BME 42-620 Engineering Molecular Cell Biology

Lecture 09:

The Cytoskeleton (I): Actin

The Cytoskeleton (II): Microtubule & Intermediate Filament

Outline

- Overview of cytoskeletal filaments
- Actin and its associated proteins
- Microtubule and its associated proteins; Centrosome
- Intermediate filament and its associated proteins

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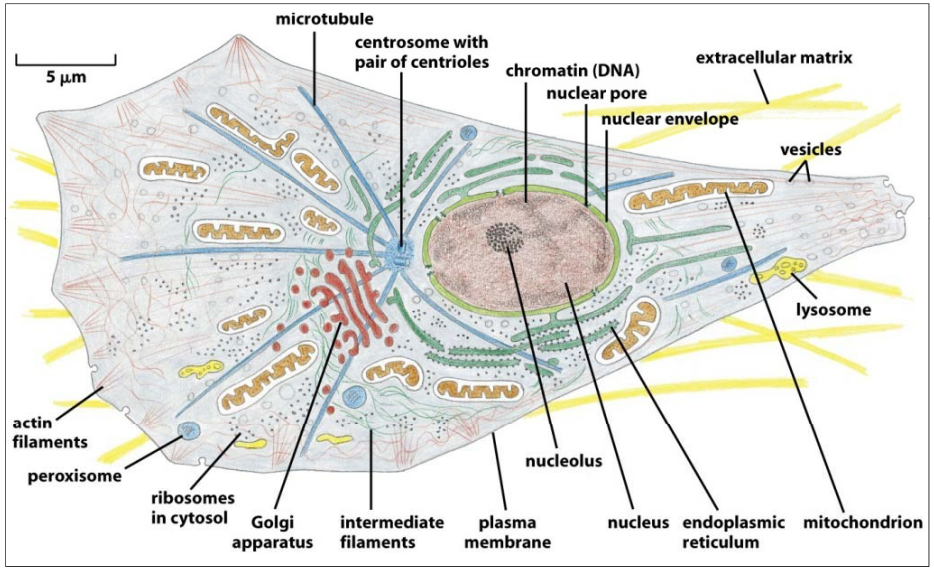
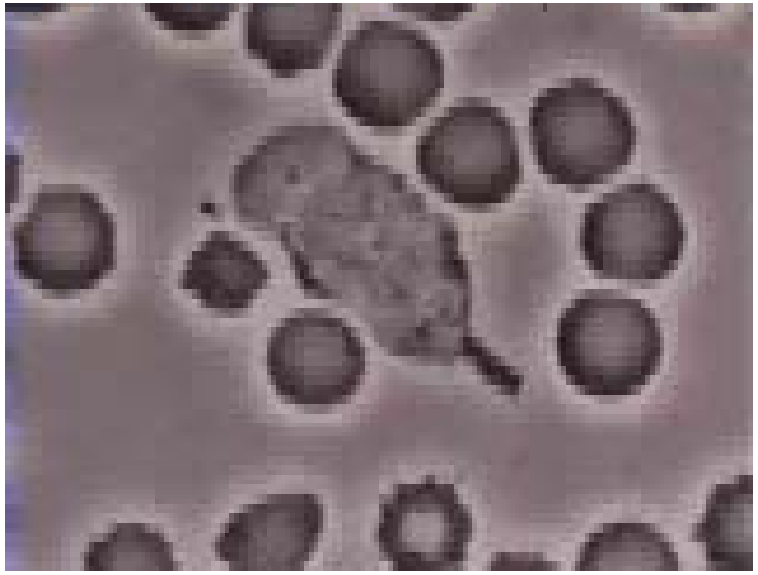
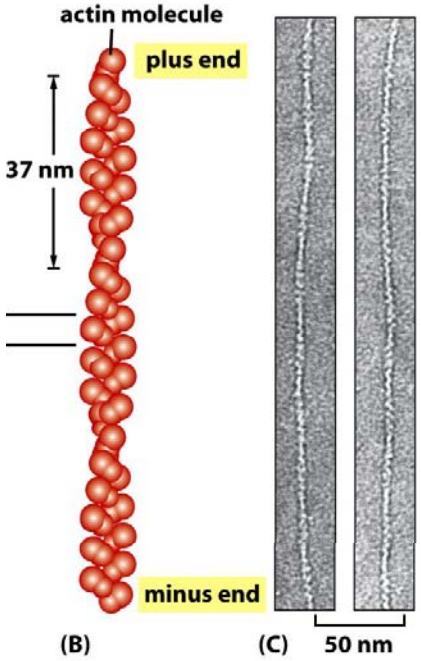
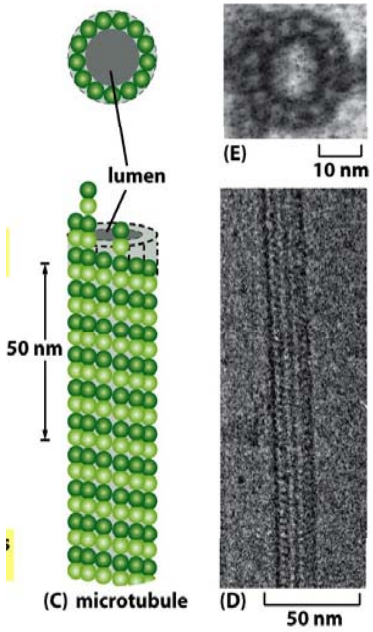
The Cytoskeleton is Highly Dynamic and Regulated

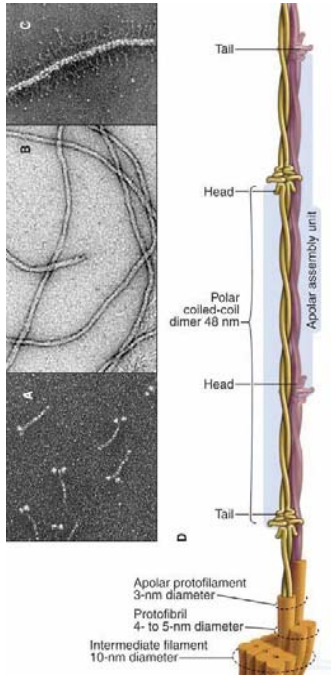




Overview of Cytoskeletal Filaments







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

Catalyzed and Regulated Self-Assembly of Macromolecules (I)

- Binding specificity between monomers is determined by multiple weak bonds on complementary surfaces.

- Demo: poliovirus capsid self assembly

Olson, A. J., Hu, Y. H. E., & Keinan, E. (2007). Chemical mimicry of viral capsid self-assembly. *Proceedings of the National Academy of Sciences*, 104(52), 20731-20736.

Catalyzed and Regulated Self-Assembly of Macromolecules (II)

- Cellular macromolecules often form through self-assembly of small subunits.

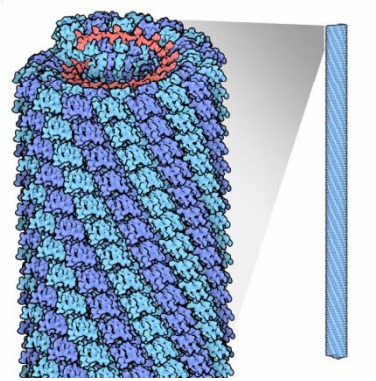
- This process requires energy and is often catalyzed and regulated.

- Advantages:

  - Reduced possibility of error
  - Possibility for repair
  - Subunit can be recycled
  - Assembly provides multiple entry points for regulation
    - *Regulation of subunit synthesis and degradation*
    - *Regulation of nucleation*
    - *Regulation by changing environmental conditions*
    - *Regulation by modifying subunits*
    - *Regulation using accessory proteins*

Example: Self-Assembly of a Tobacco Mosaic Virus

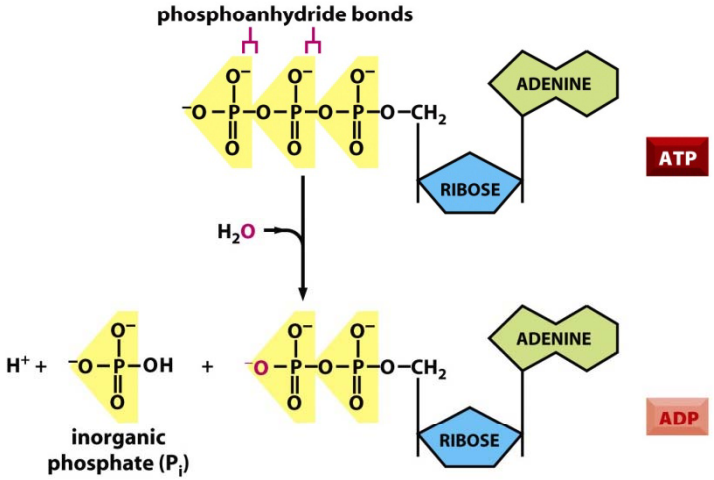
- The virus is made of 2130 protein subunits of 158 amino acids each.
- It also has a single-stranded RNA of 6390 nucleotids.
- Translation error → 1 out of 3000 amino acids (0.99967)
  Probability of making a correct TMV = 0.99967^{158}=0.949
- If instead the TMV is assembled from one large sequence of amino acid, probability of making a correct TMV = 0.99967^{336540}=$1.87X10^{-49}$
- Self-assembly is one of the central principles in biology.
- The assembly information is encoded in weak but highly specific noncovalent interactions.

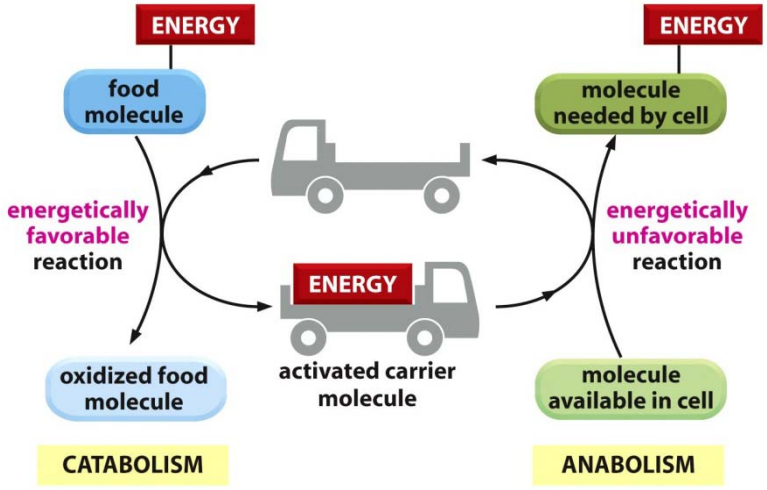


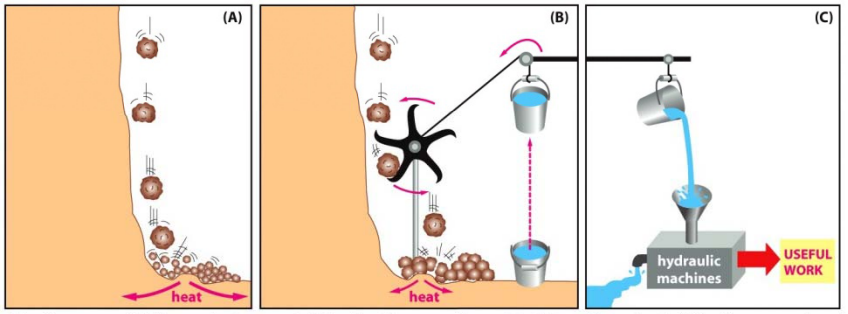
Pollard & Earnshaw, *Cell Biology*, 2/e, 2008, Chapter 5,

ATP (adenosine triphosphate) (I)

- ATP is the most widely used energy carrier in cells.







