BME 42-620 Engineering Molecular Cell Biology

Lecture 10:

The Cytoskeleton (II): Microtubule & Intermediate Filament

The Cytoskeleton (III): Molecular Motors

Outline

- Summary: actin and its associated proteins
- Microtubule and its associated proteins; Centrosome
- Intermediate filament and its associated proteins
- An overview of molecular motors

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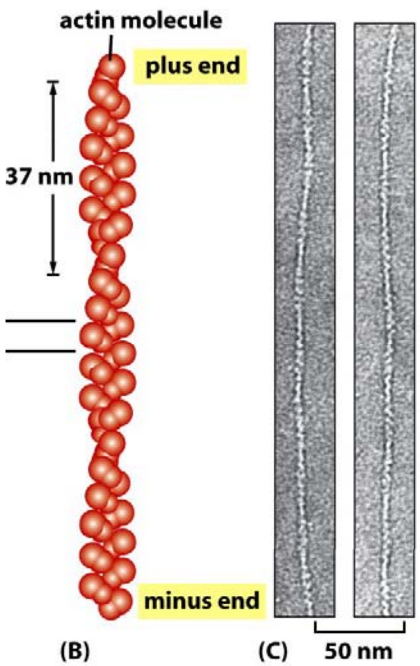
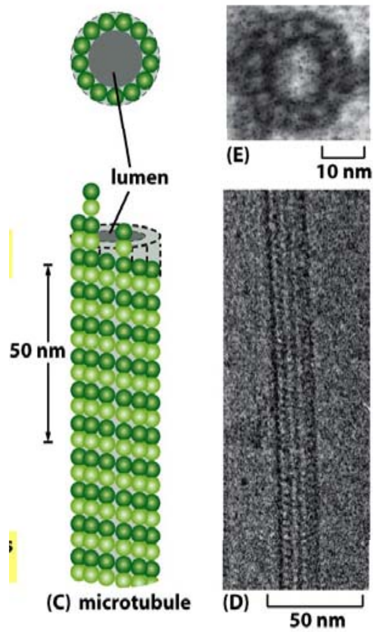
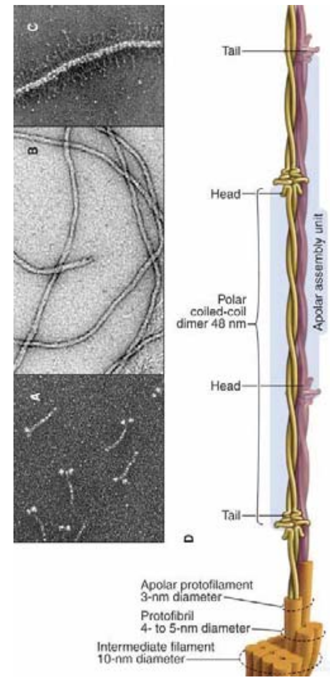
Overview of Cytoskeletal Filaments



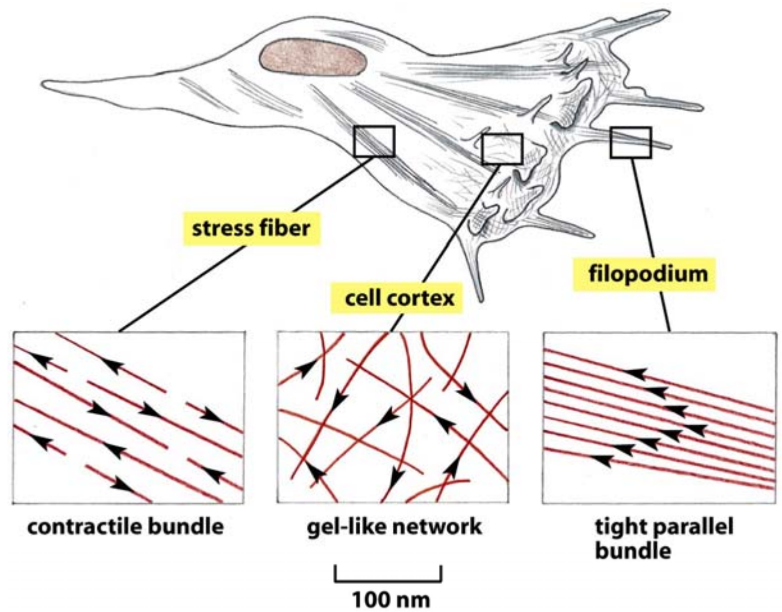




	Shape	Diameter	Subunits	Polarized
actin	cable	~6 nm	actin monomer	yes
microtubule	tube	~25nm	tubulin heterodimer	yes
intermediate filament	rope	~10nm	Various dimers	no

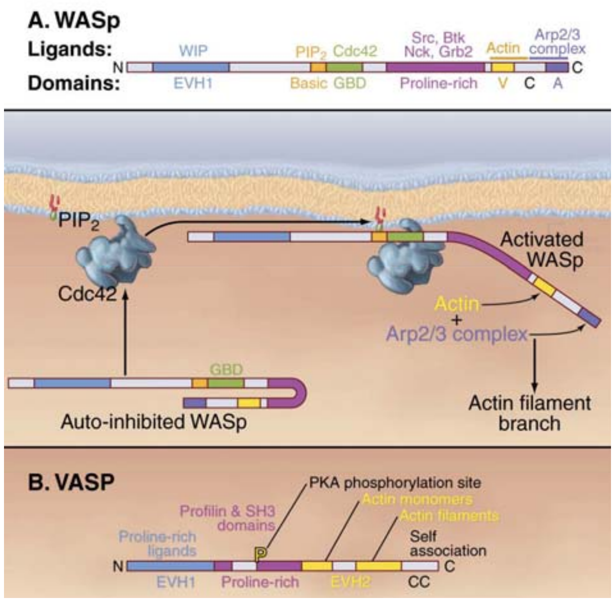
Summary: Actin and its Associated Proteins (I)

- Actin is relatively soft (we will study related quantification in later lectures).
- Actin often form bundles; their mechanical strength comes mostly from bundling and crosslinking.
- Actin function mostly to withstand tension rather than compression.
- Actin is relatively stable and easy to work with biochemically.



Summary: Actin and its Associated Proteins (II)

- Different actin associated proteins serve a broad range of functions.
- These proteins generally have multiple functional domains serving multiple functions.
- Some but not all of them are essential.
- Most of the proteins have functional overlap.
- Mathematical models are required to understand complex interactions between these proteins.



Outline

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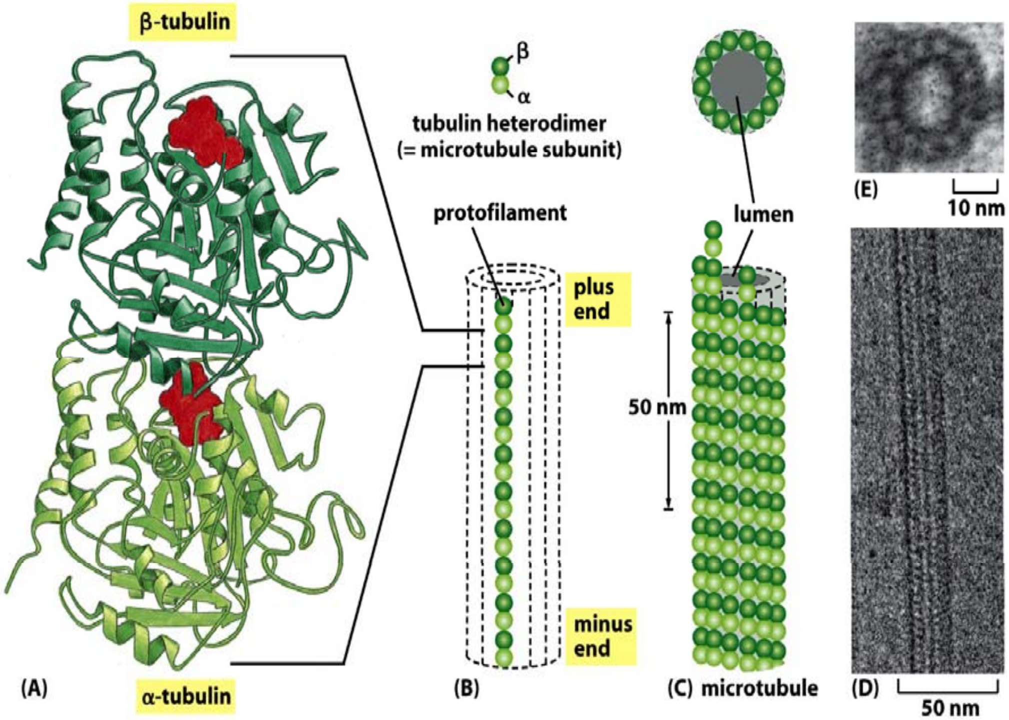
Overview of Microtubule Structure

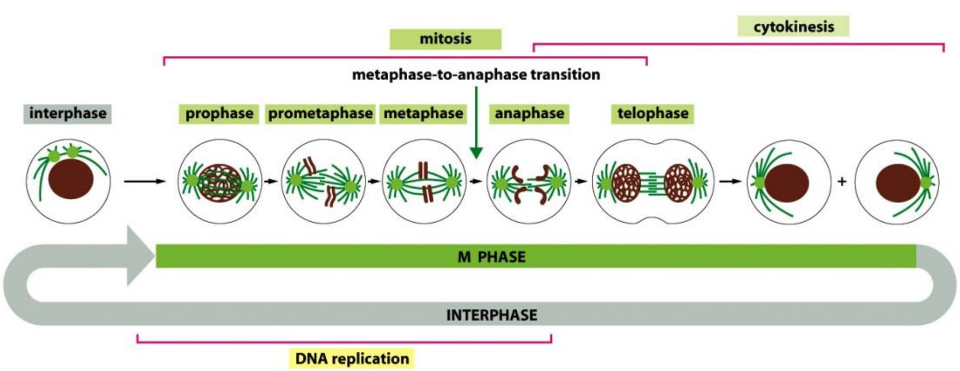
- Microtubule is polarized.
  - plus (β-tubulin) end
  - minus (α-tubulin) end
- The outer diameter of a microtubule is ~25 nm.
- A microtubule typically has 13 protofilaments; Some may have 11, 15, or even 16.
- The GTP bound to the α-tubulin monomer does not hydrolyze.



