Lecture 3

Microtubule Structure, Organizers, and Motors

Outline:

Microtubule Structure

Microtubule Organizers

Microtubule Motors

Paper:

Microtubules

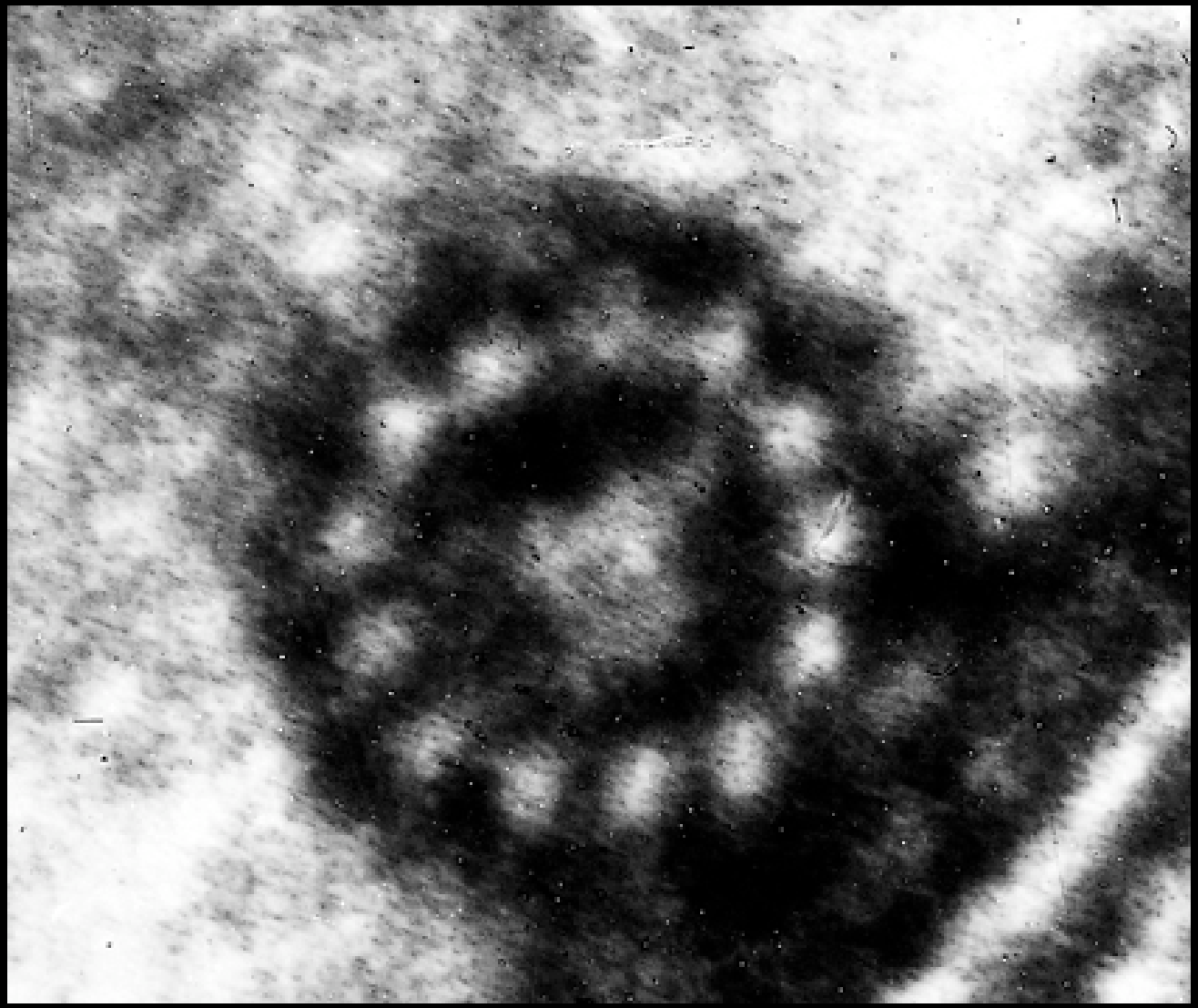
Roles:
cell organization, vesicle traffic, chromosome segregation

Subunit: tubulin
highly conserved heterodimer of α/β subunits, 50 kD each

both subunits bind GTP ⇒
α non-exchangeable; β exchangeable

10-15 linear protofilaments form microtubule

cryo-EM of pure microtubules



MT in cells:

13 protofilaments
(10-15)
25 nm diameter

Tubulin organization in a microtubule

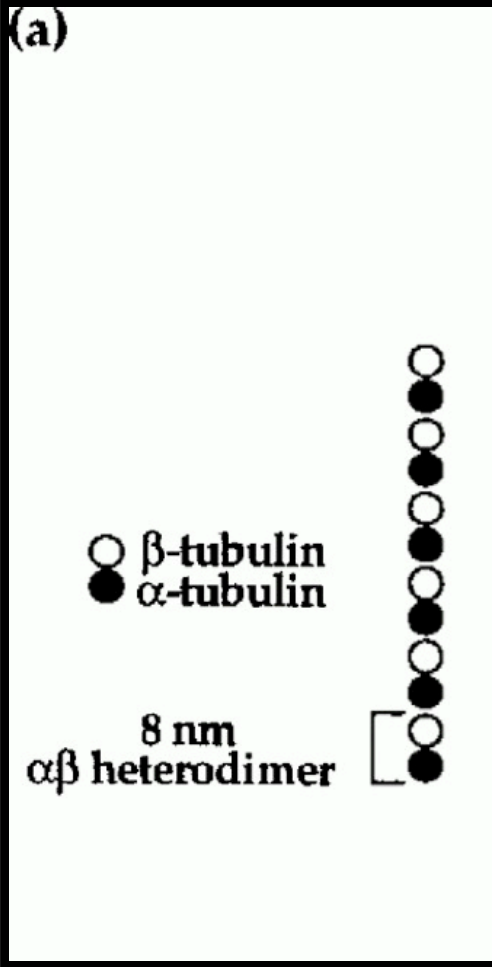
dimers arranged head-to-tail in protofilaments
-polarized structure

protofilaments associate laterally to form microtubules

(+) end = faster growing end

(-) end often embedded in microtubule-organizing center

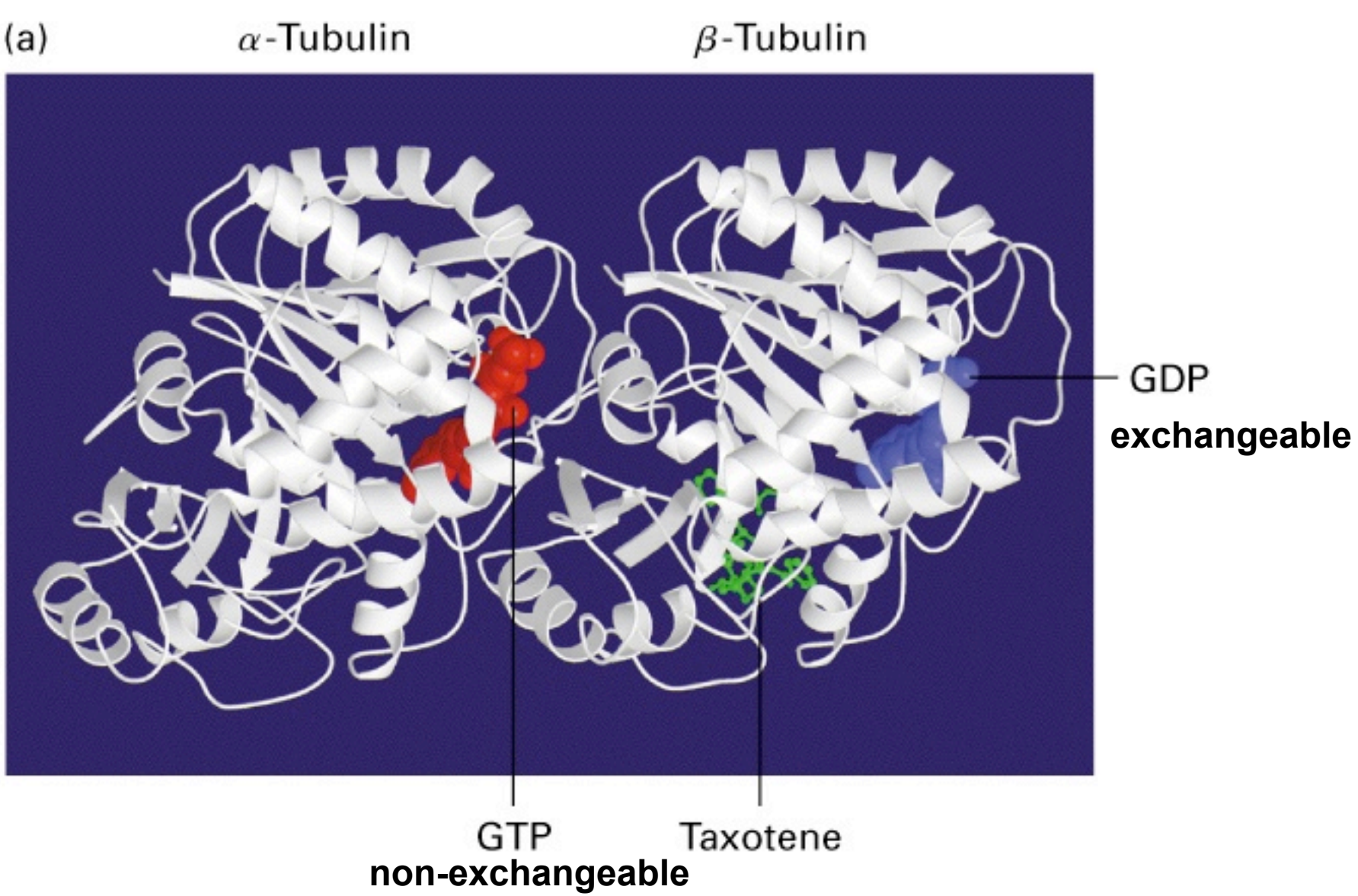
Possible lattice structures



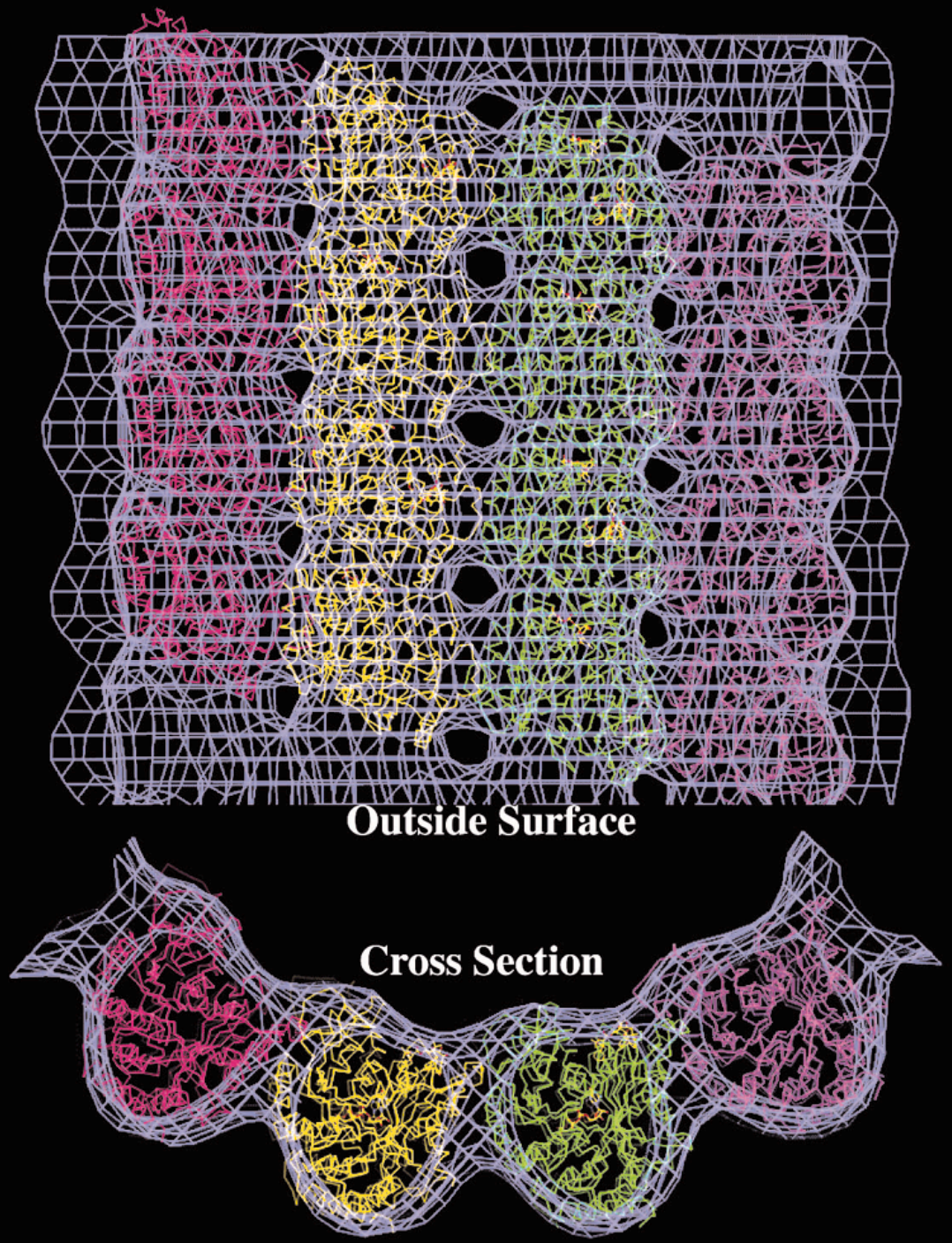
How to distinguish?

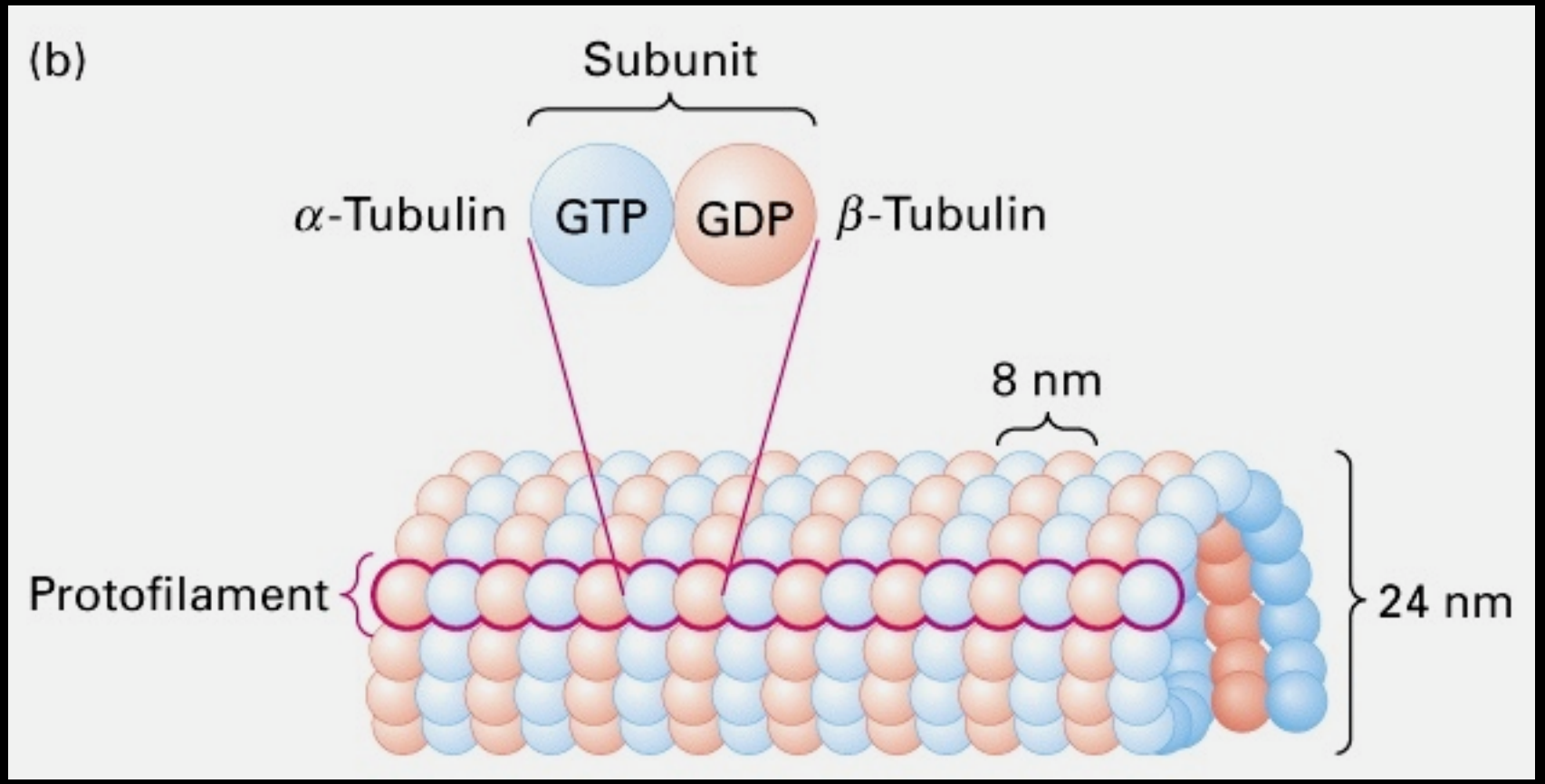
decoration by motor protein that binds β-tubulin

