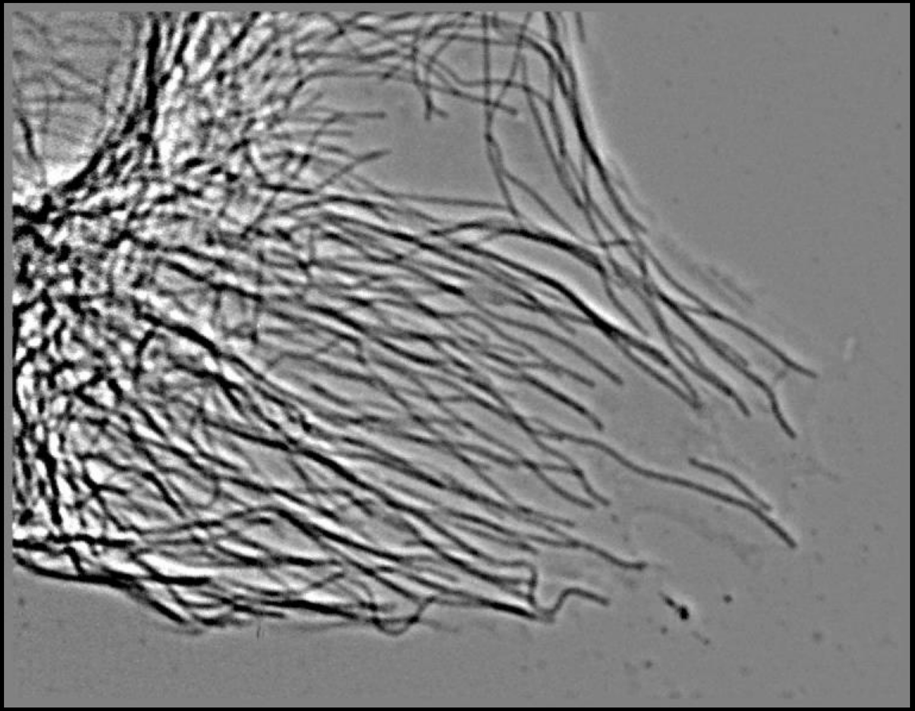
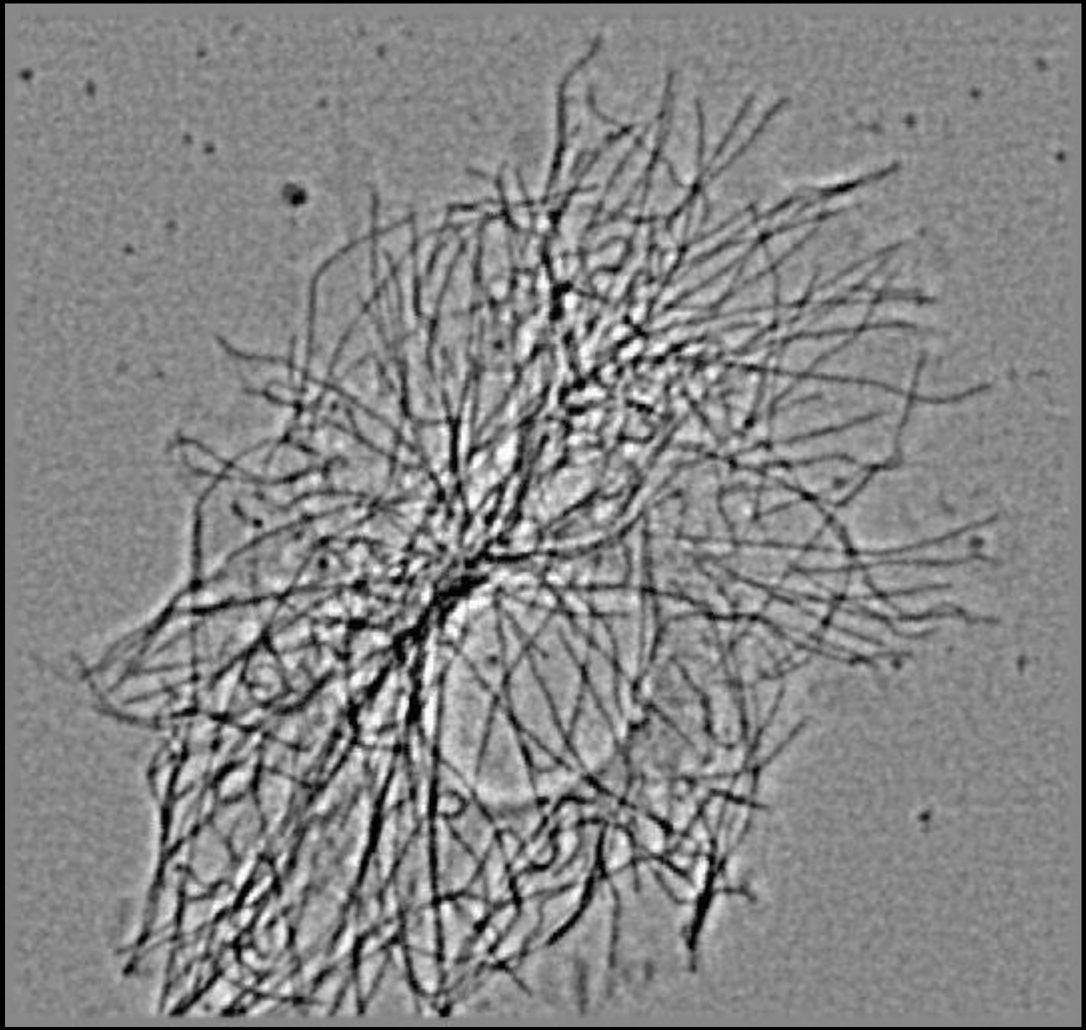
Regulation of Microtubule Dynamics



Anna Akhmanova
Cell Biology
Faculty of Science
Utrecht University
The Netherlands