Microtubule Organization at Different Stages

- Microtubule in interphase
  - Organized into a radial pattern centered at the centrosome

- Microtubule in metaphase
  - Organized into a bipolar architecture



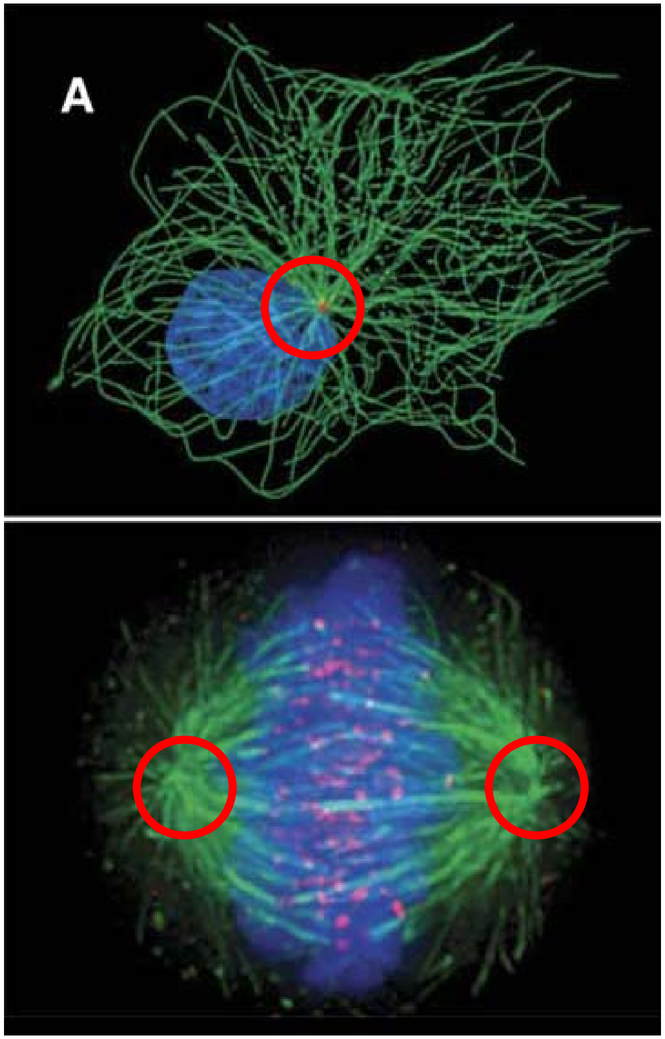


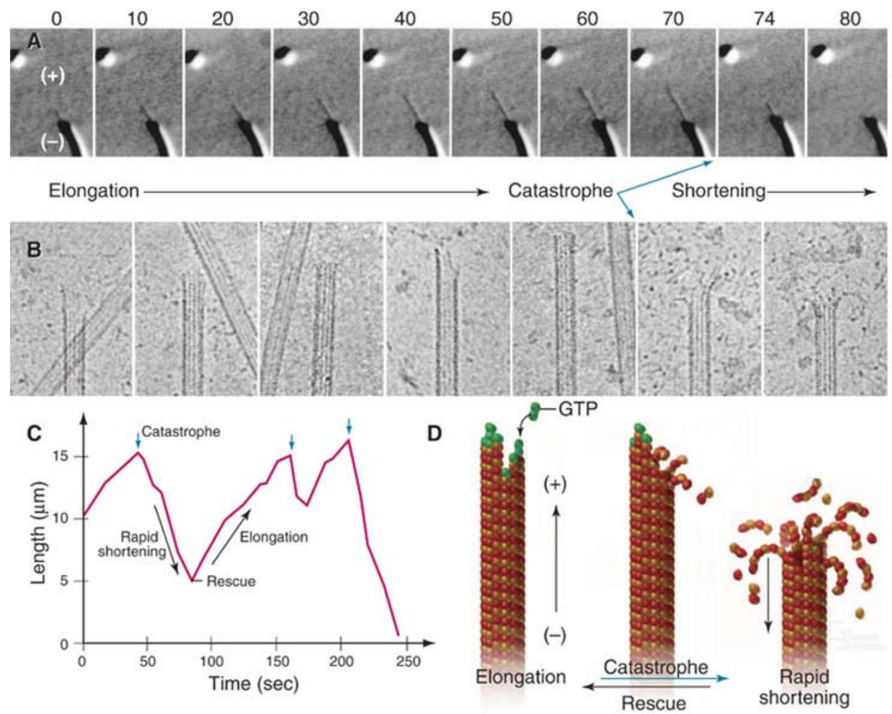
Fig. 34-1

Microtubule Dynamic Instability (I)

Video 1
(Figure 1A)

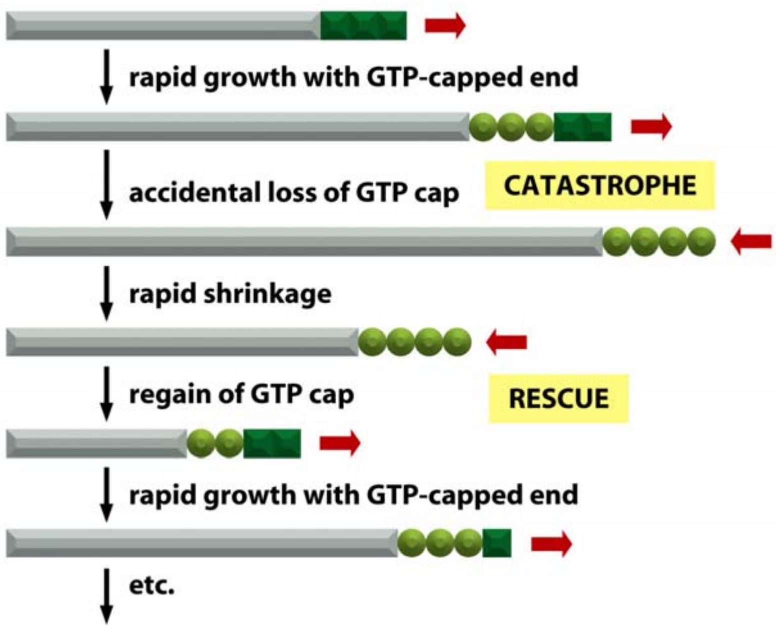
Microtubules in a PtK1 cell at the edge of an epithelial cell island. Few microtubules rapidly grow into nascent protrusions.

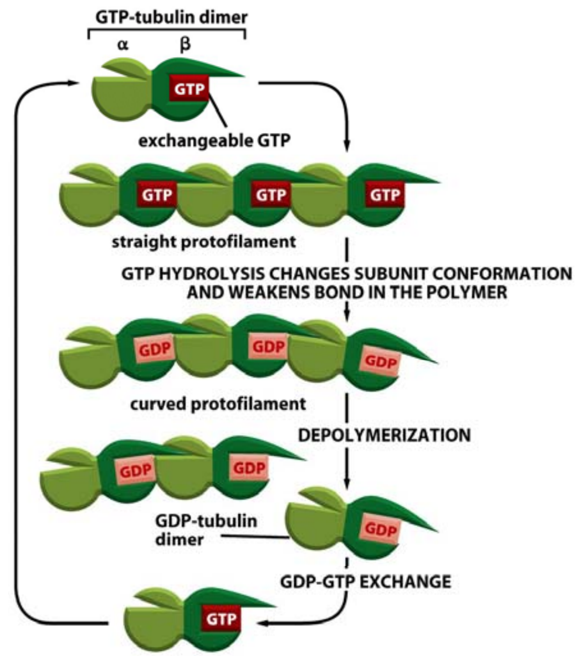
Elapsed time: 9 min 05 sec

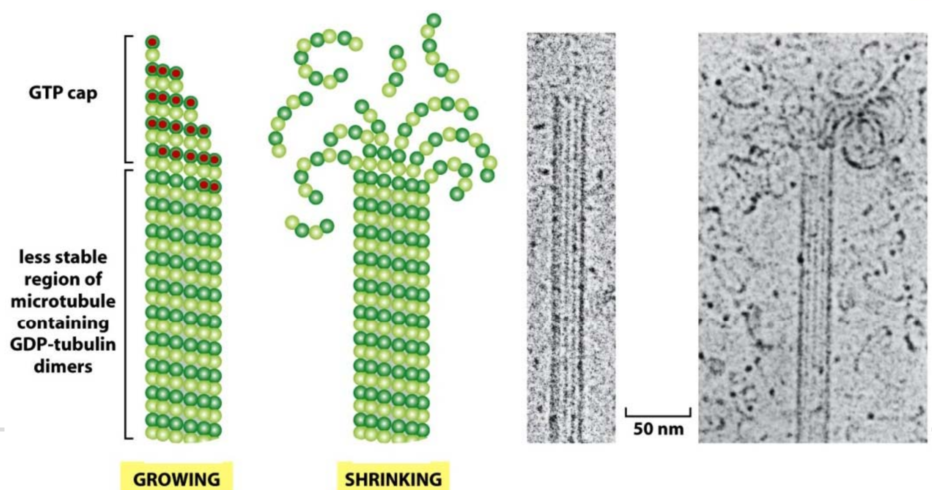


T. Wittmann et al, *J. Cell Biol.*, 161:845, 2003.

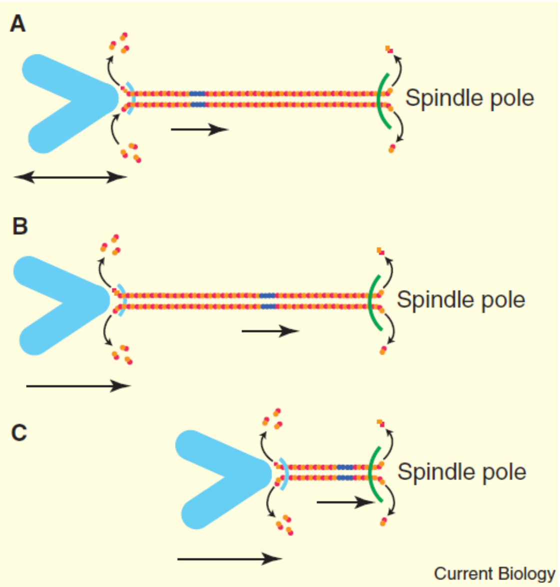
Microtubule Dynamic Instability (II)

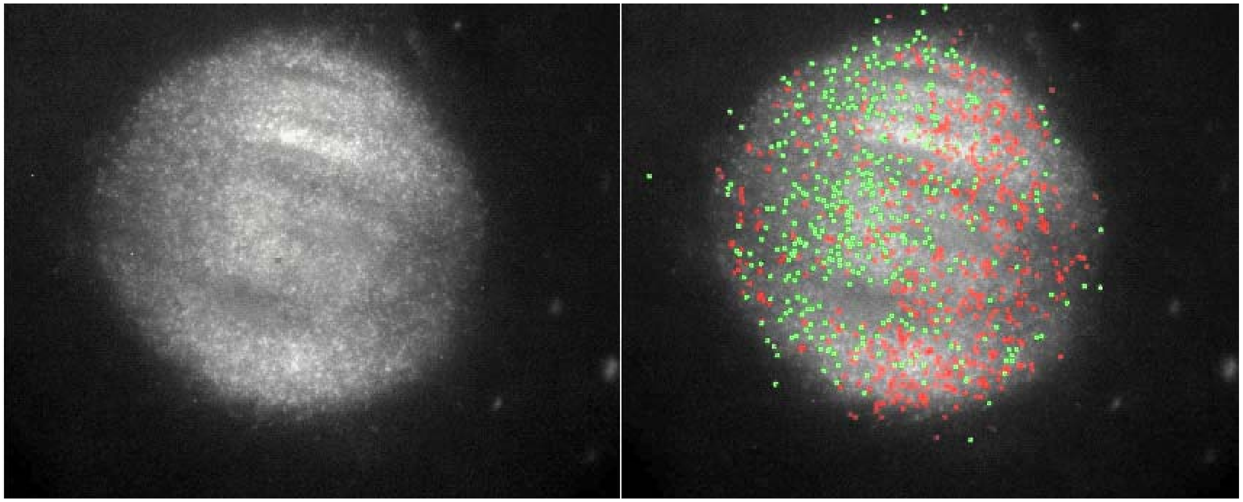






Microtubule Treadmilling vs Dynamic Instability





Khodjakov & Kapoor, *Current Biology*, 15:R966-R968, 2005.