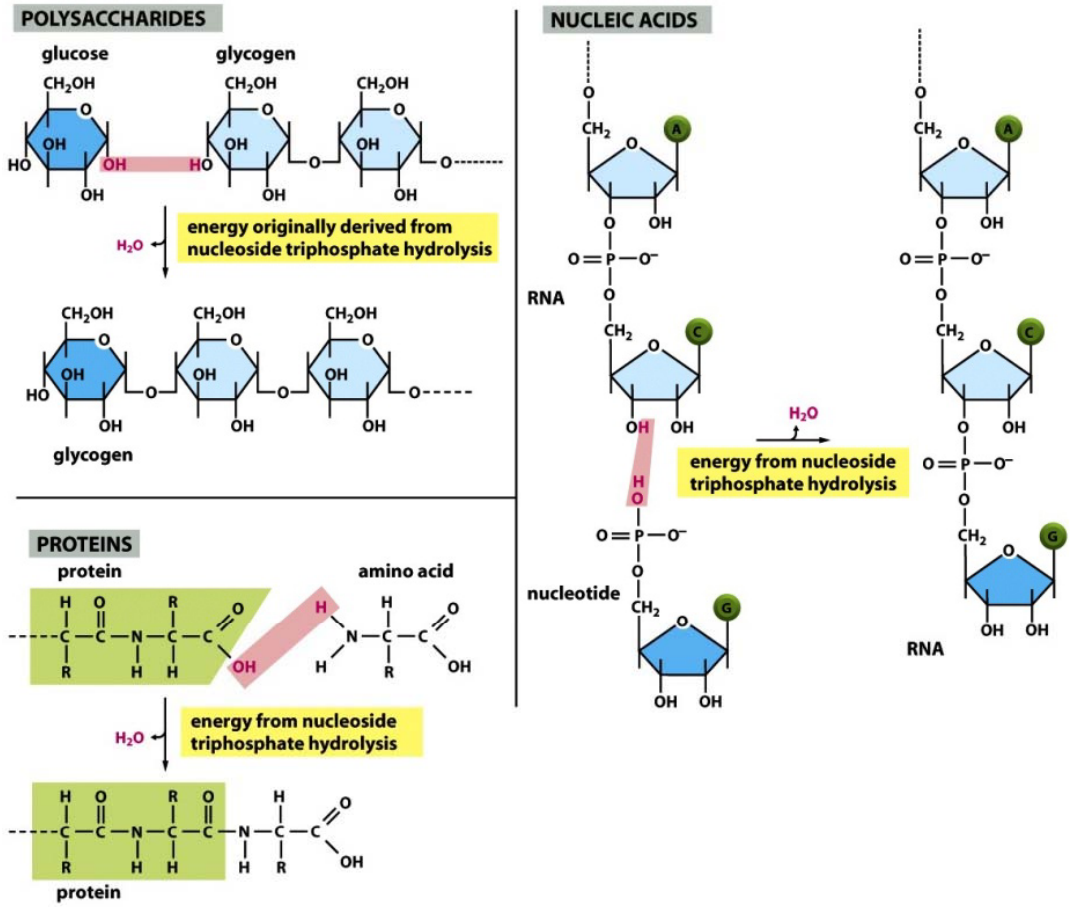
kinetic energy of falling rocks is transformed into heat energy only

part of the kinetic energy is used to lift a bucket of water, and a correspondingly smaller amount is transformed into heat

the potential kinetic energy stored in the raised bucket of water can be used to drive hydraulic machines that carry out a variety of useful tasks

ATP (adenosine triphosphate) (II)

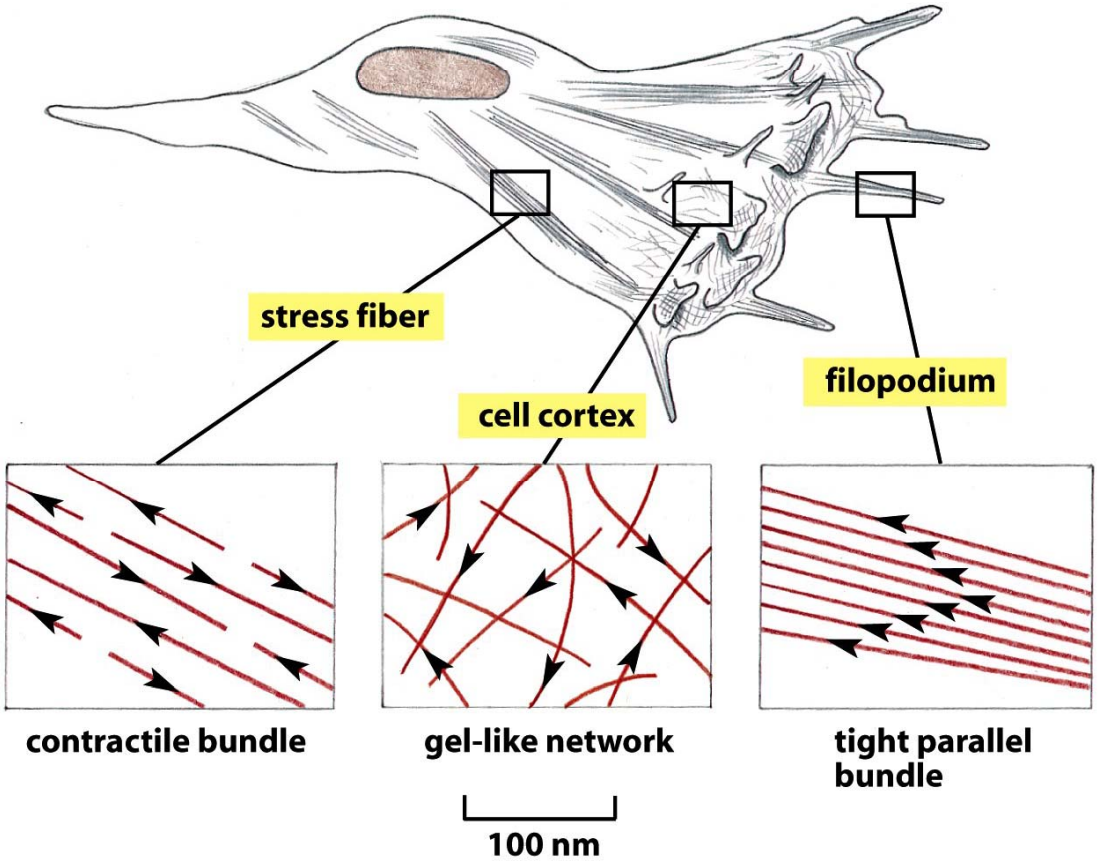
- Synthesis of biological polymers is driven by ATP hydrolysis.



Outline

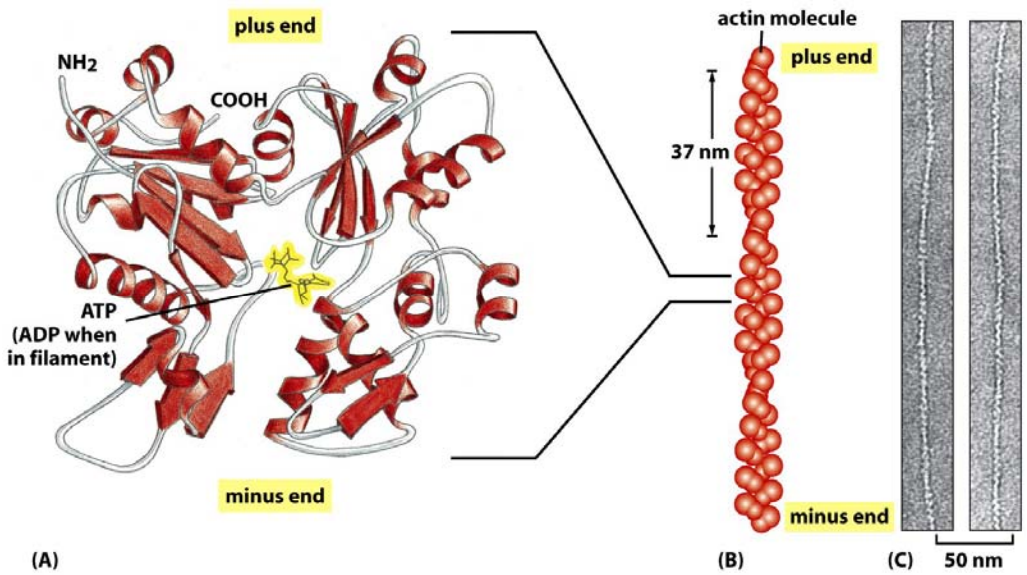
- Overview of cytoskeletal filaments
- Actin and its associated proteins
- Microtubule and its associated proteins; Centrosome
- Intermediate filament and its associated proteins

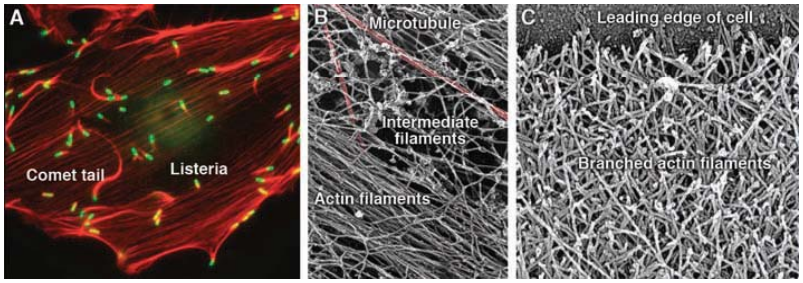
Organization of Actin with a Cell



Actin Structure and Function

- Each actin subunit is a globular monomer.
- One ATP binding site per monomer.
- Functions
  - Cell migration
  - Cell shape
  - Used as tracks for myosin for short distance transport