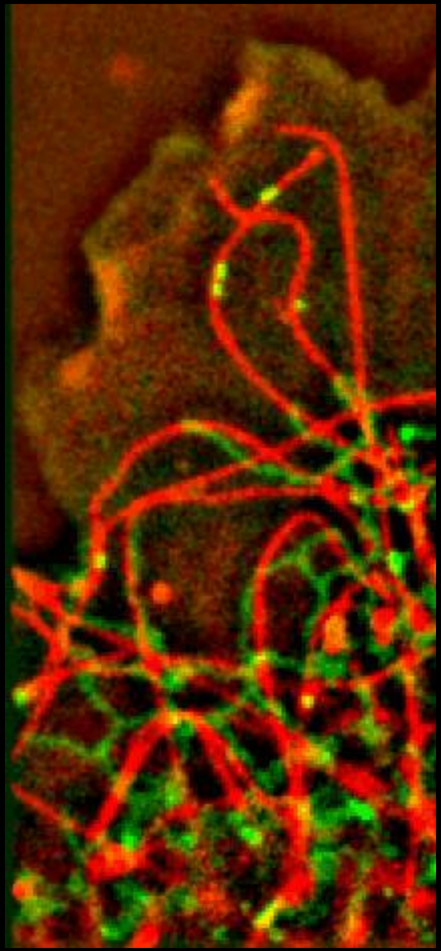
Microtubules



mCherry-α-tubulin; MRC5 human lung fibroblast

movie: Ilya Grigoriev

Microtubules are required for organelle transport and attachment

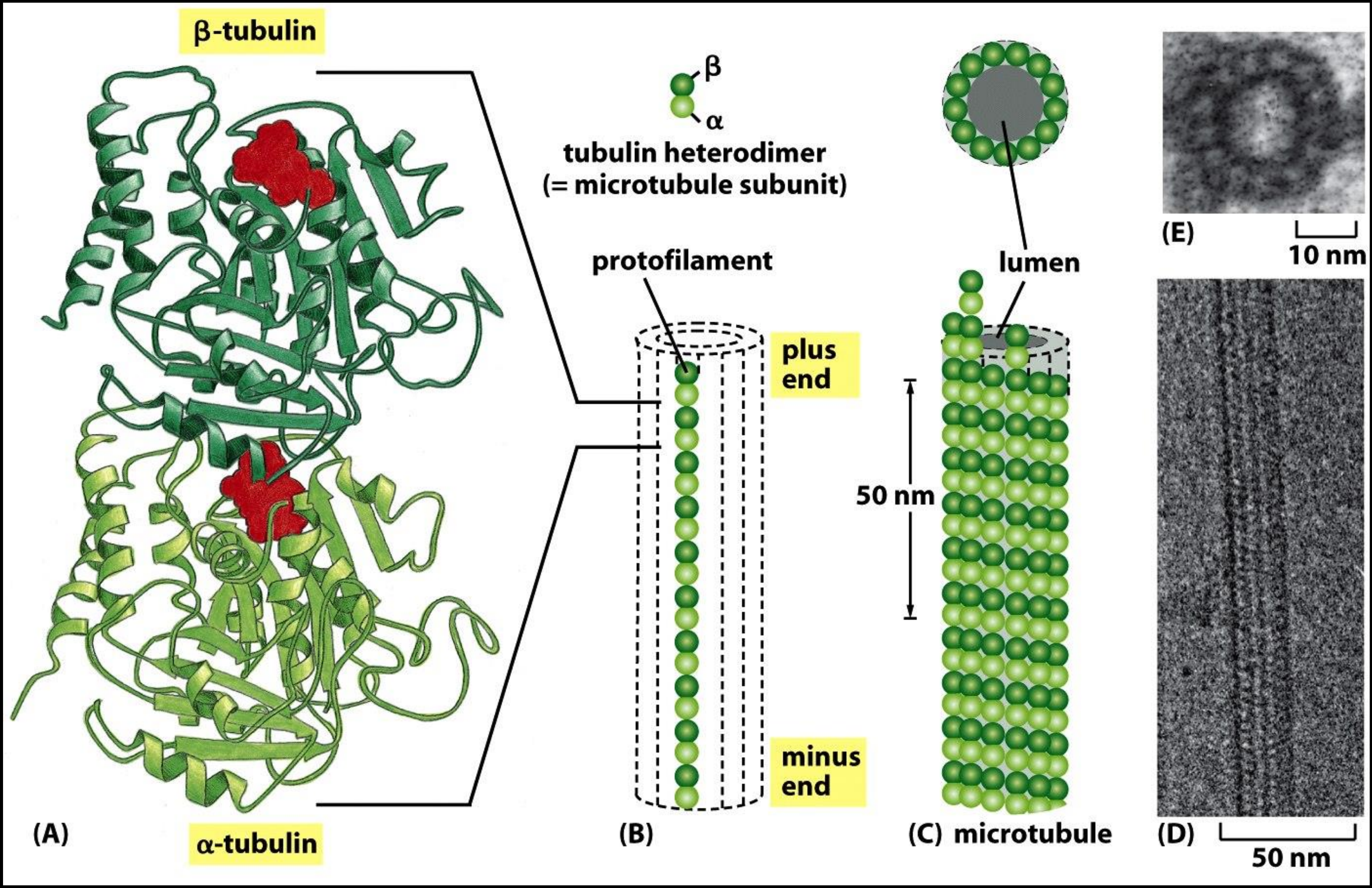


100ms/frame

GFP-Rab6A and mCherry-α-tubulin
in a MRC5 human lung fibroblast

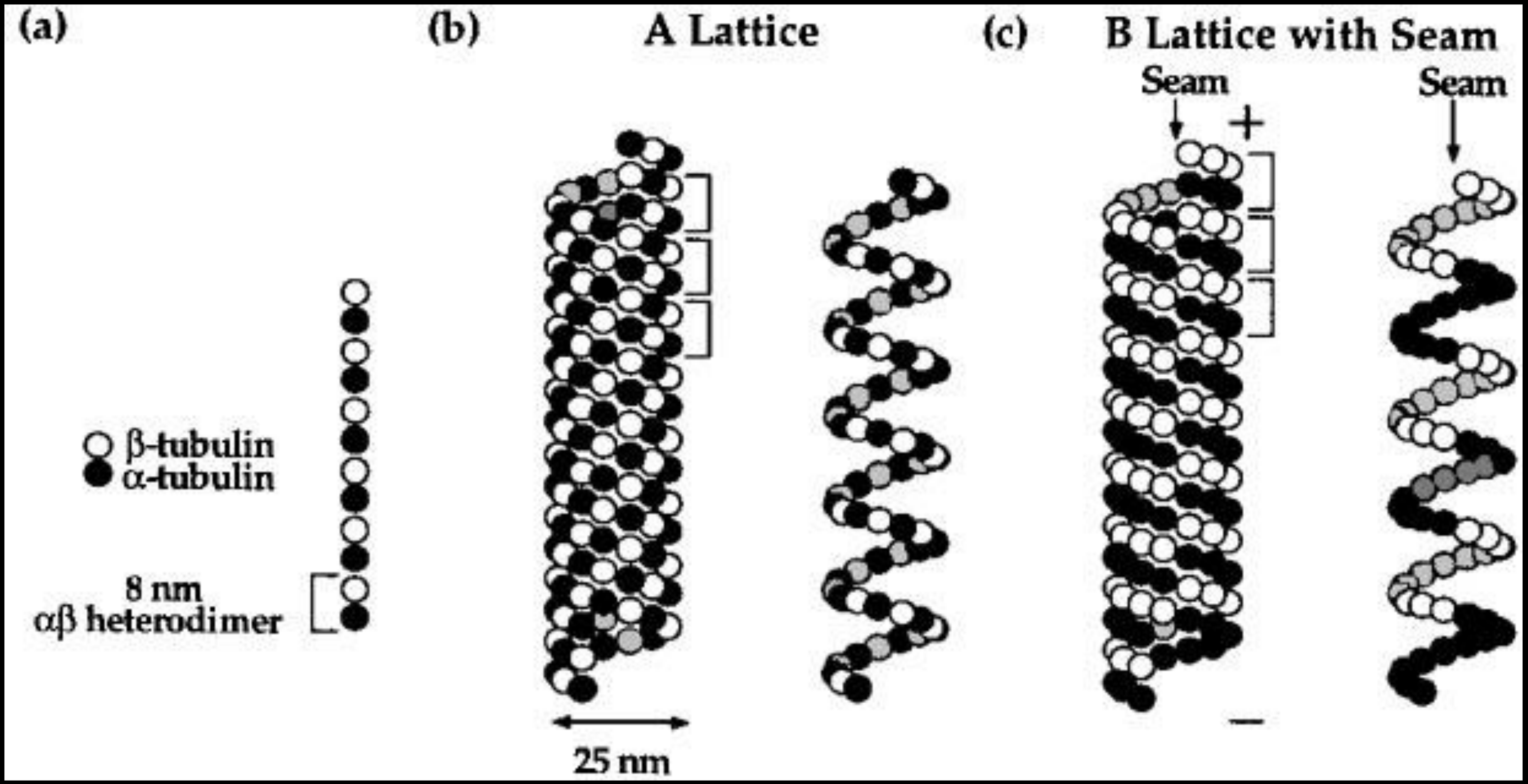
movie: Ilya Grigoriev

Microtubule Structure



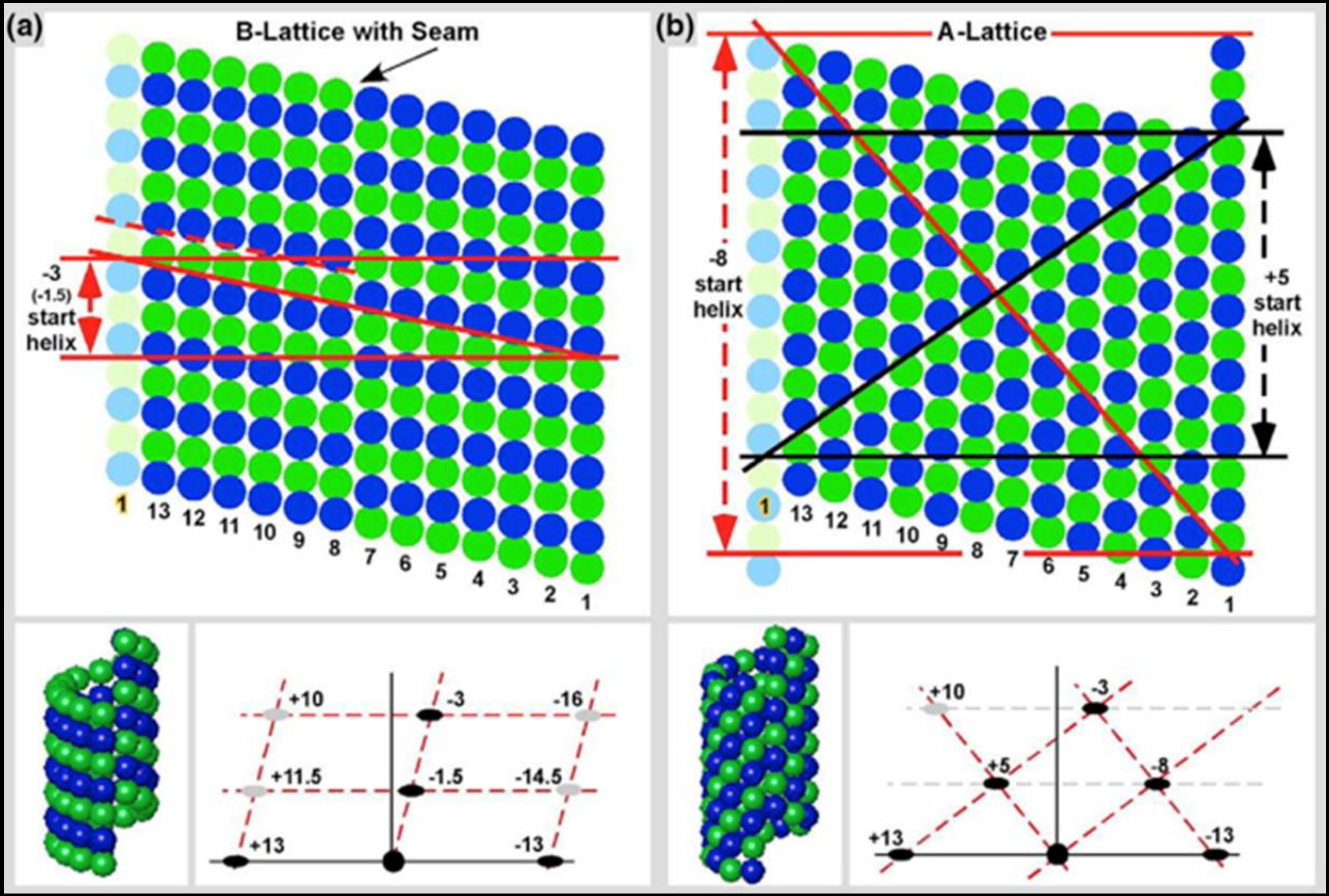
Molecular Biology of the Cell (© Garland Science 2008)

Microtubule Structure

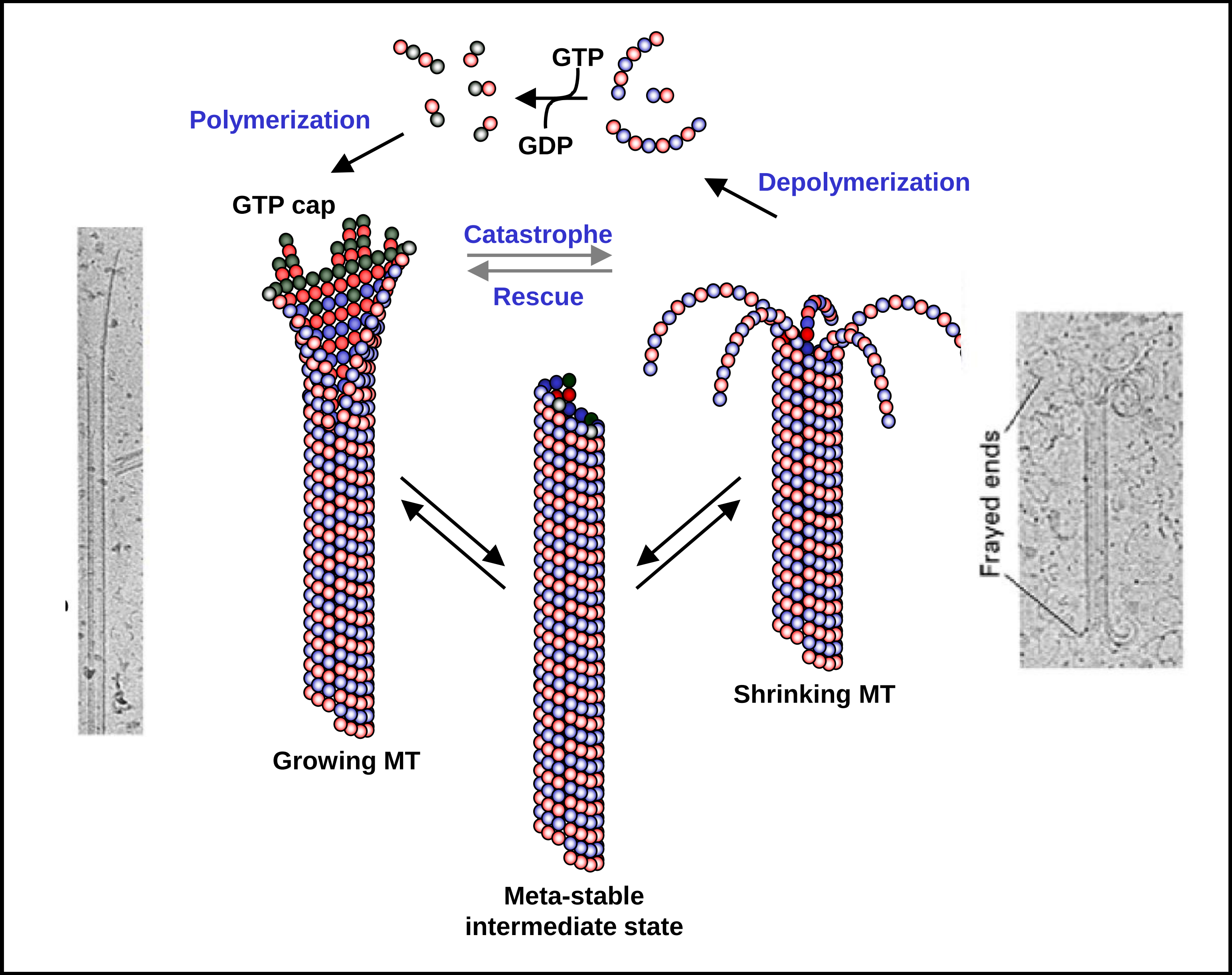


Desai and Mitchison, 1997

Microtubule Structure



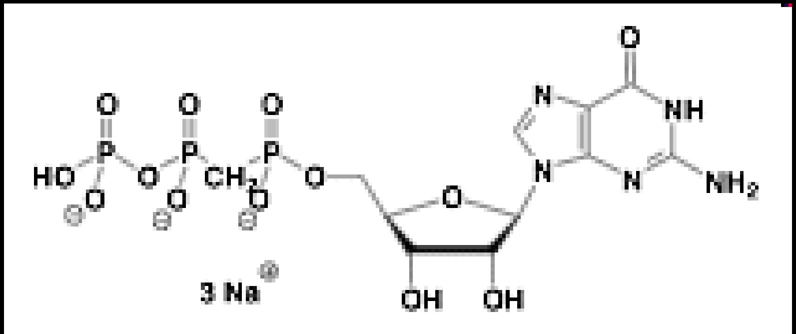
Microtubule Dynamics



The role of GTP hydrolysis:

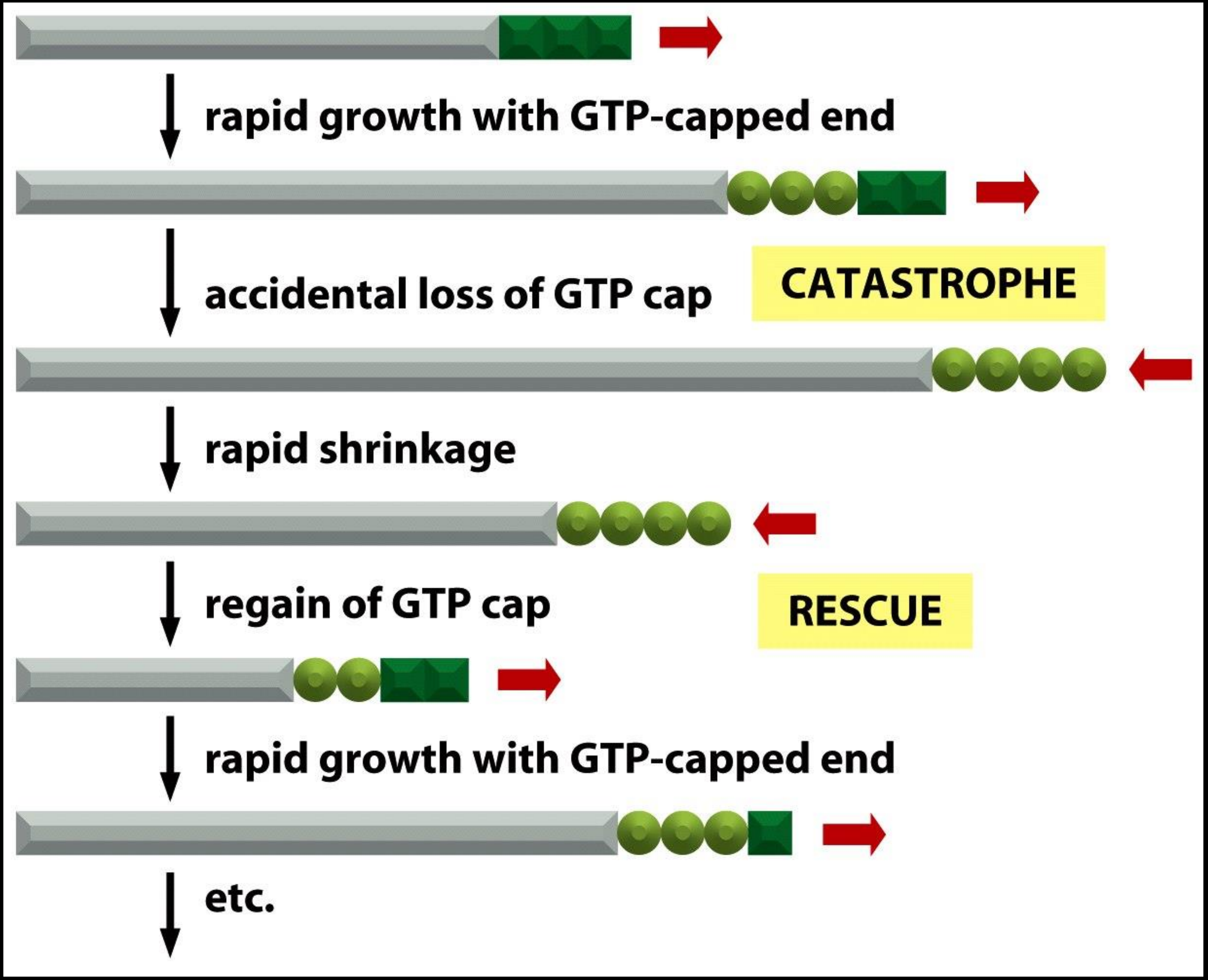
Not needed for assembly
Required for depolymerization