Representative Microtubule Dynamic Instability Parameters

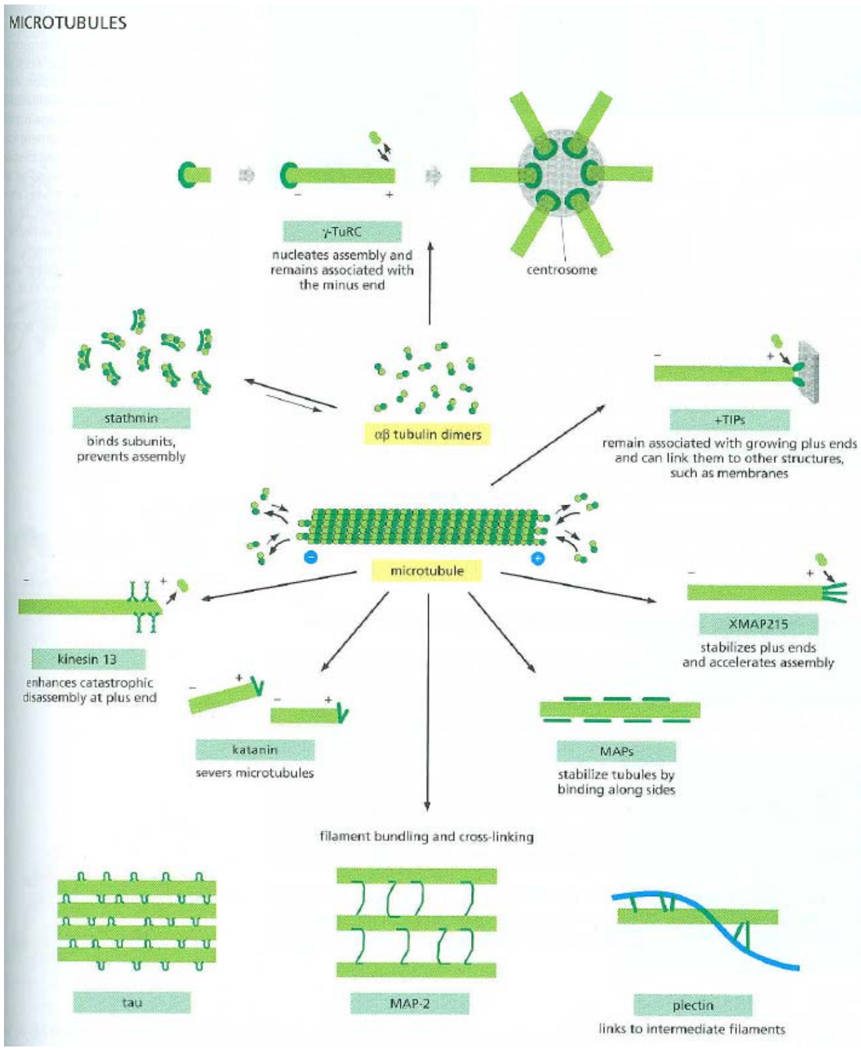
Table 1. Comparison of the parameters of microtubule dynamics in vitro and in vivo. Dashes indicate that no microtubules were formed. NA, not applicable.

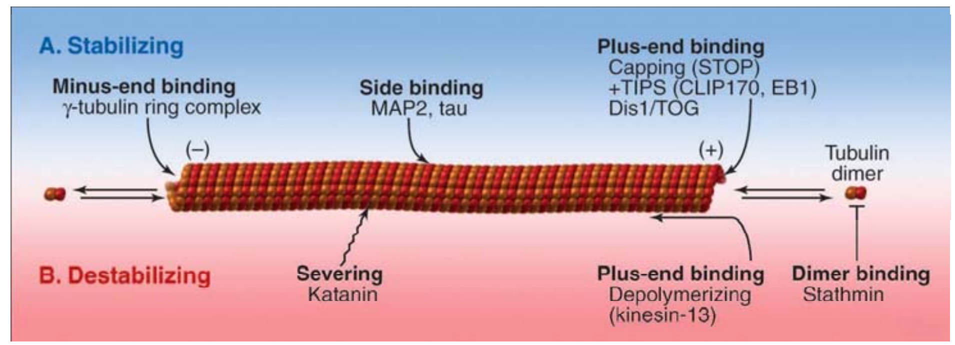
Parameter	In vitro (25 µM tubulin)				In vivo				
					Xenopus egg extracts		Newt lung cells‡	Mammalian LLCPK-1 cells§	
	Tubulin alone	+0.8 µM XMAP215	+ 0.2 µM XKCM1	+ 0.8 µM XMAP215 + 0.2 µM XKCM1	Interphase*	Mitotic†		Interphase	Mitotic
Growth (V_g) (µm/min)	2.56 (±0.75)	6.76 (±1.76)	–	8.73 (±3.72)	7.10	11.40	7.20	11.50	12.80
Shrinkage (V_s) (µm/min)	NA	NA	–	19.94 (±5.20)	9.40	13.50	17.30	13.10	14.10
Catastrophe (F_{cat}) (events/min)	0.04	0	–	1.06	0.69	2.44	0.84	1.56	3.48
Rescue (F_{res}) (events/min)	NA	NA	–	1.30	1.08	0.70	2.64	10.50	2.70

*Verde *et al.* (9). †Tournebize *et al.* (10). ‡Cassimeris *et al.* (5). §Rusan *et al.* (7).

Kinoshita et al, *Science*, 294:1340, 2001

Overview of Microtubule Associated Proteins





Microtubule Associated Proteins (I)

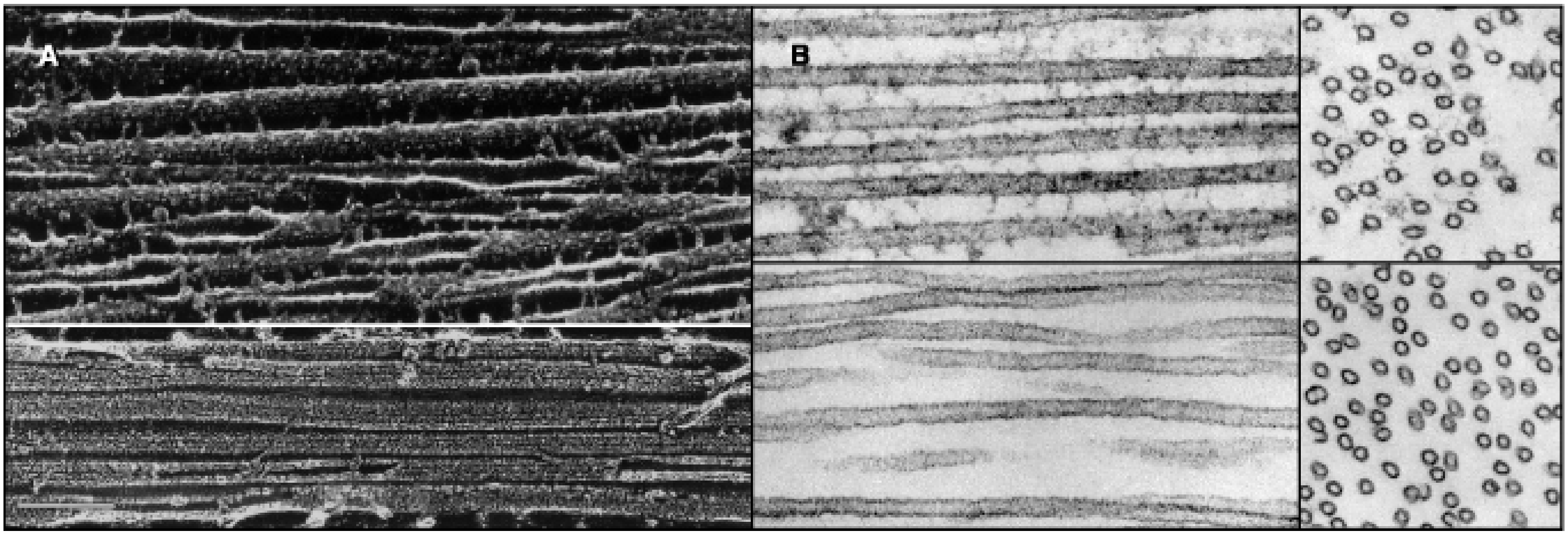
- **Stabilizing MAPs**

Tau	Binds side and stabilizes microtubules
MAP2, MAP4	Binds side and stabilizes microtubules

- **Destabilizing MAPs**

Op18/stathmin	Binds tubulin dimers & destabilizes MTs
Katanin	AAA ATPase that severs microtubules
XKCM/MCAK	Kinesin-related; destabilizes plus ends

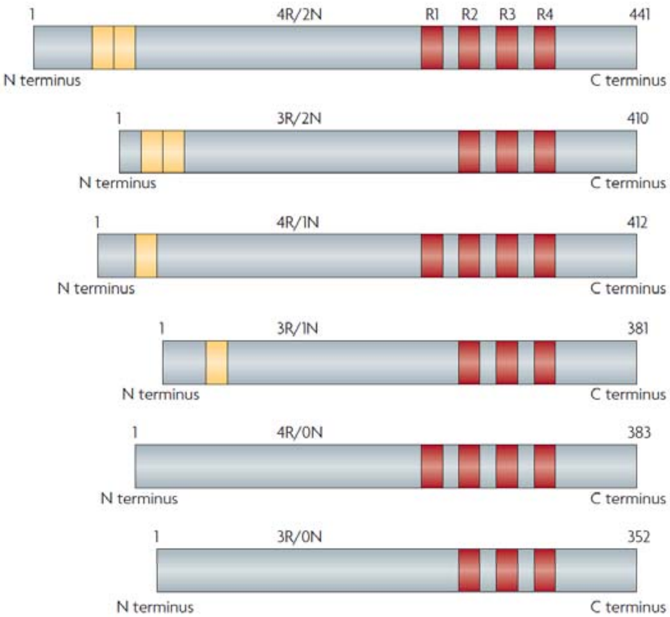
Microtubule Associated Proteins (II)