Structure solved by electron crystallography - Eva Nogales

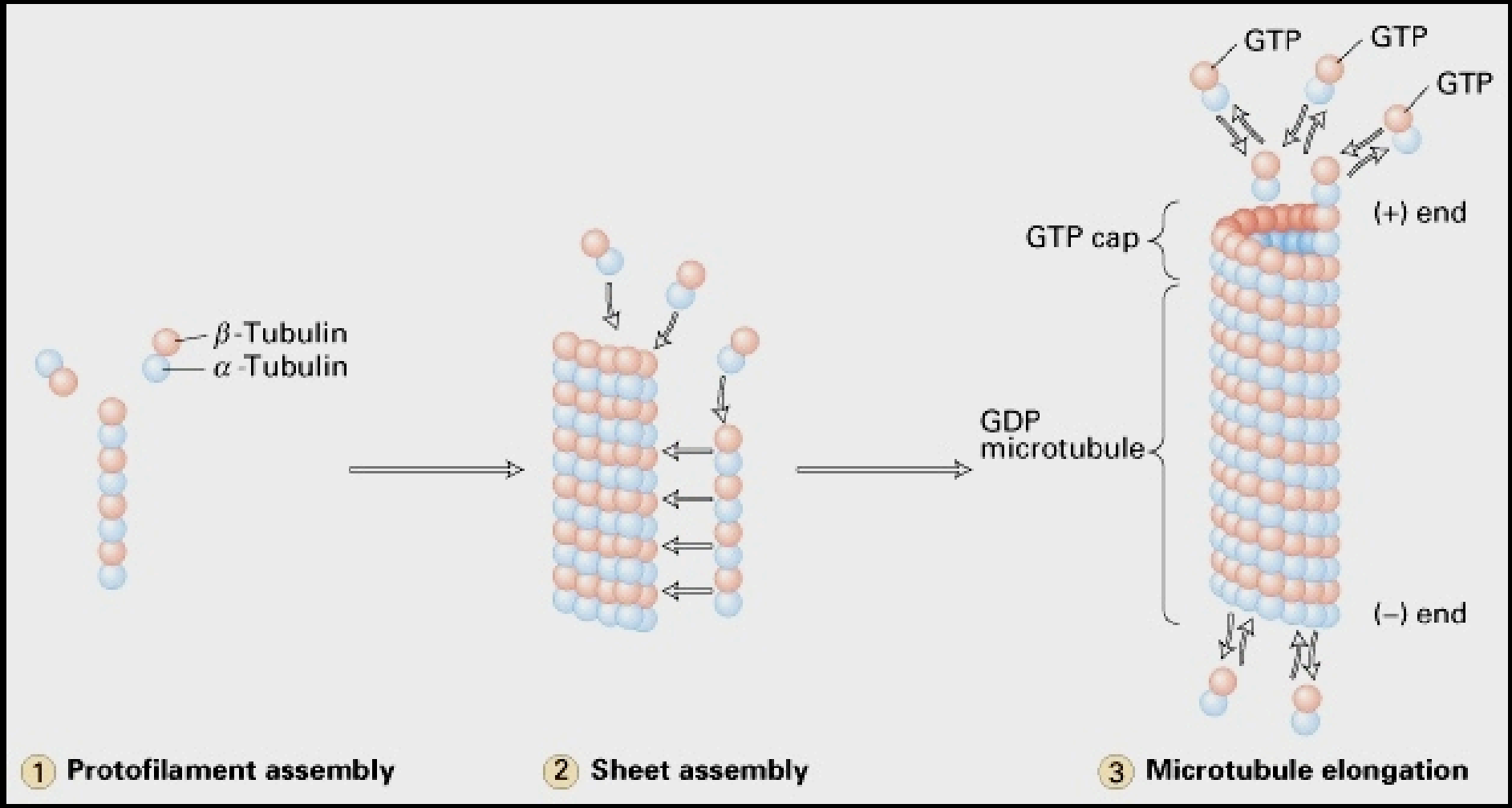


docking
of crystal
structure
onto
microtubule

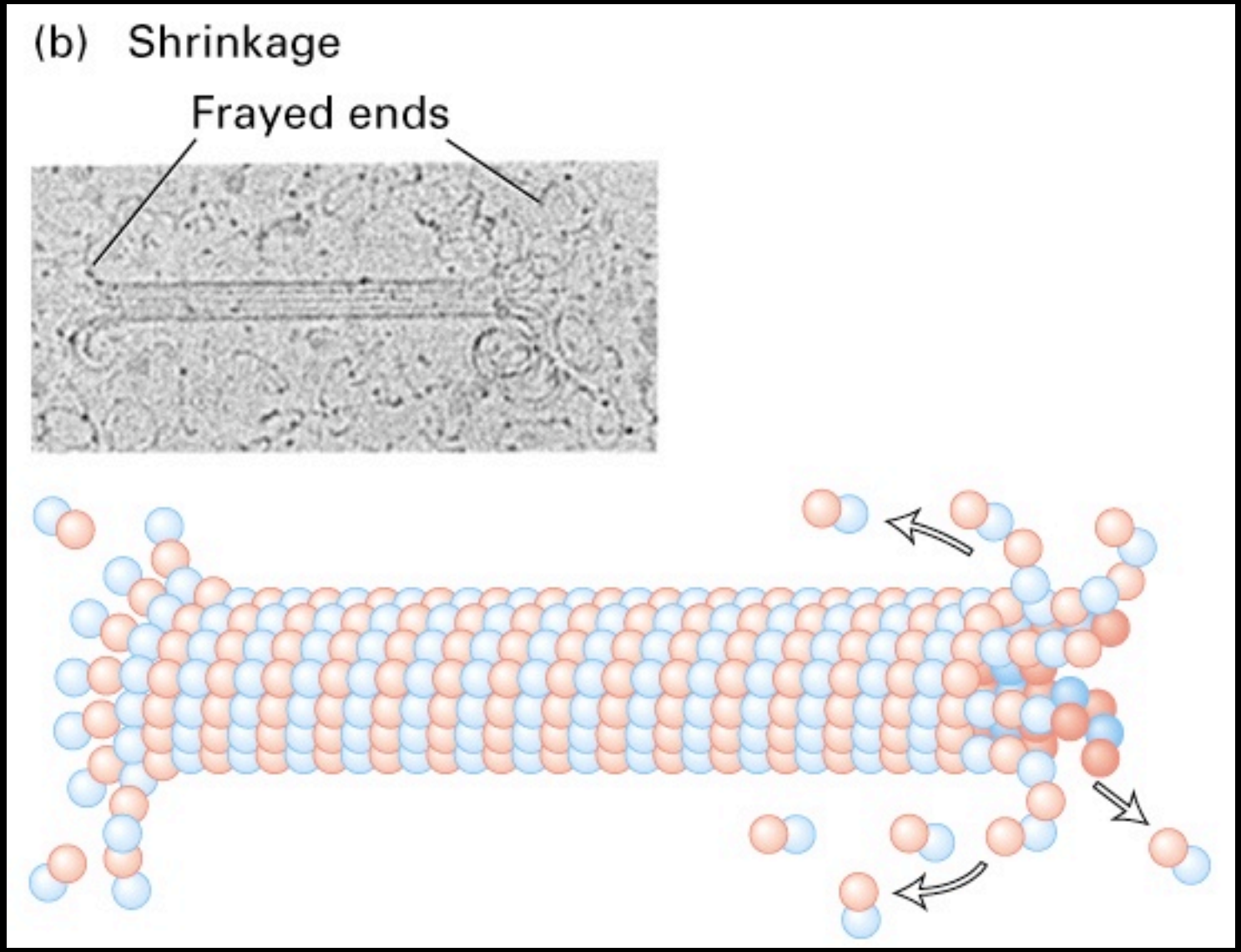




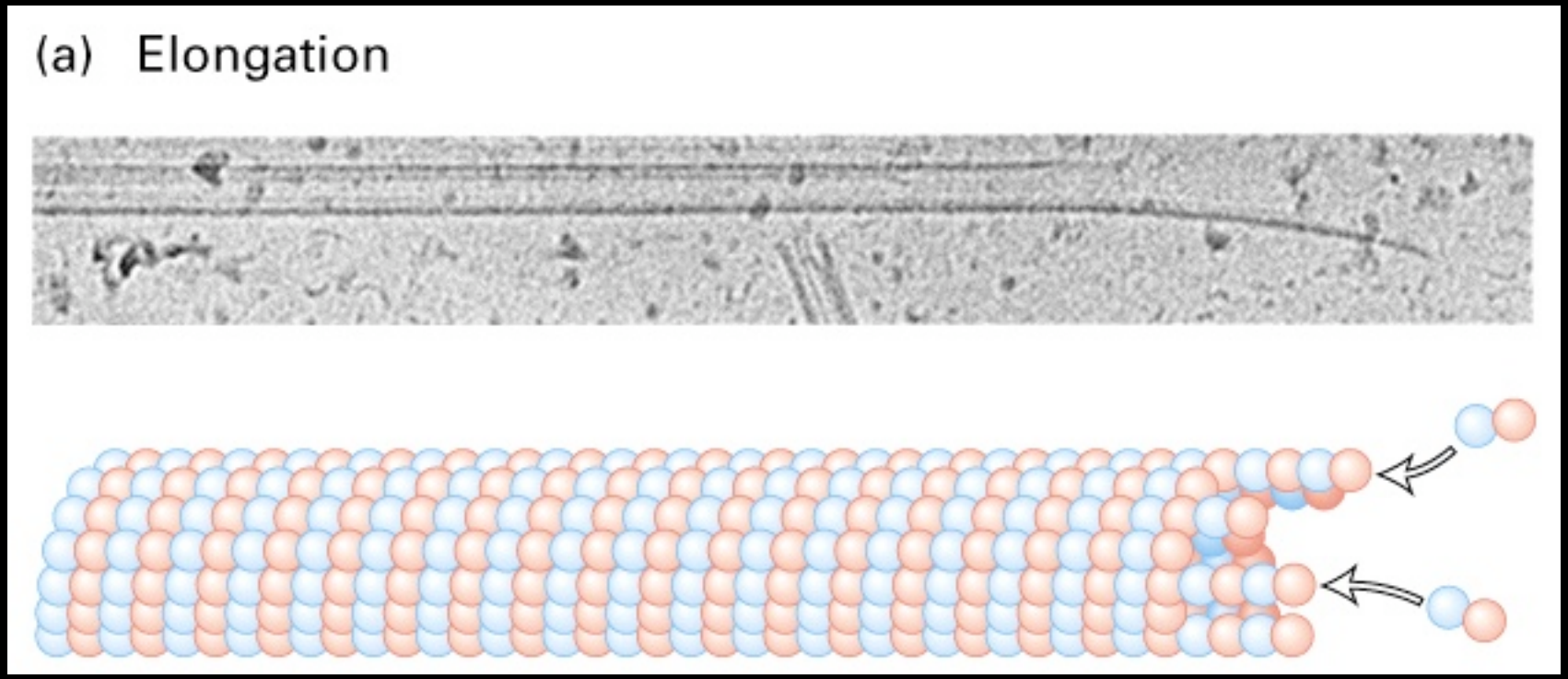
what generates dynamic behavior?



β-tubulin GTP hydrolyzed soon after polymerization



curved protofilaments ⇒ GDP-tubulin exposed



straight protofilaments ⇒ GTP-tubulin in cap

Parameters of MT dynamics: (+) end

	in vitro	*in vivo*
critical concentration	15 μM tubulin	~15 μM tubulin
growth rate	1.5 μm/min	8-15 μm/min
shrinkage rate	30 μm/min	15-30 μm/min
catastrophe frequency	0.06/min	0.5-8/min
rescue frequency	3/min	0.5-2.5/min
turnover rate	$t_{1/2}$ = 30 min	$t_{1/2}$ = 0.5-20 min

Conclusions

Microtubules in vivo:

- much higher growth rate
- many more catastrophes

Dynamic parameters of MTs *in vivo* are quite variable

Microtubule Nucleation in vivo

15 μM not high enough for “spontaneous assembly”

⇒ always “seeded”

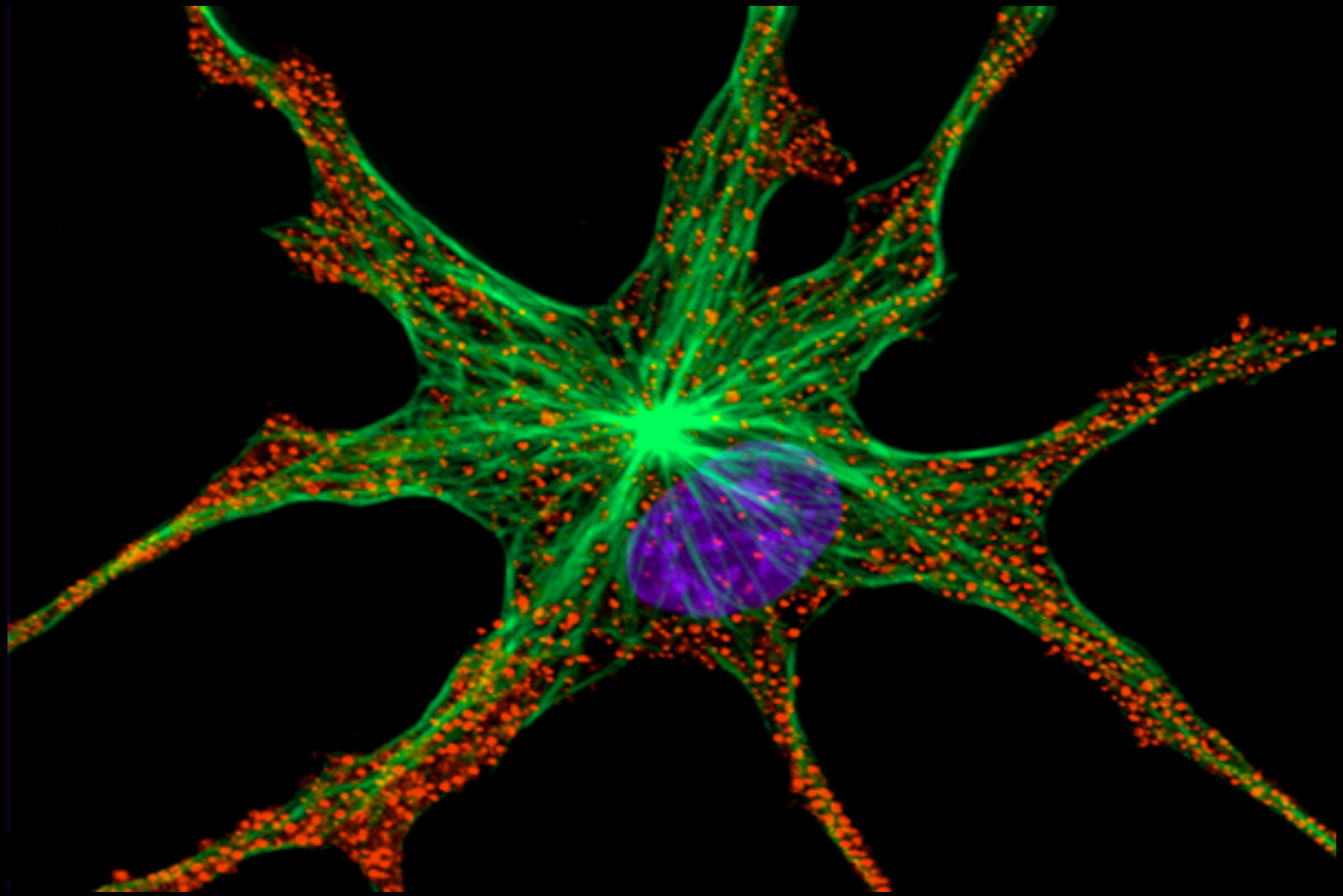
general term: microtubule organizing center “MTOC”

most cells: centrosome

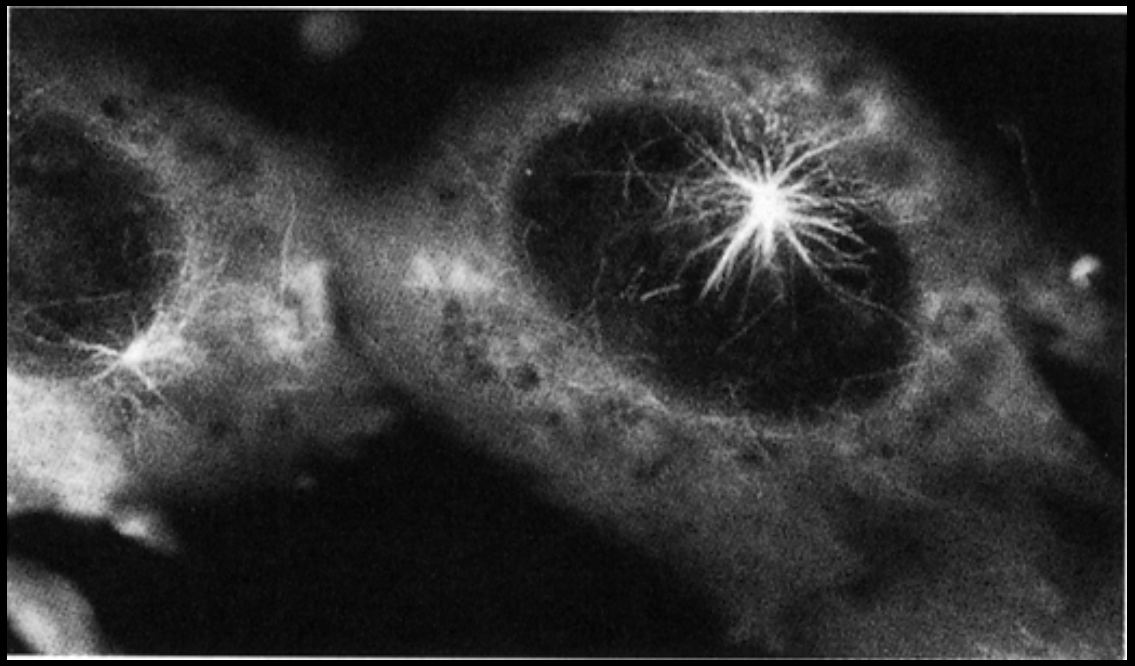
defined orientation:

microtubules (-) ends tethered, (+) ends extend outward

MTs in interphase cell by immunofluorescence



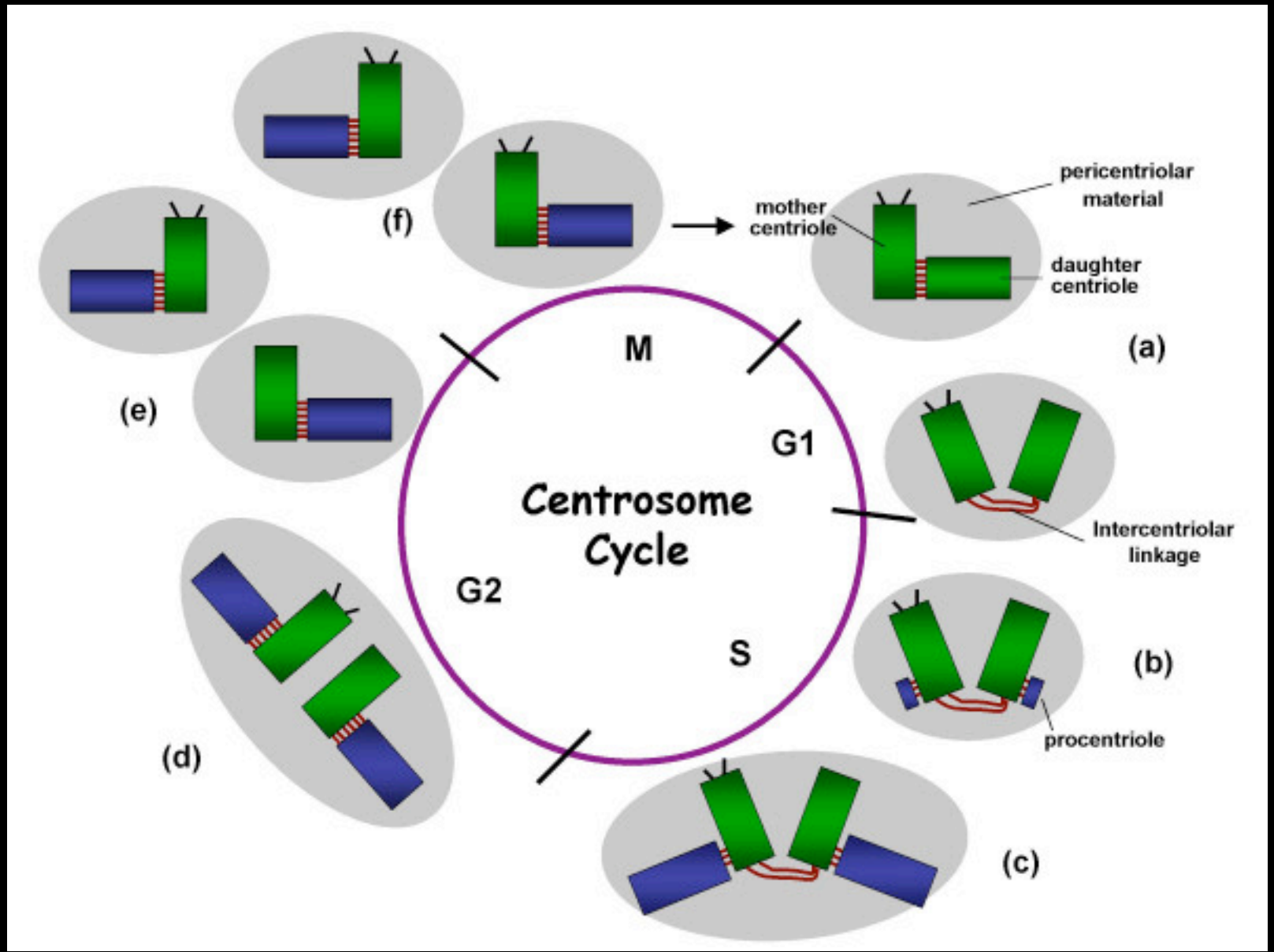
Depolymerize MTs, then allow to regrow



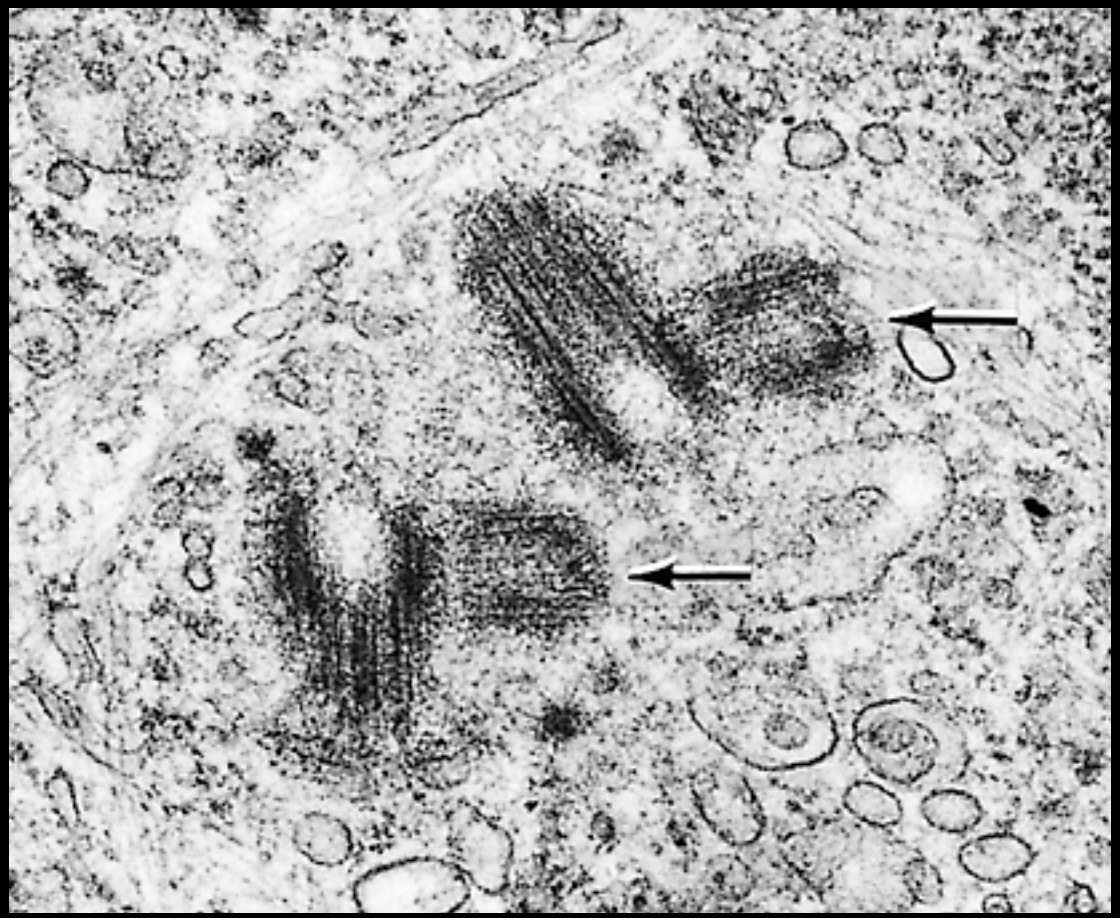
centrosome consists of:

centriole pair: similar to basal body of flagellum

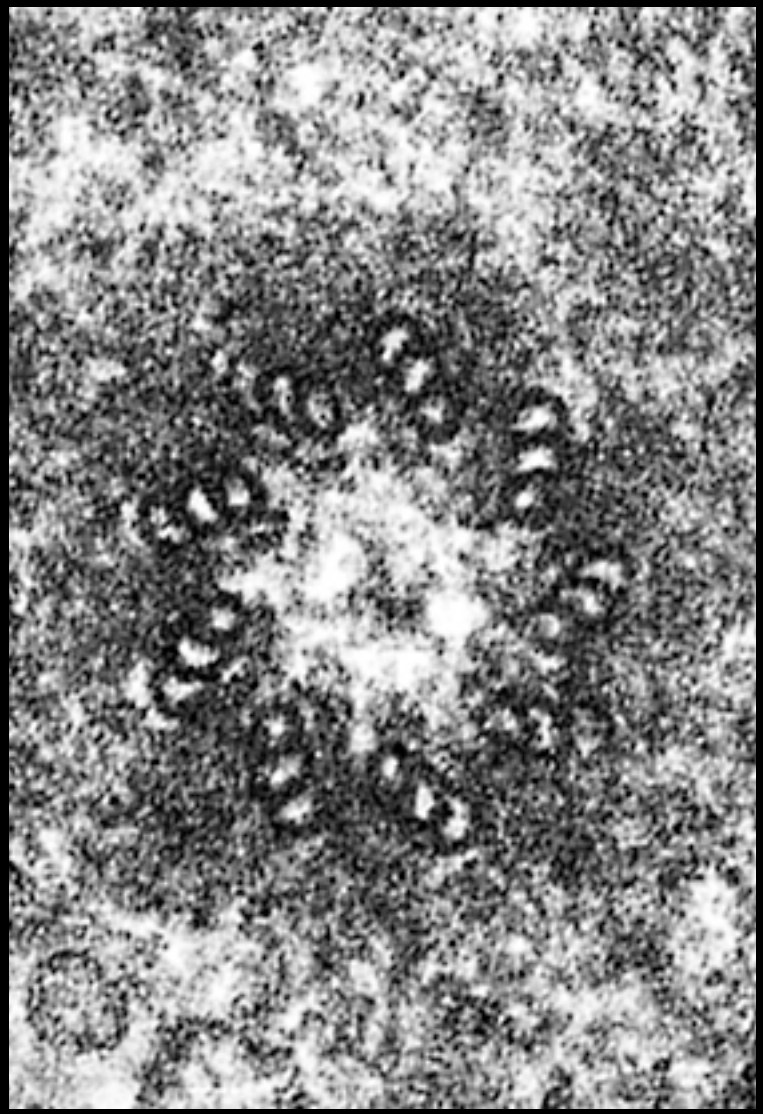
surrounded by pericentriolar material = “PCM”


Centrosome Cycle
M
G1
S
G2
(a)
(b)
(c)
(d)
(e)
(f)
mother centriole
pericentriolar material
daughter centriole
Intercentriolar linkage
procentriole

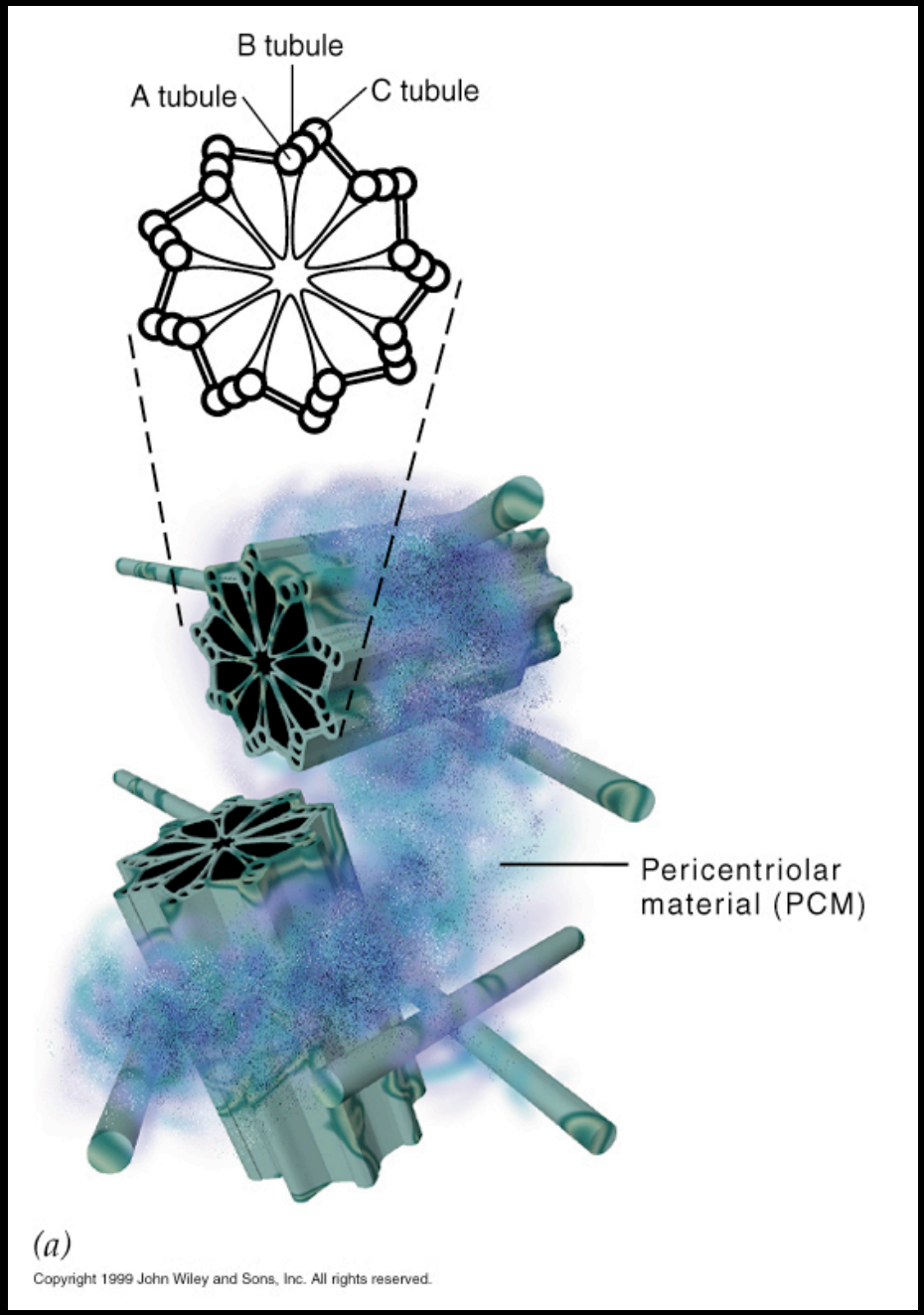
duplicating centrosome



cross-section

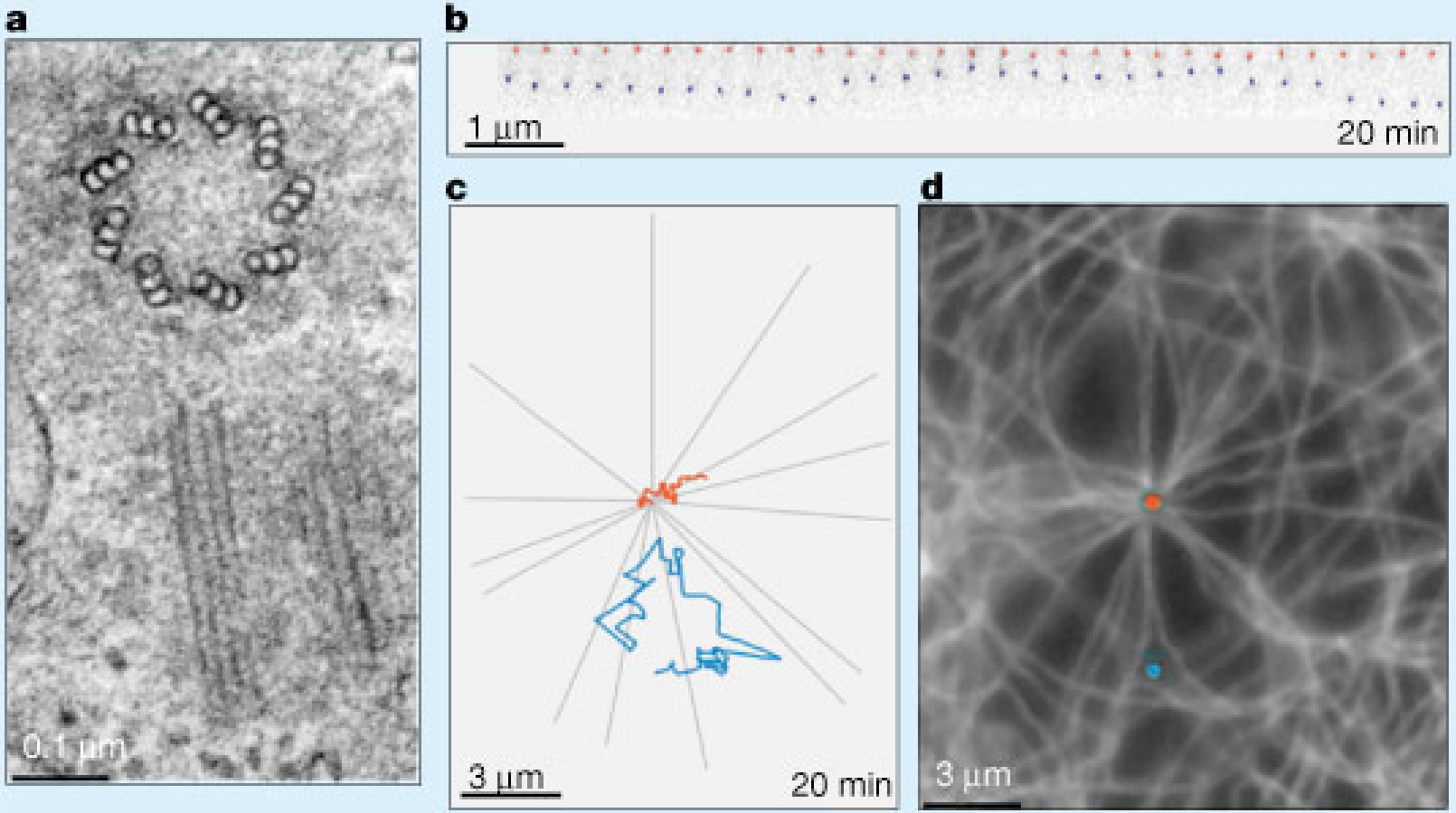


9 triplet microtubules



Centrioles go for a stroll

GFP-tag centriolar protein: daughter centriole mobile!



Piel et al., JCB 149, 317 (2000)

Centrosomal Proteins

70 by proteomic analysis, roles?

- microtubule nucleation
- centrosome duplication
- cell cycle regulation

γ-tubulin: nucleating factor

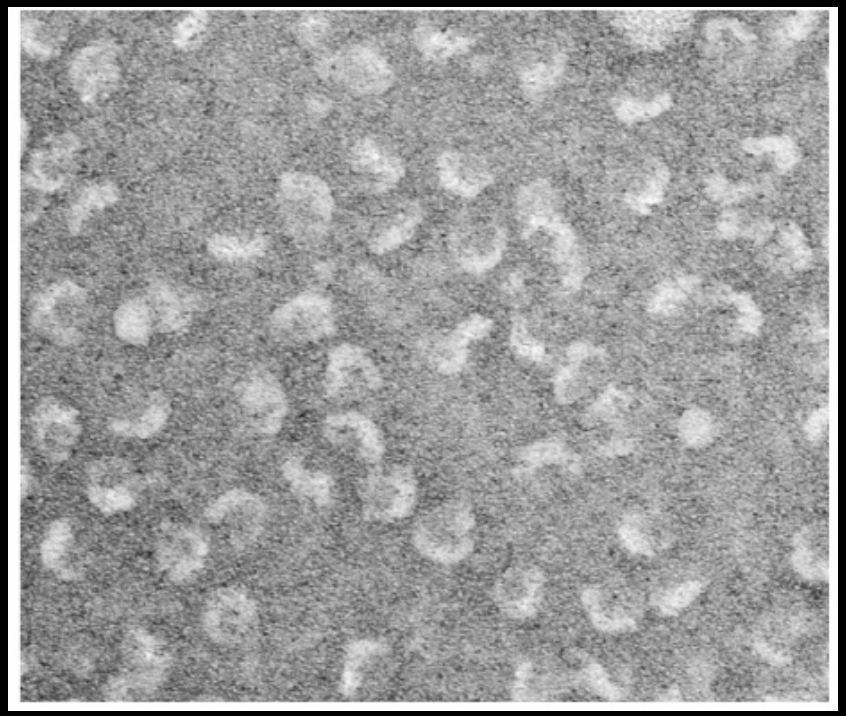
- highly conserved tubulin isoform: 30% similarity
- also found soluble in cytoplasm
- required for microtubule nucleation

purified from Xenopus egg extracts

- complex with ~7 other subunits
- forms rings!