Pollard & Cooper, Science,326-1208, 2009

Basics Terms of Chemical Reaction Kinetics

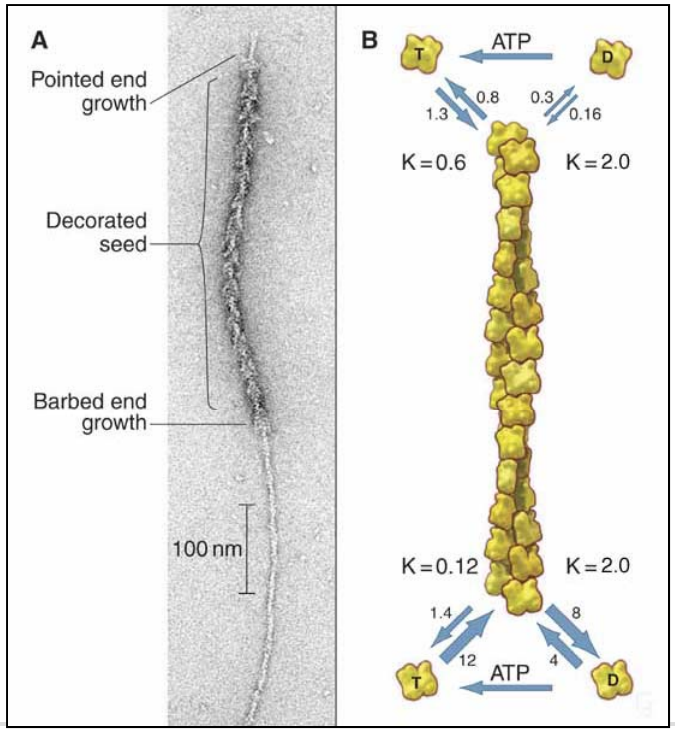
- A reversible bimolecular binding reaction

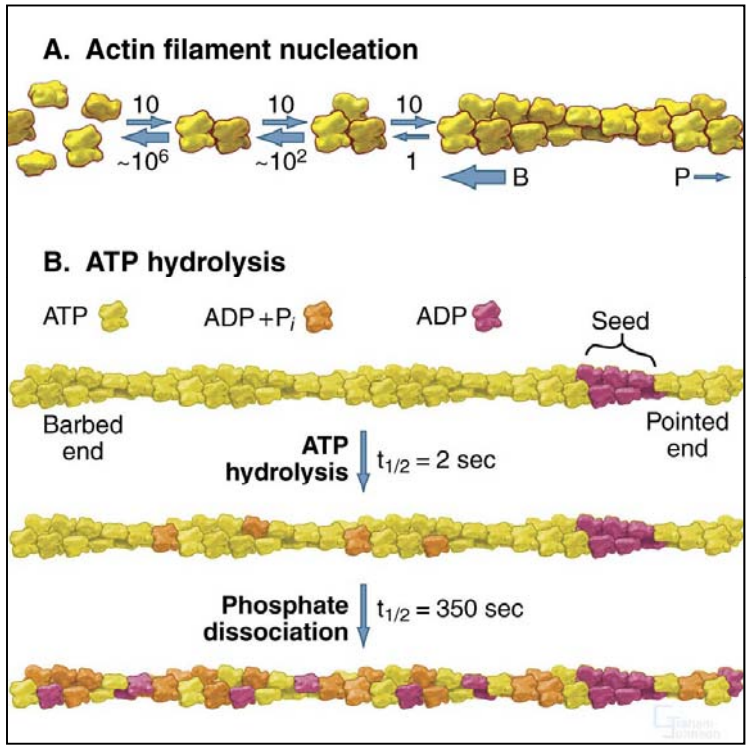
$$A + B \rightleftharpoons AB$$

- Rate of association = $k_+[A][B]$
- Rate of disassociation = $k_-[AB]$
- At equilibrium $k_+[A][B] = k_-[AB]$

Actin Nucleation and Nucleotide Hydrolysis

- Actin polymerizes and depolymerizes substantially faster at the plus end (barbed end) than at the minus end (pointed end).



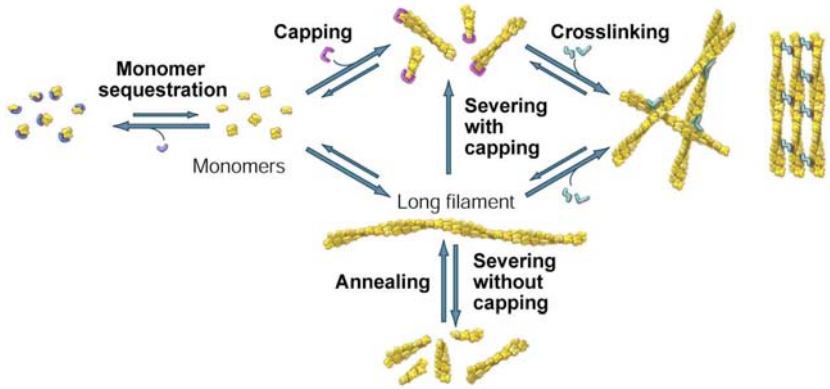


Actin Associated Proteins (I)

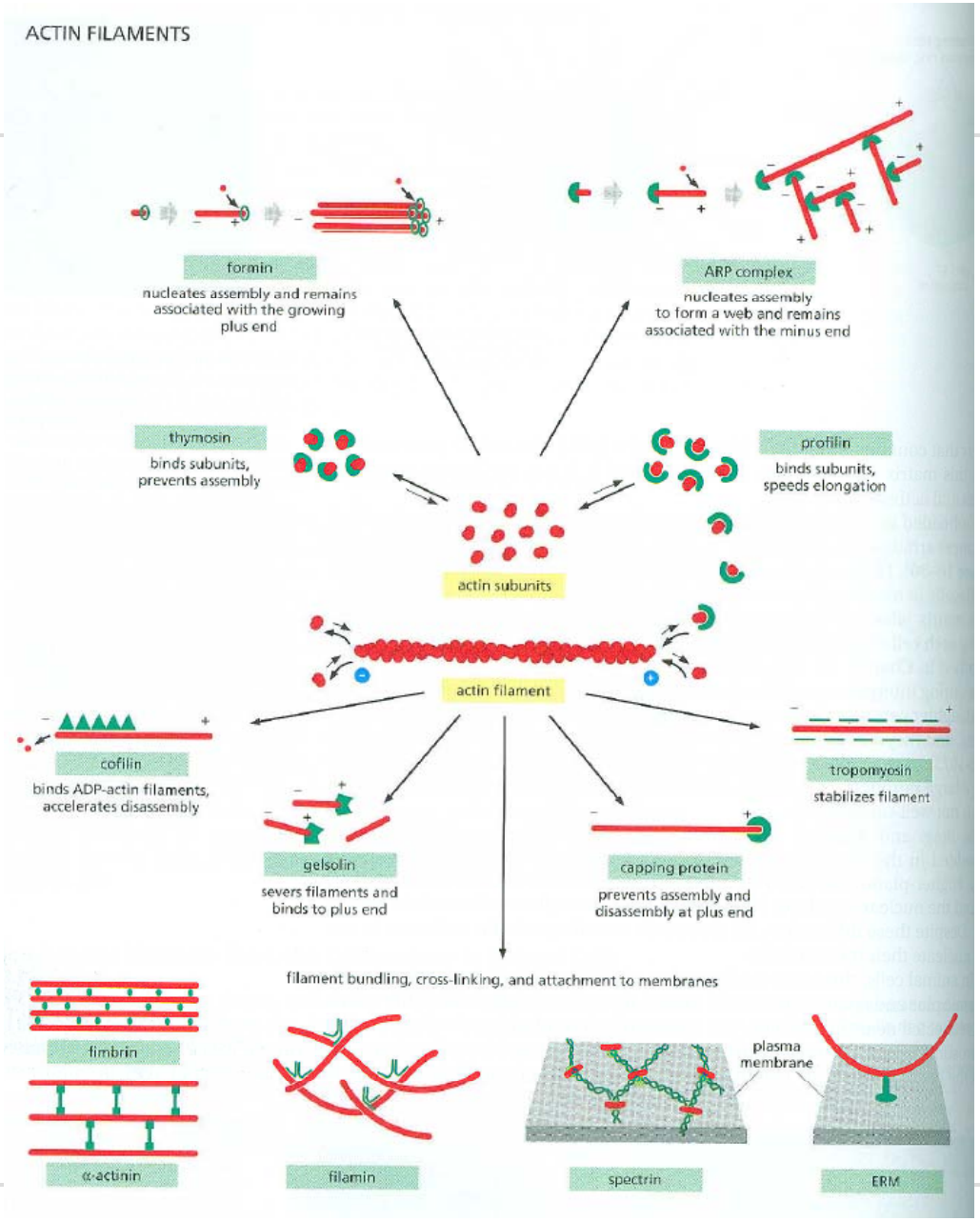
- More than 100 associated proteins identified so far.

- Functions
  - Monomer binding
  - Nucleation
  - Filament capping
  - Filament severing
  - Filament side-binding and supporting
  - Filament crosslinking

  - Signaling adapter

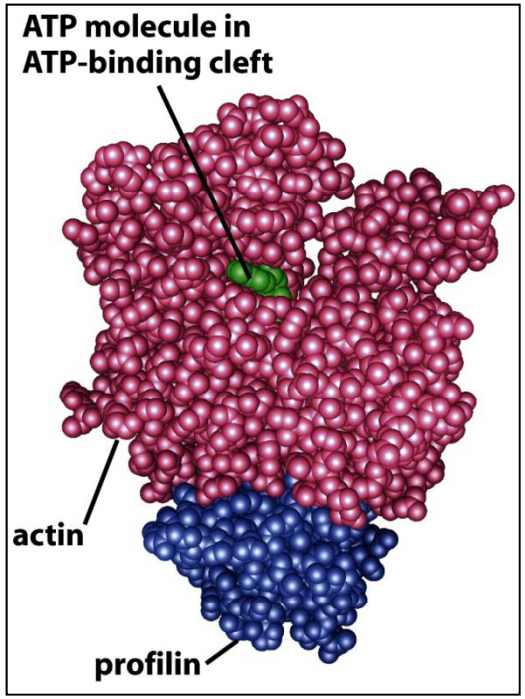


- Functional overlap and collaboration between actin-binding proteins


ACTIN FILAMENTS
formin
nucleates assembly and remains associated with the growing plus end
ARP complex
nucleates assembly to form a web and remains associated with the minus end
thymosin
binds subunits, prevents assembly
profilin
binds subunits, speeds elongation
actin subunits
actin filament
cofilin
binds ADP-actin filaments, accelerates disassembly
tropomyosin
stabilizes filament
gelsolin
severs filaments and binds to plus end
capping protein
prevents assembly and disassembly at plus end
filament bundling, cross-linking, and attachment to membranes
fimbrin
α-actinin
filamin
plasma membrane
spectrin
ERM