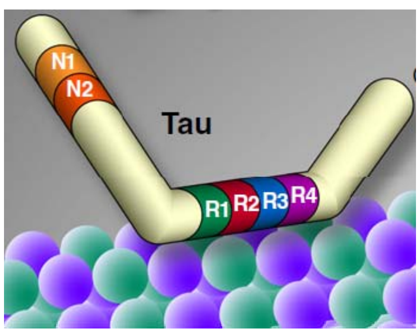


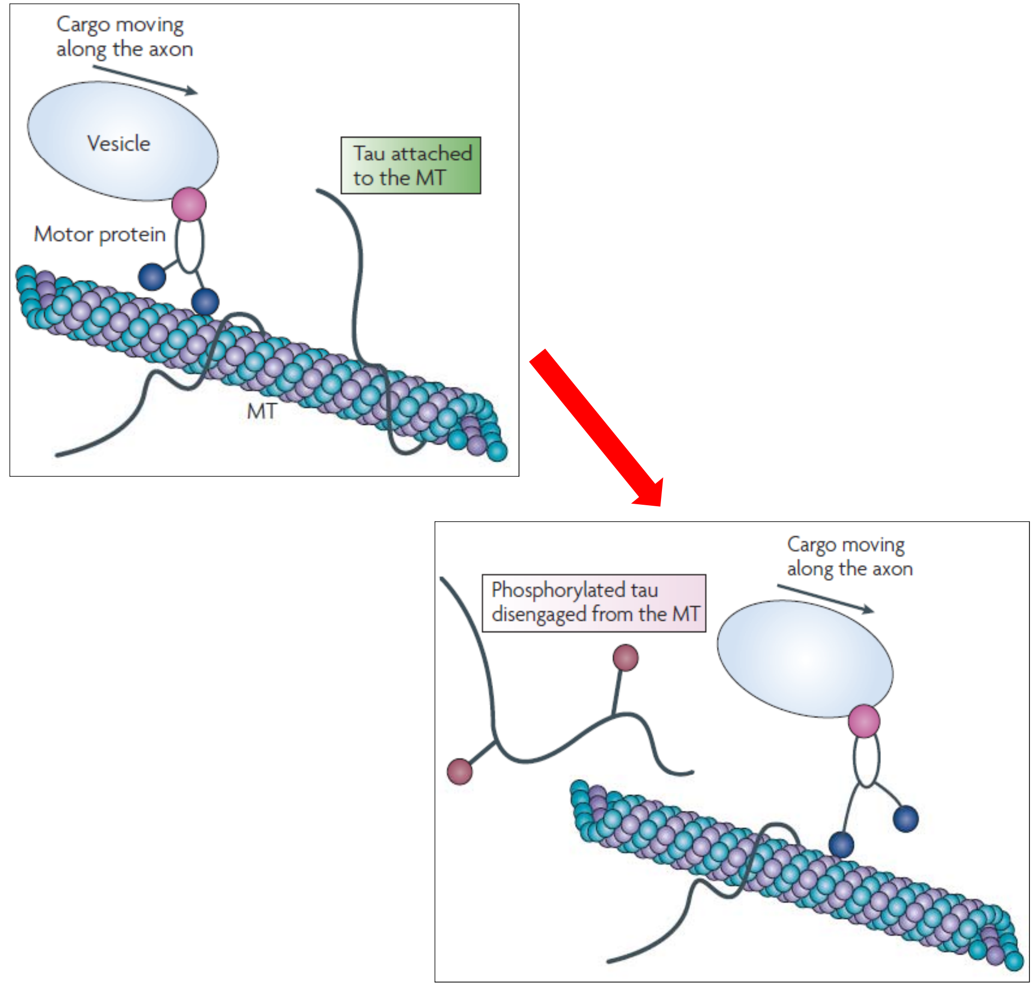
Frozen deep-etched, shadowed microtubules with (upper) and without (lower) tau

Fixed, embedded sections of microtubules with (upper) and without (lower) MAP2

Microtubule Associated Proteins (III): Tau



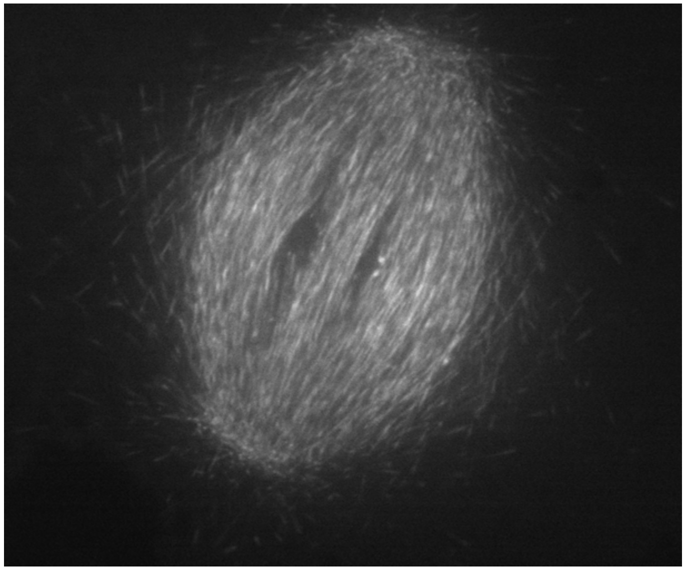


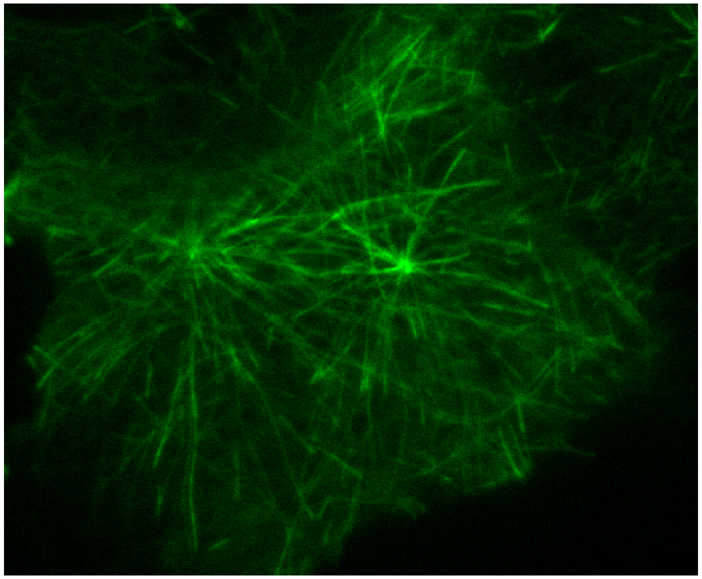


Ballatore et al, *Nat. Rev. Neurosci.* 2007
Morris et al, *Neuron*, 2011

Microtubule Associated Proteins (IV)

- CLIP170 is the first microtubule plus end tracking protein identified.
  - →Links membranes to growing plus ends.
  - →Binds the microtubule plus end to reduce catastrophe.
- EB1 is another microtubule plus end tracking protein identified.
  - → Binds the microtubule plus end to reduce catastrophe.



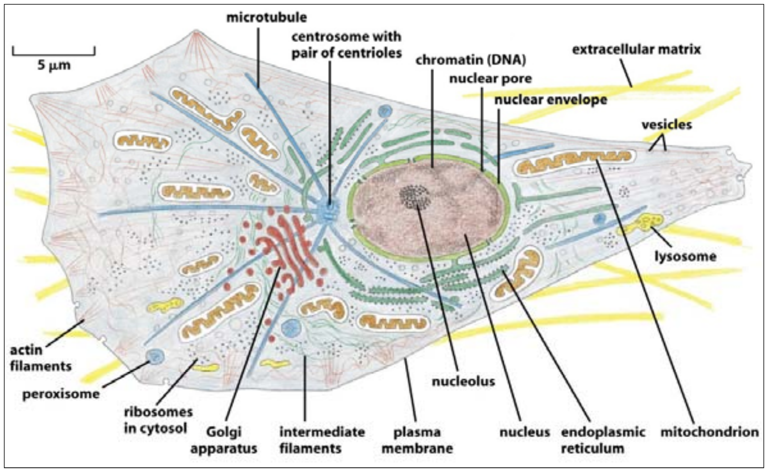


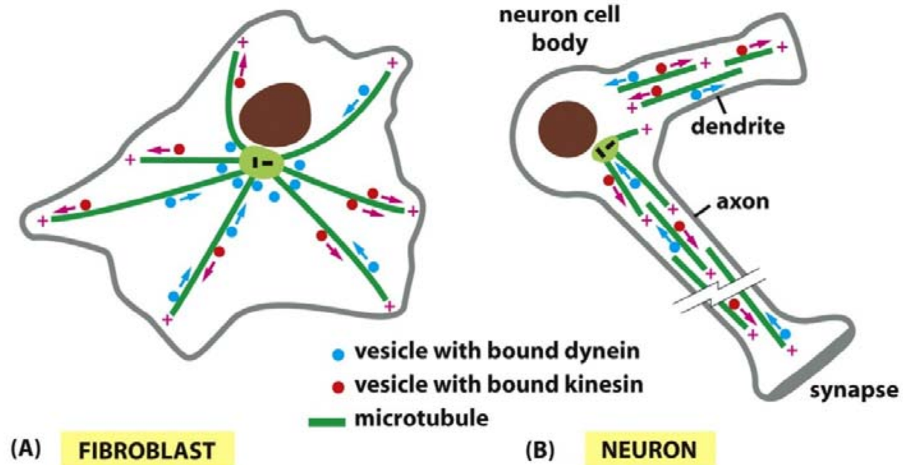
Dynamics and mechanics of the microtubule plus end. Howard J, Hyman AA. *Nature*. 2003 422(6933):753-8.