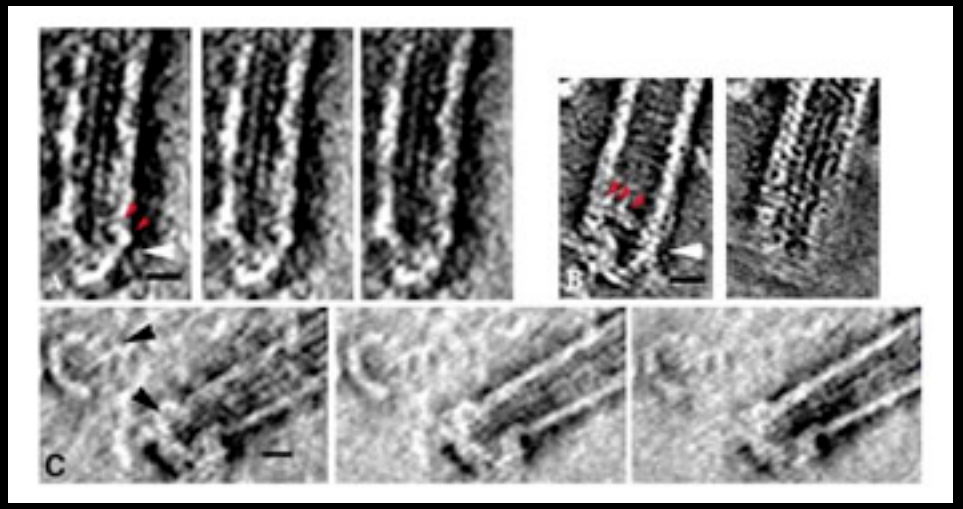
called γ-tubulin ring complex (γ-TuRC)

purified γ-TuRCs



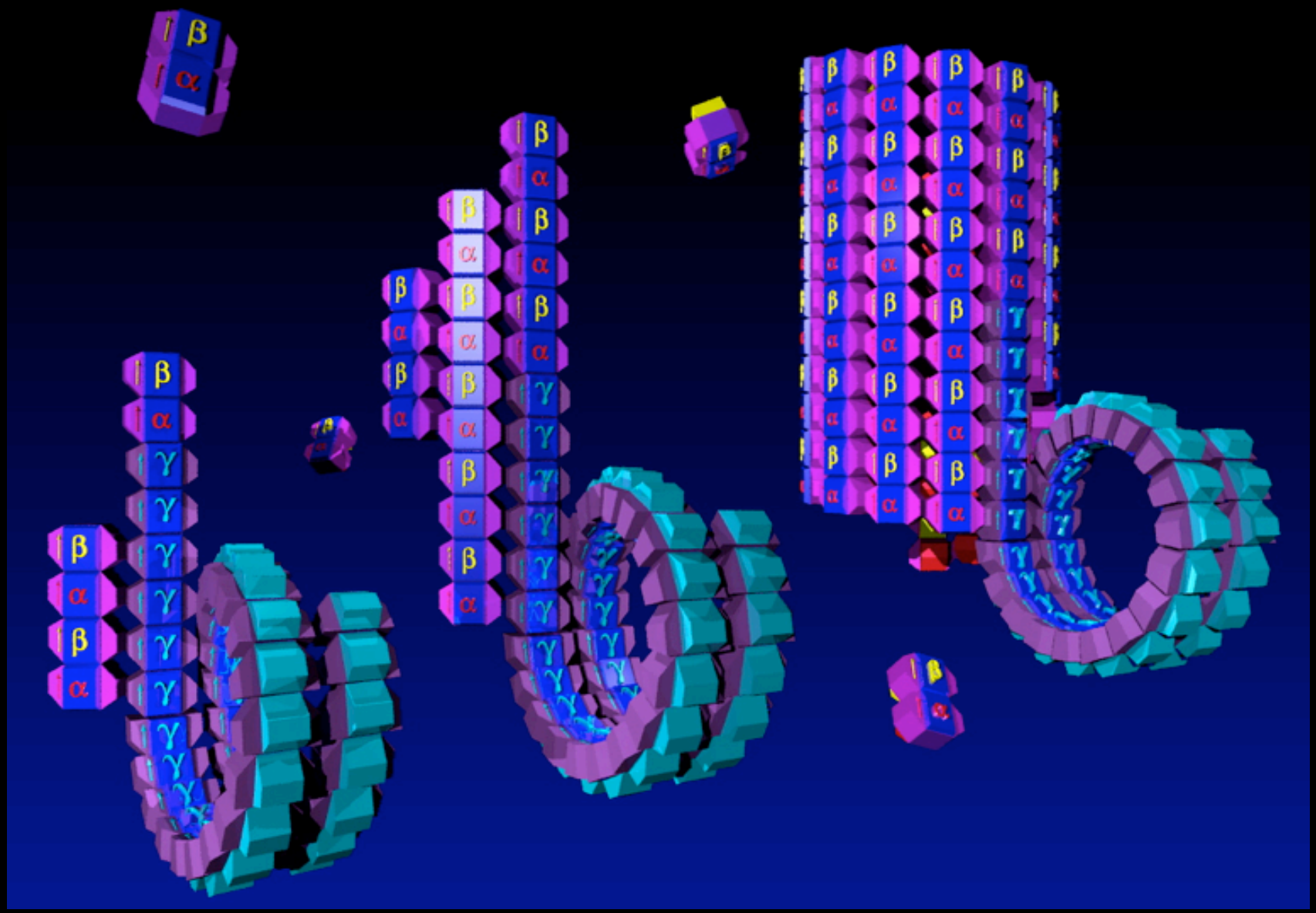
MTs nucleated by γ-TuRCs

in vitro



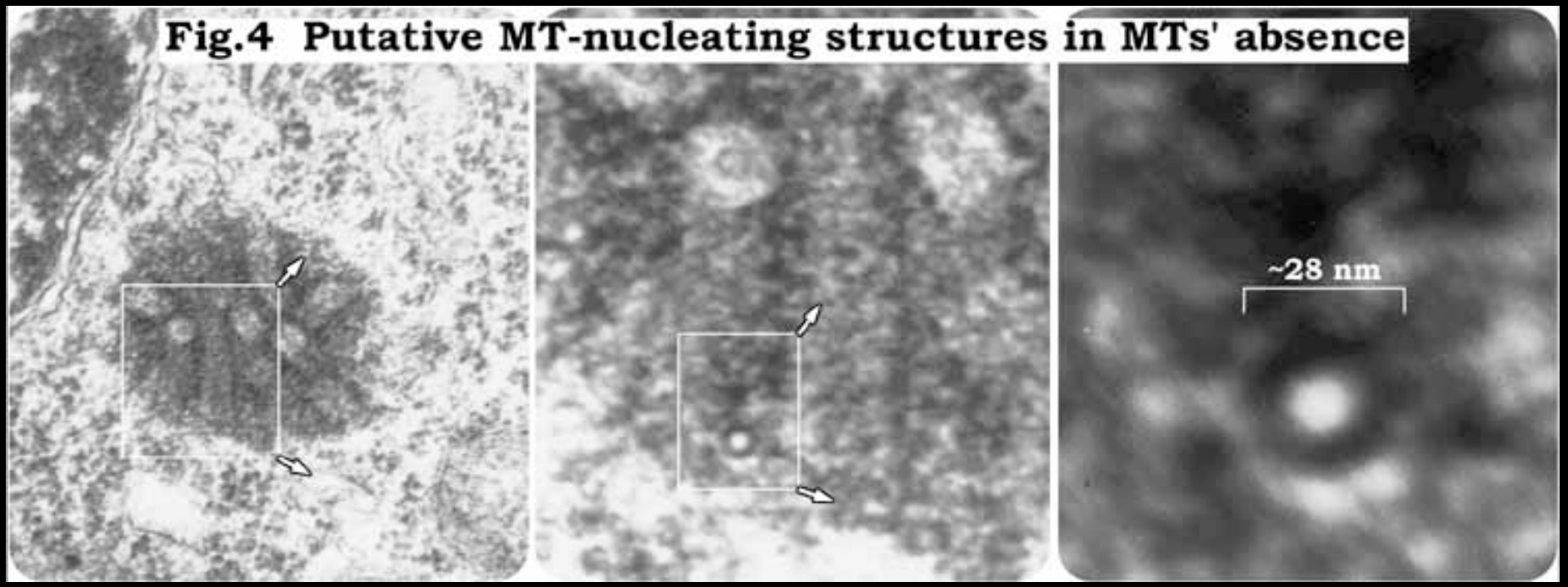
12-14 γ-tubulin molecules/ring

Model for nucleation by γ-TuRCs



Not all nucleation centers have centrioles

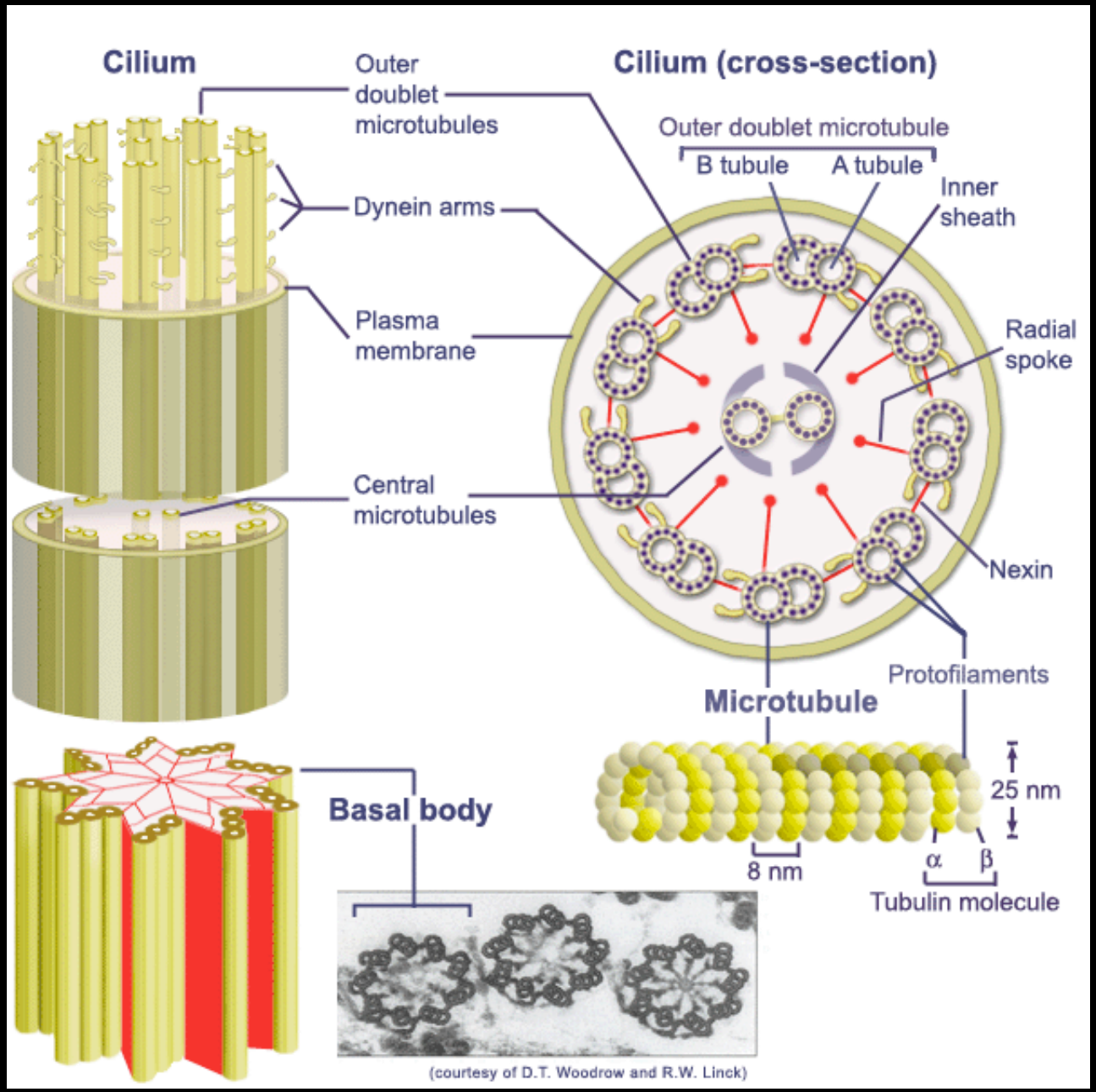
plant MTOC



Not all nucleation centers have centrioles

RAN GAP and chromosomes
many meiotic cells acentriolar-MT bundling by motors

will discuss when talking about chromosomes next week


Cilium
Outer doublet microtubules
Dynein arms
Plasma membrane
Central microtubules
Basal body
Cilium (cross-section)
Outer doublet microtubule
B tubule
A tubule
Inner sheath
Radial spoke
Nexin
Protofilaments
Microtubule
25 nm
8 nm
α
β
Tubulin molecule
(courtesy of D.T. Woodrow and R.W. Linck)

Microtubule Stability Regulators *in vivo*

Stabilizing:

Microtubule-associated proteins (MAPs)

- lattice-binding ⇒ stabilizing
- end-binding ⇒ attachment to targets

Destabilizing:

Catastrophe-promoting factors:

- Stathmin/Op18
- Kinl kinesins

Severing factors:

- Katanin

Lattice-binding (textbook) MAPs

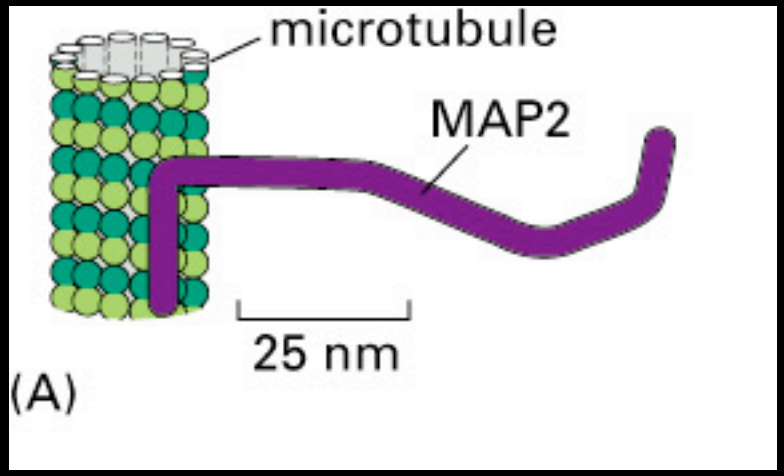
TABLE 19-1 Major Microtubule-Associated Proteins

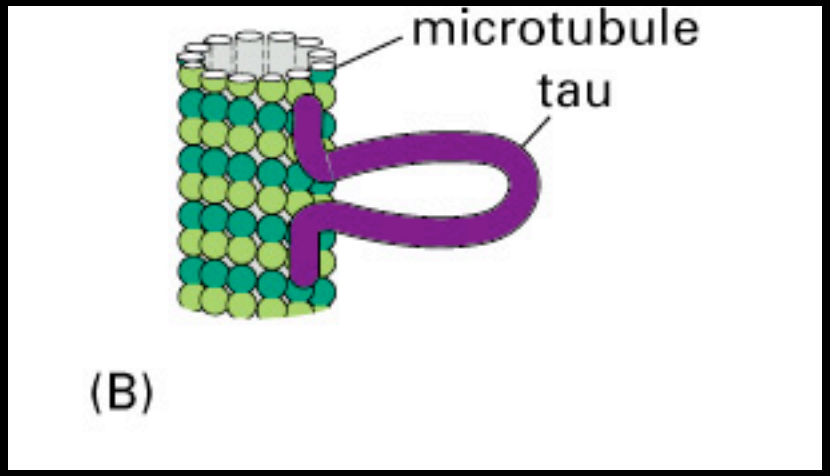
Protein	MW	Domain Organization*	Location
TYPE I			
MAP1A	300,000 heavy chain		Dendrites and axons
MAP1B	255,000		Dendrites and axons
TYPE II			
MAP2a	280,000		Dendrites
MAP2b	200,000		Dendrites
MAP2c	42,000		Embryonic dendrites
MAP4	210,000		Non-neuronal cells
Tau	55,000–62,000		Dendrites and axons

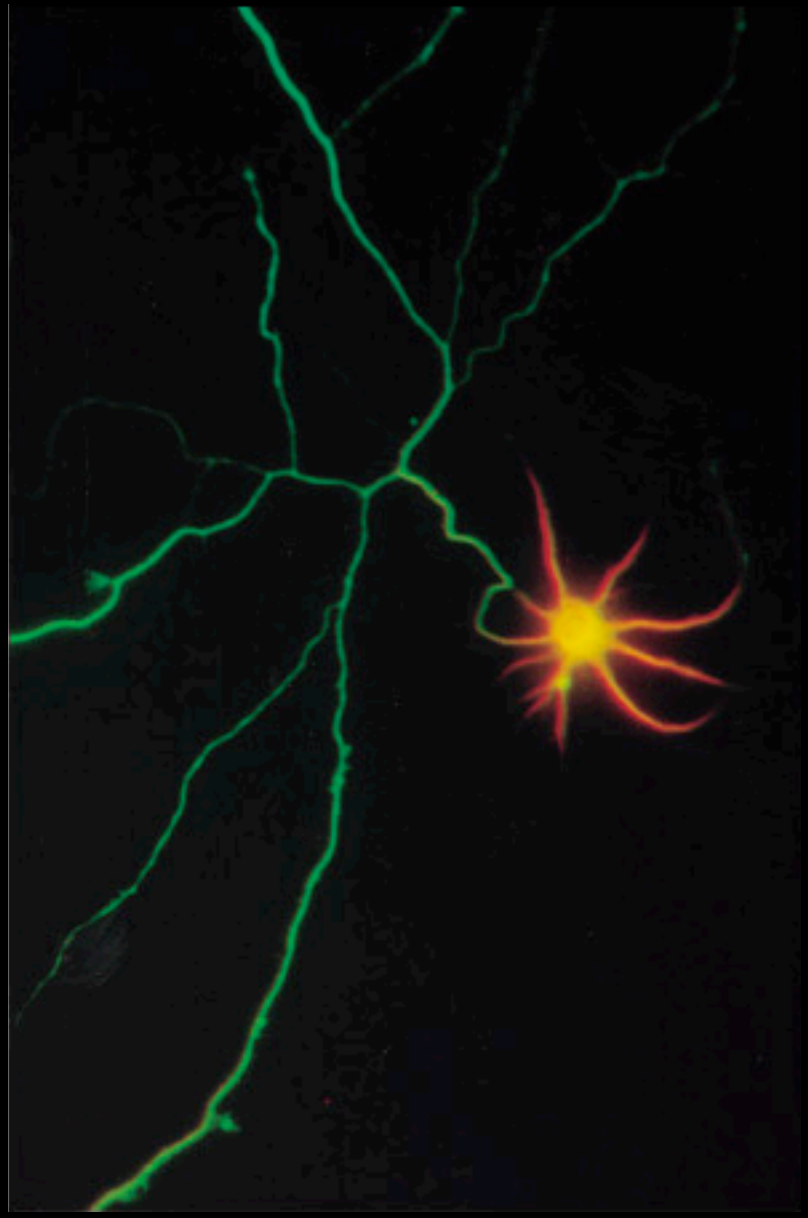
**Yellow, microtubule-binding domain; pink, projection domain; green, 18 amino acid repeats.*