GMPCPP



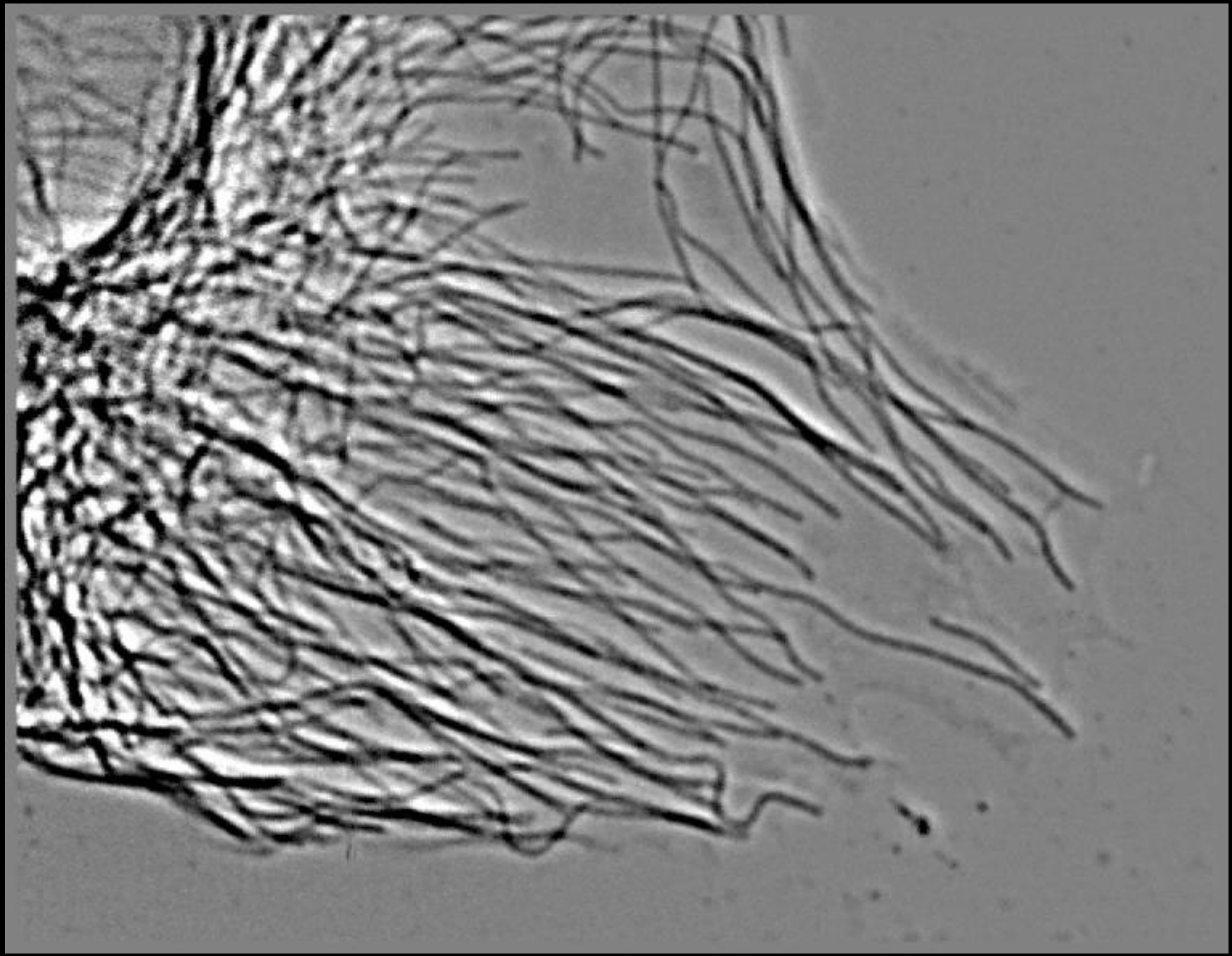
Although the β–γ linkage is normal, tubulin doesn’t hydrolyse it under standard conditions

Microtubule Dynamics



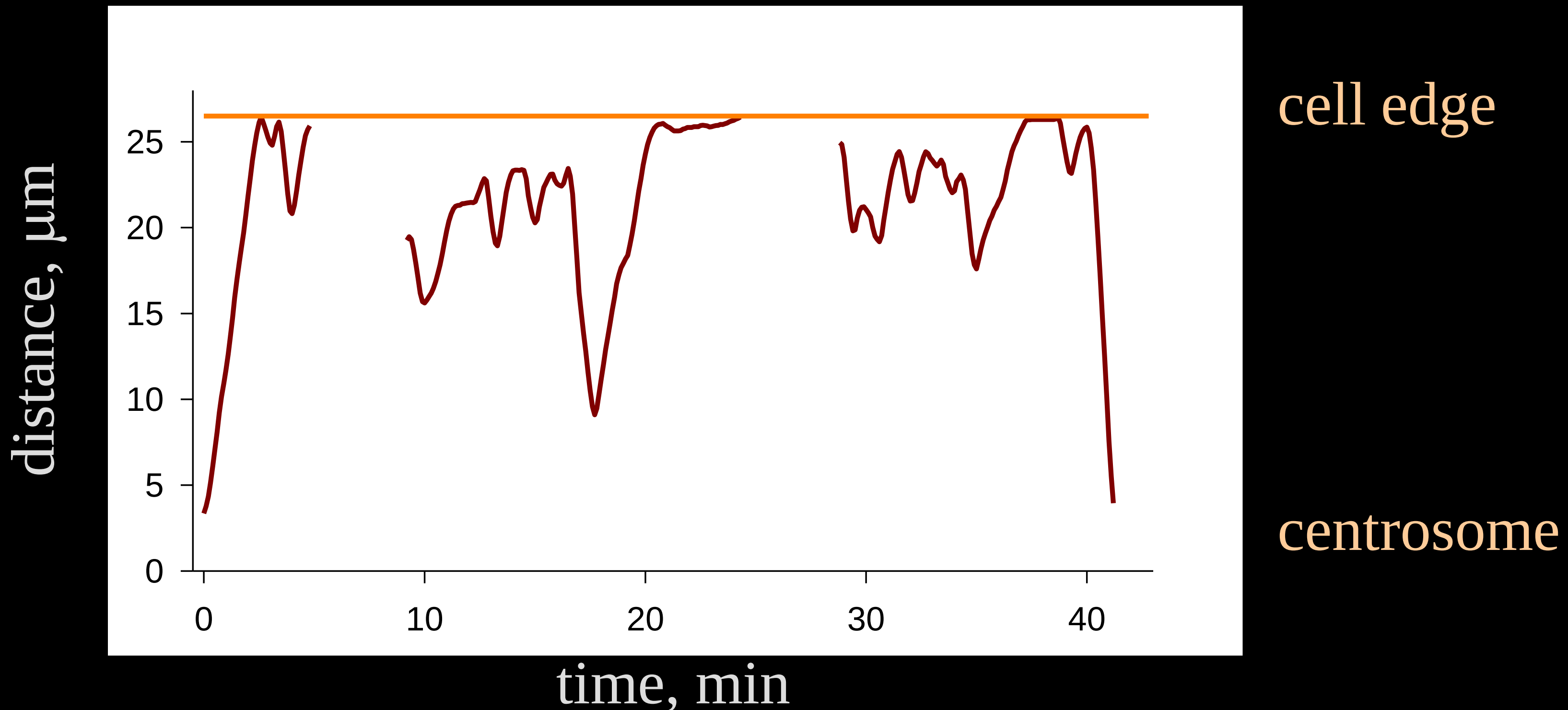
Molecular Biology of the Cell (© Garland Science 2008)

Dynamic instability in living cells



Dynamic microtubules visualized with Cy3-tubulin
in 3T3 mouse fibroblast

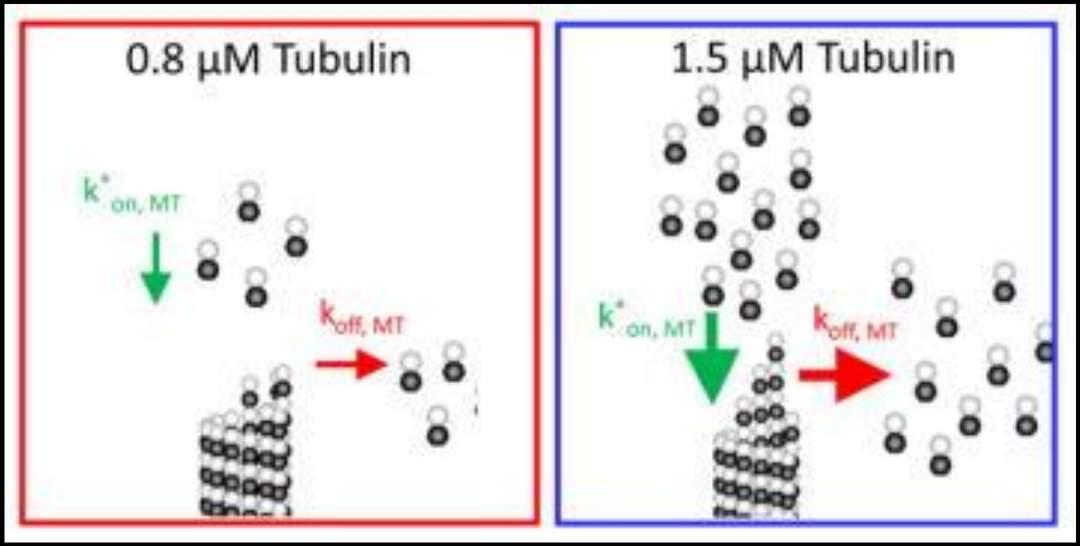
Dynamic instability in living cells



	in vitro	in vivo
rate of growth	<3-4 µm/min	10-25 µm/min
rate of shortening	15–100 µm/min	12–40 µm/min

Microtubule polymerization kinetics

Does k_{off} depend on the tubulin concentration?

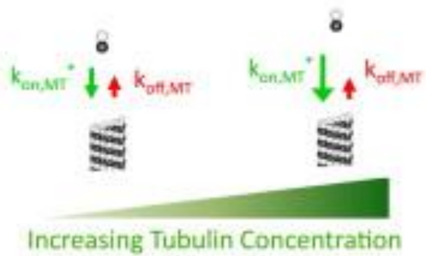
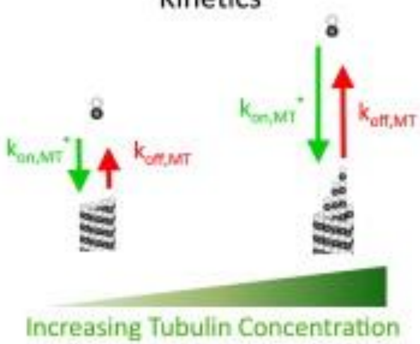


Gardner et al, Cell 2011

Microtubule polymerization kinetics

Does k_{off} depend on the tubulin concentration?

The Kinetics of Microtubule Assembly

	Previously	This Study
$k_{on,MT}$	~5 $\mu M^{-1}s^{-1}$	~58 ± 4 $\mu M^{-1}s^{-1}$
$k_{off,MT}$	Constant	Concentration Dependent
Kinetics	Slow Kinetics 	Rapid Tip-State Dependent Kinetics 

Gardner et al, Cell 2011

Microtubule-regulating factors

nucleation – γ-tubulin ring complex

polymerization – XMAP215/ch-TOG

minus end anchoring/stabilization – ninein, CAMSAP

severing – katanin, spastin

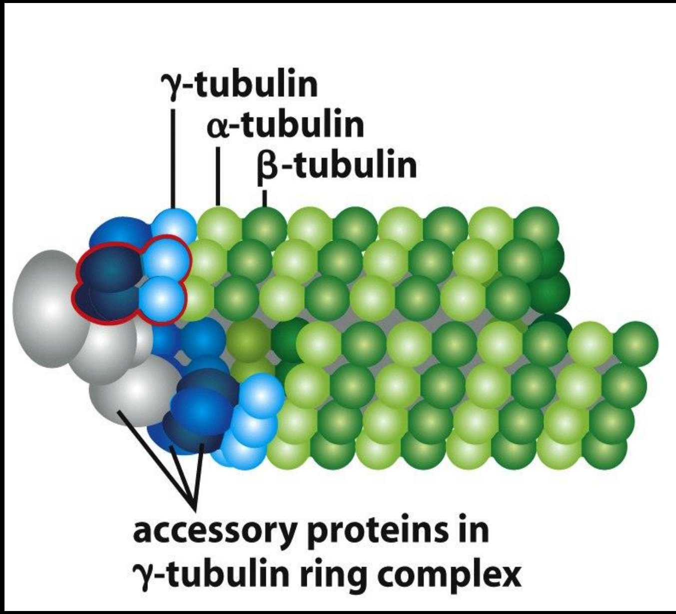
depolymerization – stathmin, kinesin-13 (MCAK)

stabilization – MAPs (tau, MAP2, MAP4)