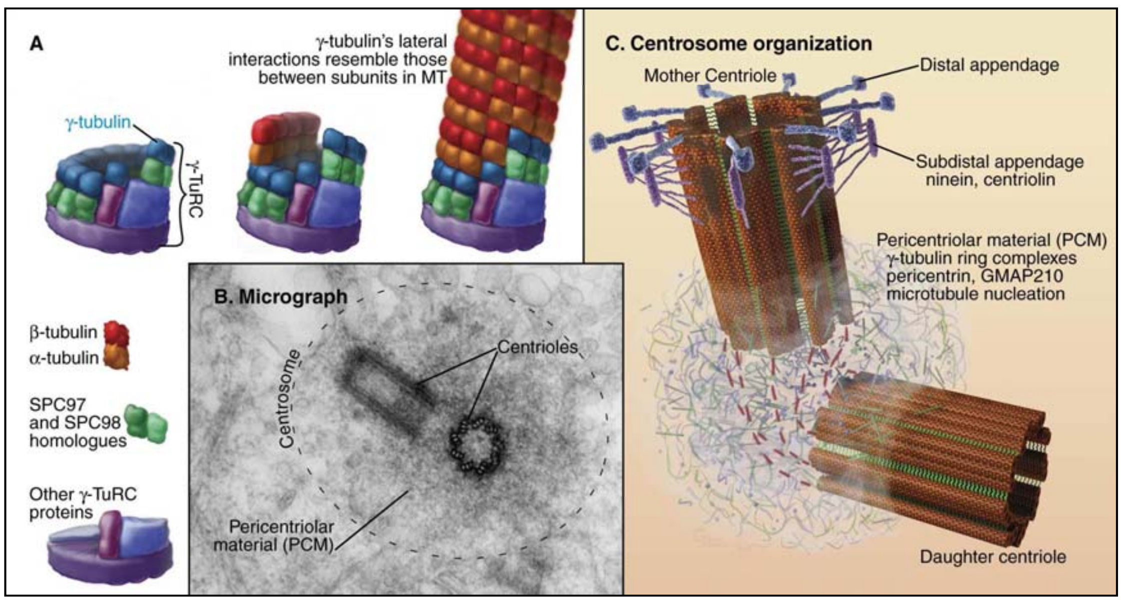
Summary: Microtubule

- Microtubule is relatively rigid (quantification in following lectures).
- Microtubule often forms bundles. Mechanical strength of microtubule networks comes mostly from bundling and crosslinking.
- Microtubule can withstand compression.
- Microtubule can denature easily and is therefore difficult to work with biochemically.

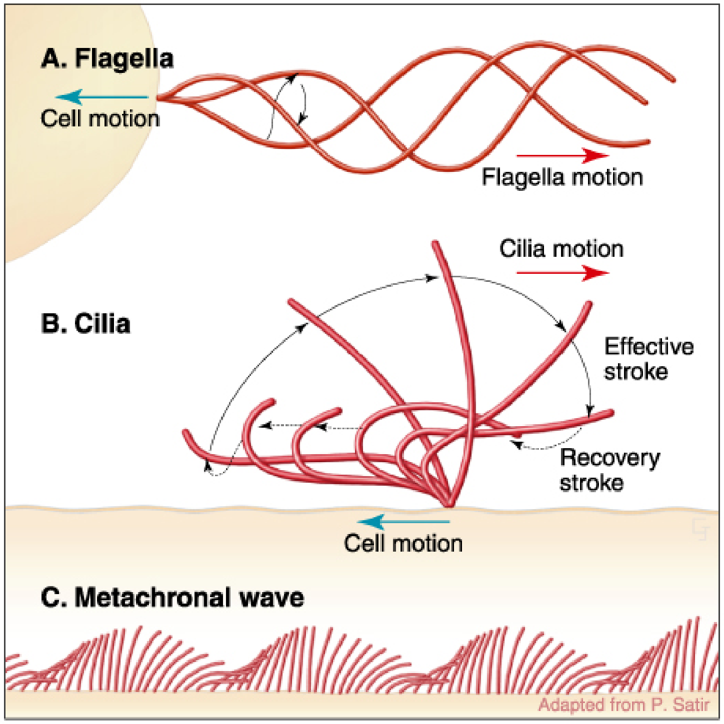
Centrosome and Centrioles



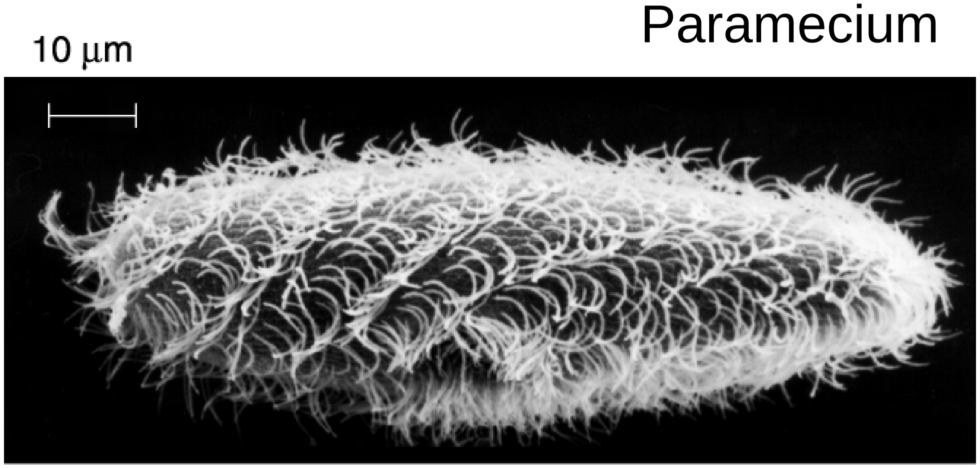




Centrioles can Form Basal Bodies for Cilia &Flagella



Paramecium



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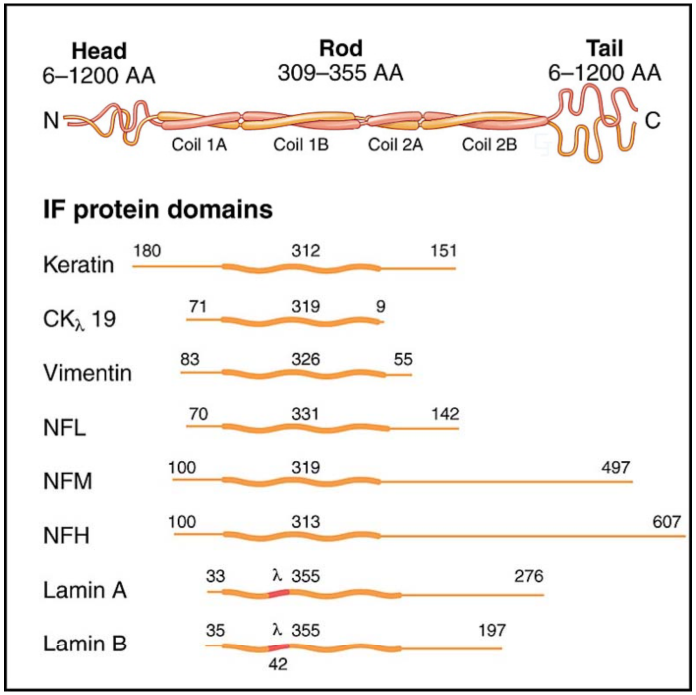
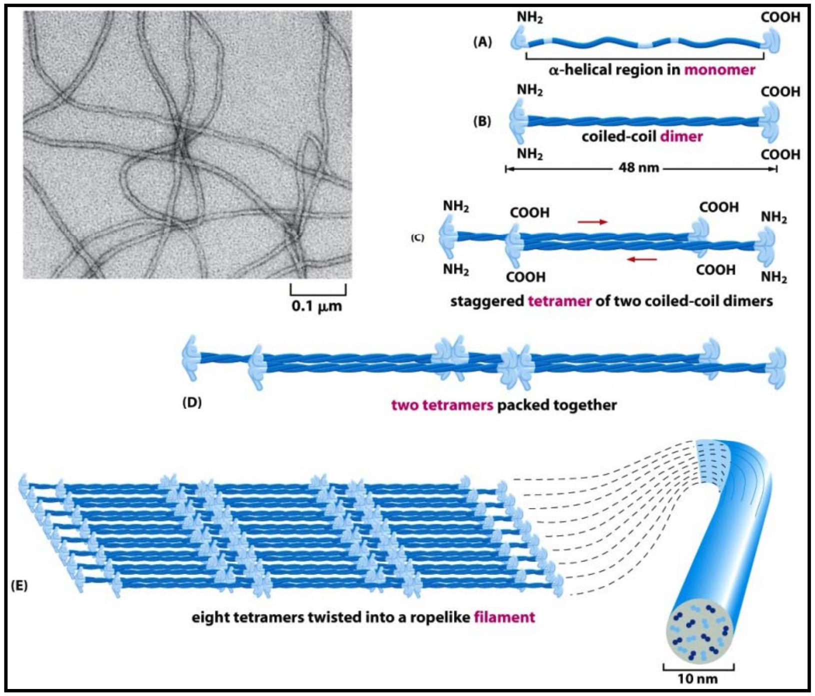
- Summary: actin and its associated proteins
- Microtubule and its associated proteins; Centrosome
- Intermediate filament and its associated proteins
- An overview of molecular motors

Intermediate Filament

- So named because of its diameter in striated muscles (diameter ~10nm).
- Its core structure is an α-helical coiled coil.
- N- and C-terminal domains vary considerably in size.





Intermediate Filament Classification

Table 35-1

CLASSIFICATION OF INTERMEDIATE FILAMENT PROTEINS BASED ON ROD DOMAIN SEQUENCES

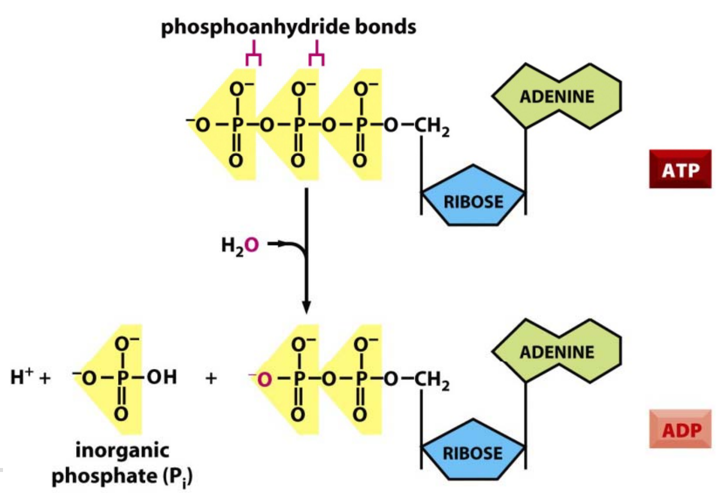
Class	Type	Genes	Molecule	Distribution	Diseases
I	Acidic keratin	>15	40–65 kD, obligate heterodimer with class II	Epithelial cells	Blistering skin, corneal dystrophy, brittle hair and nails
II	Basic keratin	>15	51–68 kD, obligate heterodimer with class I	Epithelial cells	Similar to class I
III	Desmin	1	53 kD, homopolymers	Muscle cells	Cardiac and skeletal myopathies
	GFAP	1	50 kD, homopolymers	Glial cells	Alexander disease; mouse null viable
	Peripherin	1	57 kD	Peripheral > CNS neurons	
	Synemin	1	190 kD, interacts with other class III IFs	Muscle cells	
	Vimentin	1	54 kD, homopolymers and heteropolymers	Mesenchymal cells	Mouse null viable
IV	Neurofilament				
	NFL	1	Obligate heteropolymers with NFM, NFH	Neurons	Mouse null viable; neuropathies
	NFM	1	Obligate heteropolymers with NFL, NFH	Neurons	
	NFH	1	Obligate heteropolymers with NFL, NFM	Neurons	Mutations a risk factor in amyotrophic lateral sclerosis
	α-Internexin	1	55 kD, homopolymers	Embryonic neurons	
V	Lamins	4	7 Isoforms, 62–72 kD, homodimers	Animal, plant nuclei	Cardiomyopathy, lipodystrophy, one form of Emery-Dreifuss muscular dystrophy, two forms of progeria plus many others
VI	Nestin	1	230 kD, homopolymers	Embryonic neurons, muscle, other cells	