co-purify with microtubules

MAP binding regulated by phosphorylation

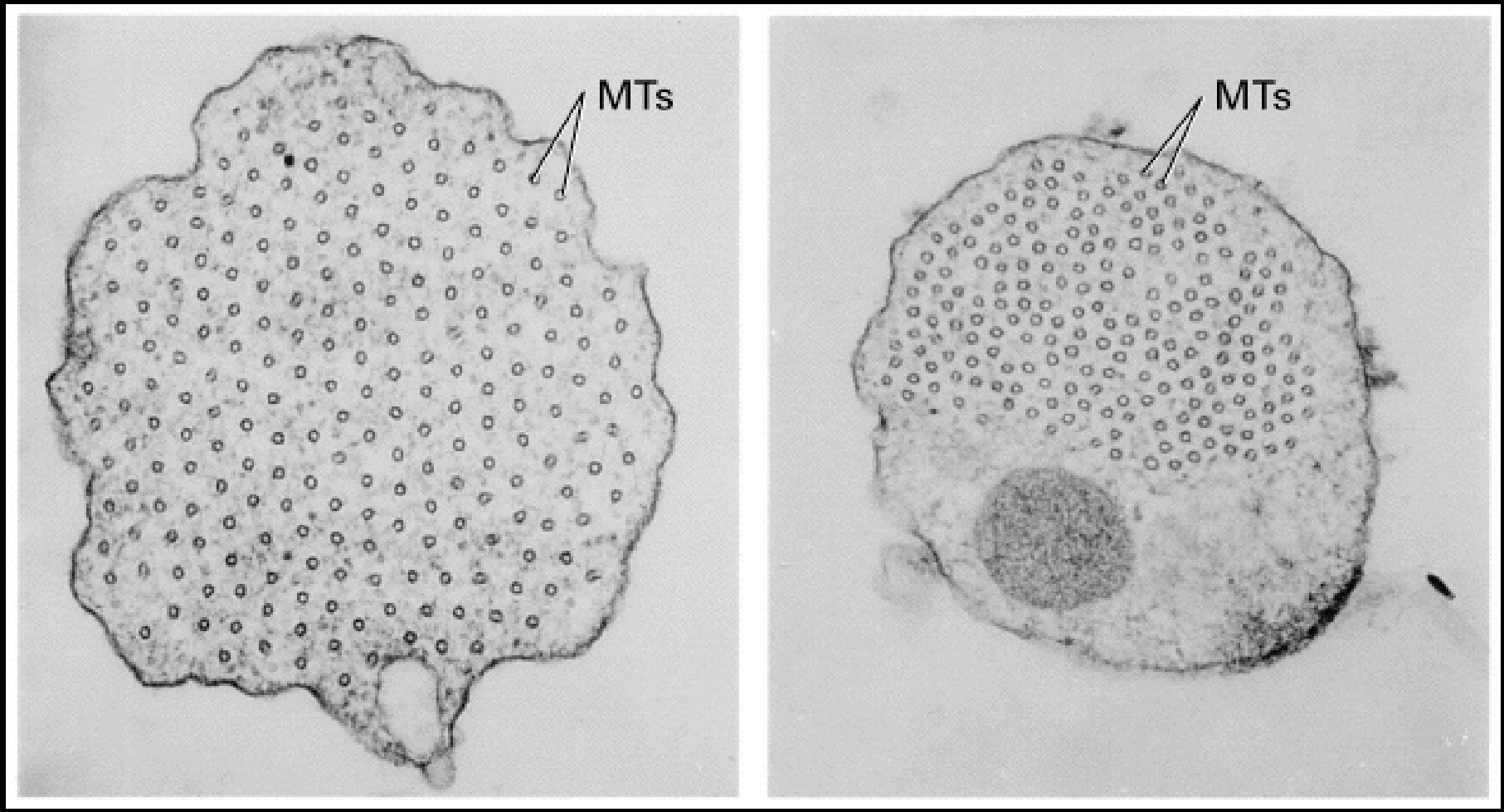






Green - phospho-tau
Red - MAP4

Express brain MAPs in non-neuronal cells - processes form



MAP2 tau

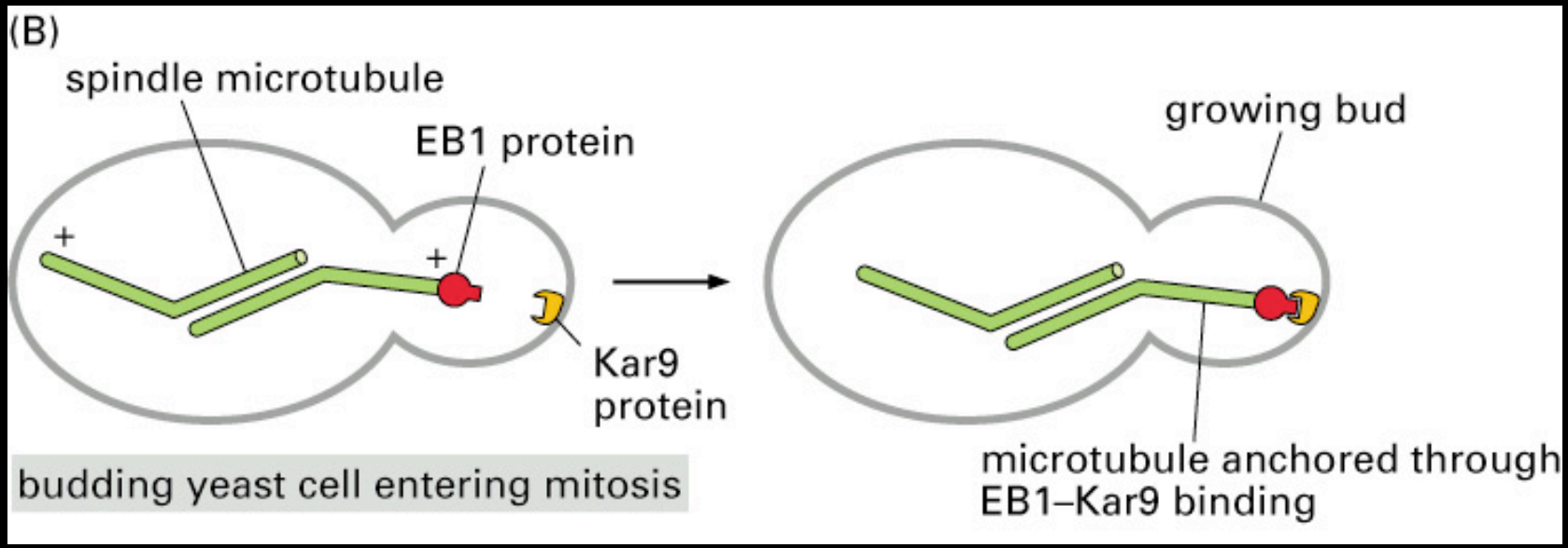
MT spacing determined by size of projection domain

End-binding MAPs

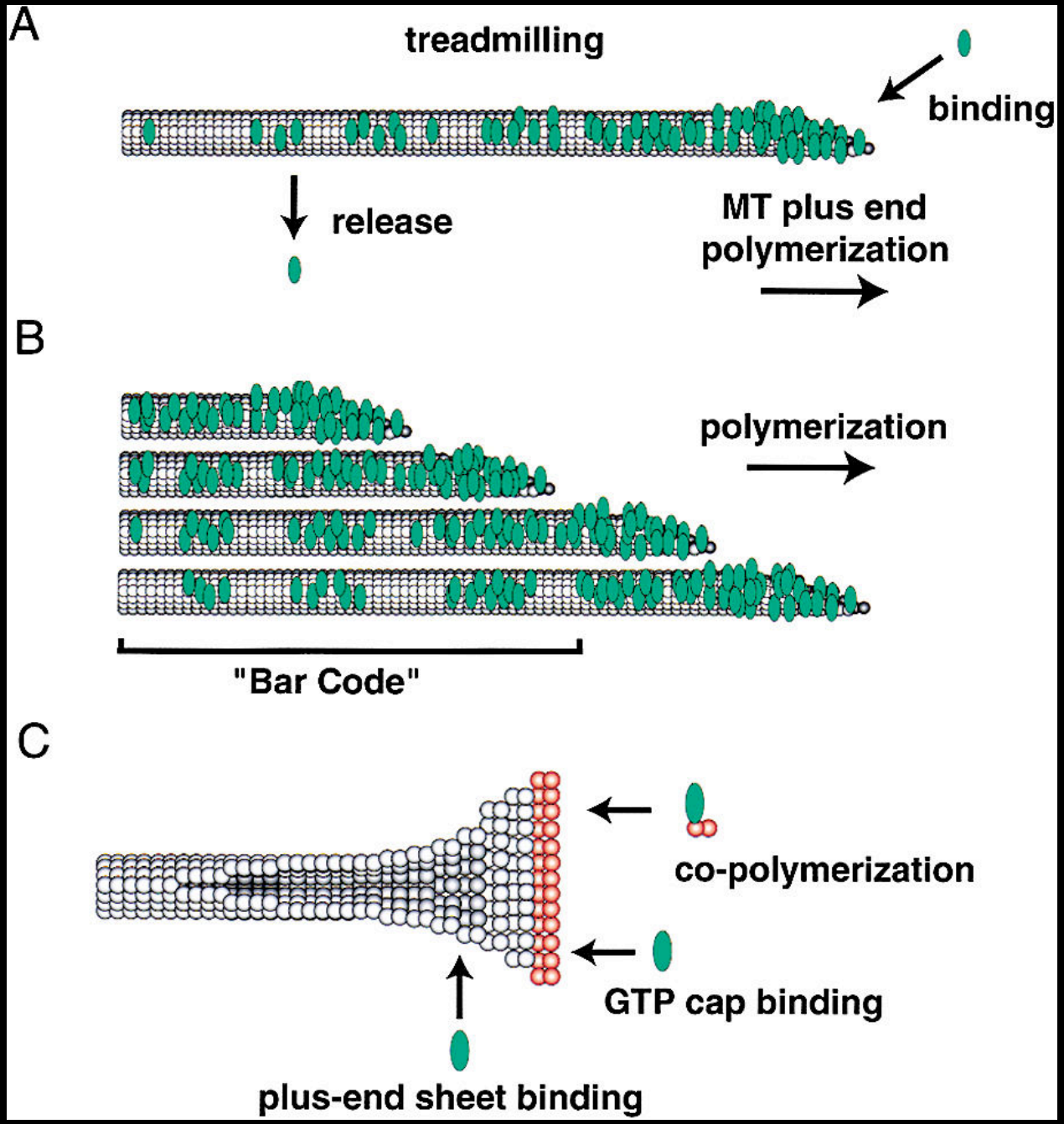
CLIP-170
EB-1

Associate with growing (+) ends of microtubules

Associate with other proteins on cellular targets:
cell cortex, kinetochore



Plus end treadmilling of End-binding MAP:



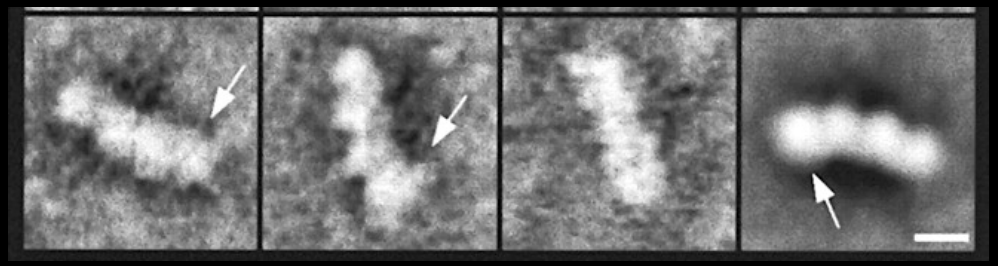
Microtubule-destabilizing proteins

Stathmin/Op18:

may sequester tubulin dimers
promotes GTP hydrolysis

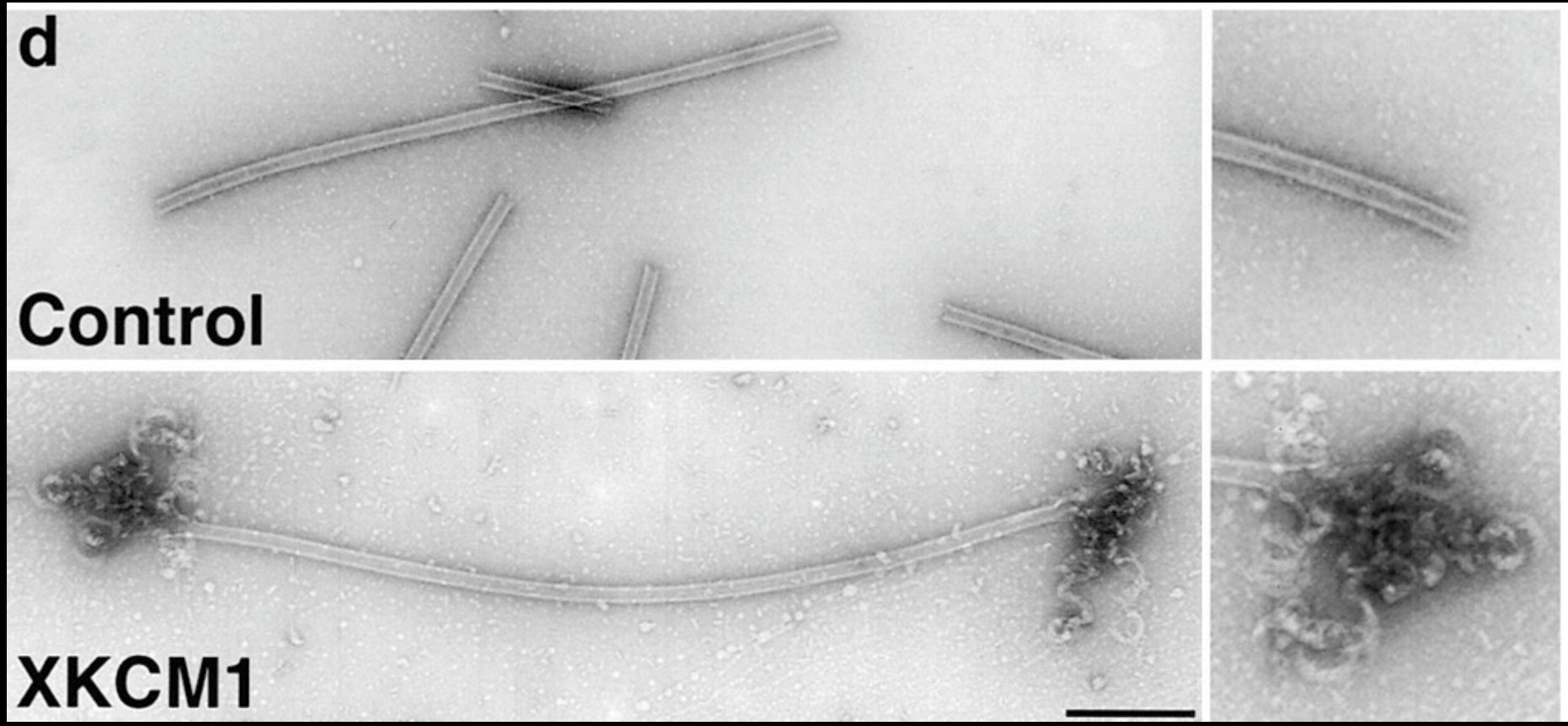
binds to two tubulin dimers:

Steinmetz et al.
EMBO J 19, 572 (2000)

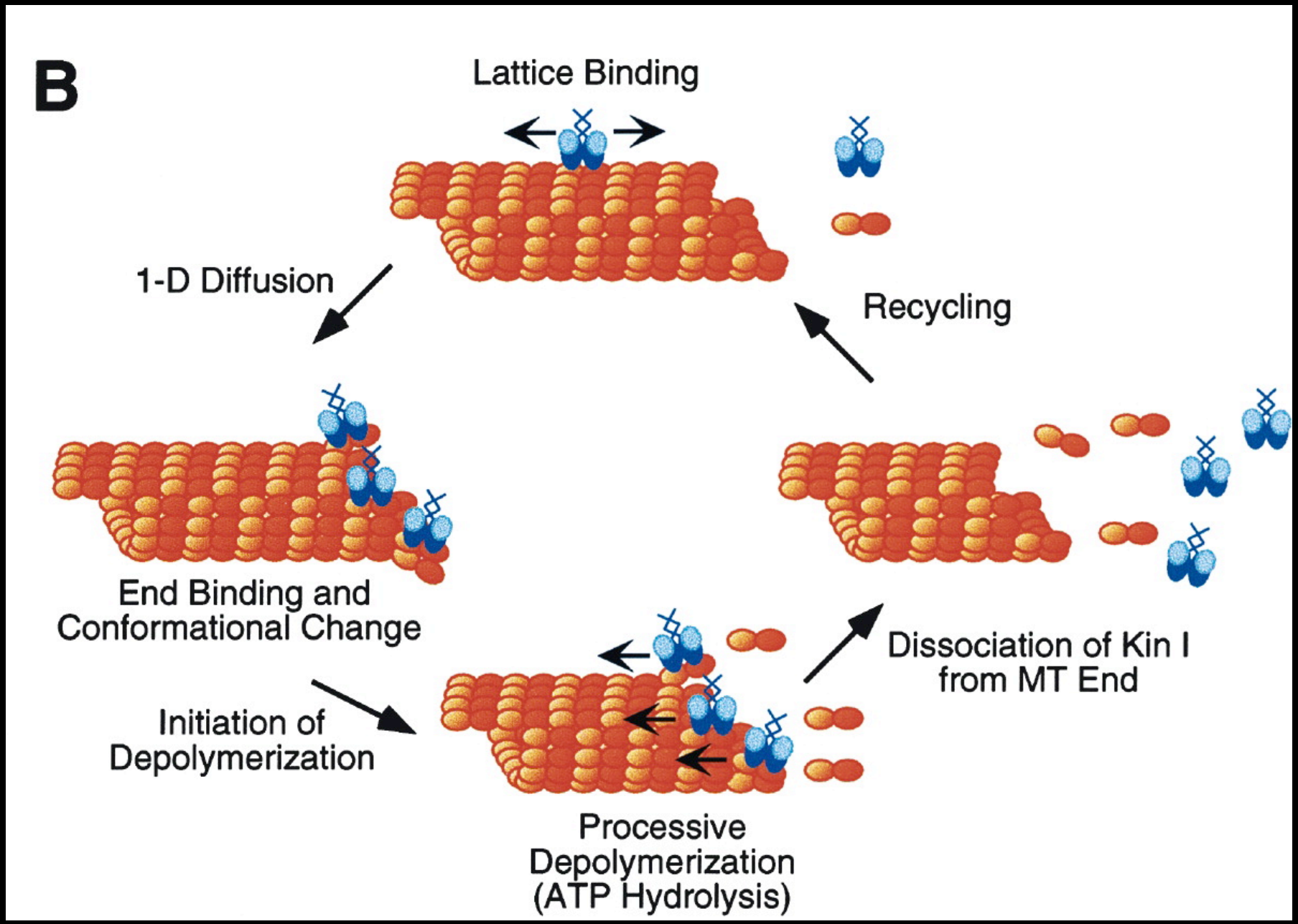


Stathmin, a gene enriched in the amygdala, controls both learned and innate fear.
Cell 2005 123:697-709.

Kinesin 13 family



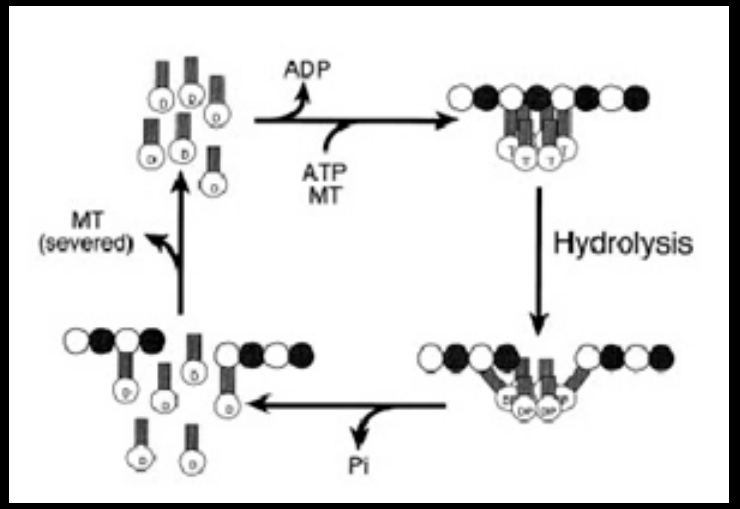
Peels protofilaments at both plus and minus ends

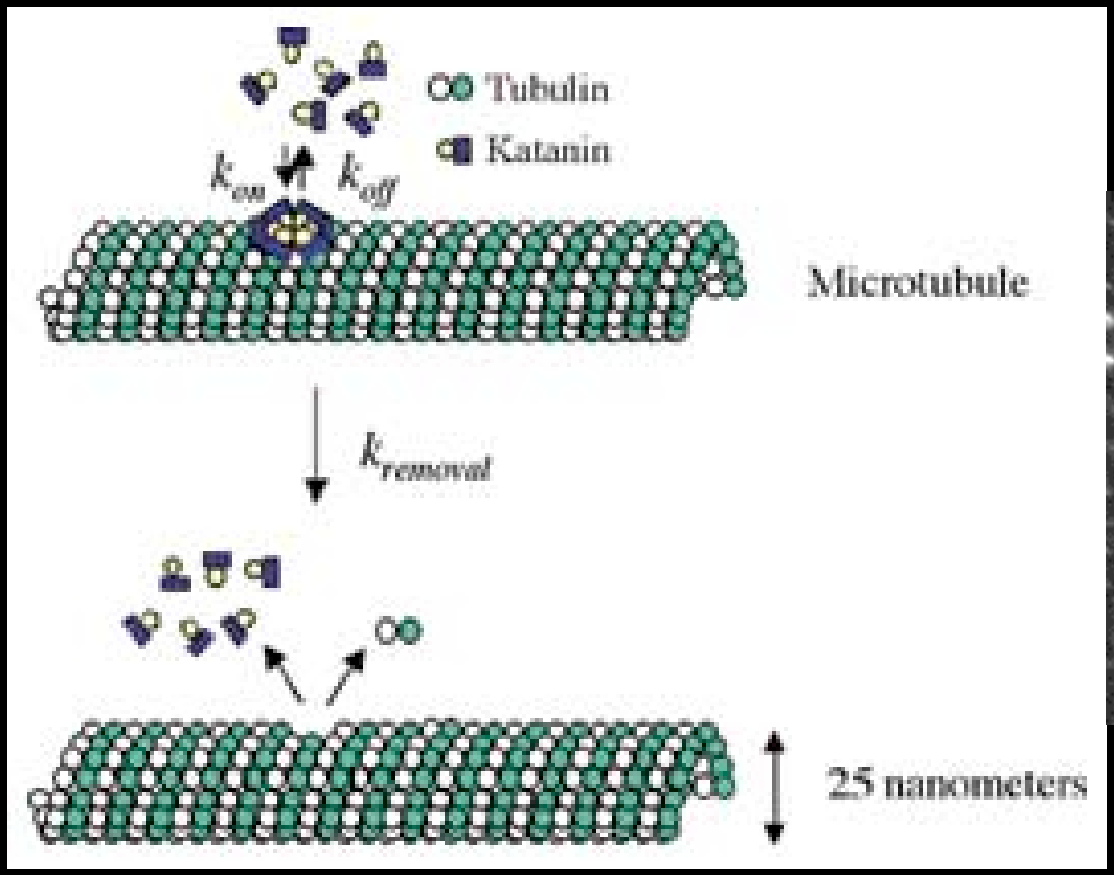

B
Lattice Binding
1-D Diffusion
Recycling
End Binding and
Conformational Change
Dissociation of Kin I
from MT End
Initiation of
Depolymerization
Processive
Depolymerization
(ATP Hydrolysis)