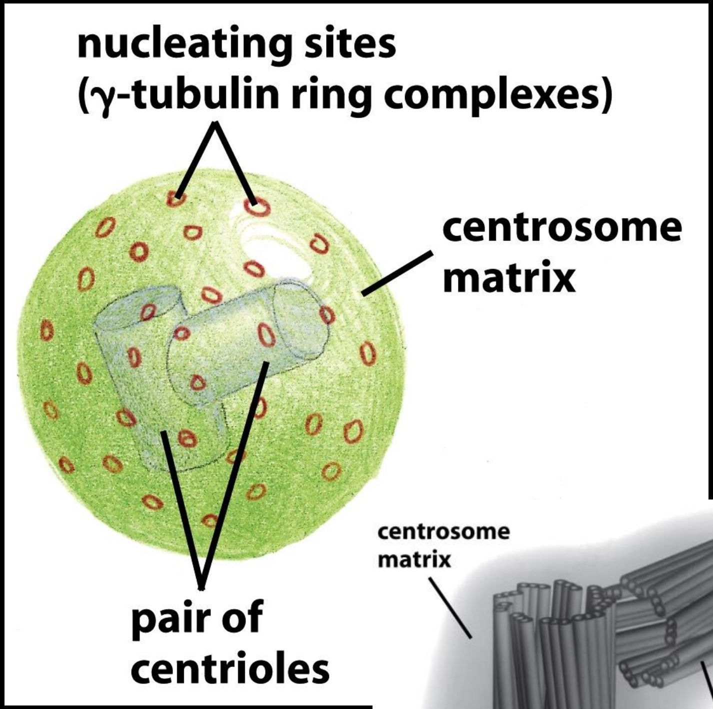
MT nucleation

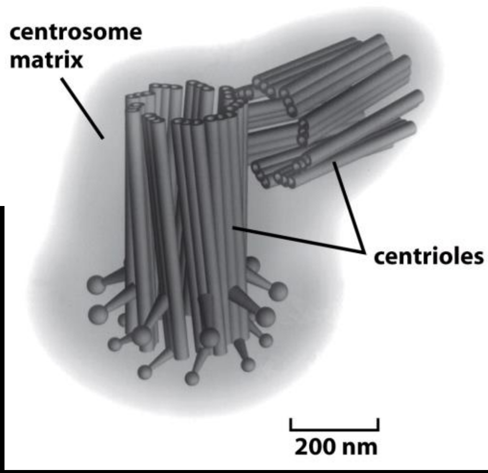
Microtubule nucleation: γ-tubulin

γ-tubulin

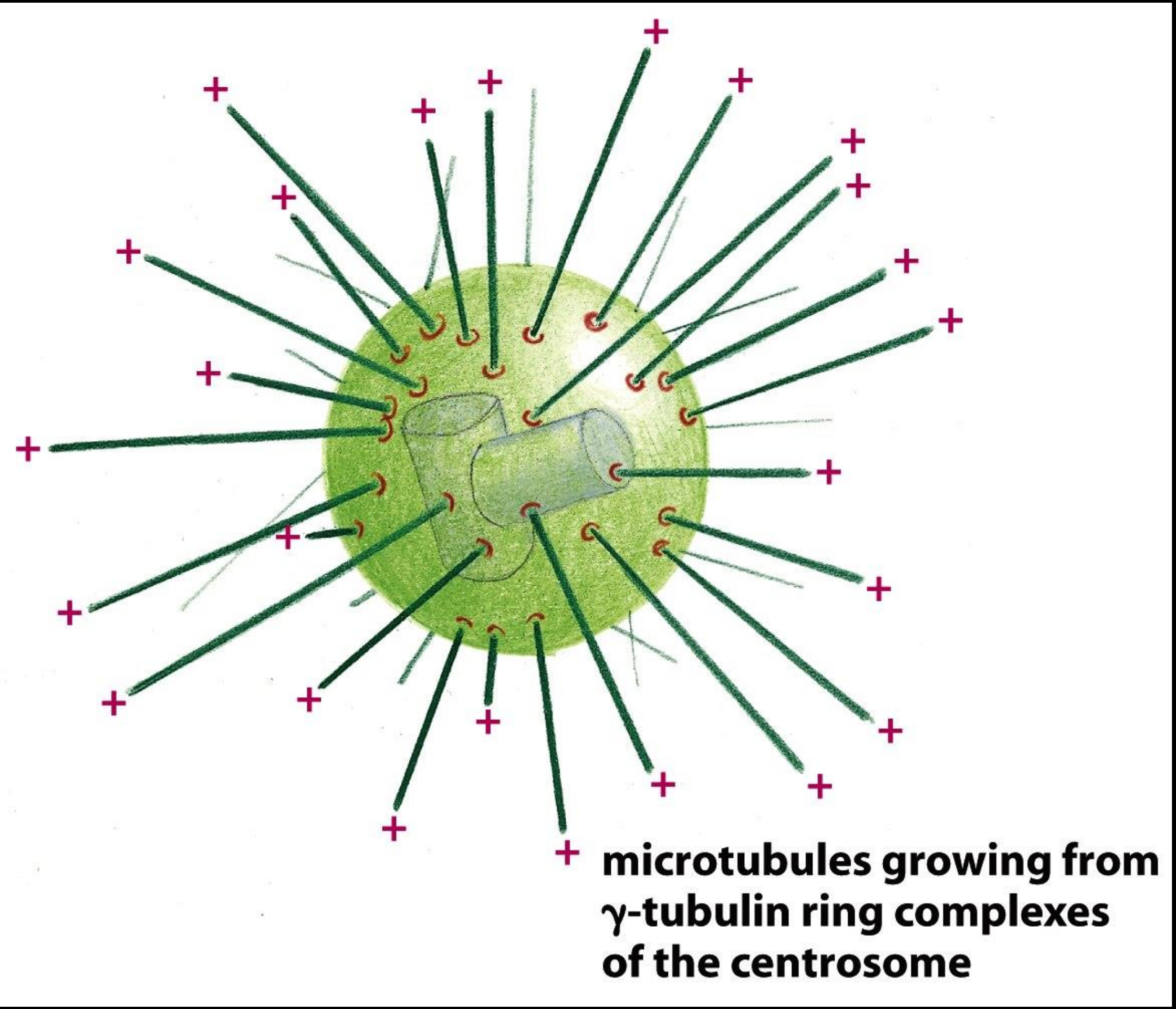


centrosome



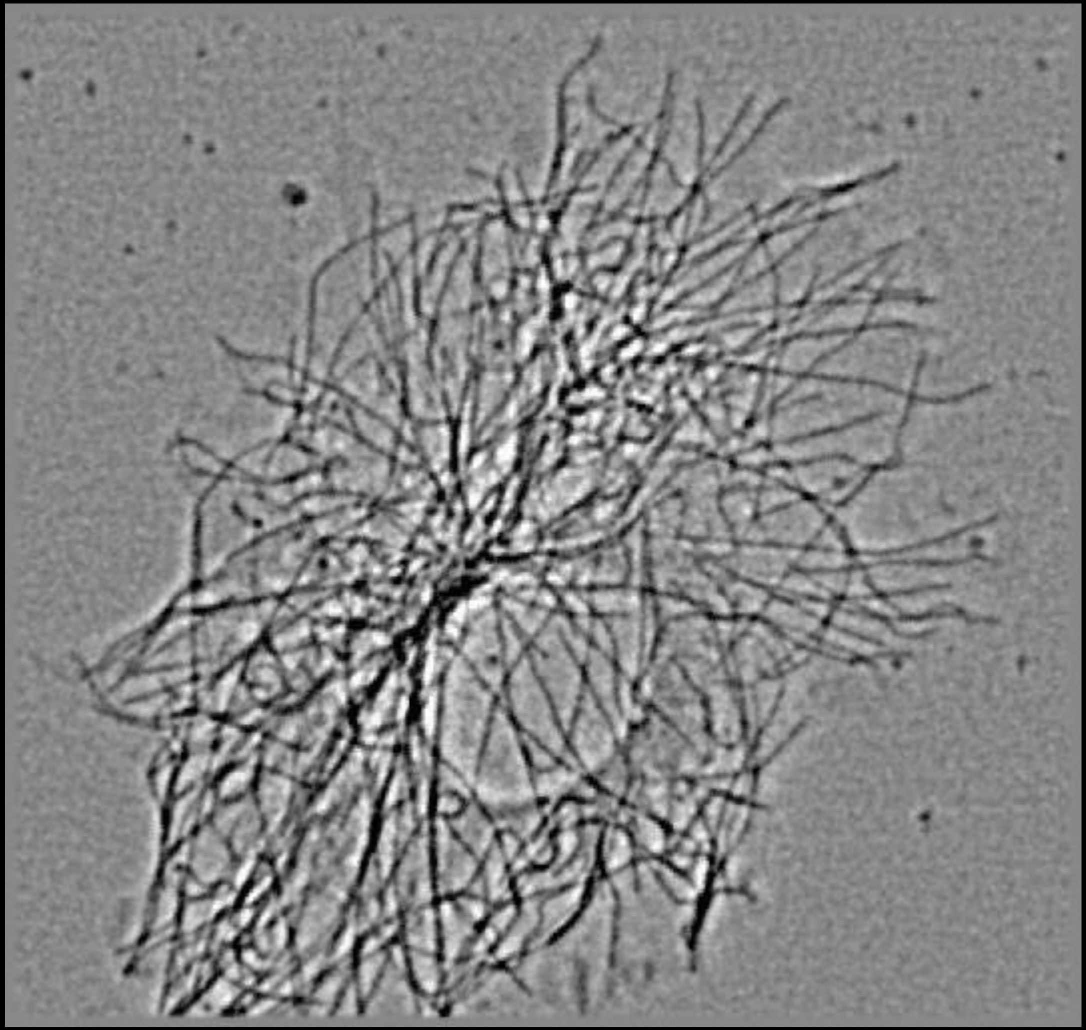


Microtubule nucleation: γ-tubulin



Molecular Biology of the Cell (© Garland Science 2008)

Microtubule organization

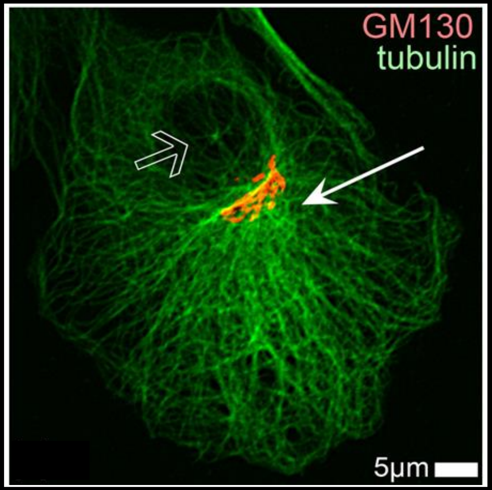


Dynamic microtubules visualized with mCherry-tubulin
in MRC5 human lung fibroblast

Microtubules
in interphase

movie: Ilya Grigoriev

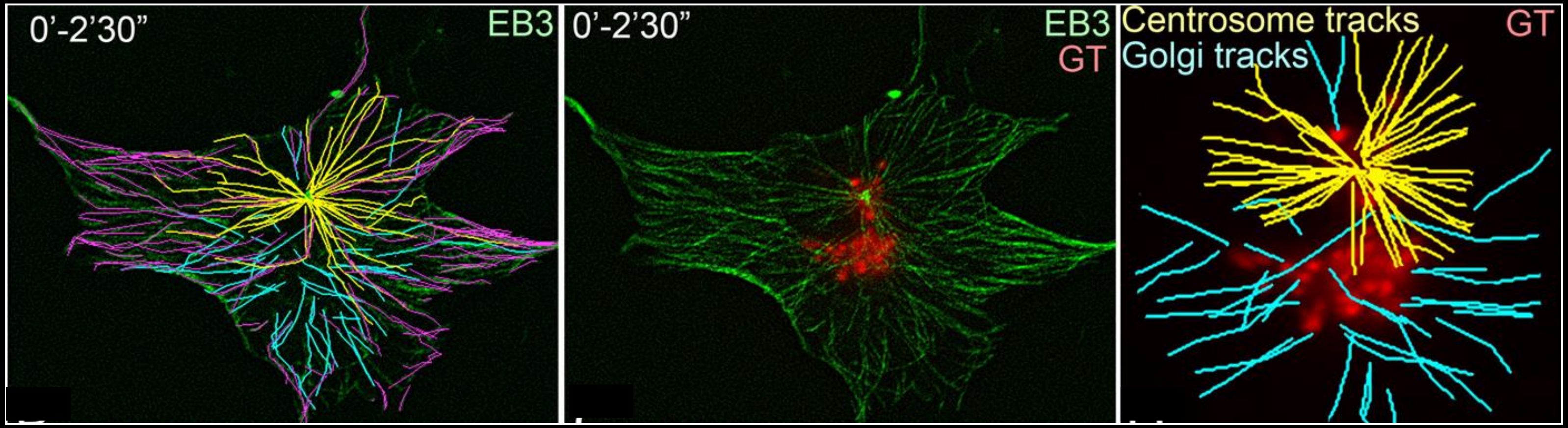
Non-centrosomal microtubule nucleation



MTs can originate from the Golgi

proteins involved: CLASP, AKAP450

Efimov et al., 2007, Rivero et al., 2009



Non-centrosomal microtubule nucleation

Nuclear envelope in muscle cells

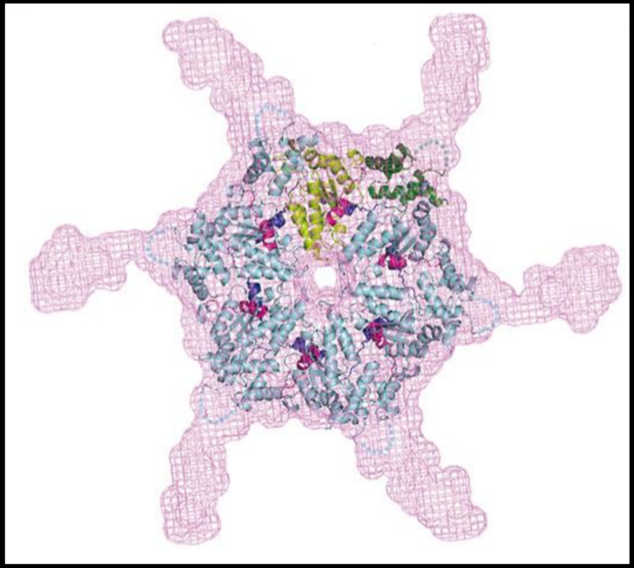
Other microtubules (plants, fission yeast)

