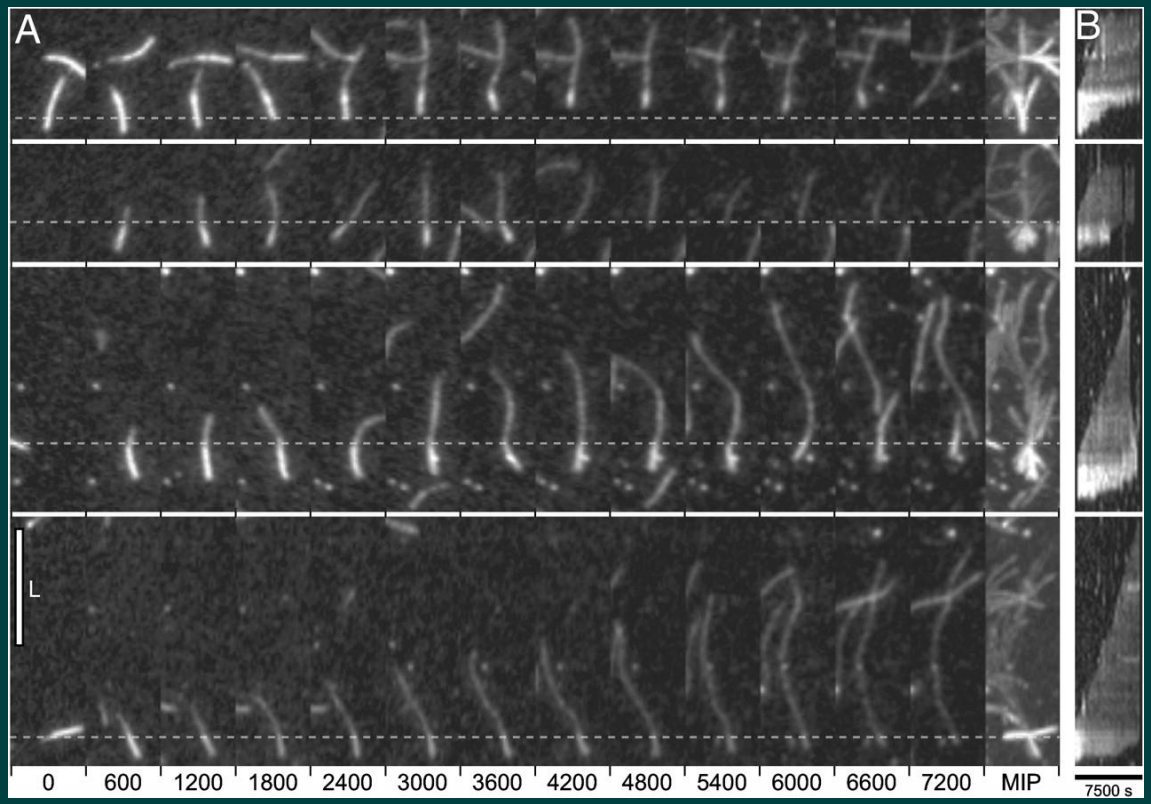
*Estimated from the analysis of length fluctuation in the steady-state phase in single-molecule analysis.

†Estimated from the average length change in the polymerization phase in single-molecule analysis.

‡,§Obtained from the initial rate of increase in the fluorescence intensity in solution in the presence‡ or absence§ of methylcellulose.

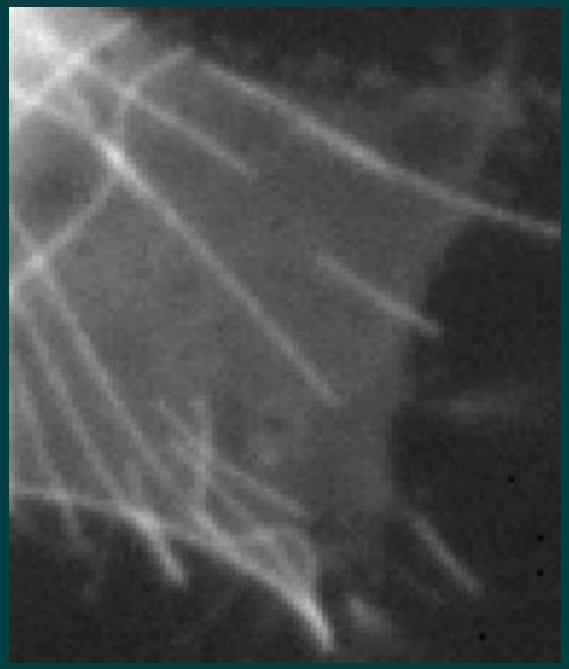
TIRF measurement of actin treadmilling in vitro





Kuhn JR, Pollard TD. Biophys J. 2005 Feb;88(2):1387-402.

Dynamics of individual microtubules in cells



From Rodionov and Borisy, 1997, 1999

microtubule moves – how can we distinguish if this is transport or treadmilling?