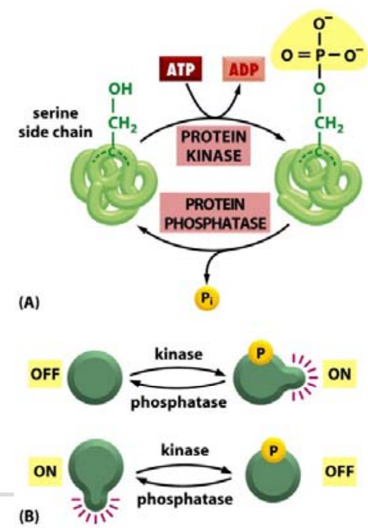
IF, intermediate filament; NFH, neurofilament heavy; NFL, neurofilament light; NFM, neurofilament medium.

Reference: Omary MB, Coulombe PA, McLean WHI: Intermediate filament proteins and their associated diseases. New Engl J Med 351:2087–2100, 2004.

Intermediate Filament

- Biochemically most stable among the three cytoskeletal filaments.
- Assembly disassembly regulated by phosphorylation
  - Phosphorylation: reaction in which a phosphate group is covalently coupled to another molecule.
  - Kinase: an enzyme that catalyzes the addition of phosphate groups
  - Phosphatase: an enzyme that catalyzes the removal of phosphate groups
- Phosphorylation may stabilize or destabilize intermediate filaments.





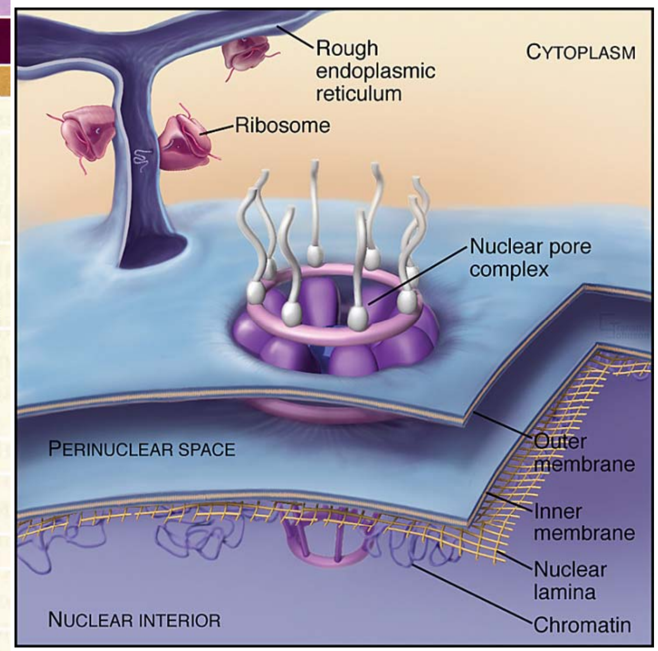
Intermediate Filament Associated Proteins

Table 35-2

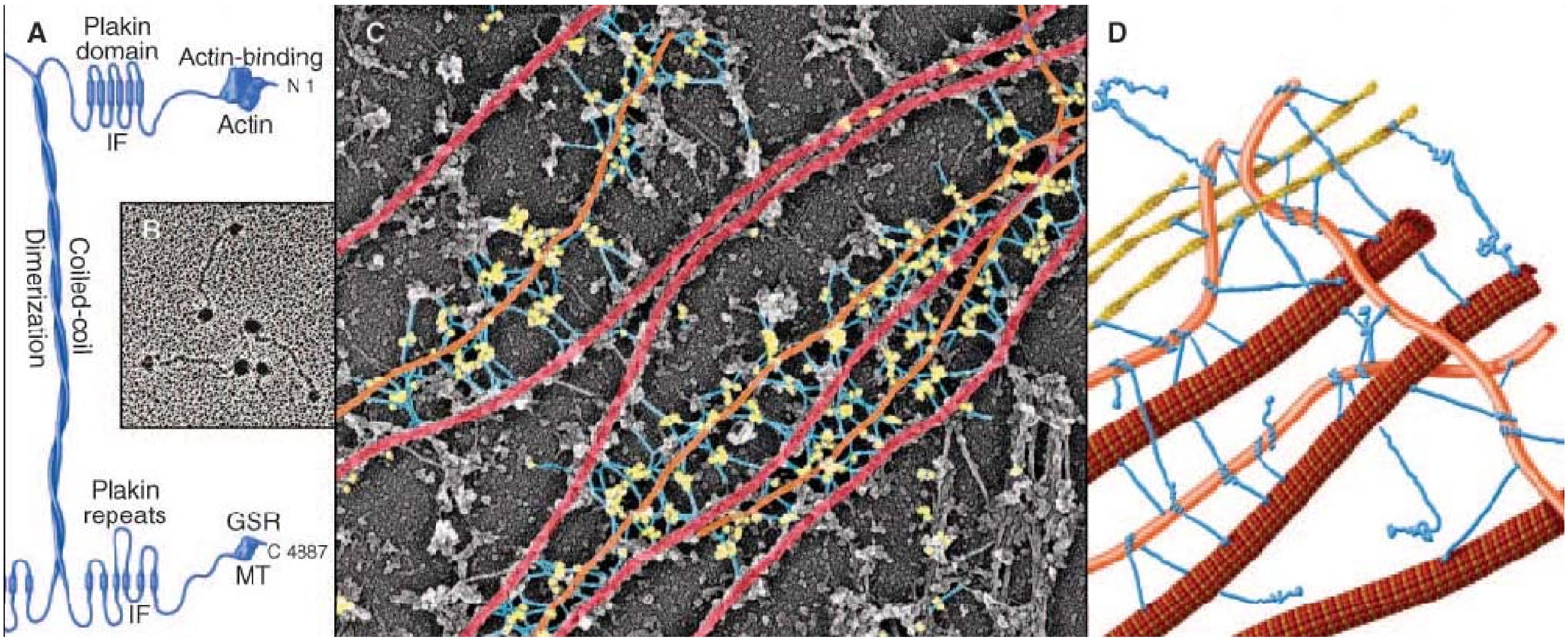
PROTEINS ASSOCIATED WITH INTERMEDIATE FILAMENTS

Name	Molecule	Distribution	Partners	Diseases
Plakins				
BPAG-1	Multiple splice isoforms (a, b, e, n) with ABDs and plakin domains ± spectrin and plakin repeats	a: Hemidesmosomes b: Muscle, cartilage e: Epithelial hemidesmosomes n: Neurons	IFs, MTs, actin	Autoimmune bullous pemphigoid
Desmoplakin	Two splice isoforms with plakin and coiled-coil domains and plakin repeats	Desmosomes	IFs; cadherin and other desmosome proteins	Autoimmune pemphigus; genetic striate palmoplantar keratoderma
Plectin	Multiple splice isoforms; ABD, plakin domain and plakin repeats	Most tissues except neurons	IFs, actin, MTs, spectrin, β4 integrin	Autoimmune pemphigus; genetic epidermolysis bullosa with muscular dystrophy
Epidermal				
Filaggrin	Ten 37-kD filaggrins cut by proteolysis from profilaggrin	Cornified epithelia	Aggregates keratin	?
Lamin Associated				
LAP1	57–70 kD isoforms	Integral nuclear membrane proteins	Binds laminin to nuclear envelope	
LAP2	50 kD	Integral nuclear membrane protein	Binds laminin to nuclear envelope	
LBR	73 kD	Integral nuclear membrane protein	Binds laminin to nuclear envelope	Pelger-Huët anomaly; Greenberg skeletal dysplasia
Emerin	34 kD	? Peripheral protein of the inner nuclear membrane	? Nucleates and binds actin filaments to the nuclear envelope	Emery-Dreifuss muscular dystrophy

ABD, actin binding domain; IFs, intermediate filaments; MTs, microtubules.



Plectin Connects IFs to Actin & Microtubules



Microtubule: red
Intermediate filament: orange

Intermediate Filaments

• Inter mediate filaments are flexible but stable.

• Primary assumed function of intermediate filaments is to prevent excessive stretching.

• In general, much less is known about intermediate filaments compared to actin and microtubule.