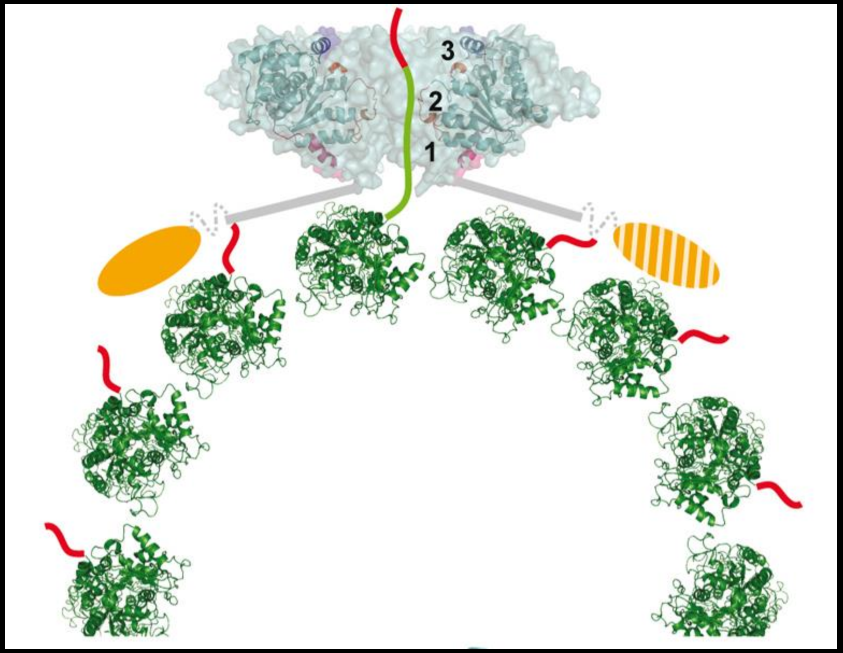
Non-centrosomal arrays in insect cells

MT severing proteins

Spastin, Katanin

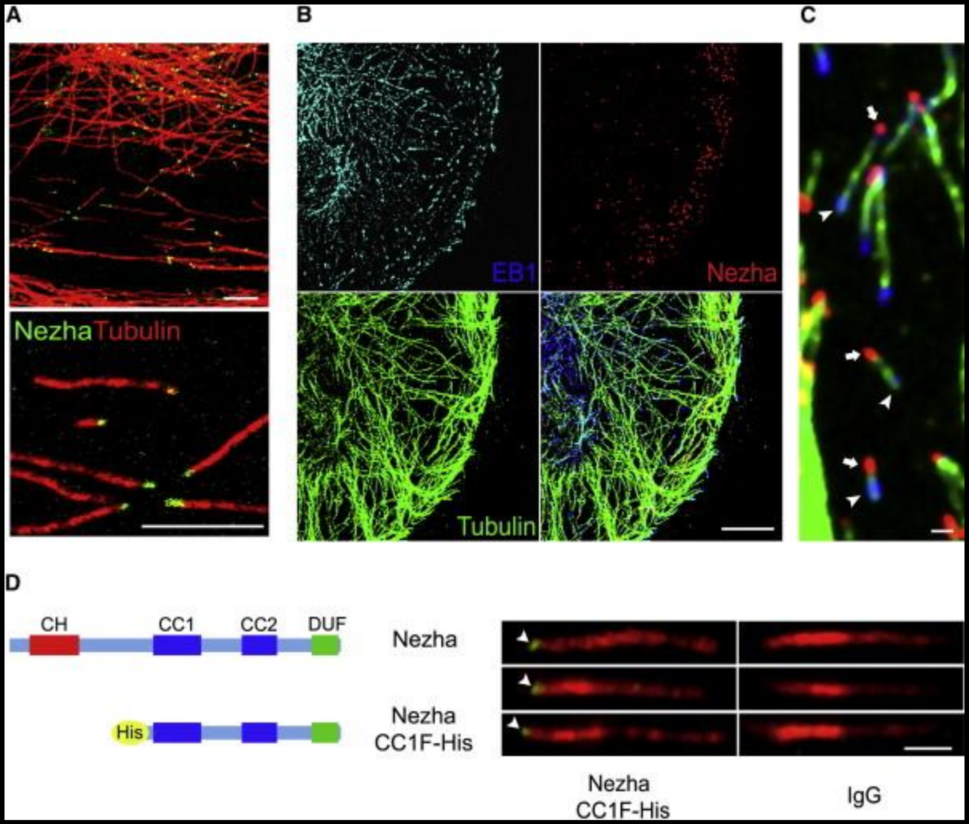
Spastin





Roll-Mecak and Vale, Nature 2008

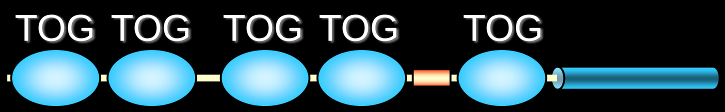
MT minus end-stabilizing proteins: CAMSAP/Nezha/Patronin



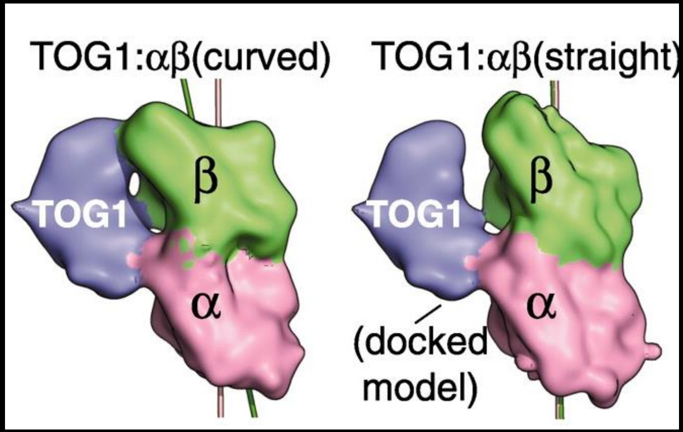
Meng et al., Cell 2008
Goodwin and Vale 2010
Tanaka et al., PNAS 2012

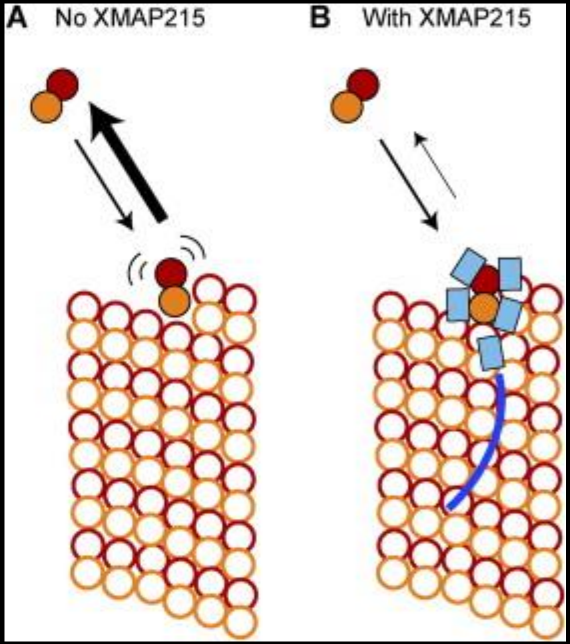
Regulation of Microtubule Growth

Polymerization: XMAP215(ch-TOG)