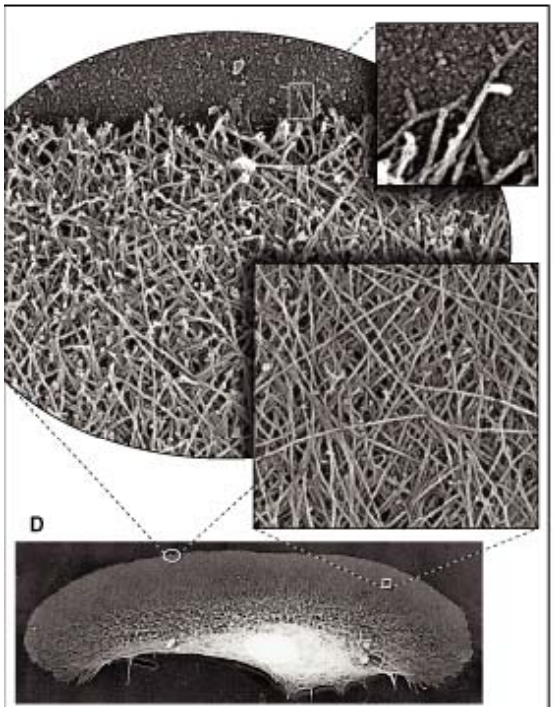
Actin Associated Proteins (II)

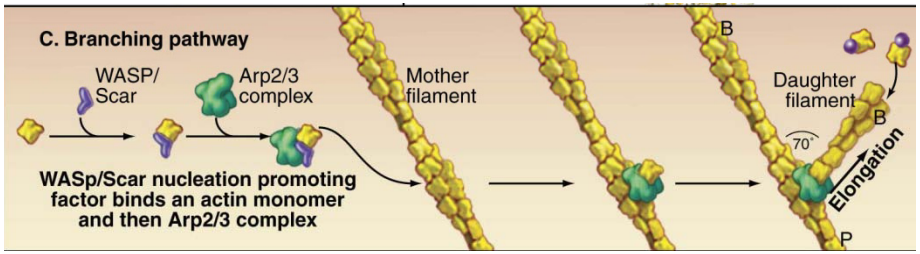
- Monomer binding proteins
  - profilin: to bind actin monomer and accelerate elongation
  - thymosin: to bind and lock actin monomer
  - ADF/cofilin: to bind and destabilize ADP-actin filaments

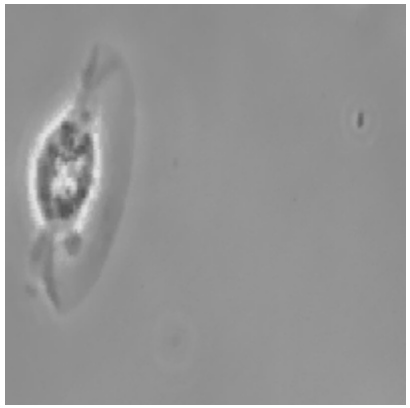


Actin Associated Proteins (III)

- Actin nucleation
  - Formins: to initiate unbranched actin filaments
  - Arp2/3: to bind the side of actin and initiate branching







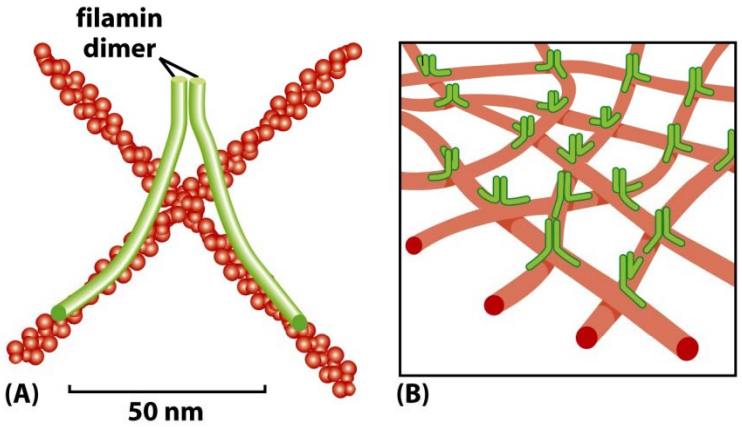
Actin Associated Proteins (IV)

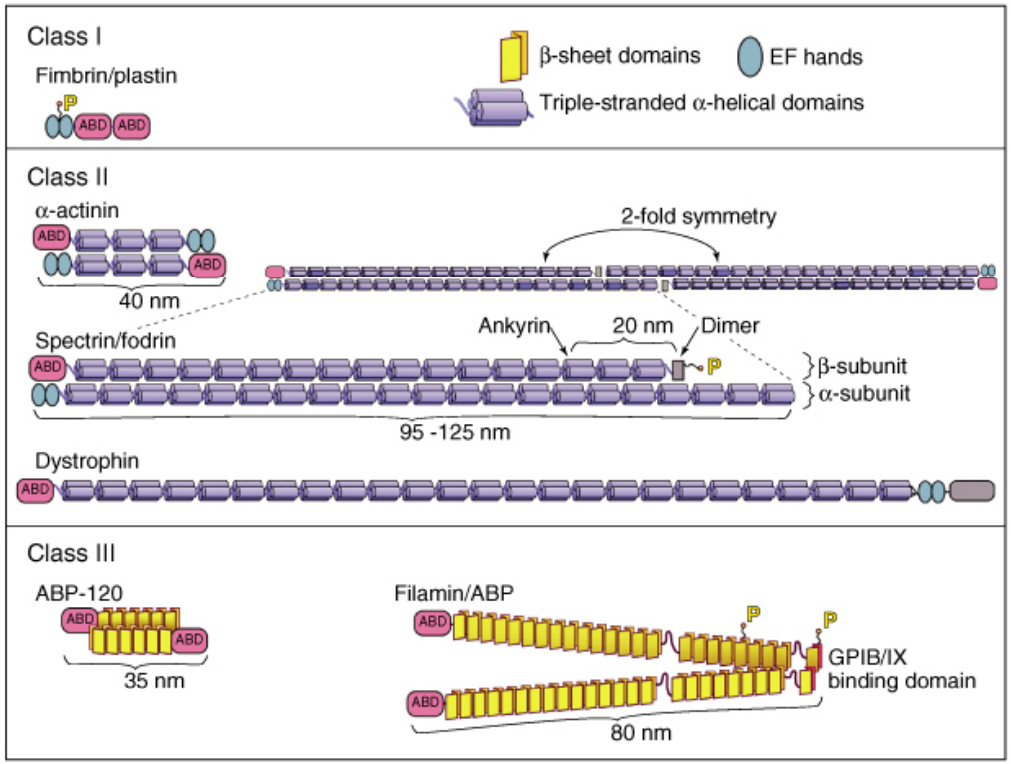
- Actin capping protein
  - Blocks subunit association and disassociation
- Actin severing protein
- Three families of proteins perform both functions
  - Gelsolin
  - Fragmin-severin
  - ADF/cofilin

Actin Associated Proteins (V)

- Actin side-binding proteins
  tropomyosin, nebulin, caldesmon

- Actin crosslinking pro
  - α-actinin
  - filamin
  - spectrin
  - ERM

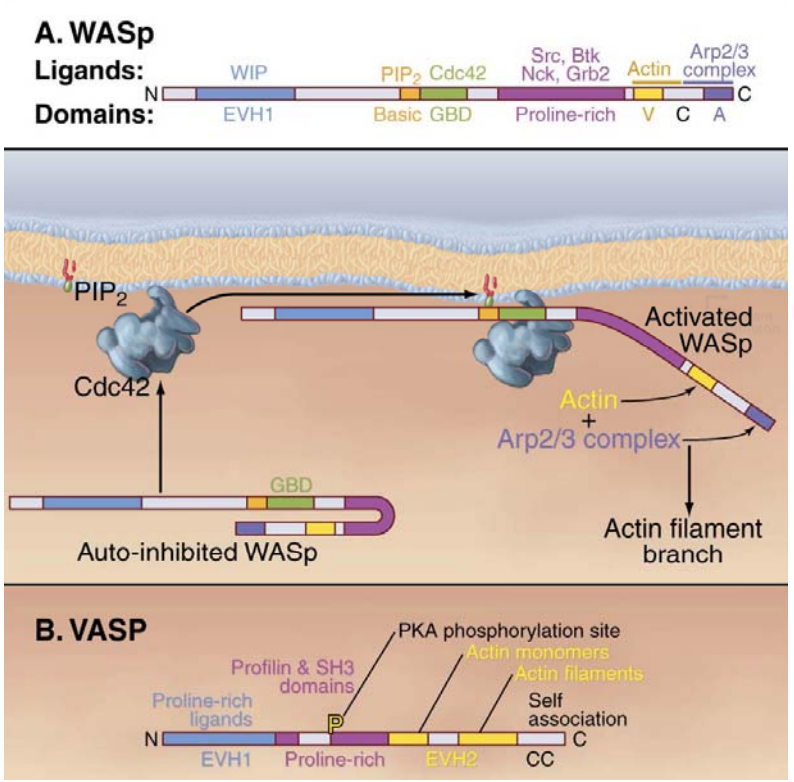




Actin Adapter Protein

- Adaptor proteins such as WASP (a branching mediating factor) & VASP (a polymerization mediating factor) server as connectors between signaling pathways and actin assembly.