Severing factors

Katanin

AAA ATPase family subunits form ring localizes to centrosome





Microtubule-based Motor Proteins

Dyneins:

axonemal - flagellar motor

cytoplasmic - vesicle traffic, mitosis, morphogenesis

one major isoform - multiple functions

Kinesins:

multiple isoforms - separable functions:

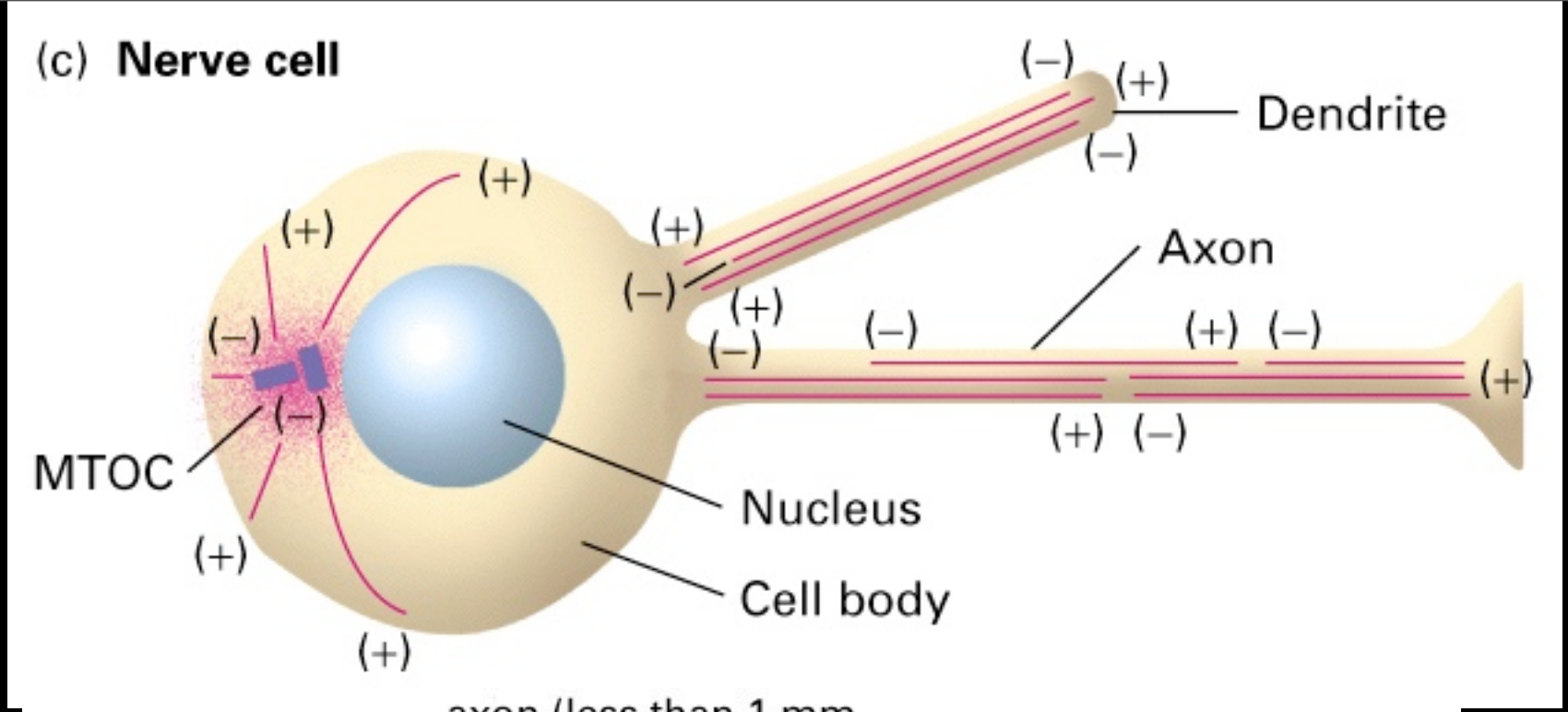
vesicle traffic, mitosis, morphogenesis

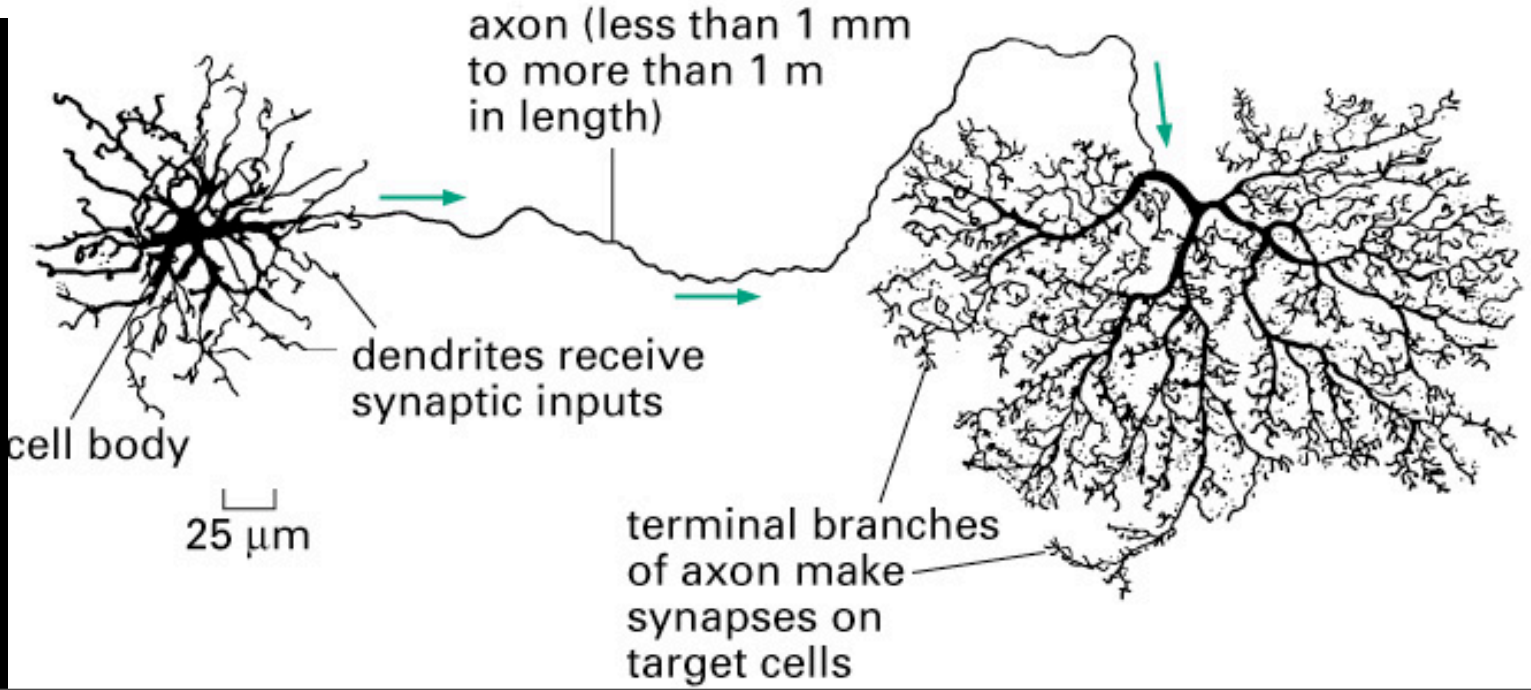
10 major subfamilies

motor functions:

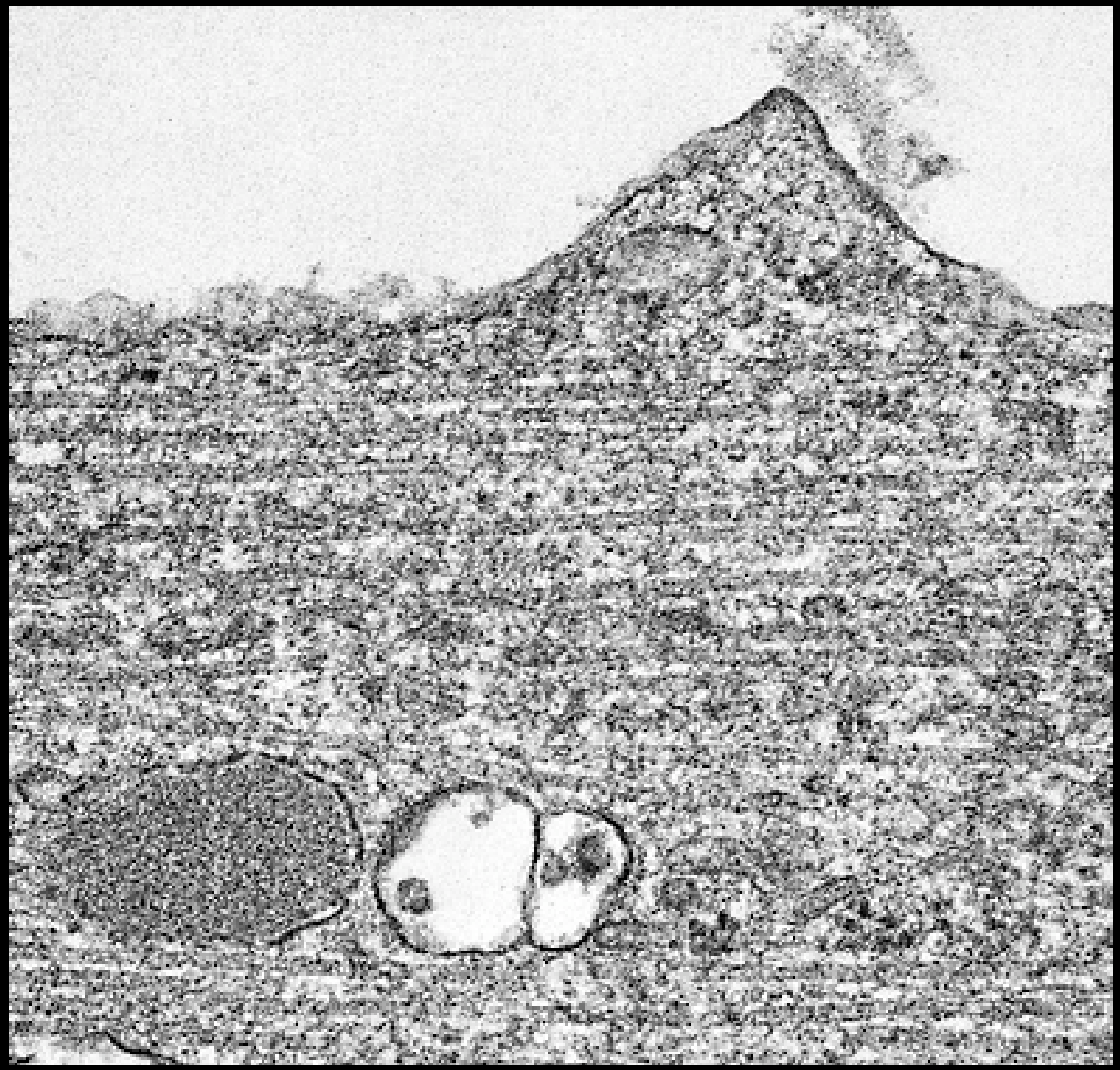
move cargo along microtubule

move microtubules


(c) Nerve cell
(–)
(+)
Dendrite
(–)
(+)
(+)
(+)
(+)
(–)
(+)
Axon
(–)
(+)
(–)
(–)
(+)
(–)
(+)
(+)
(–)
MTOC
(–)
Nucleus
(+)
Cell body
(+)


axon (less than 1 mm
to more than 1 m
in length)
dendrites receive
synaptic inputs
cell body
25 µm
terminal branches
of axon make
synapses on
target cells

axon MTs



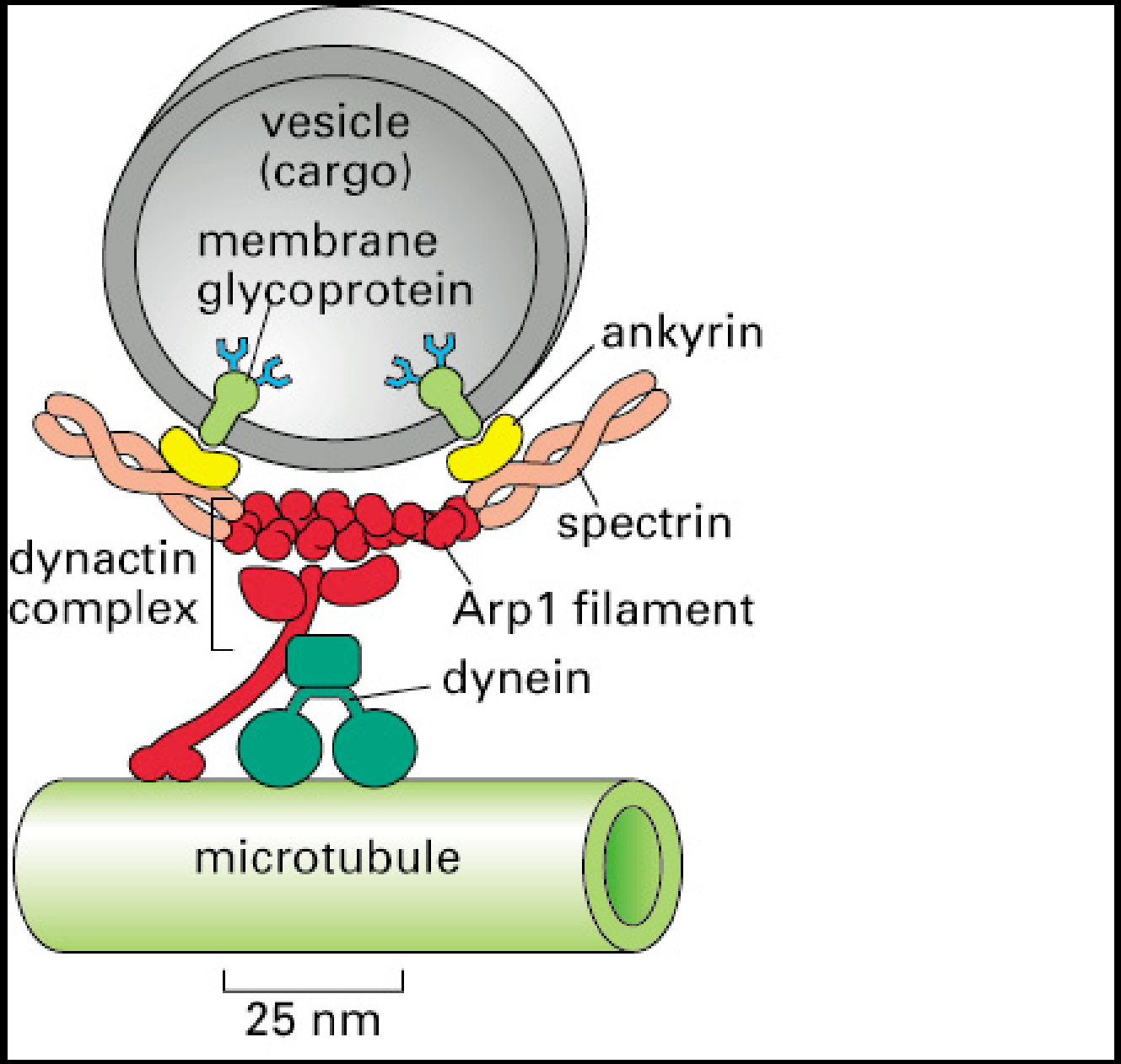
Cytoplasmic Dynein

500 kD heavy chain - AAA ATPase:
multiple ATP and MT binding sites,
intermediate and light chains