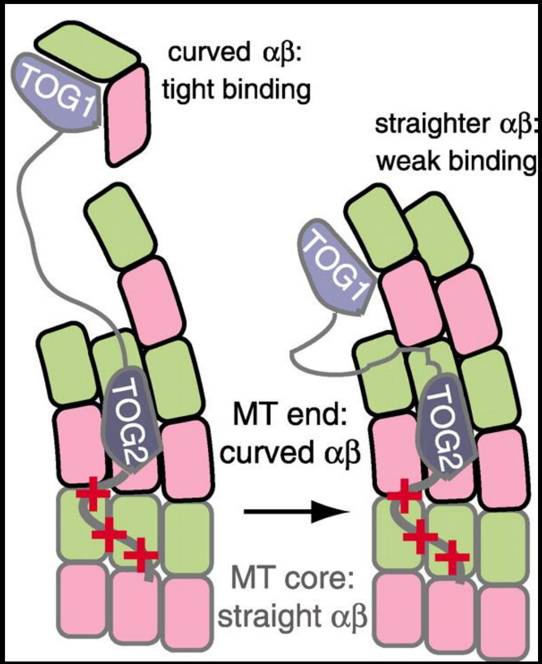


Accelerates MT growth rate in vitro
Suppresses catastrophes





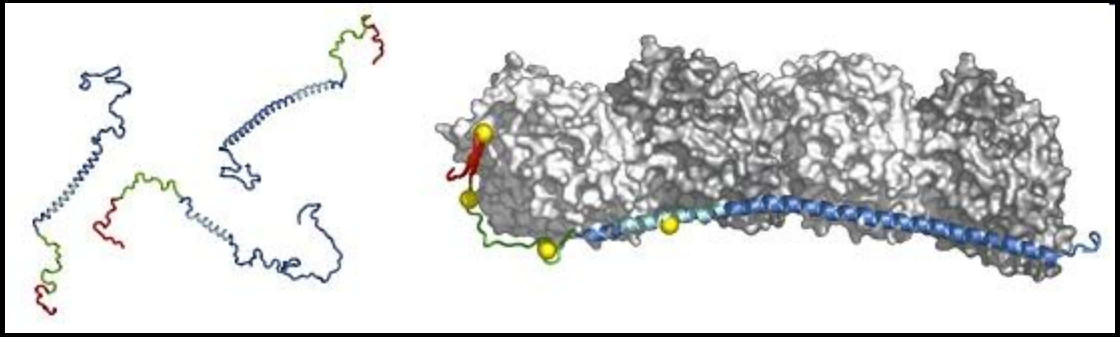
Brouhard et al, Cell 2008



Ayaz et al, Science 2012

Destabilization: Stathmin

Oncoprotein 18/stathmin; RB3, SCLIP, SCG10

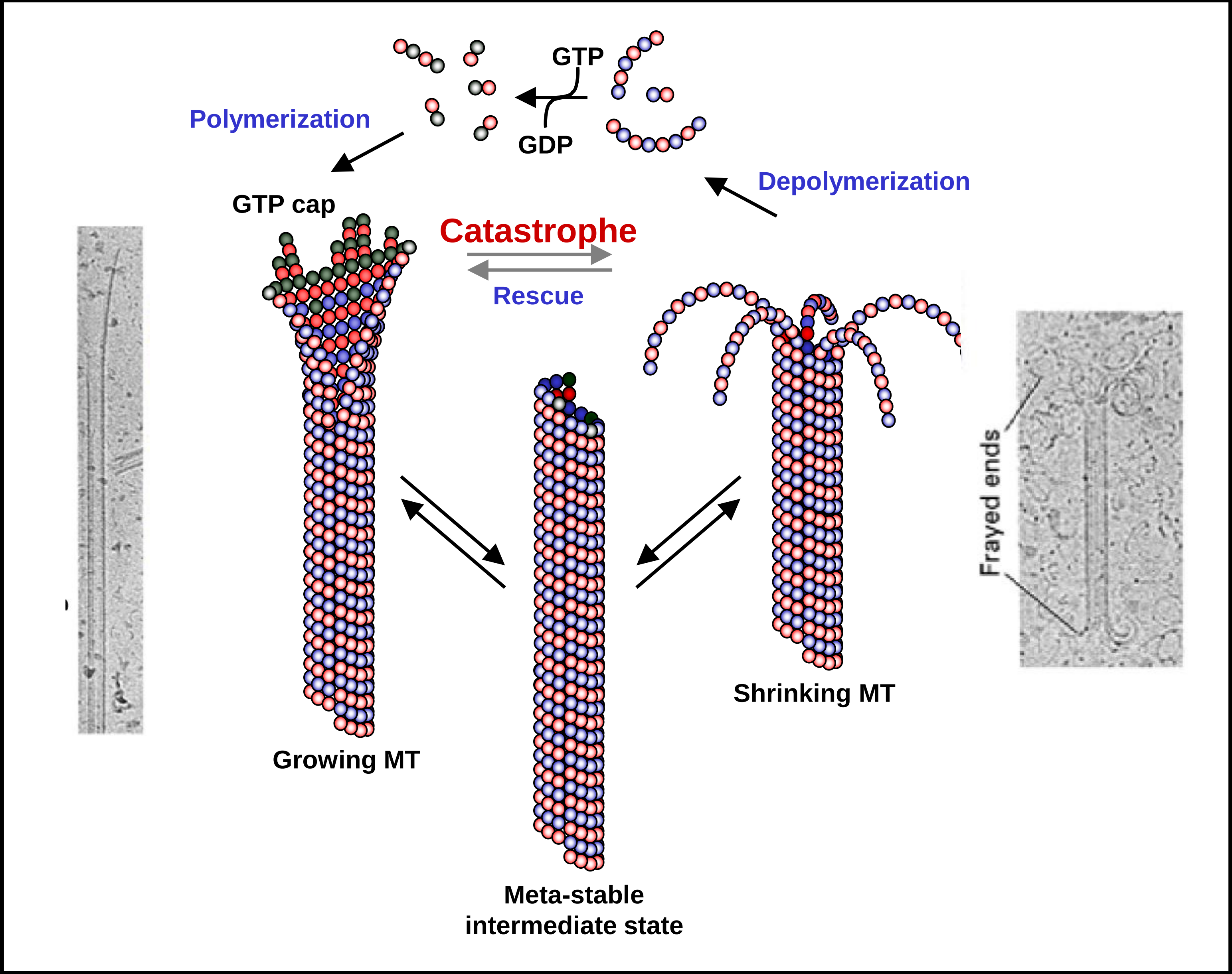


3.5 A resolution structure of tubulin in complex with colchicine and with the stathmin-like domain (SLD) of RB3

Ravelli et al, Nature 2004

Catastrophe

Catastrophe induction



Catastrophe induction

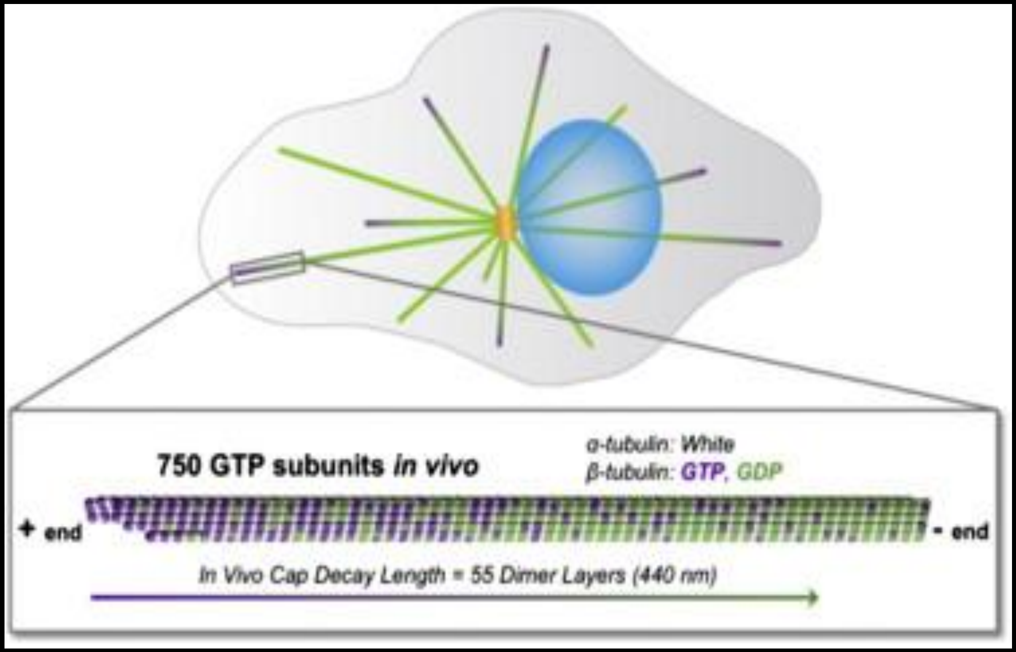
Loss of GTP cap

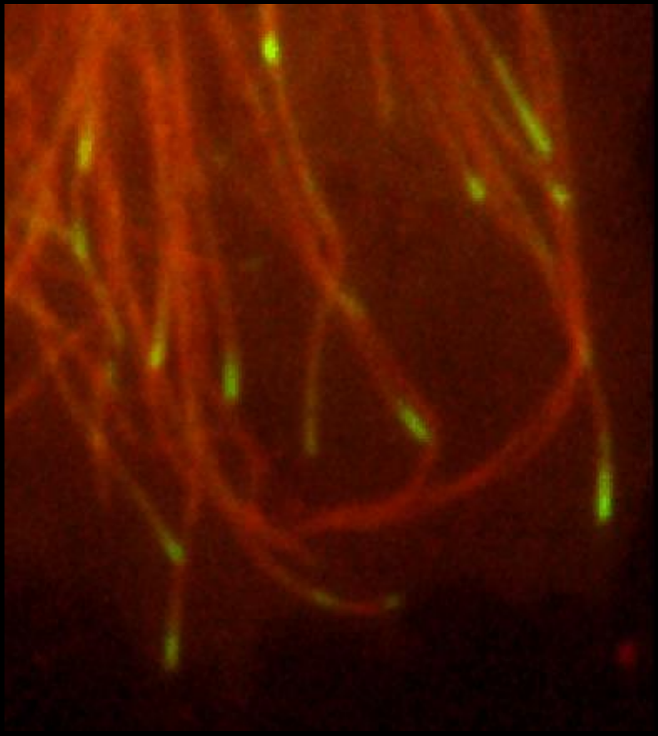
Slow growth, obstacles (barriers)

Catastrophe inducing factors

How long is the GTP cap?

End Binding (EB) proteins are proposed to recognize the cap





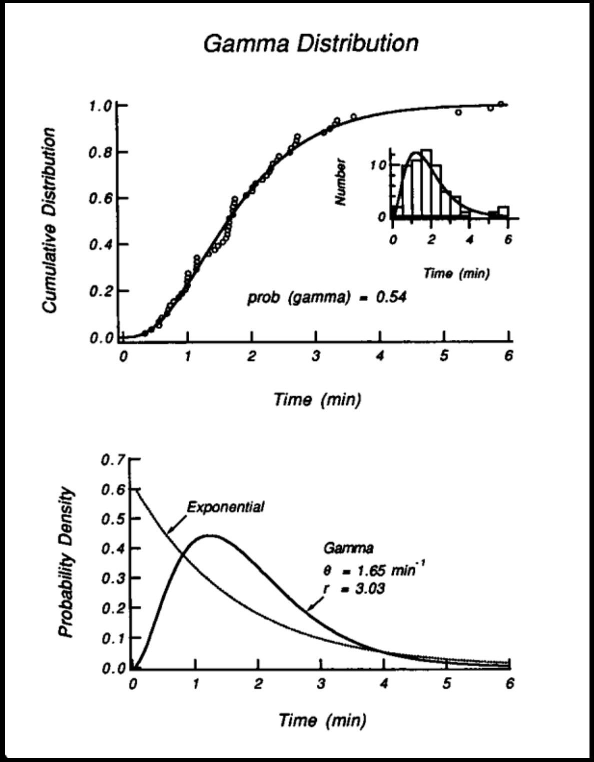
GFP-EB3 and mCherry-α-tubulin in a MRC5 human lung fibroblast

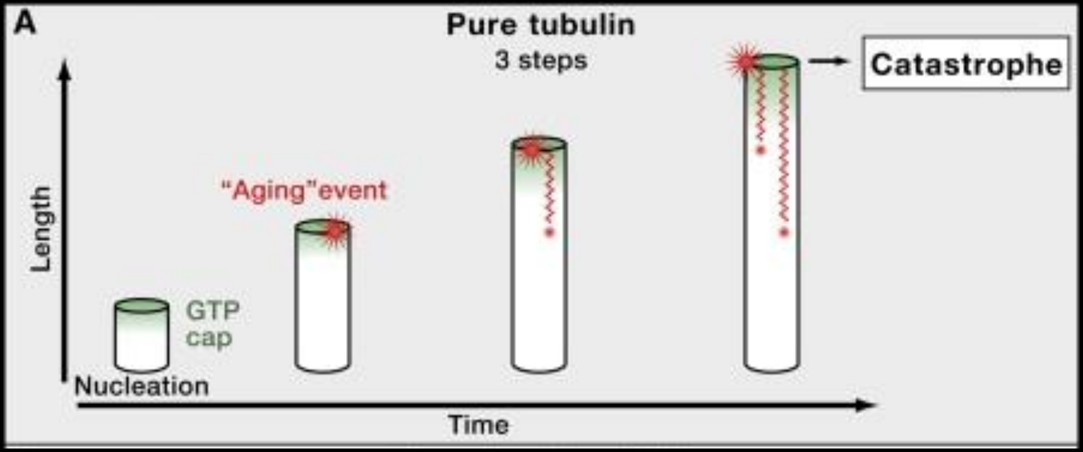
Seetapun et al., Curr Biol 2012

Catastrophe is likely to be a multistep process

Catastrophes do not follow first-order kinetics

Catastrophes occur after several intermediate steps

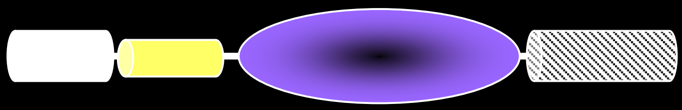


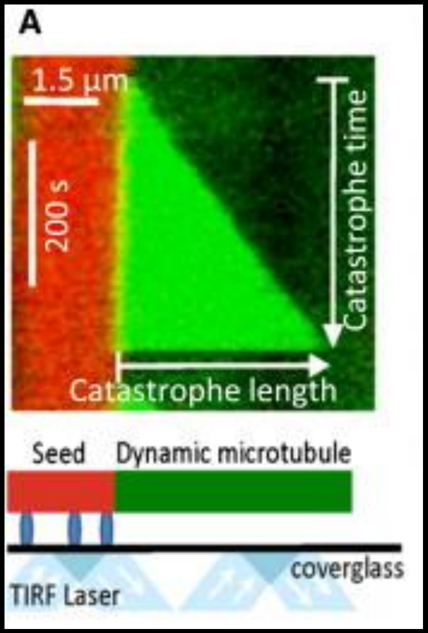


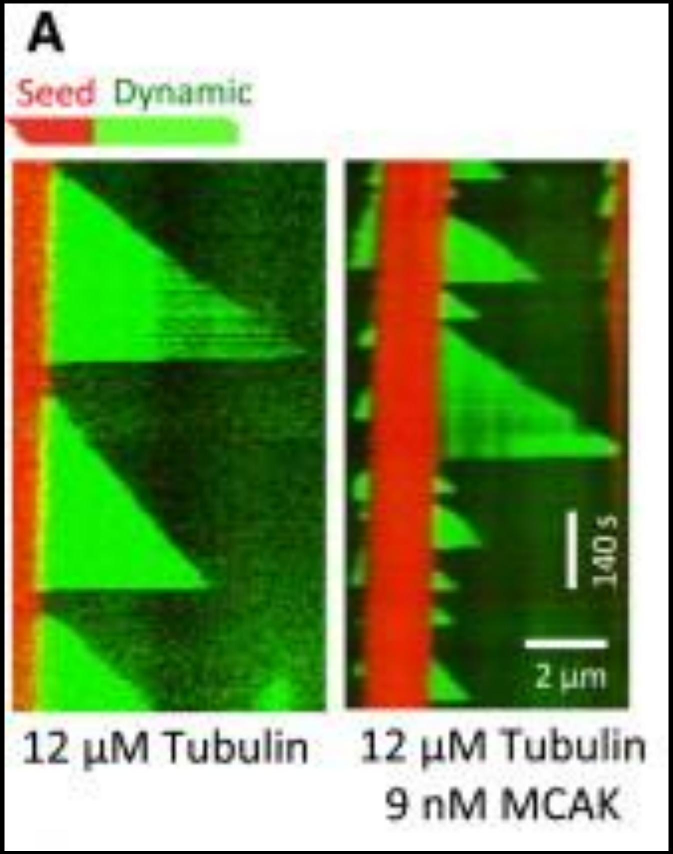
Odde et al., Biophys J 1995

Catastrophe induction: Kinesin-13

MCAK (XKCM1)=KIF2C
KIF2A, KIF2B



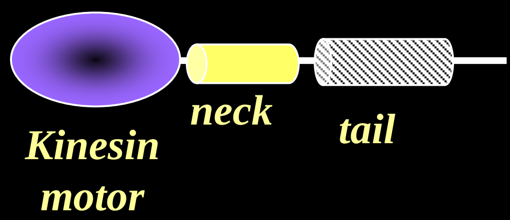


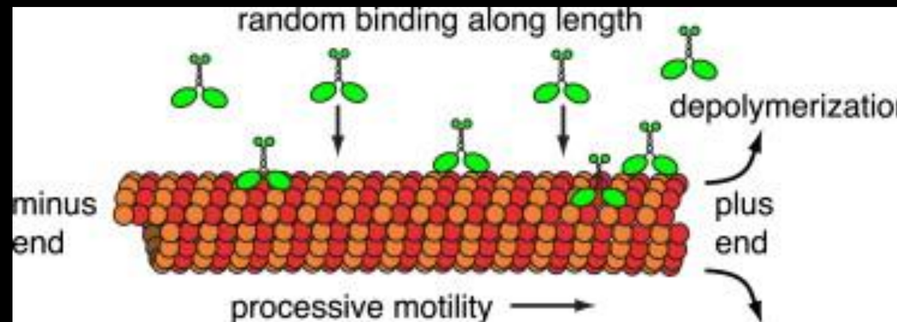


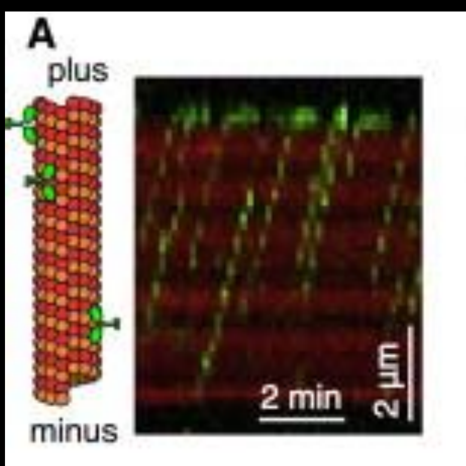
Gardner et al, Cell 2011

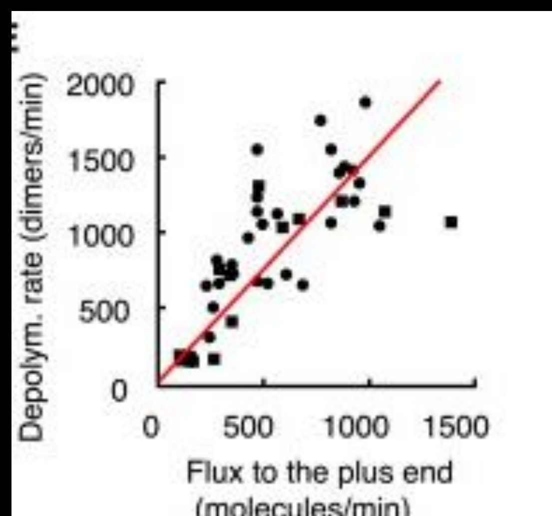
Kinesin-8 family –motile microtubule destabilizing proteins