- WASP: Wiskott-Aldrich syndrome protein



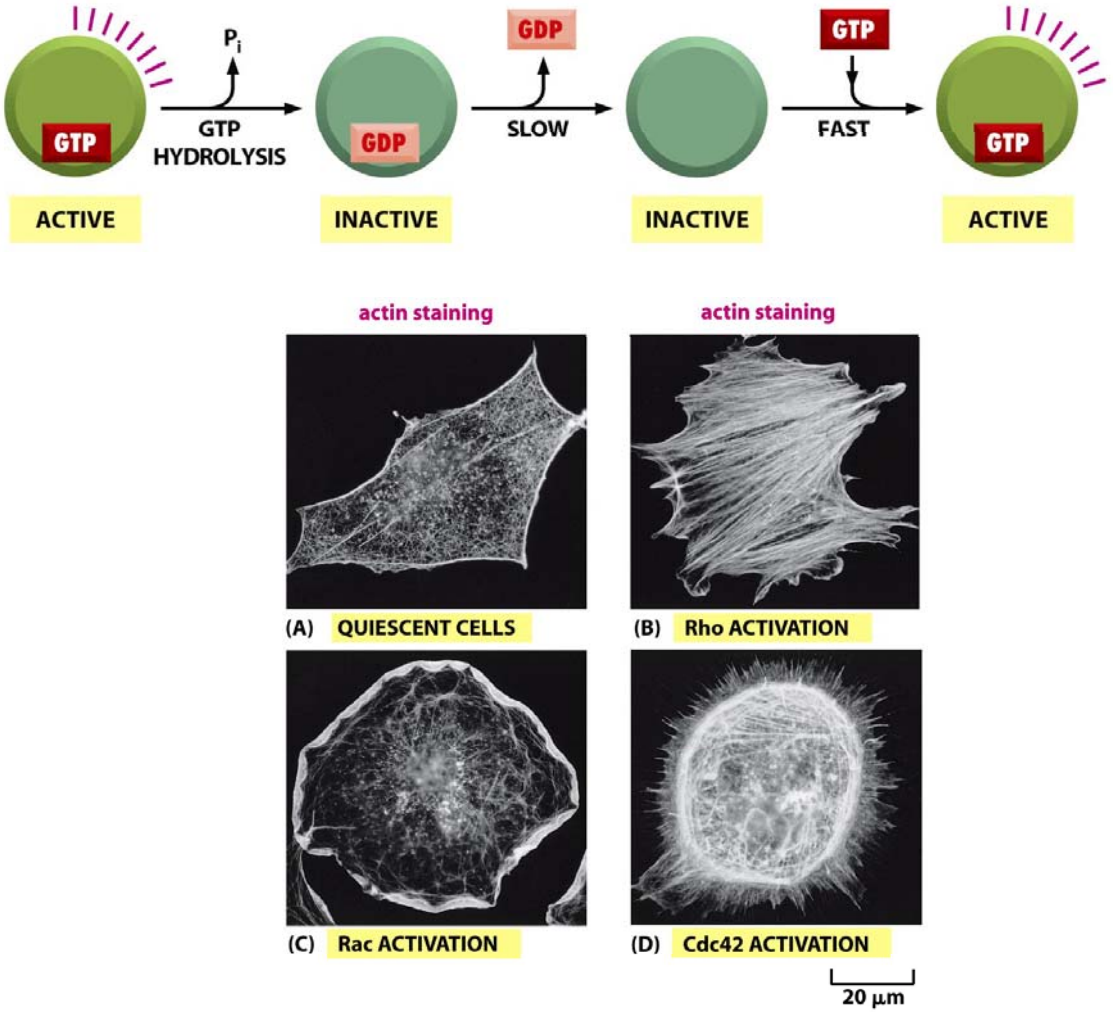
Actin Regulation

- GTPase: Molecule switch; Family of enzymes that are activated by GTP binding and inactivated by GTP hydrolysis and phosphate dissociation.

- GTPases:
  cdc42: its activation triggers actin polymerization and bundling at filopodia.

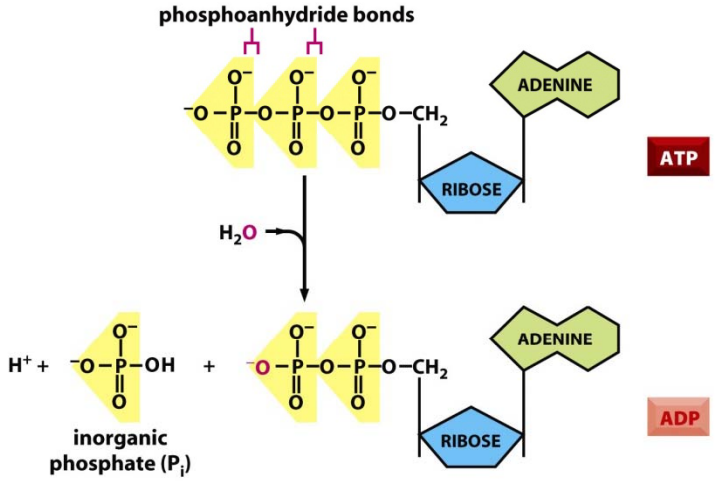
  Rho: its activation promotes actin bundling.

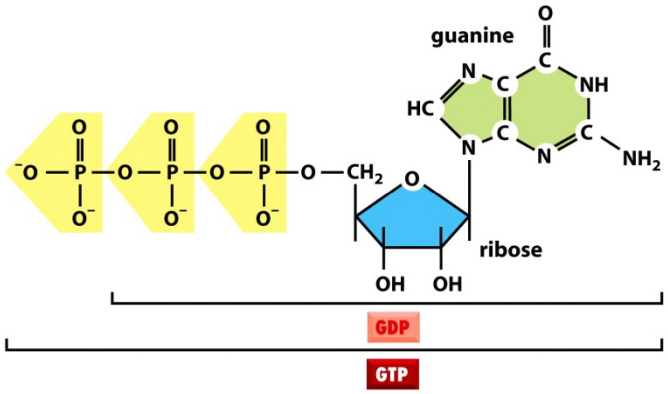
  Rac: its activation promotes polymerization at the cell periphery.

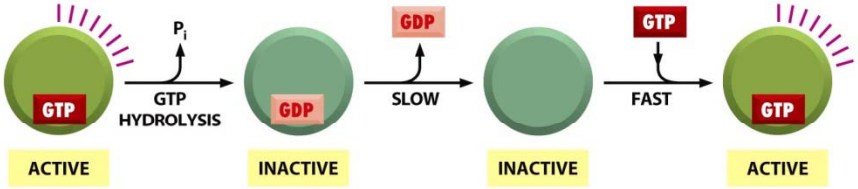


GTP (guanosine triphosphate)

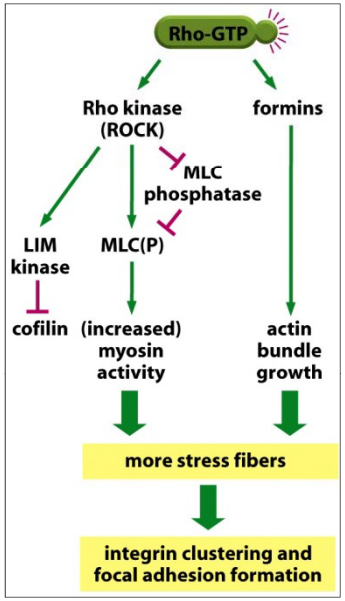
- GTP-binding proteins (GTPases) are important signaling molecules.
- When GTP is hydrolyzed into GDP, the GTP binding domain undergoes a conformation change that inactivates the GTPase.

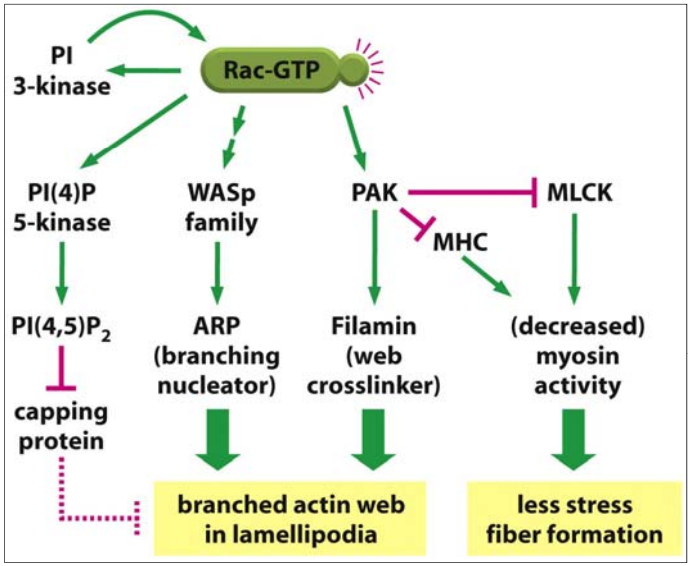


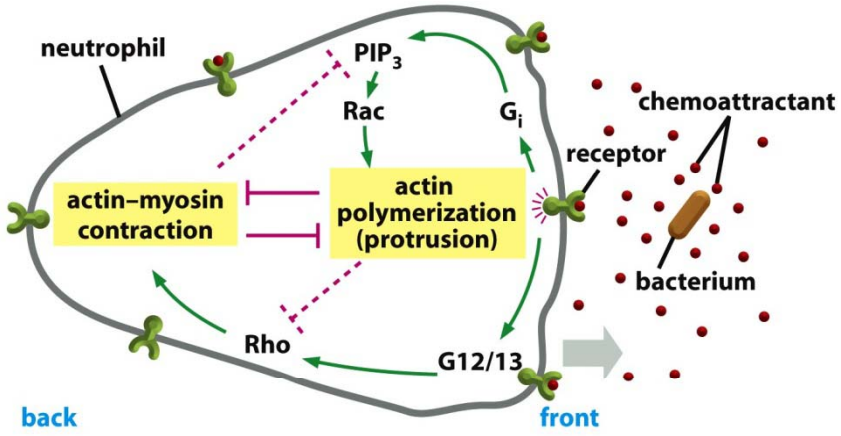


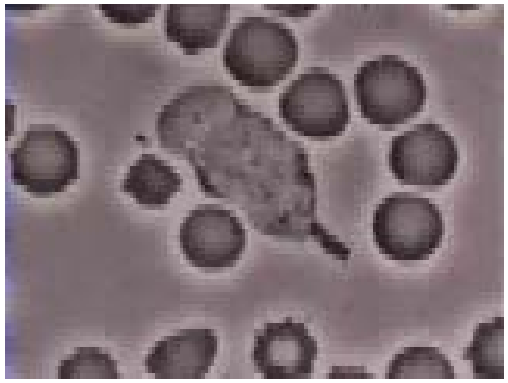


Rac on Actin Organization









Summary: actin

- Relatively soft (quantification to follow in later lectures).
- Often form bundles; mechanical strength comes mostly from bundling and crosslinking.
- Mostly function to withstand tension rather than compression.
- Relatively stable and easy to work with (biochemically).