Minus end-directed

activating complex required to interact with cargoes
= dynactin complex

Dynein/dynactin attaching to vesicle



Overexpression of dynactin subunit disrupts Golgi localization

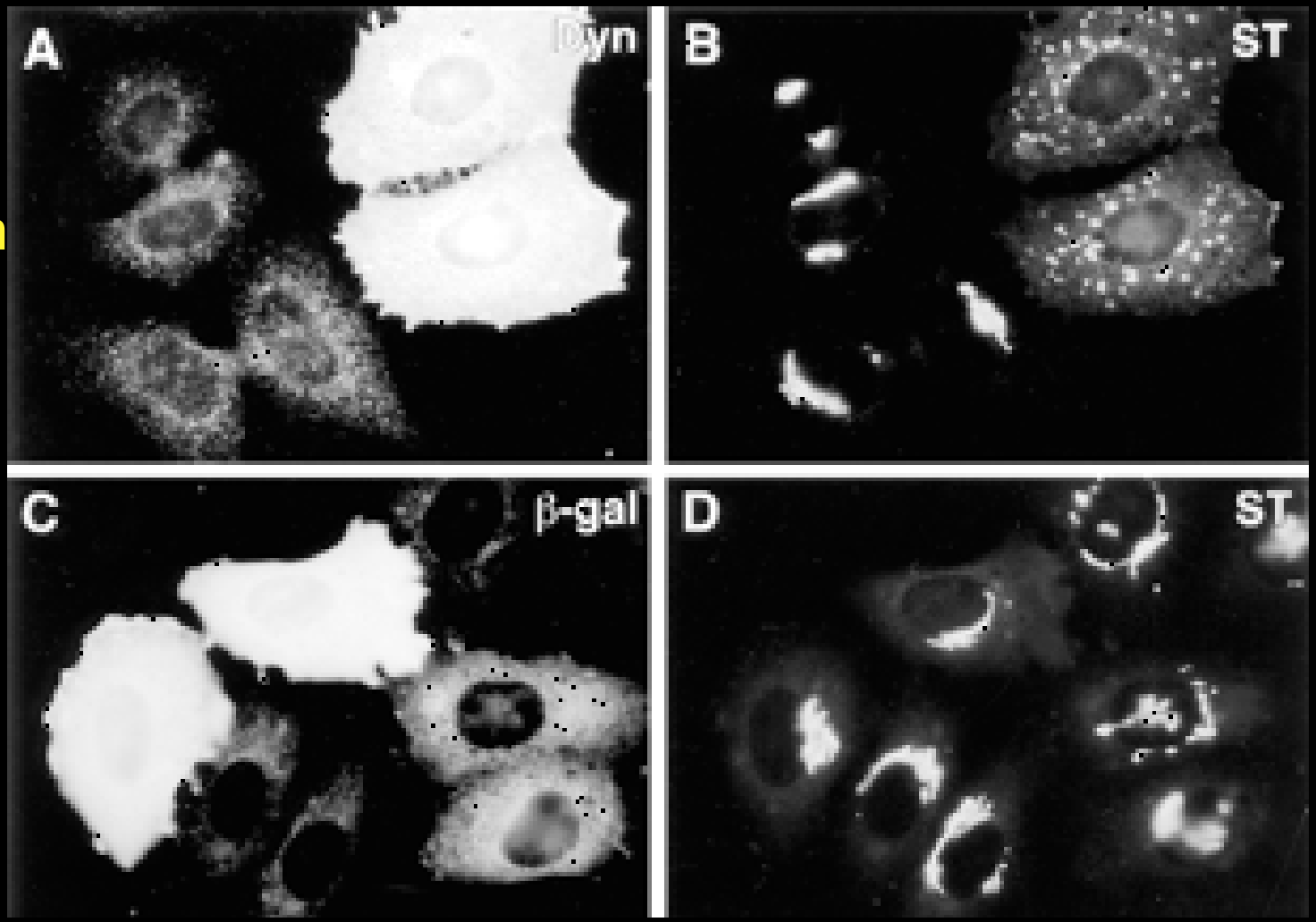
expressed protein

transfected cells

Golgi marker

p50 dynamitin

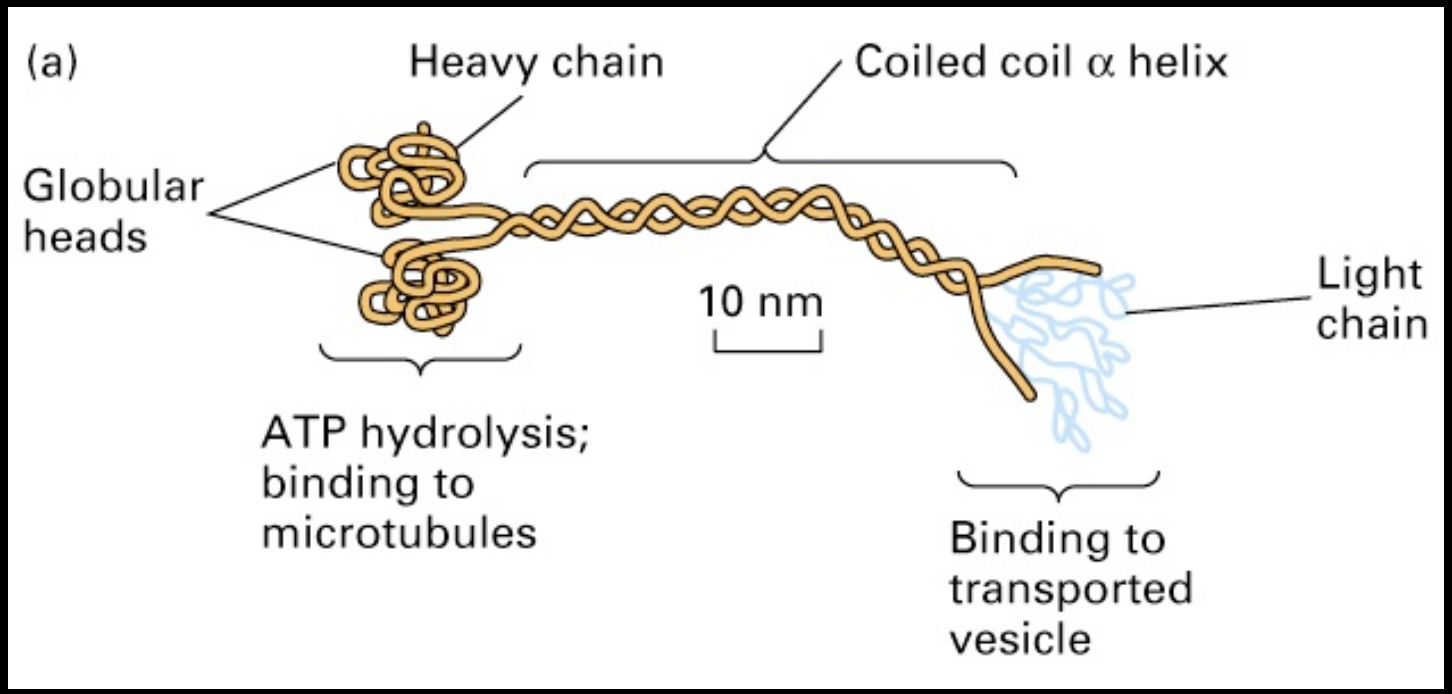
β-gal



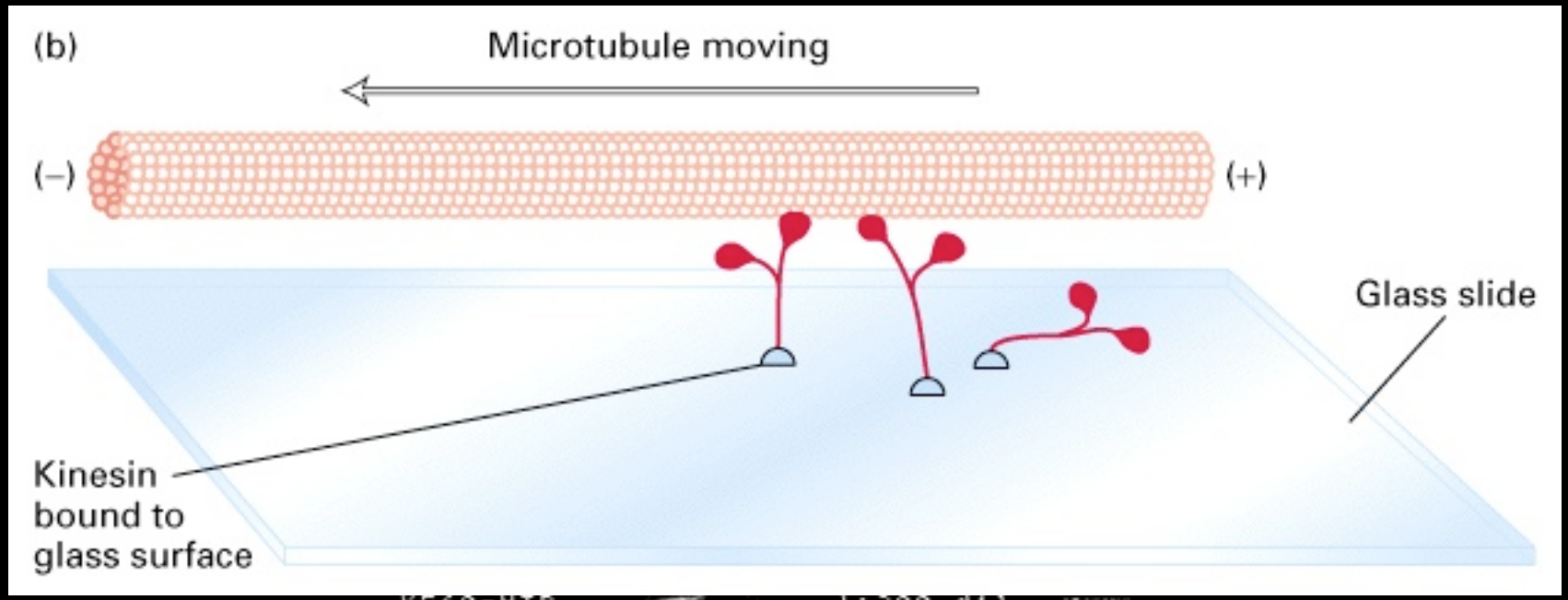
Burkhardt et al., JCB 139, 469 (1997)

Kinesin

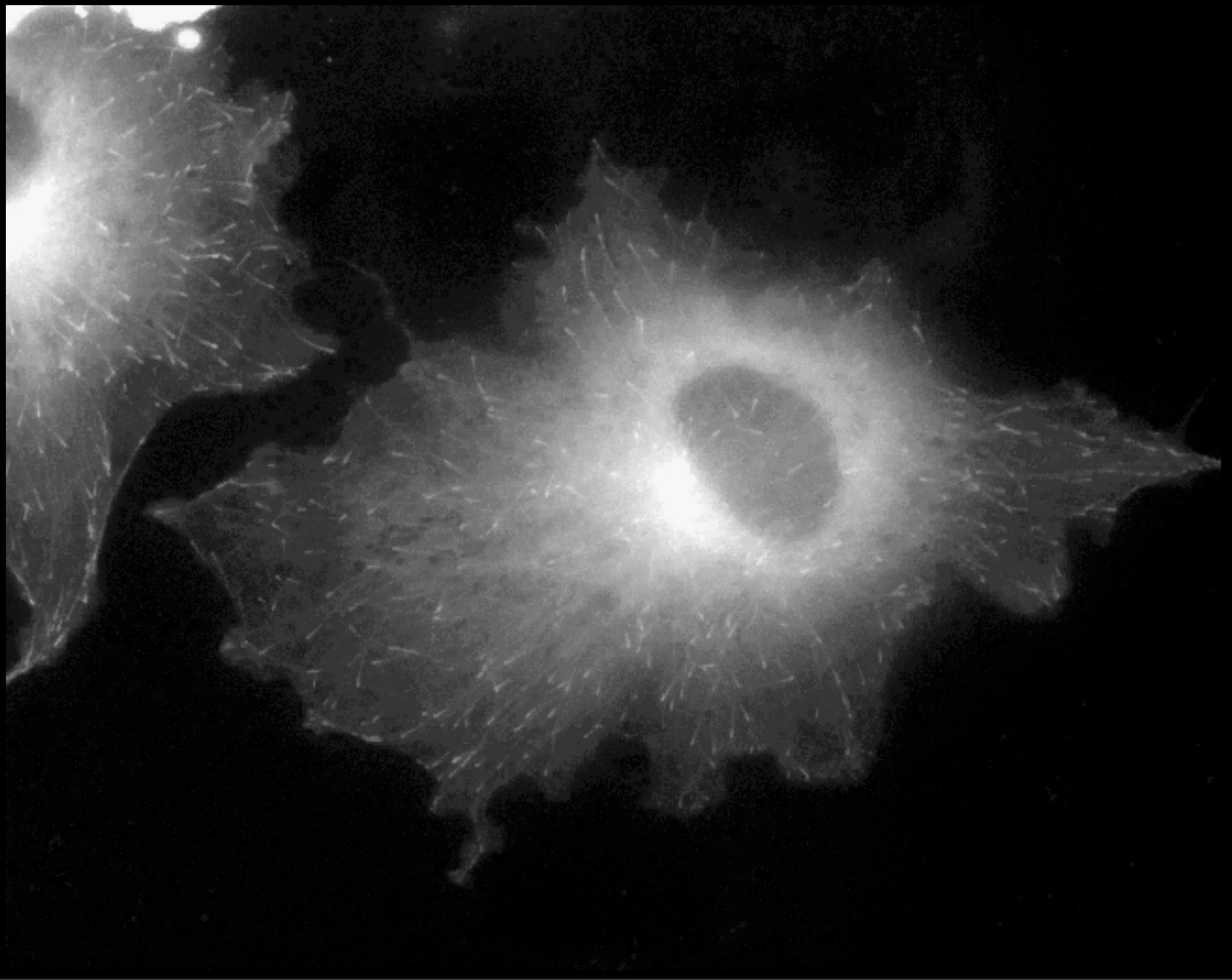
First isoform called “conventional” kinesin
(+) end-directed
purified from squid giant axon
assay : microtubule gliding



Kinesins are plus end motors *in vitro*



Kinesins are plus end motors *in vivo*



Kinesins are plus end motors *in vivo*

axonemes kinesin in vitro made kinesin

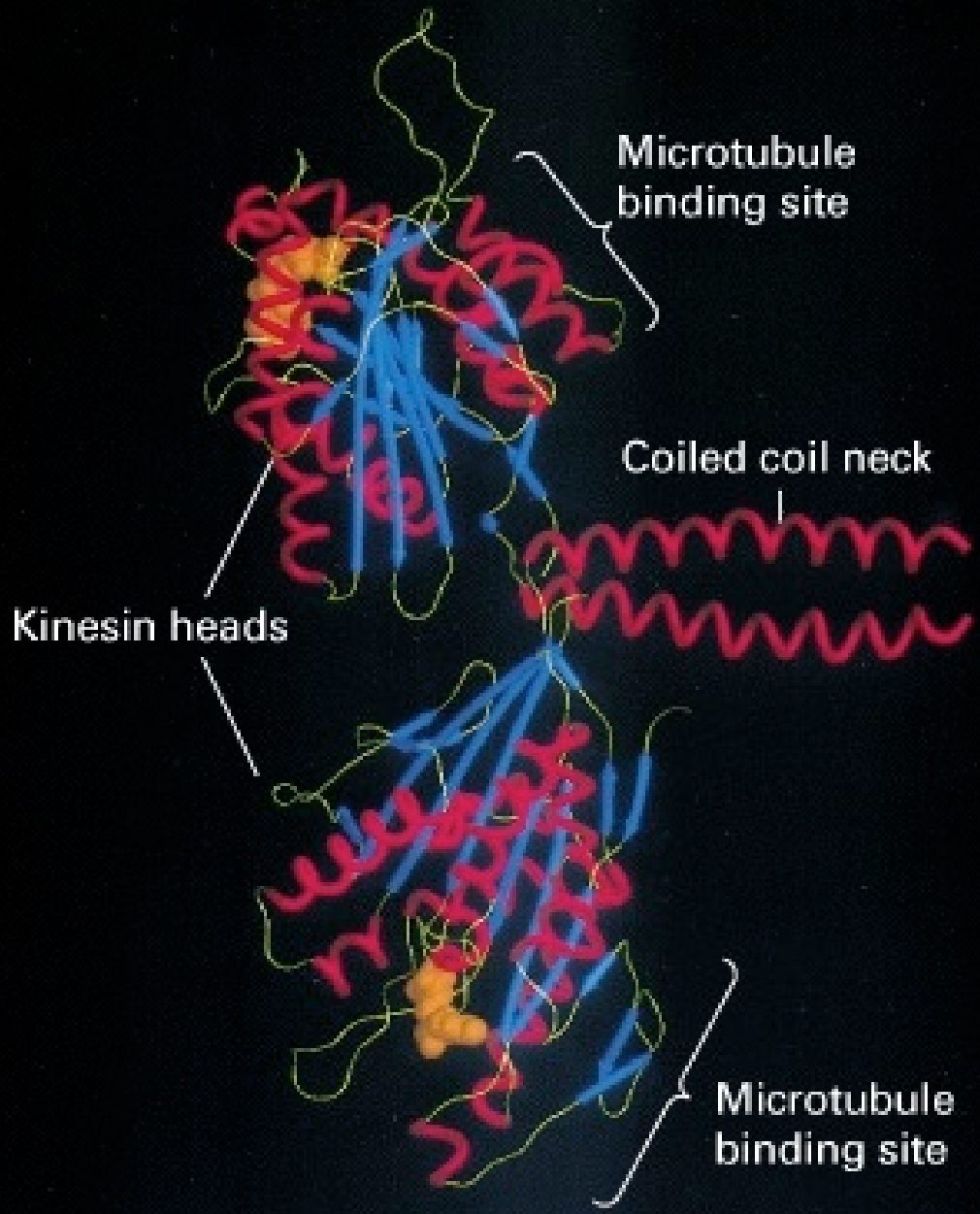
ATP hydrolysis cycle ⇒ conformational change cycle

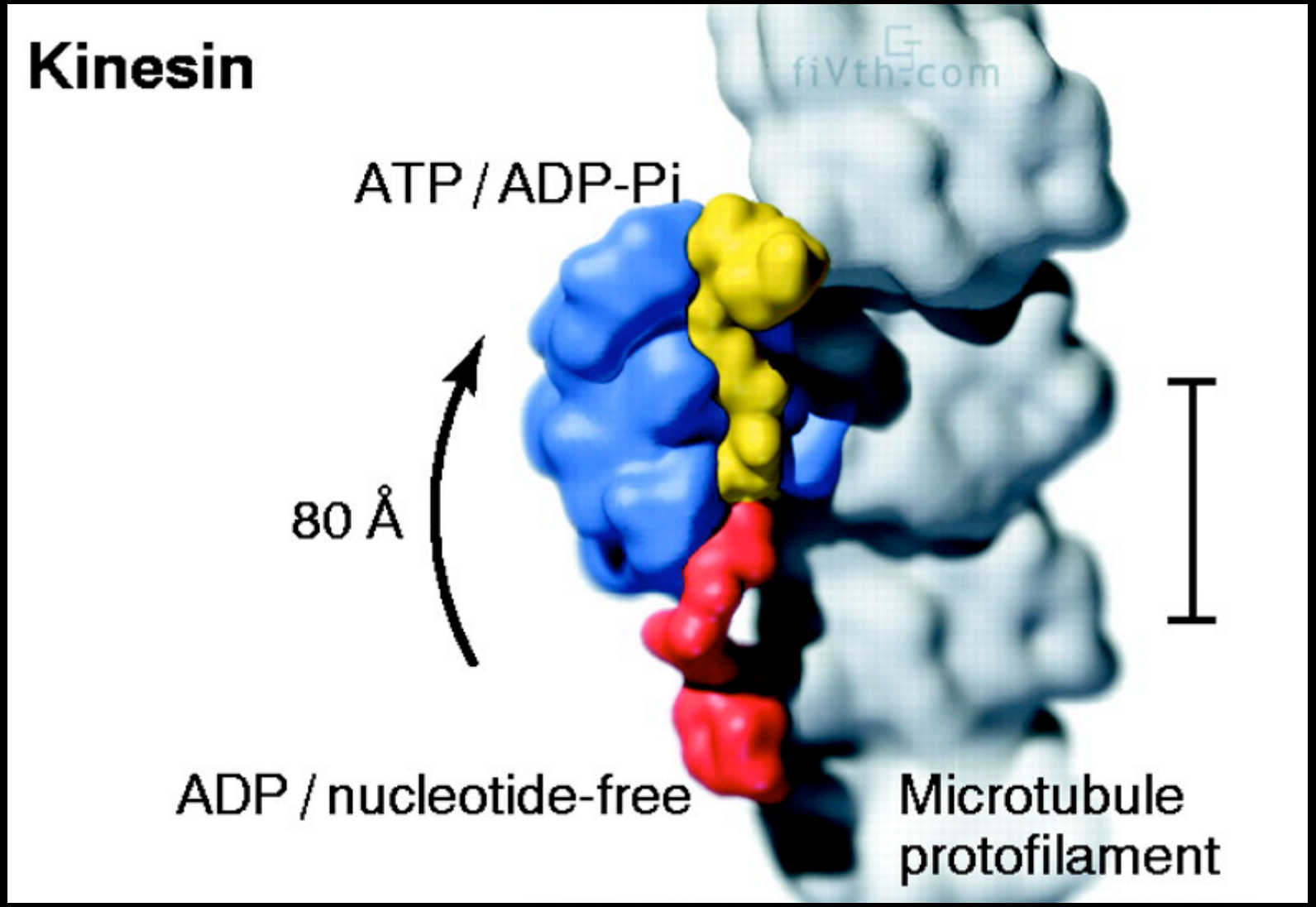
kinesin-MTs

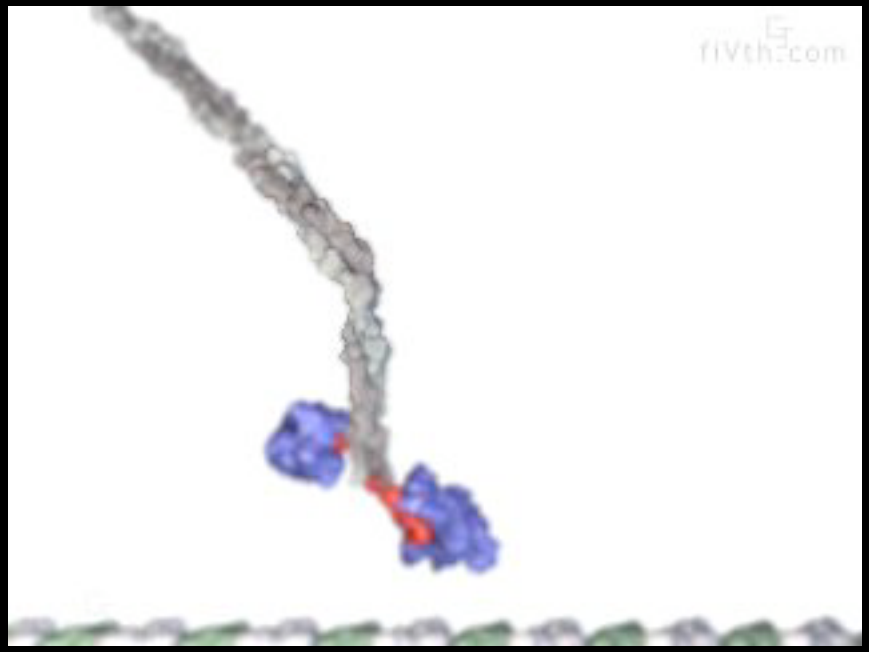
lots of steps, then dissociate (processive)