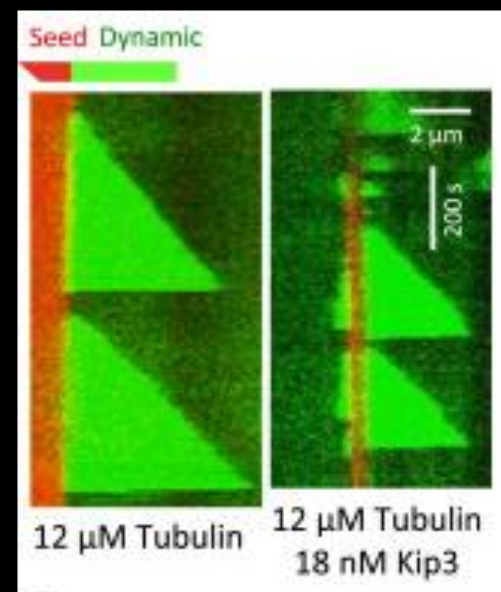
Kip3 (yeast)
KIF18A/B (mammals)





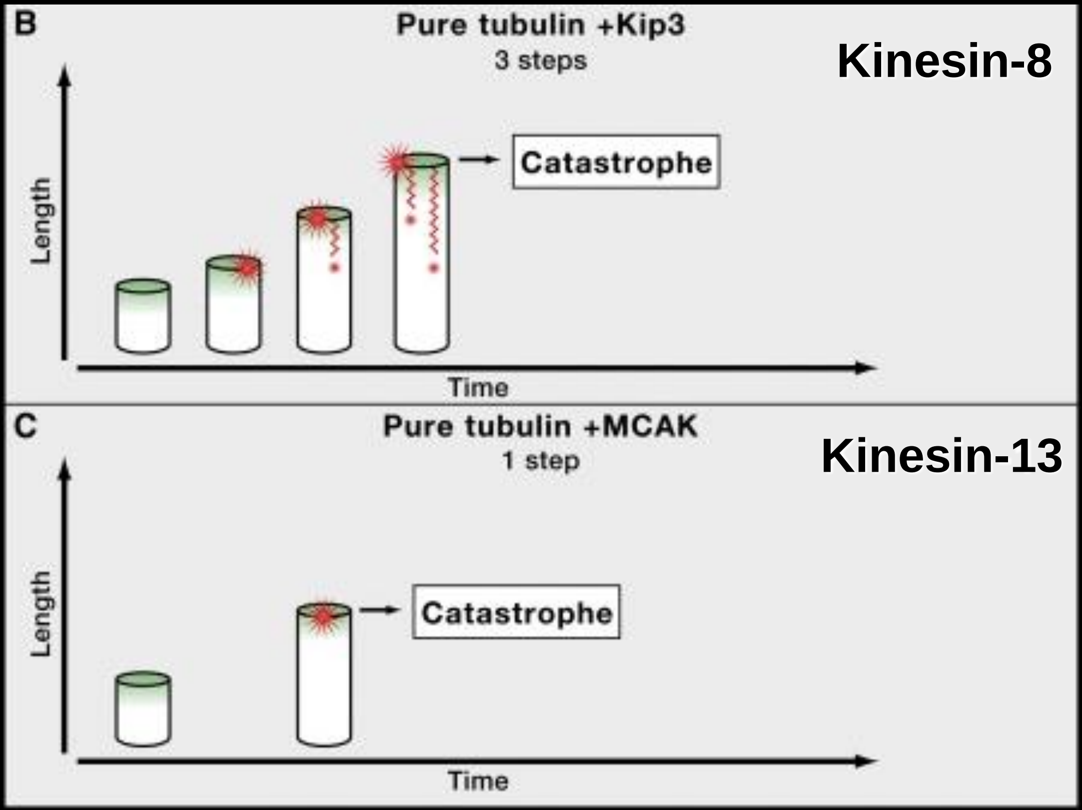






Varga et al, Cell 2009
Gardner et al, Cell 2011

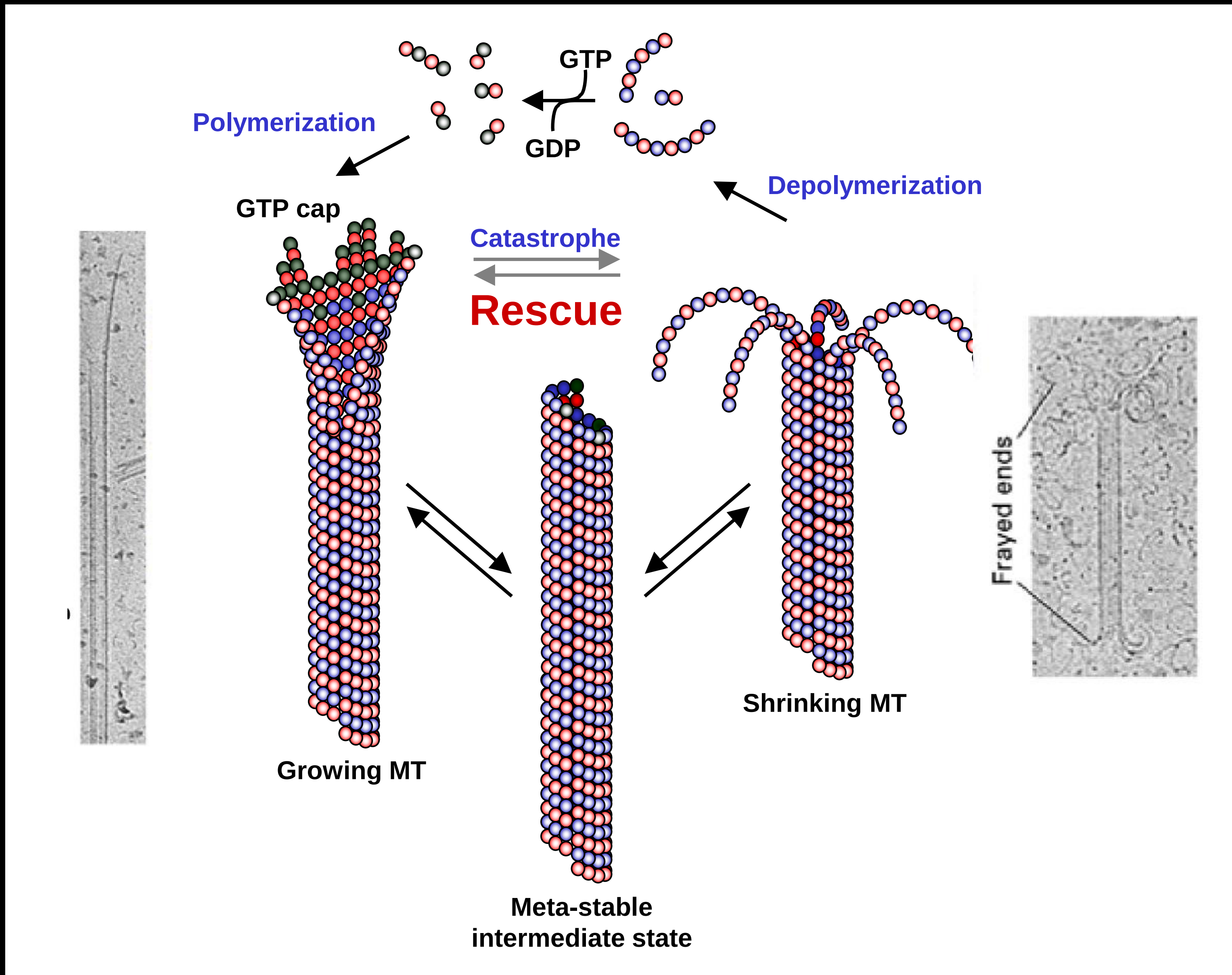
Kinesins affect “microtubule aging” in a different way



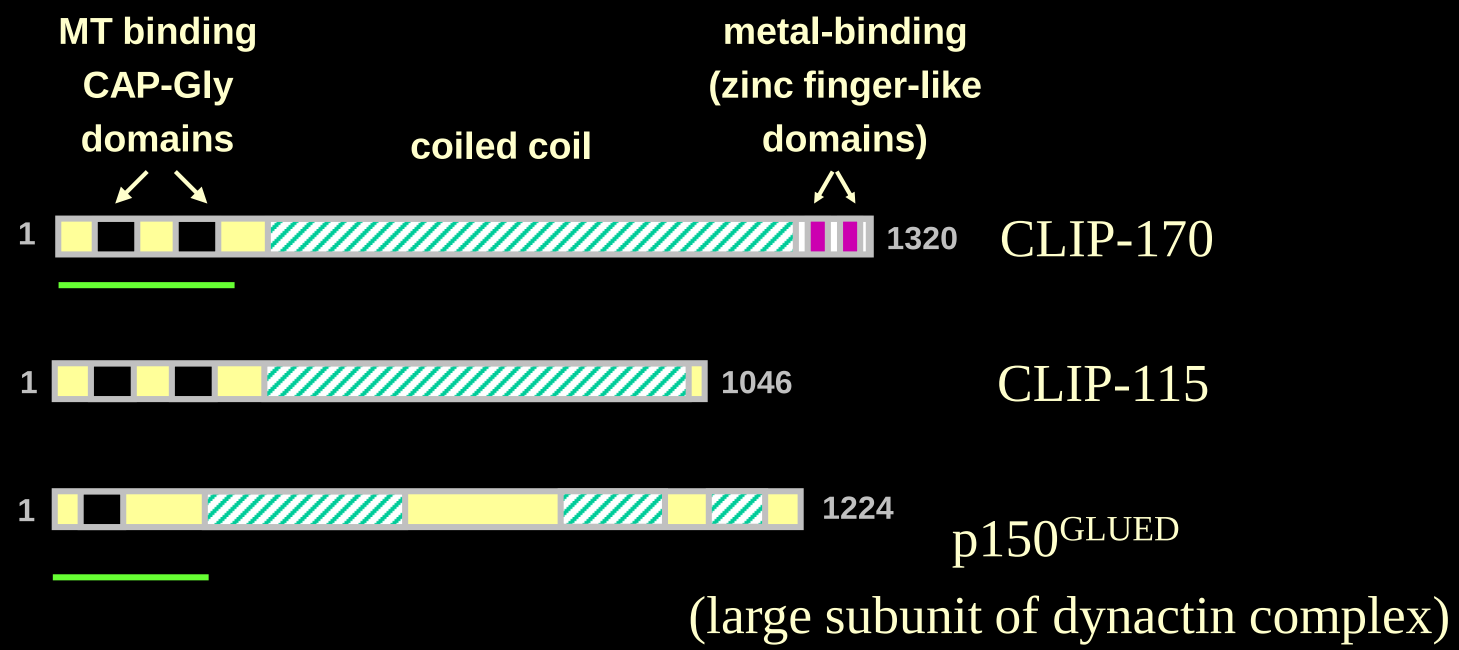
Gardner et al, Cell 2011

Rescue

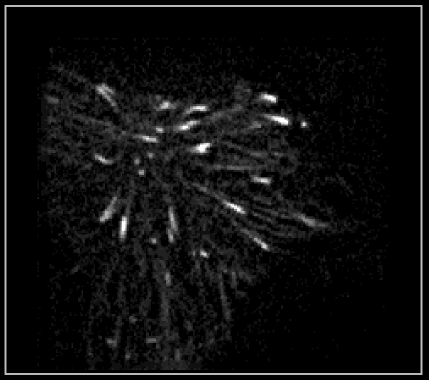
Rescue factors



CAP-Gly family of microtubule plus end tracking proteins



GFP-CLIP-170-N



Movie:Y.Komarova

Monomeric N-terminal head domain
of CLIP-170 or p150^{GLUED}
is sufficient for microtubule plus end tracking

(Perez et al., 1999, Diamantopoulos et al, 1999,
Vaughan et al., 2002)