Summary: actin associated proteins

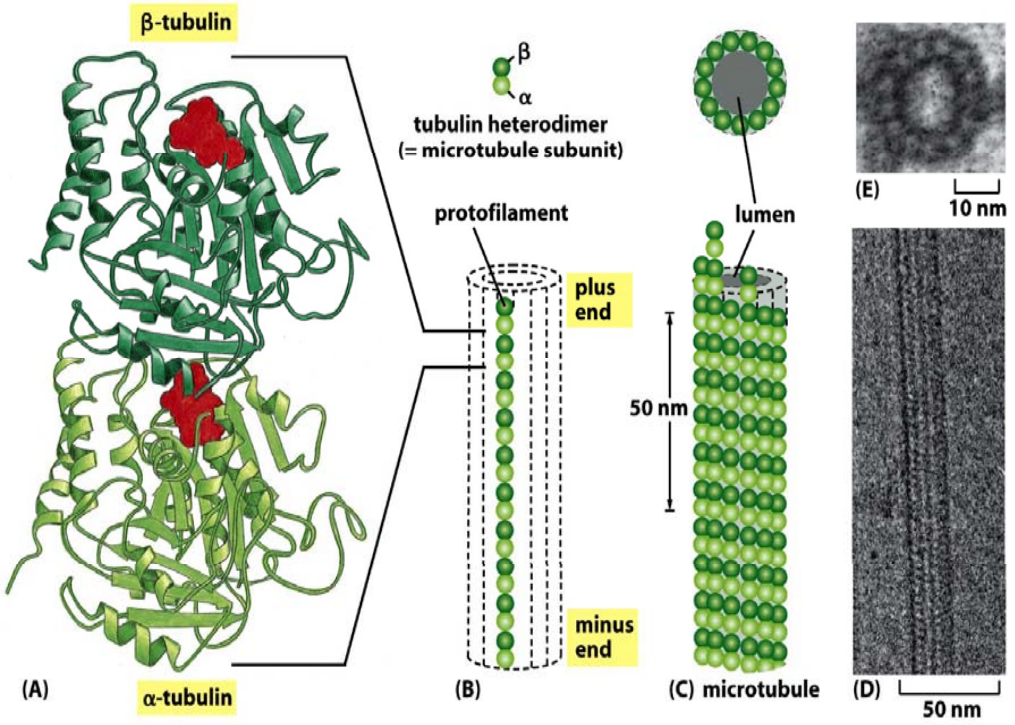
- Different associated proteins serve a broad range of functions.
- Proteins with multiple functional domains can have multiple functions.
- Some but not all of them are essential.
- Most of the proteins have functional overlap.

Outline

- Overview of cytoskeletal filaments
- Actin and its associated proteins
- Microtubule and its associated proteins; Centrosome
- Intermediate filament and its associated proteins

Microtubule Structure

- Polarized
  - plus (β-tubulin) end
  - minus (α-tubulin) end
- Outer diameter: 25 nm
- Typically 13 protofilaments;
  Some have 11, 15, 16, etc



Microtubule Organization

- During interphase
  - Radial pattern organized around the centrosome
- During metaphase
  - Bipolar architecture

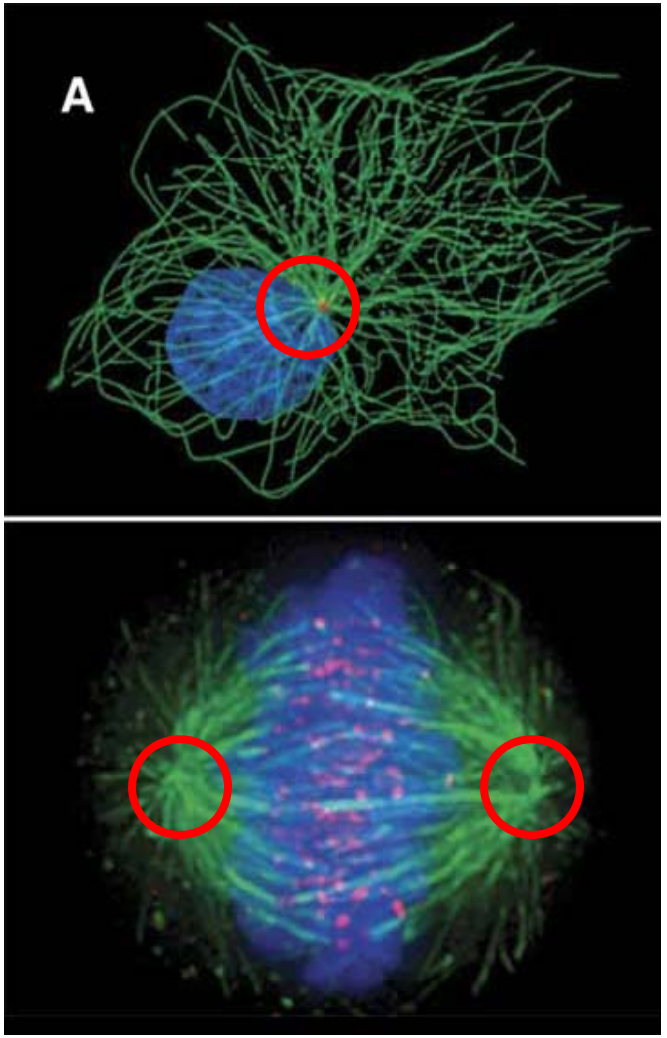
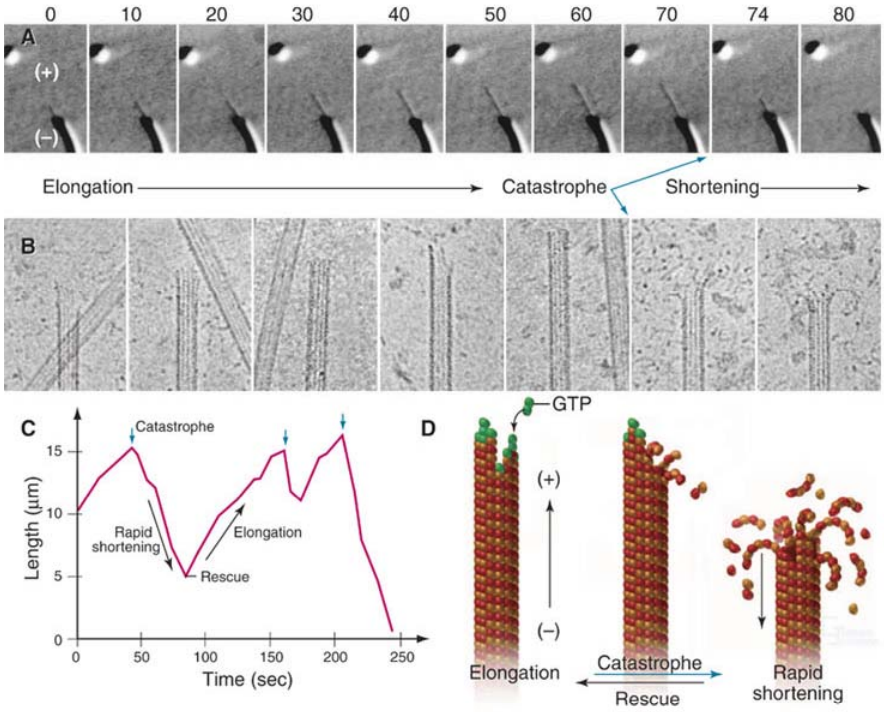
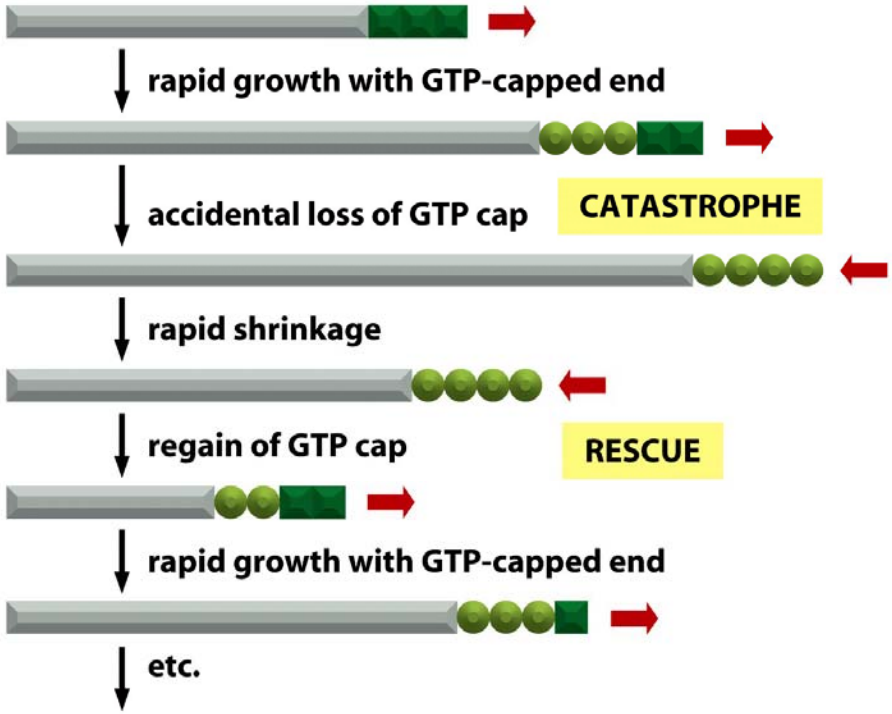