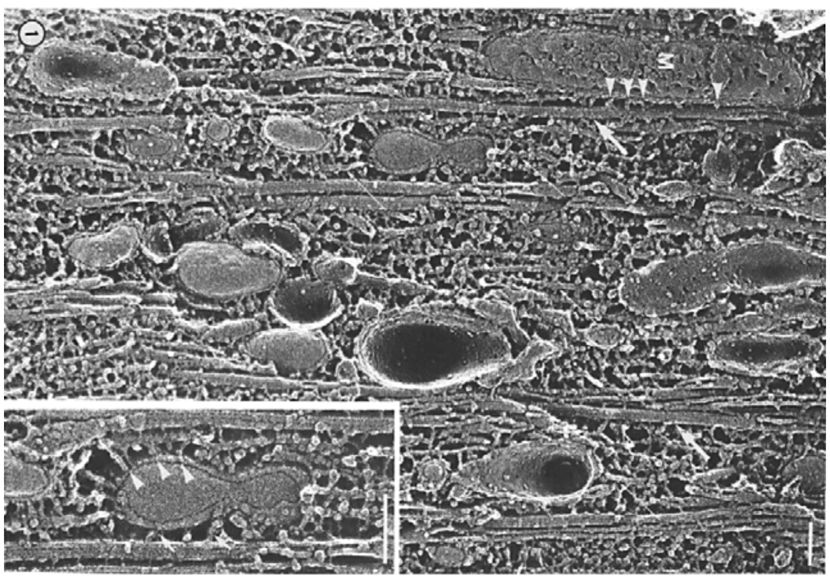
An Example: Microtubule, Intermediate Filament & Organelles in a Frog Axon

Cross-linker System between Neurofilaments, Microtubules, and Membranous Organelles in Frog Axons Revealed by the Quick-freeze, Deep-etching Method

NOBUTAKA HIROKAWA
Department of Physiology and Biophysics, Washington University School of Medicine, St. Louis, Missouri 63110

N. Hirokawa, J. Cell Biol. 94:129, 1982

Nobutaka Hirokawa
University of Tokyo



Bar: 0.1μm

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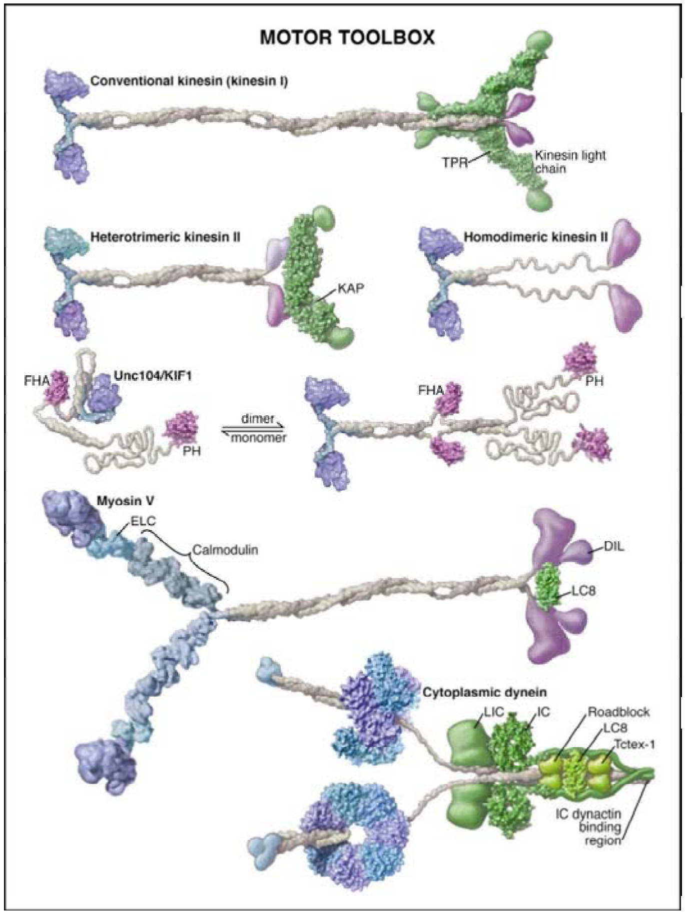
Overview of Molecular Motors

- Myosin binds and walks on actin.
- Kinesin and dynein binds and walks on microtubule.



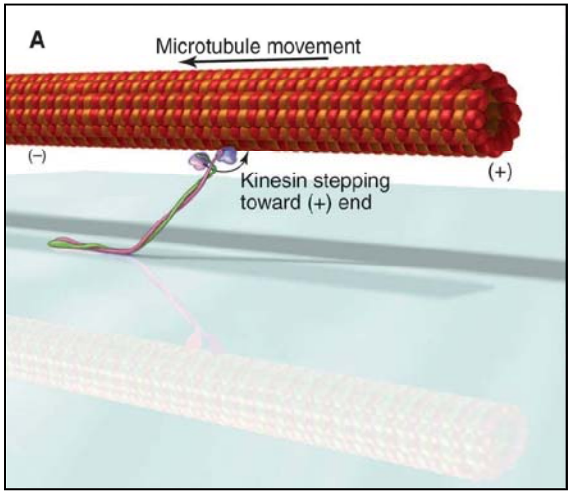
Vale RD, *Cell*, 112:467,2003

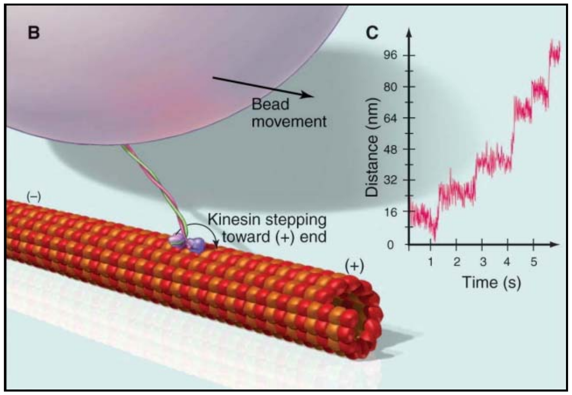
Molecular Motors and Motility Assays

- Actin motor
  - myosin
  - Usually for short-distance movement

- Microtubule motors
  - kinesin
  - dynein
  - Usually for long-distance movement

- In vitro motility assays
  - bead assay
  - microtubule sliding assay





Bead Motility Assay Video

kinesin-coated bead moves along a microtubule

Block Lab, Stanford

Many Molecules Generate Intracellular Force & Motion

Table 36-1

EXAMPLES OF MECHANOCHEMICAL ATPASES AND OTHER SYSTEMS

Families	Track	Direction	Cargo	Energy
ATPases				
Myosins				
Muscle myosin	Actin	Barbed end	Myosin filament	ATP
Myosin II	Actin	Barbed end	Myosin, actin	ATP
Myosin I	Actin	Barbed end	Membranes	ATP
Myosin V	Actin	Barbed end	Organelles	ATP
Myosin VI	Actin	Pointed end	Endocytic vesicles	ATP
Dyneins				
Axonemal	Microtubule	Minus end	Microtubules	ATP
Cytoplasmic	Microtubule	Minus end	Membranes, chromosomes	ATP
Kinesins				
Conventional	Microtubule	Plus end	Membranes, intermediate filaments	ATP
Ncd	Microtubule	Minus end	? Microtubules	ATP
Other Mechanochemical Systems				
Polymerases				
Ribosome	mRNA	5′ to 3′	None	GTP
DNA polymerase	DNA	5′ to 3′	None	ATP
RNA polymerase	DNA	5′ to 3′	None	ATP
Conformational System				
Spasmin/centrin	None	None	Cell, basal body	Ca^{2+}
Polymerizing Systems				
Actin filaments	None	Barbed end	Membranes	ATP
Microtubules	None	Plus end	Chromosomes	GTP
Worm sperm MSP	None	Not polar	Cytoskeleton	
Rotary Motors				
Bacterial flagella	None	Bidirectional	Cell	H^+ or Na^+ gradient
F-type ATPase	None	Bidirectional	None	H^+ or ATP
V-type ATPase pump	None		None	ATP

mRNA, messenger RNA; MSP, major sperm protein.

Relations Between Molecular Motors and Cytoskeleton Polymers

- Interactions between motors and cytoskeletal polymers are dynamic and complex.

- Cytoskeletal polymers provide dynamic tracks for molecular motors to walk on.

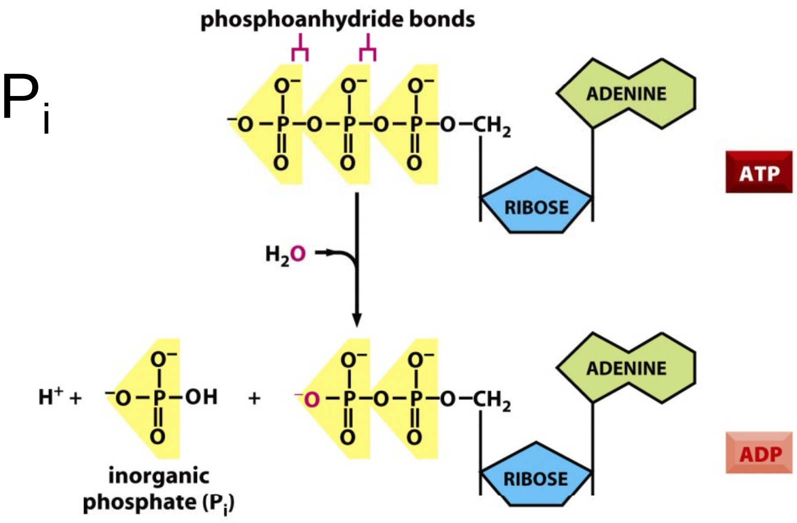
- Molecular motors active interacts with cytoskeletal polymers. For example,
    - Molecular motors transport cytoskeletal polymers, especially in neurons.
    - Molecular motors, e.g. MCAK, regulate cytoskeletal polymer dynamics.

Molecular Motors Are ATP-Hydrolysis Enzymes

- Molecular motors convert chemical energy derived from ATP hydrolysis directly into mechanical work.

- ATP (adenosine triphosphate) hydrolysis

$$ATP + H_2O \rightleftharpoons ADP + P_i$$



Motor Behavior Parameters

- Parameters that characterizing motor behaviors
  - processivity: run-length, number of steps
  - step size
  - stall force
- Myosin is nonprocessive.
- Kinesin and dynein are both processive. Processivity of dynein is weaker.
- Motors walk nano-meter scale steps of specific lengths.
- Stall force is on the pico-Newton level.

Analyzing Motor Movement at Nanometer Resolution

- Nanometer-resolution measurement of step sizes
  - First implemented in late 1980's
  - Based on fitting of point spread function

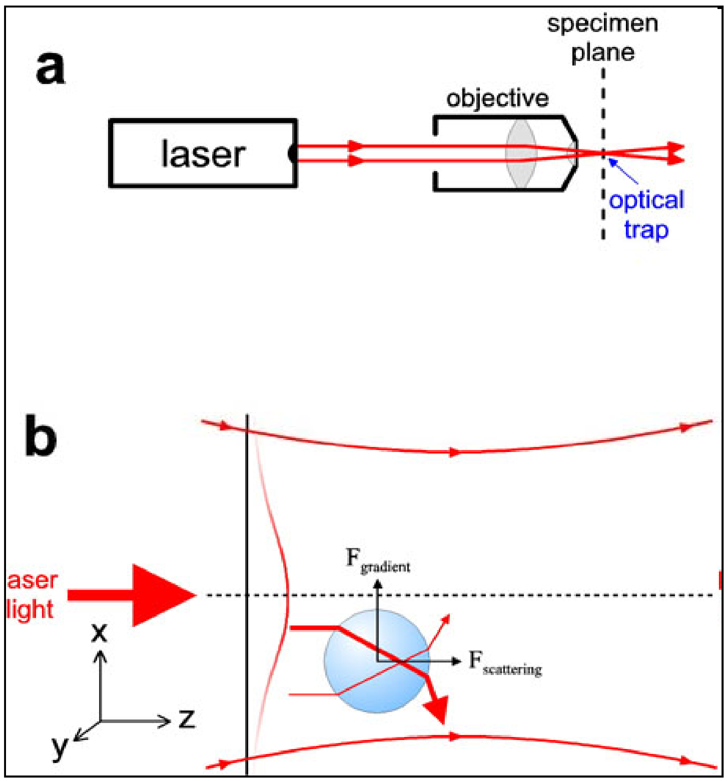
  Tracking kinesin-driven movements with nanometre-scale precision. Gelles J, Schnapp BJ, Sheetz MP. *Nature*. 331:450-3 (1988).