two heads highly coordinated

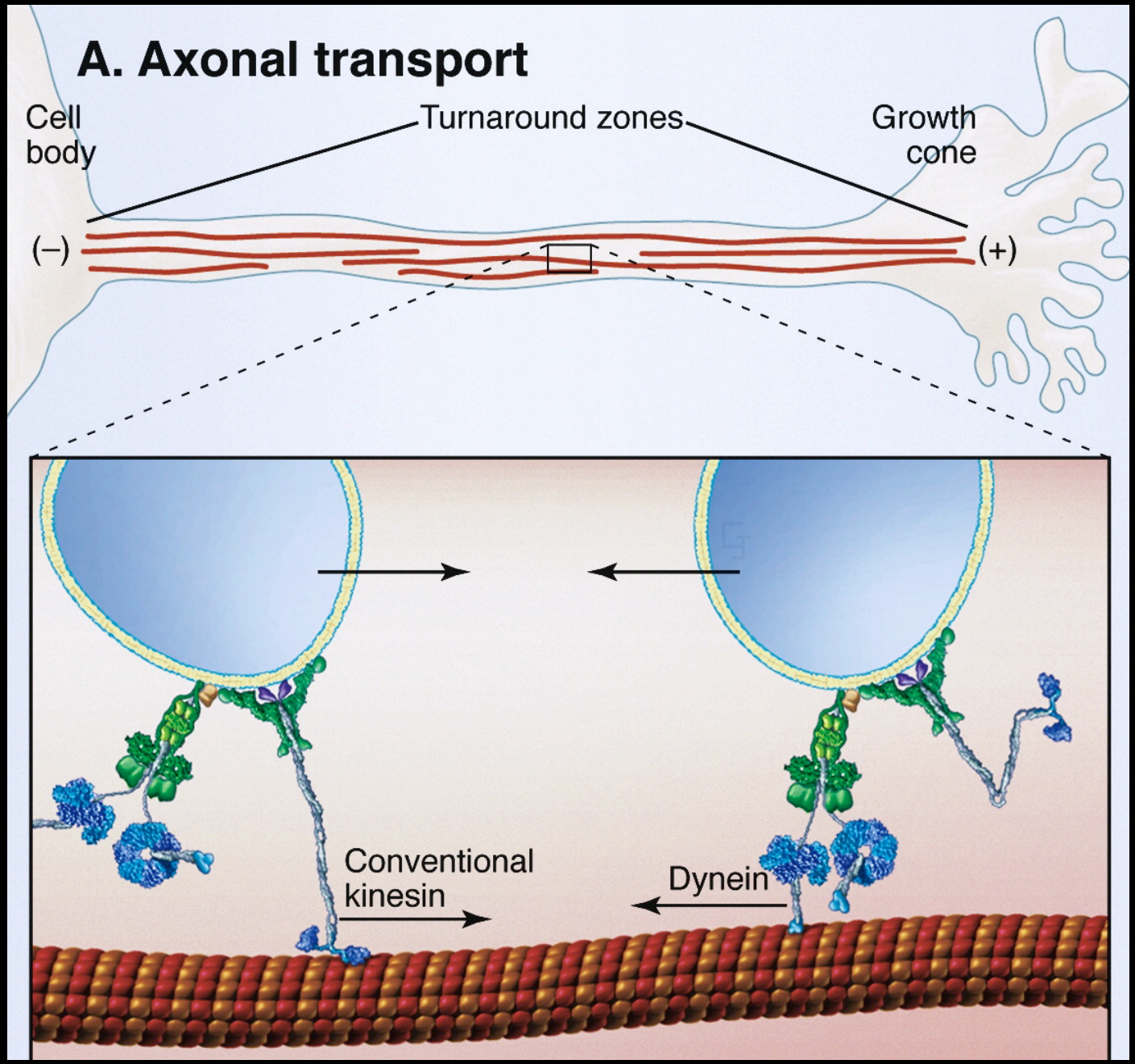
force mechanism: neck linker


Microtubule
binding site
Coiled coil neck
Kinesin heads
Microtubule
binding site

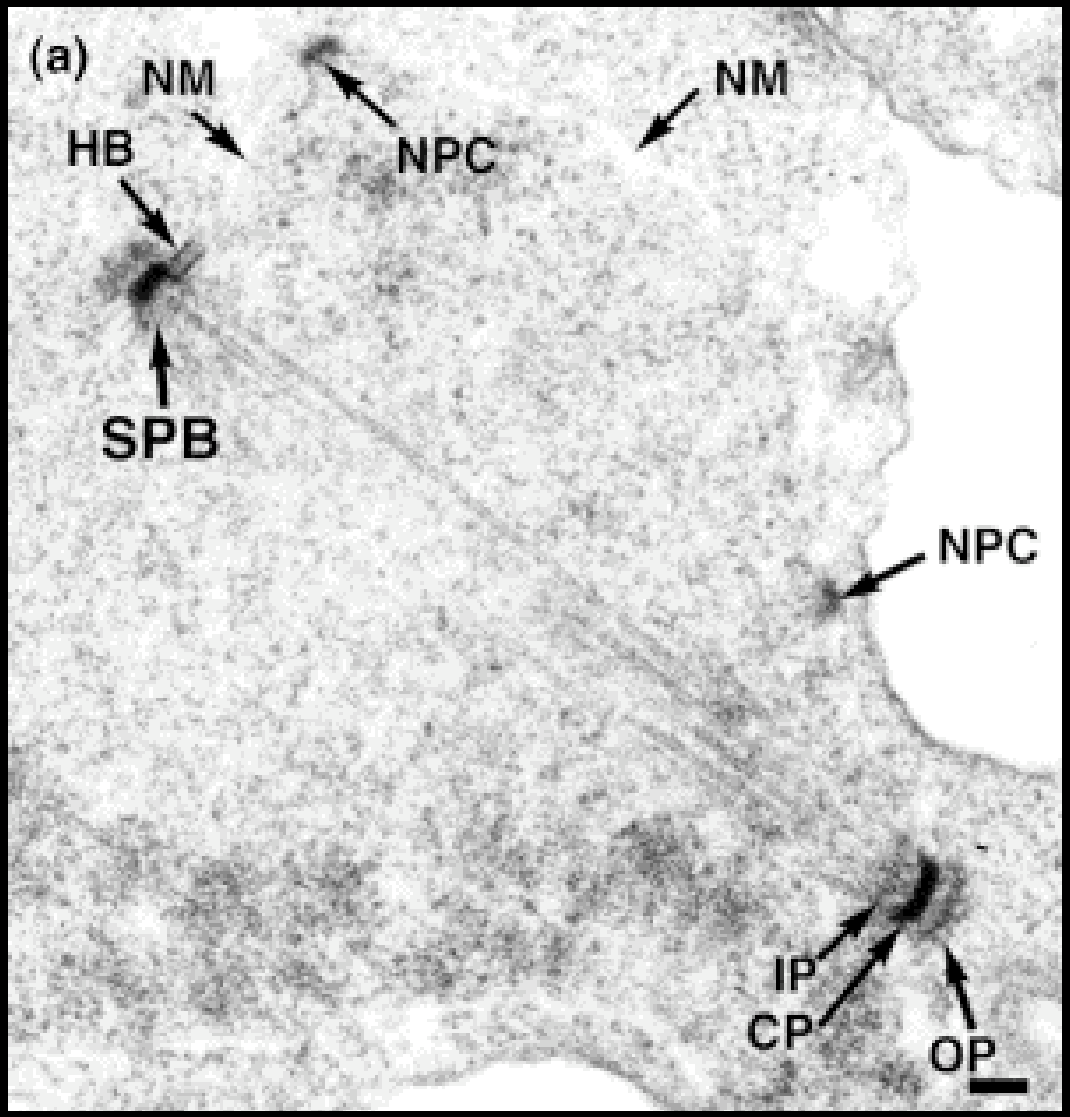

Kinesin
fiVth.com
ATP / ADP-Pi
80 Å
ADP / nucleotide-free
Microtubule
protofilament


fiVth.com

Model for bi-directional transport of vesicles

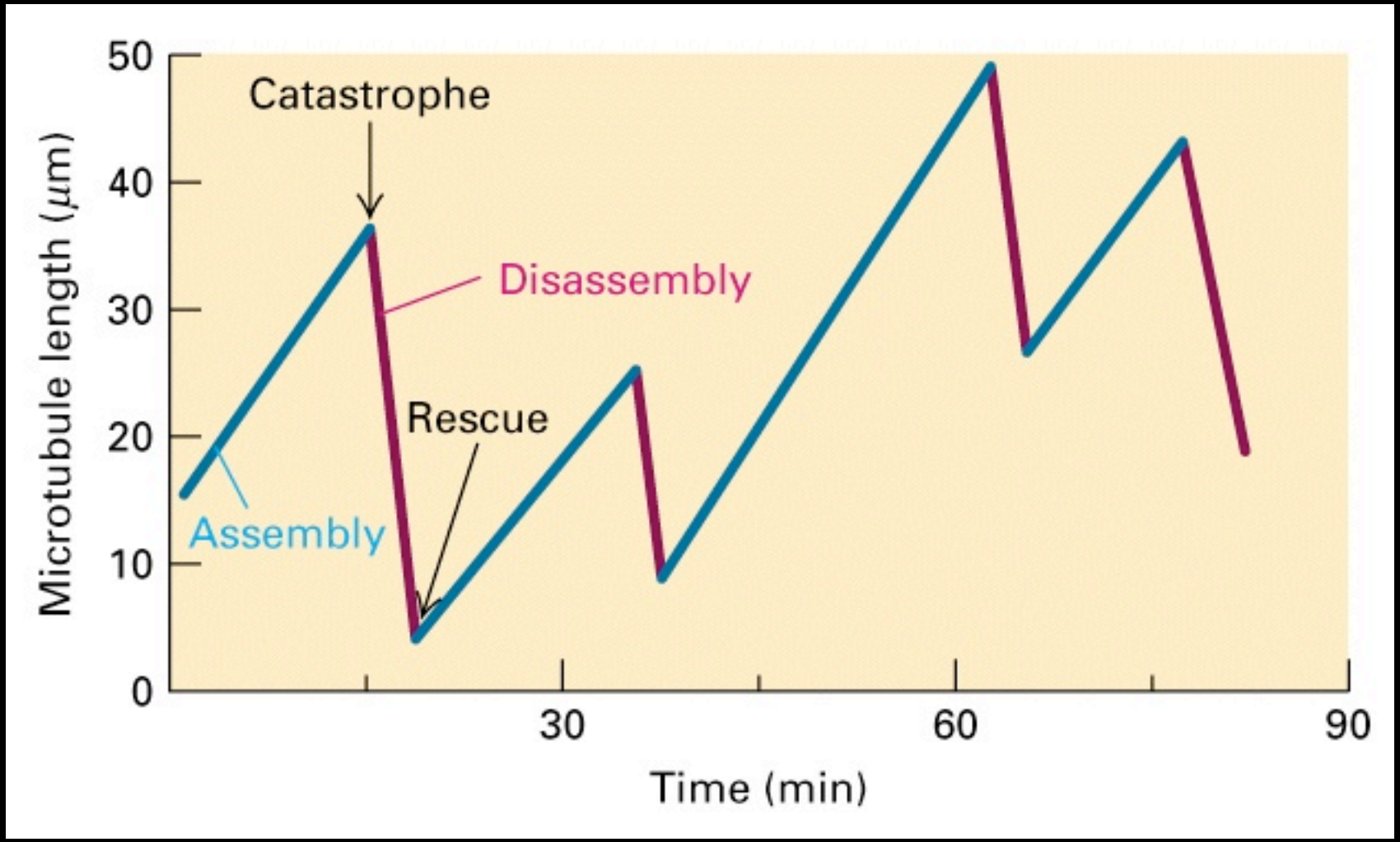


Another model system: yeast spindle pole body



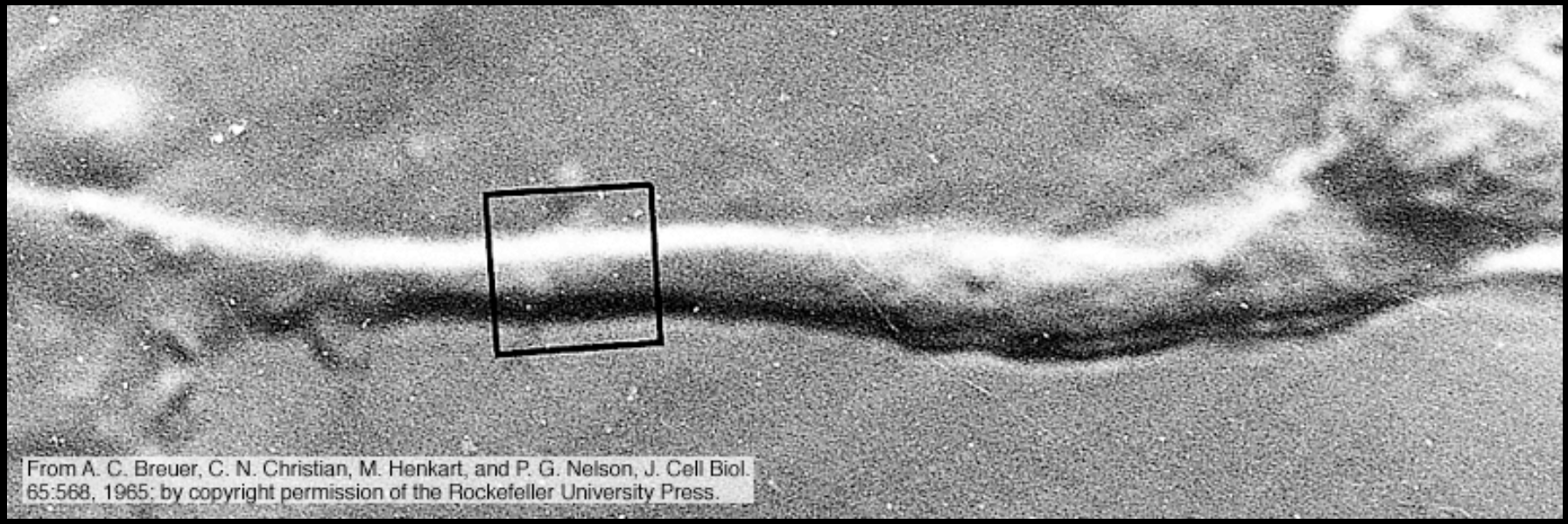
busy life of an individual microtubule at ~steady state

[tubulin] ~ 14 μM



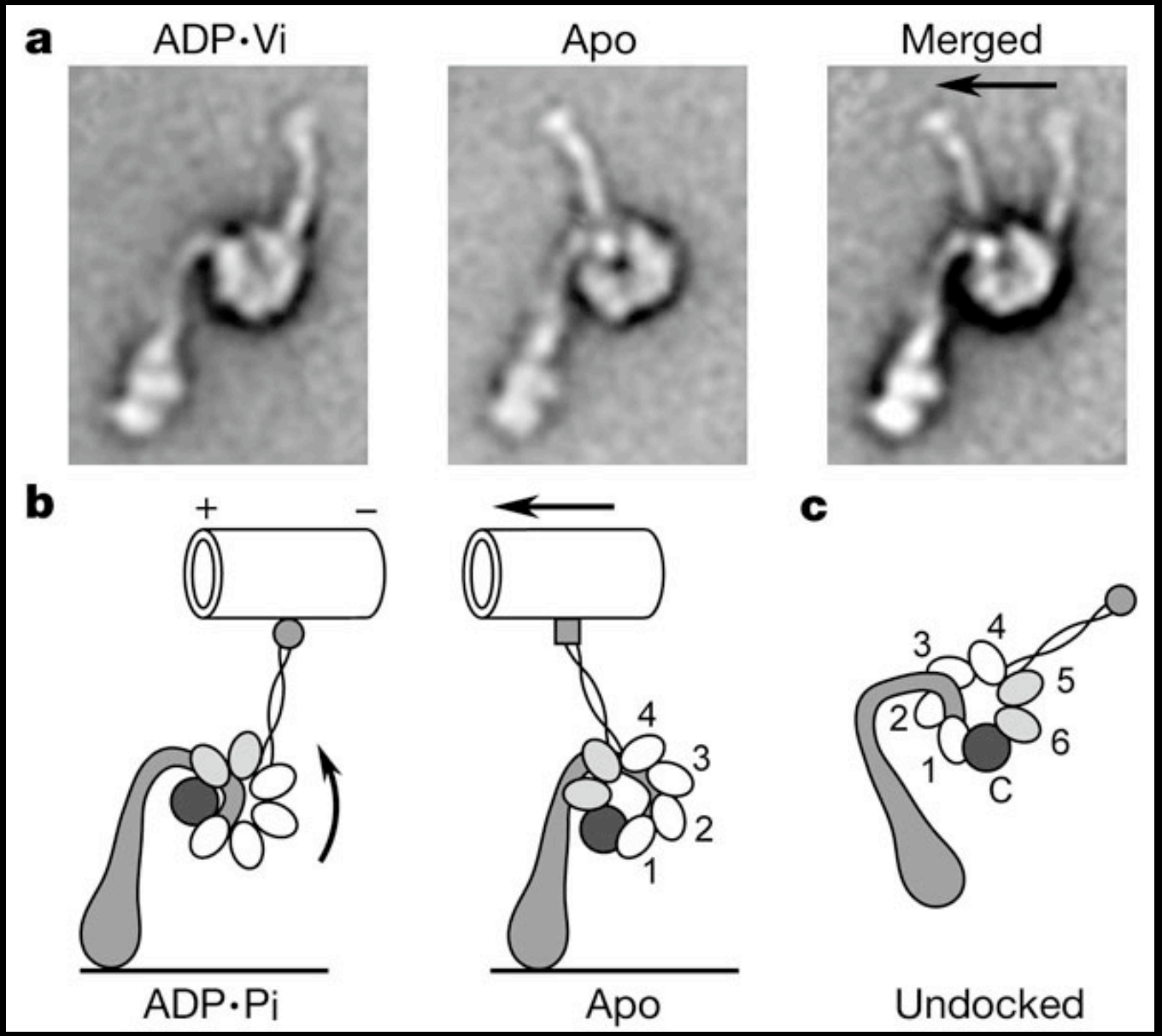
chick neuronal process (axon)

growth cone



cell body

Flagellar dynein structure and power stroke



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