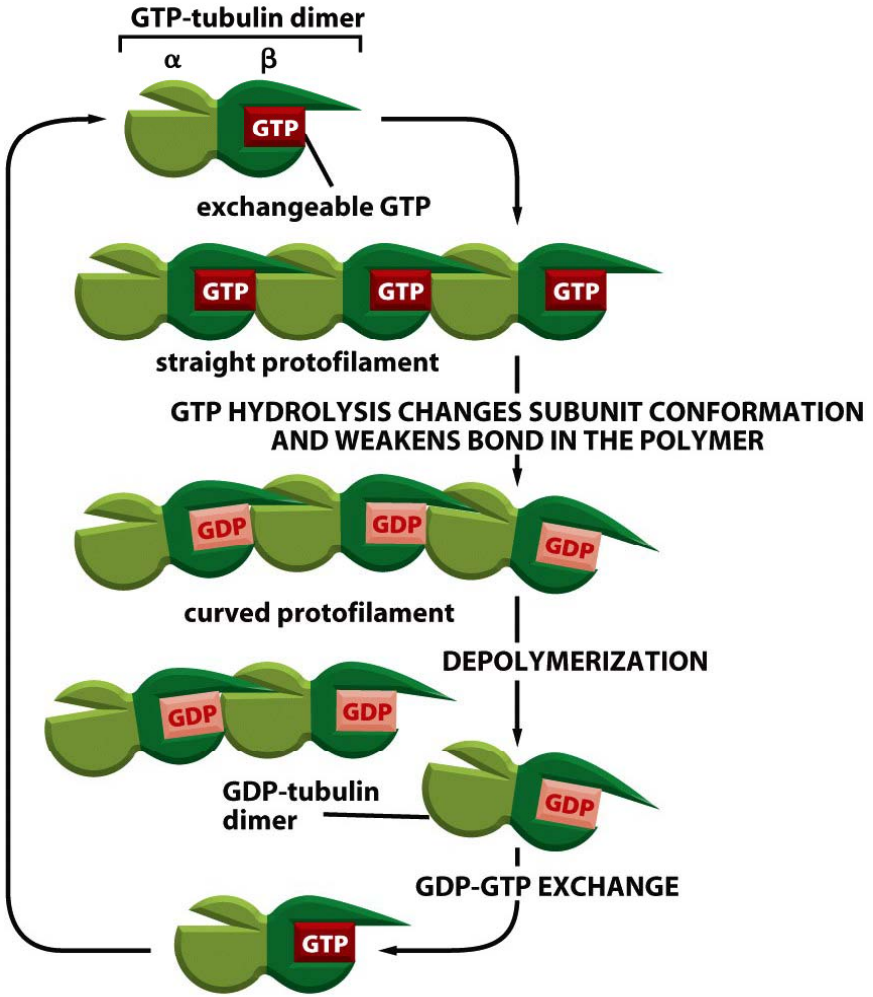
Fig. 34-1

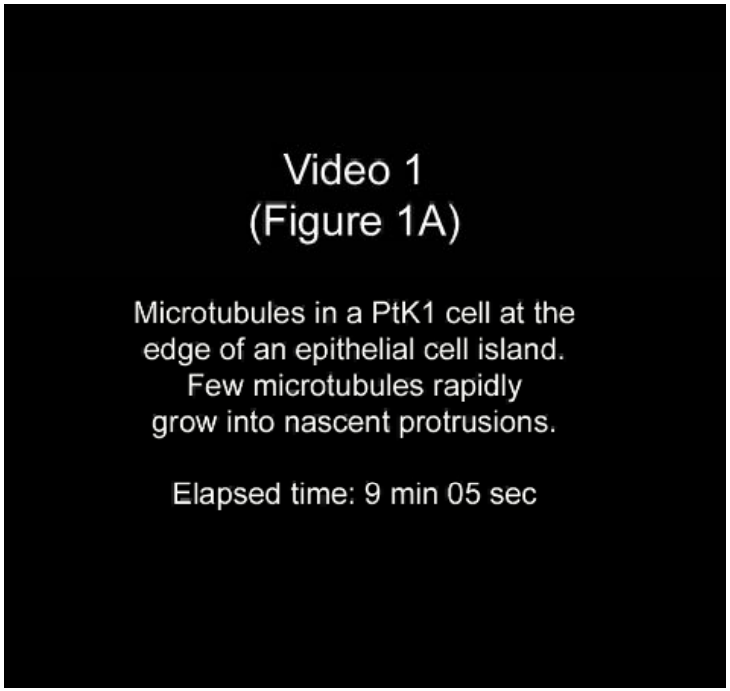
Microtubule Dynamic Instability





Microtubule Dynamic Instability





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

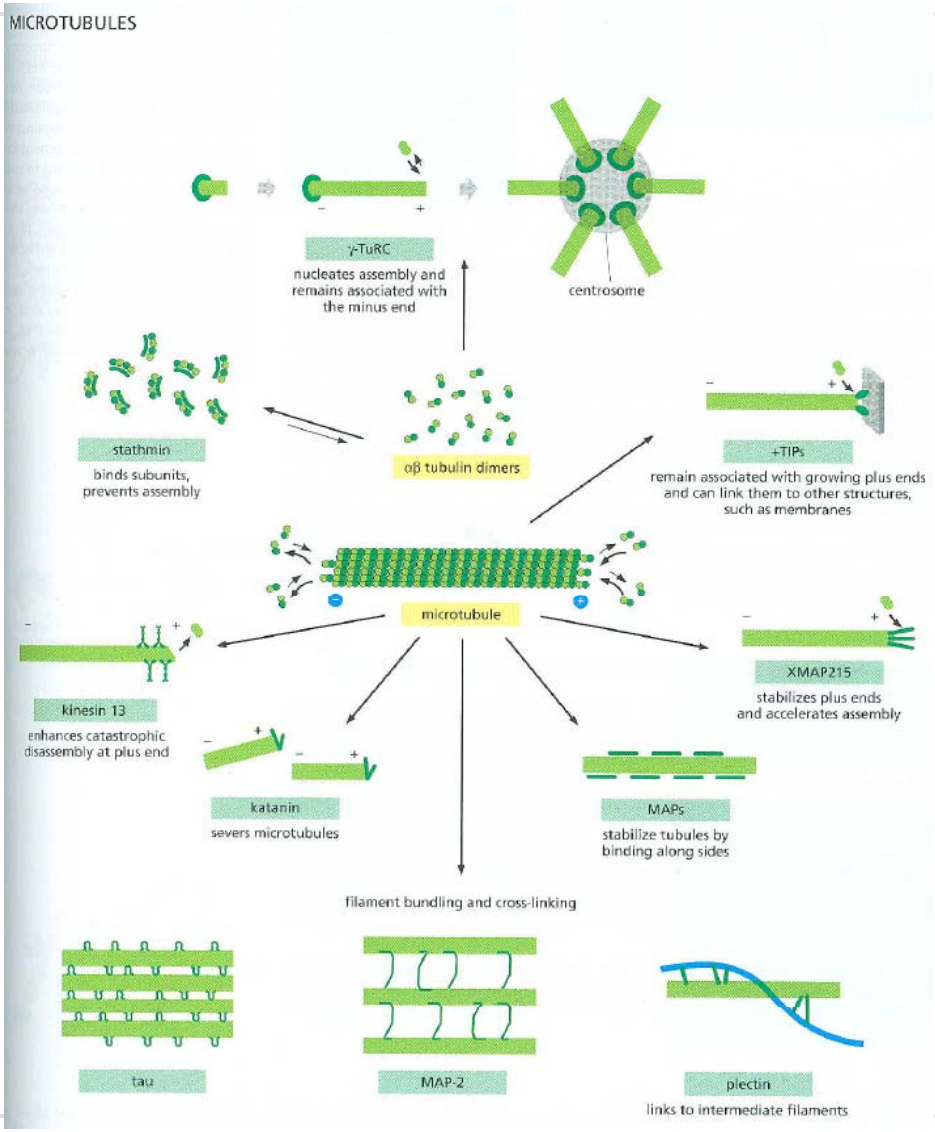
Microtubule Dynamic Instability Parameters

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


MICROTUBULES
γ-TuRC
nucleates assembly and remains associated with the minus end
centrosome
stathmin
binds subunits, prevents assembly
αβ tubulin dimers
+TIPs
remain associated with growing plus ends and can link them to other structures, such as membranes
microtubule
kinesin 13
enhances catastrophic disassembly at plus end
XMAP215
stabilizes plus ends and accelerates assembly
katanin
severs microtubules
MAPs
stabillze tubules by binding along sides
filament bundling and cross-linking
tau
MAP-2
plectin
links to intermediate filaments

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

Electron Microscopy of Microtubules Decorated with MAPs

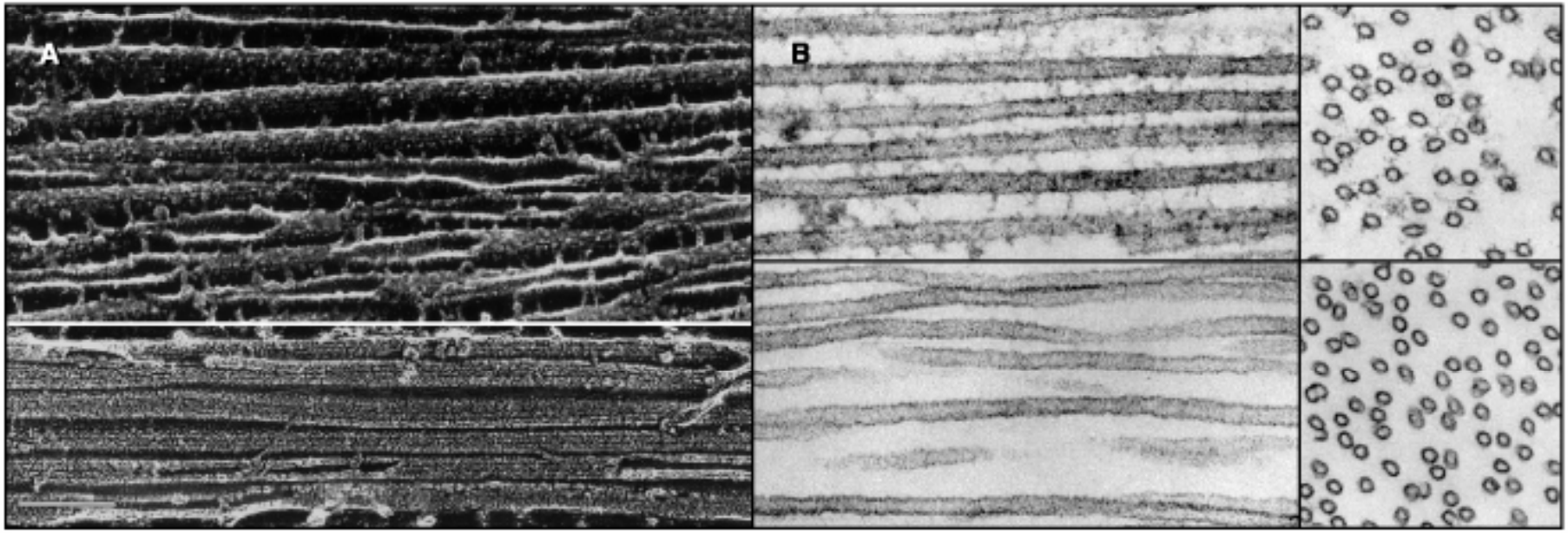


Fig. 34-11

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

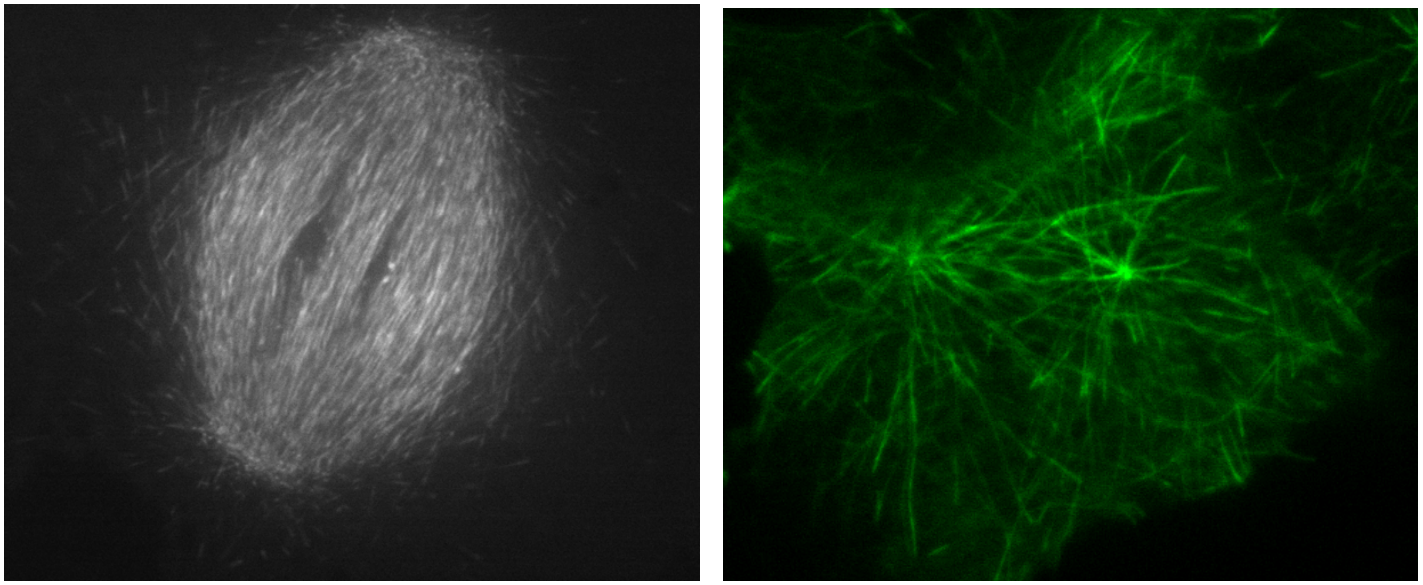
Fixed, embedded sections of microtubules with (upper) and without (lower) MAP2