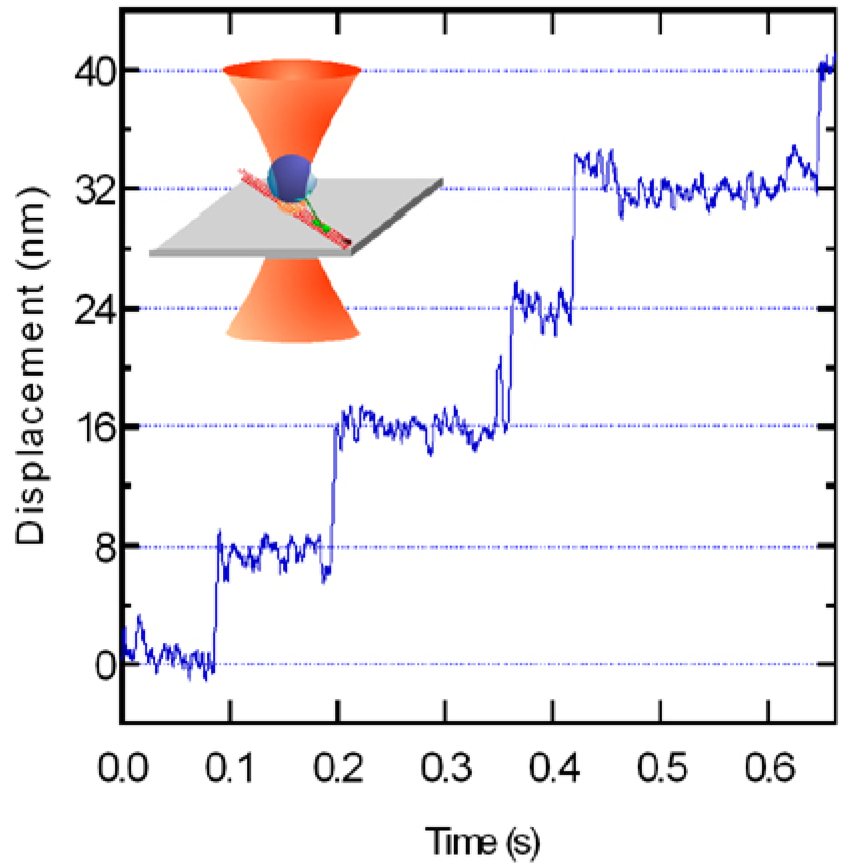
- Further improved by many others
  - Up to 1nm resolution

  Ahmet Yildiz, Paul R.Selvin. Fluorescence Imaging with One Nanometer Accuracy (FIONA): Application to Molecular Motors, *Accounts of Chemical Research*,38(7), 574-82 (2005)

Analyzing Motor Force at Piconewton Resolution

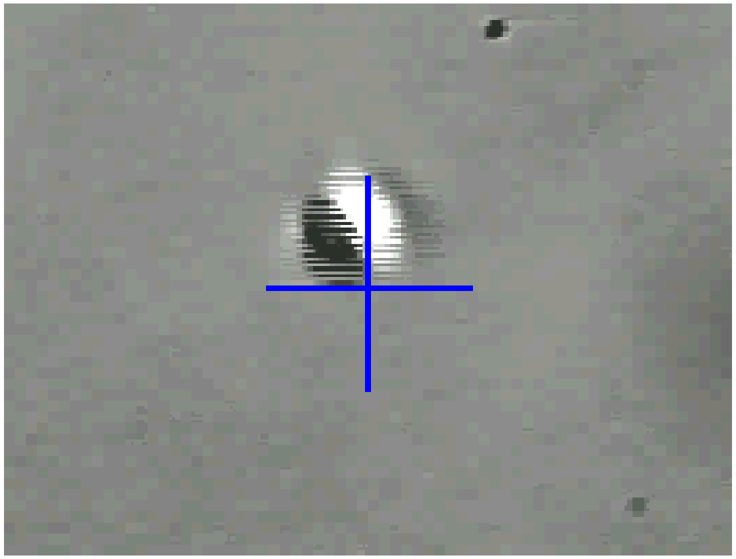
- Optical tweezer is used to generate and measure motor stall force.





From Steven Block Lab webpage
http://www.stanford.edu/group/blocklab/

Laser Force Trap Video

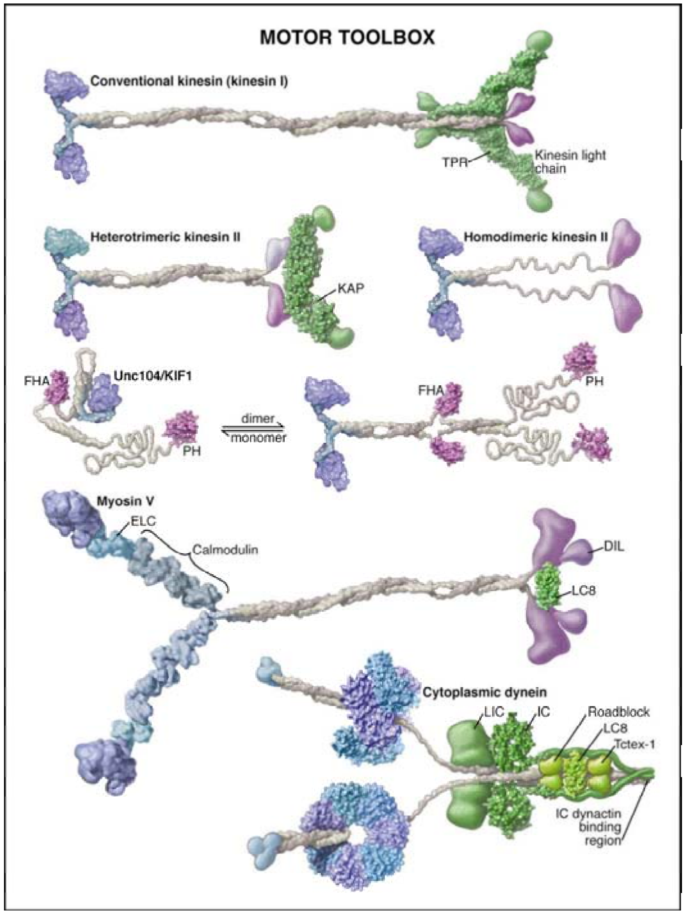


kinesin-coated bead moves in a force trap

Block Lab, Stanford

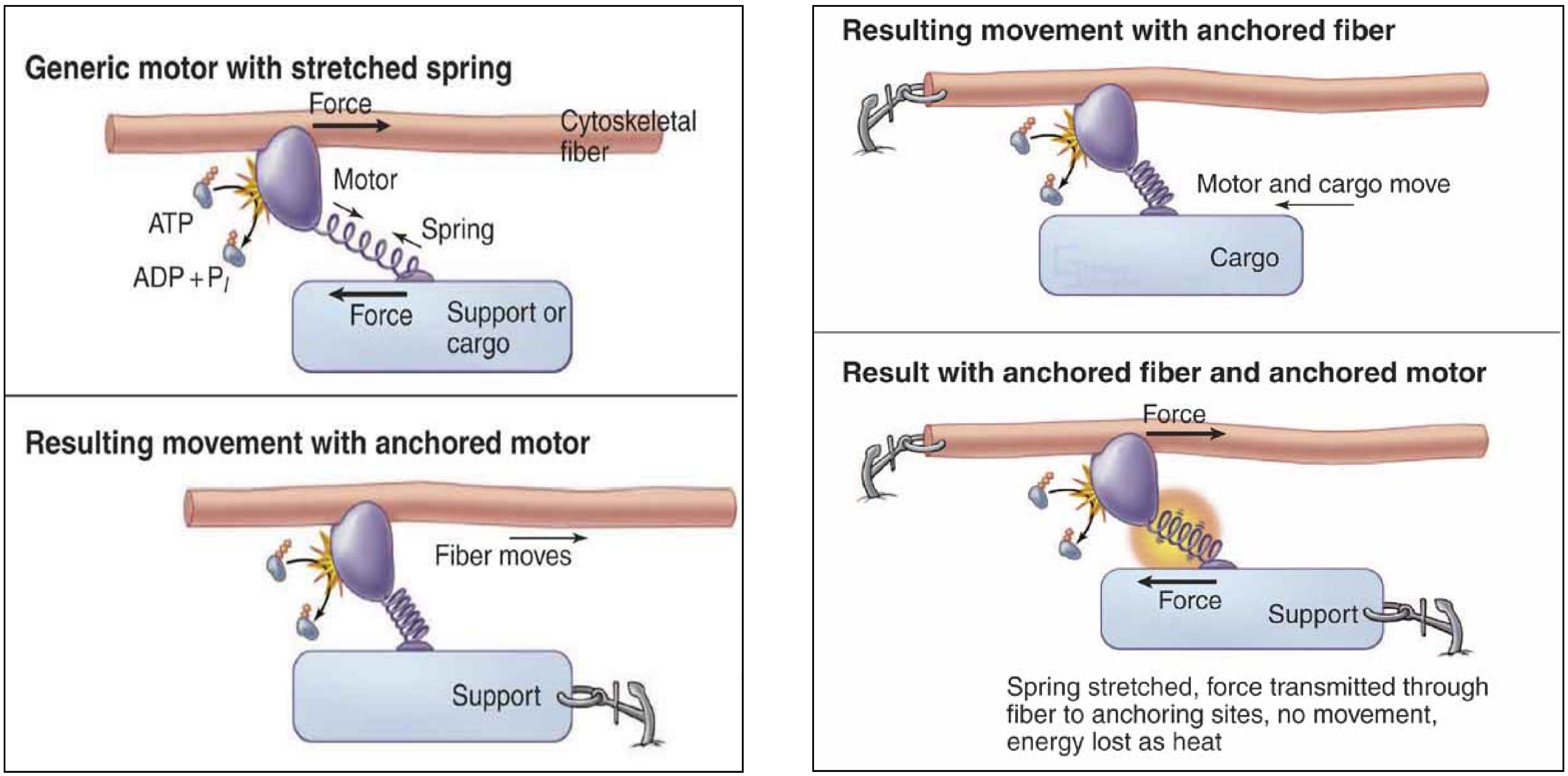
General Motor Structure

- Motor (head) domain
  - Produces force and motion
- Tail domain
  - Adapts to different cargoes



Vale RD, *Cell*, 112:467,2003

Different Motility Schemes



Required Reading

- Chapters 2 & 16

Questions ?