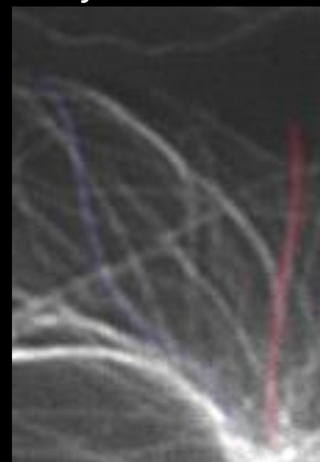
Microtubule rescue -CLIPs

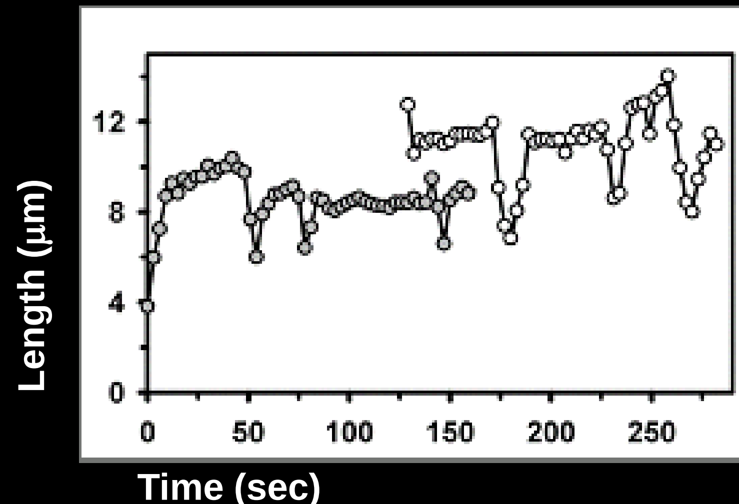
MT dynamics

Life history plots

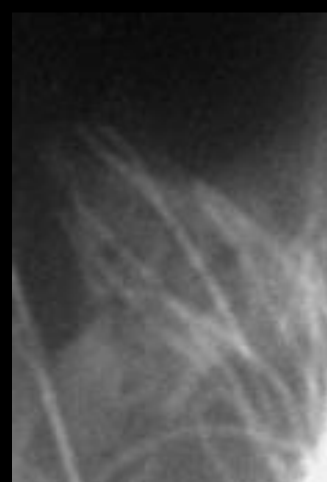
Cy3-tubulin

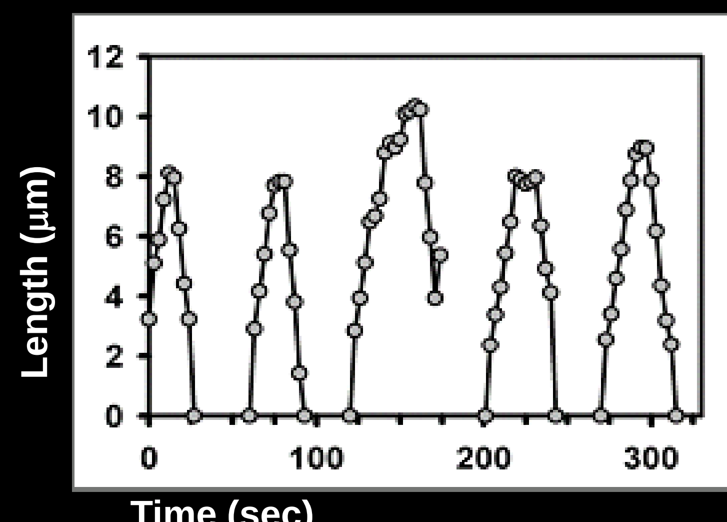
control





dominant negative CLIP-170

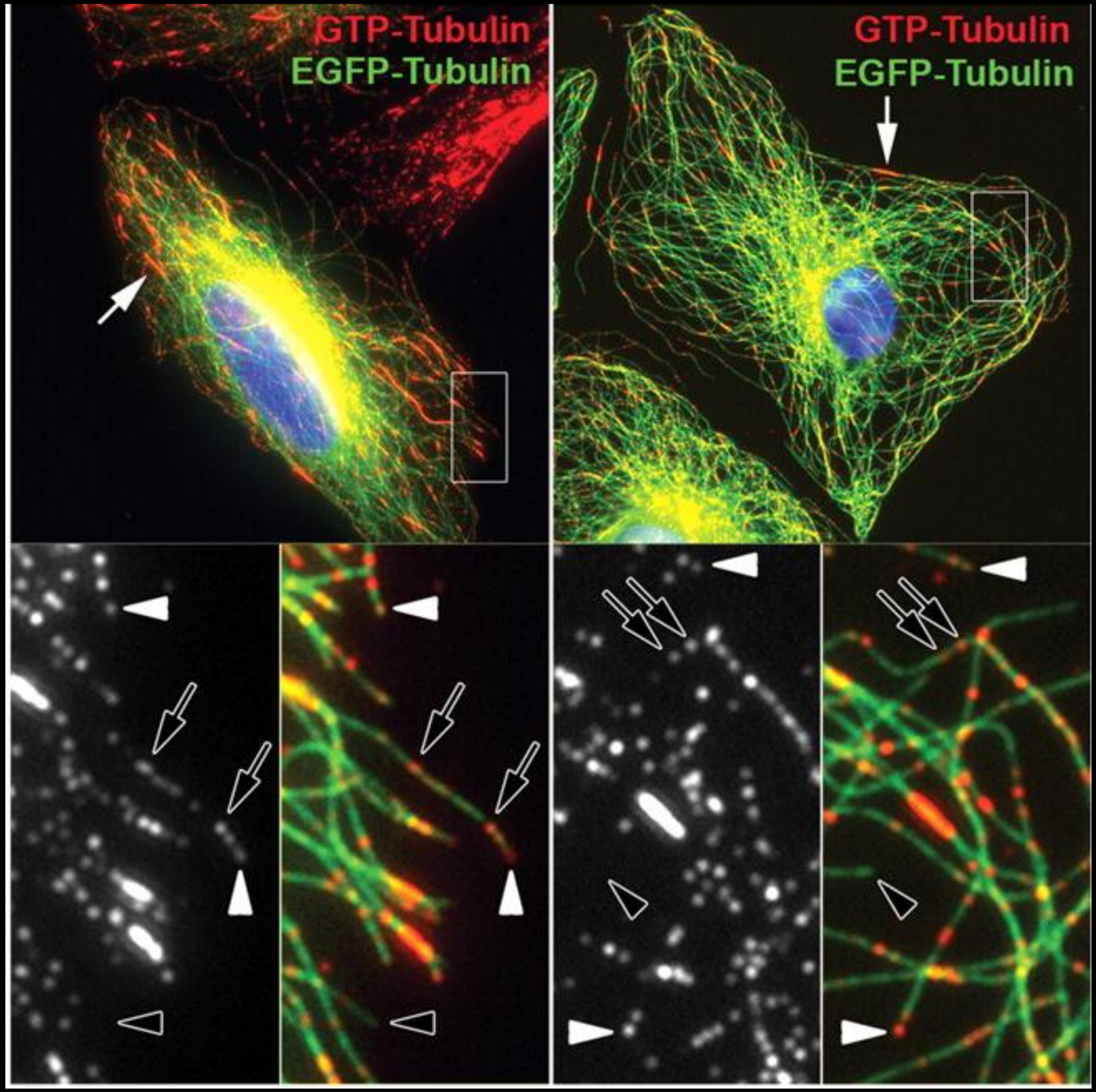




Komarova et al., JCB 2002

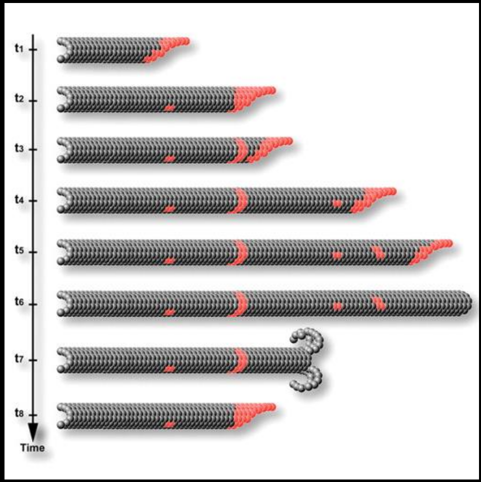
Rescue at GTP remnants

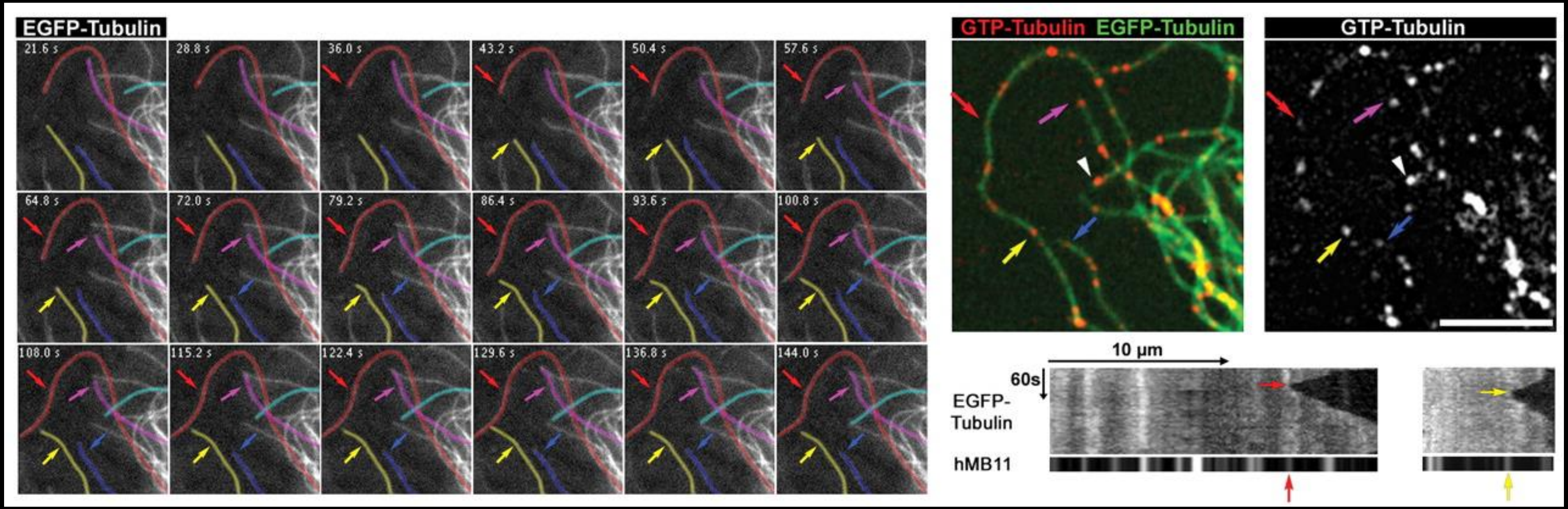
Recombinant antibody against GTP-tubulin



Dimitrov et al., Science 2008

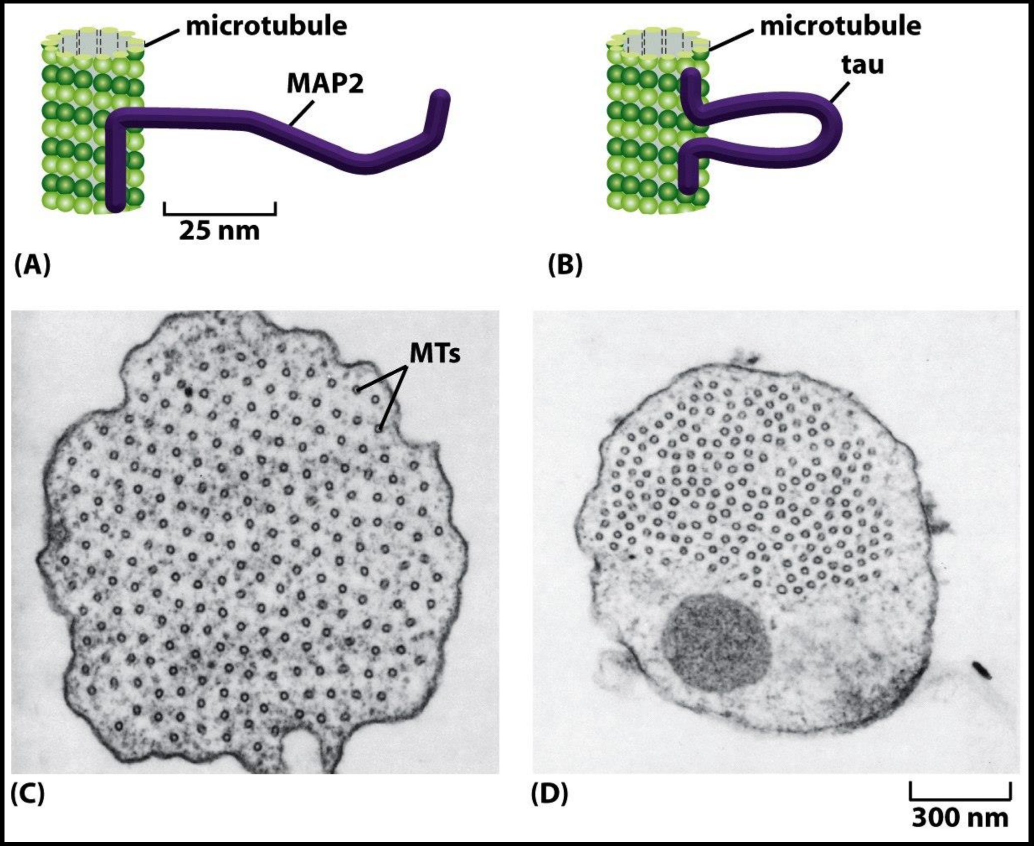
Rescue at GTP remnants





Dimitrov et al., Science 2008

MT stabilization- classical MAPs



Conclusion

Multiple factors control different aspects of microtubule dynamics

We still do not understand the mechanistic basis of transitions between microtubule growth and shortening