Microtubule Associated Proteins (II)

Plus end track protein

CLIP170 Links membranes to growing plus ends

EB1 Links APC to MT plus ends

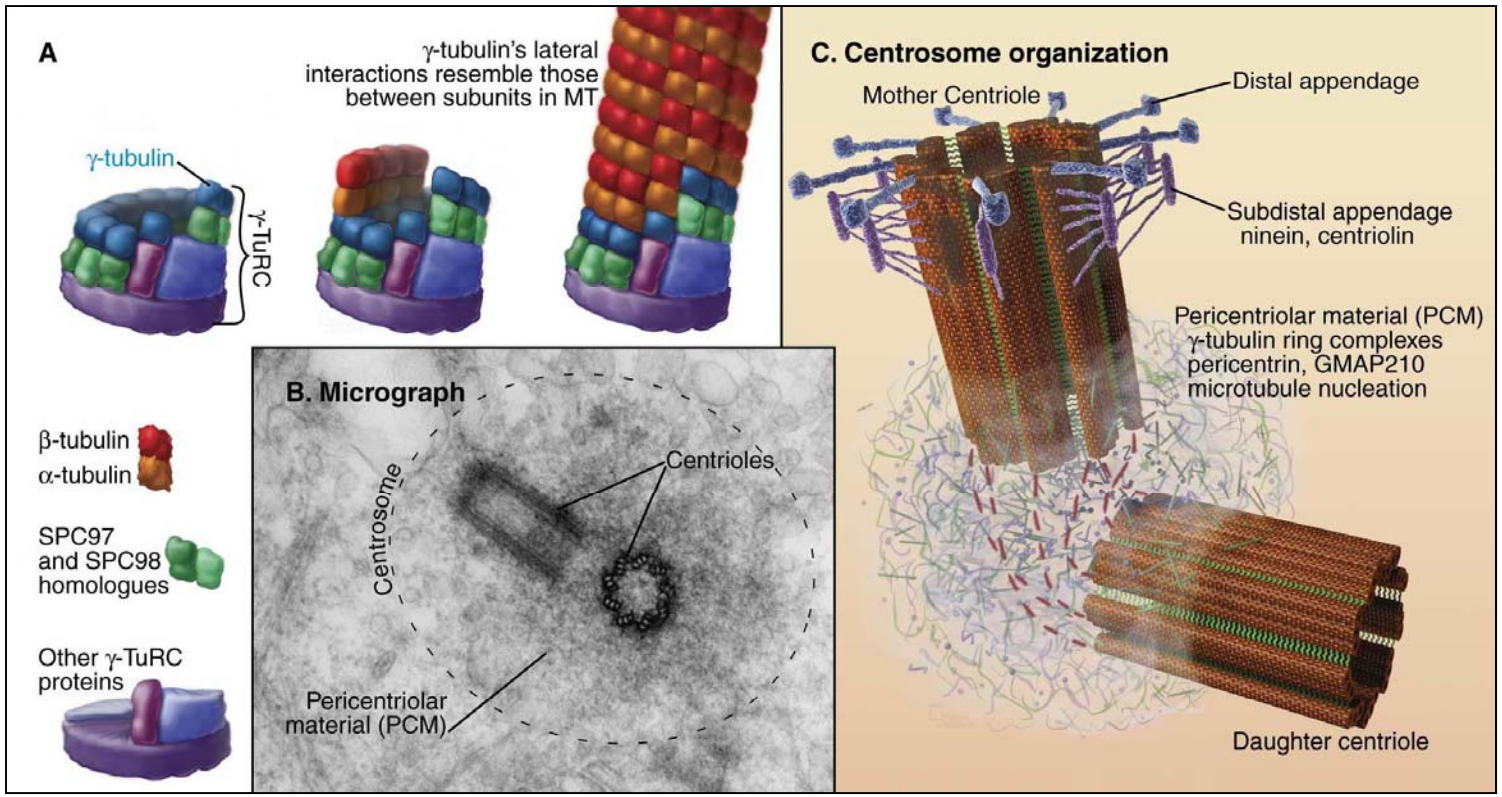


Dynamics and mechanics of the microtubule plus end. Howard J, Hyman AA. *Nature*. 2003 422(6933):753-8.

Summary: Microtubule

- Relatively rigid (quantification in following lectures).
- Often form bundles; mechanical strength comes mostly from bundling and crosslinking.
- Can withstand compression.
- Can denature easily; Difficult to work with (biochemically).

Centrosome

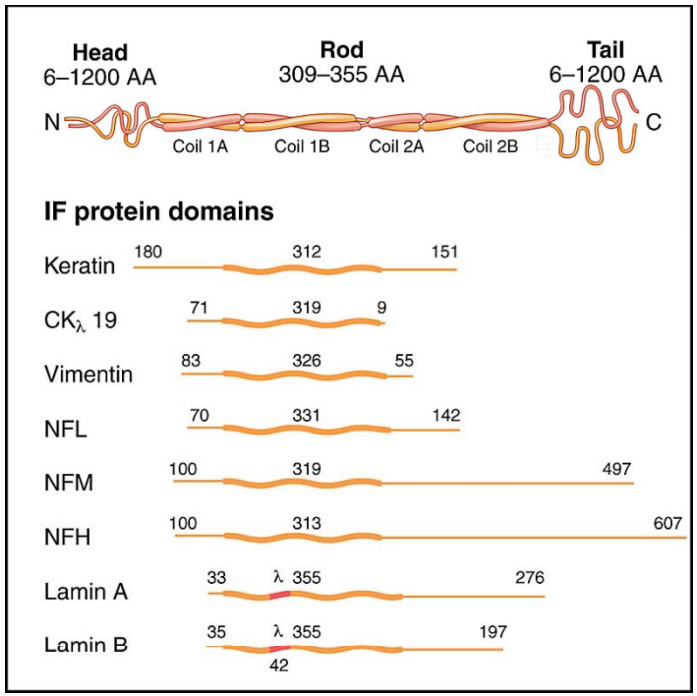

A
γ-tubulin's lateral interactions resemble those between subunits in MT
γ-tubulin
γ-TuRC
β-tubulin
α-tubulin
SPC97 and SPC98 homologues
Other γ-TuRC proteins
B. Micrograph
Centrosome
Centrioles
Pericentriolar material (PCM)
C. Centrosome organization
Mother Centriole
Distal appendage
Subdistal appendage ninein, centriolin
Pericentriolar material (PCM) γ-tubulin ring complexes pericentrin, GMAP210 microtubule nucleation
Daughter centriole

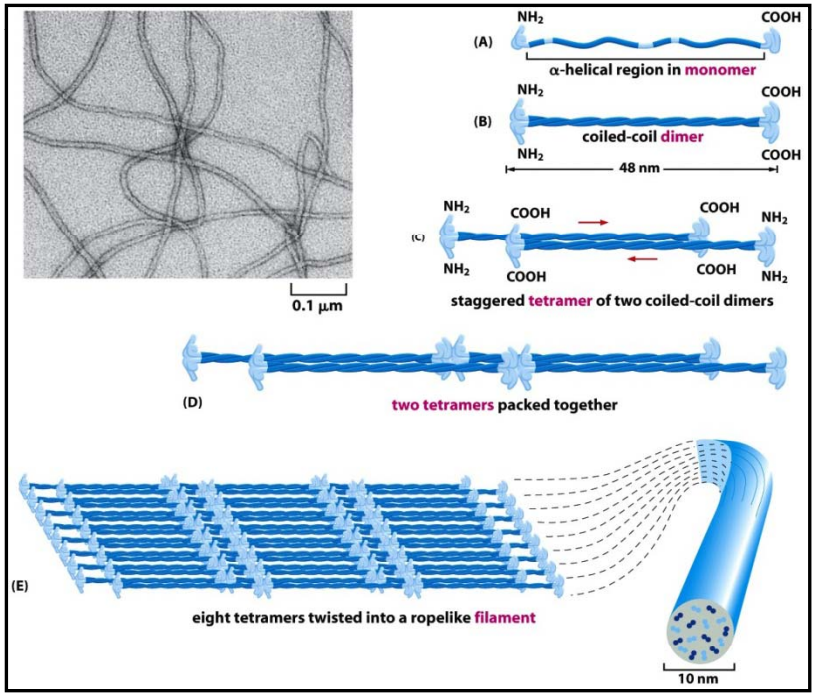
Outline

- Overview of cytoskeletal filaments
- Actin and its associated proteins
- Microtubule and its associated proteins; Centrosome
- Intermediate filament and its associated proteins

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 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.

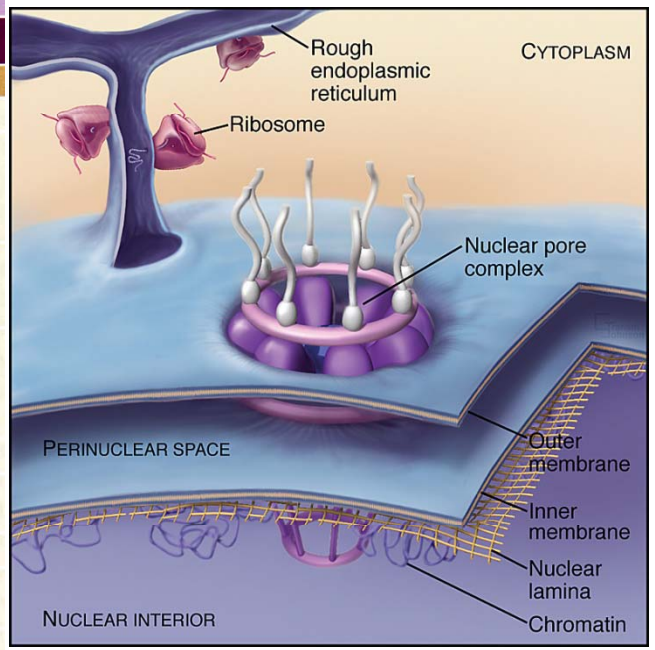
Intermediate Filament Associated Proteins

Table 35-2

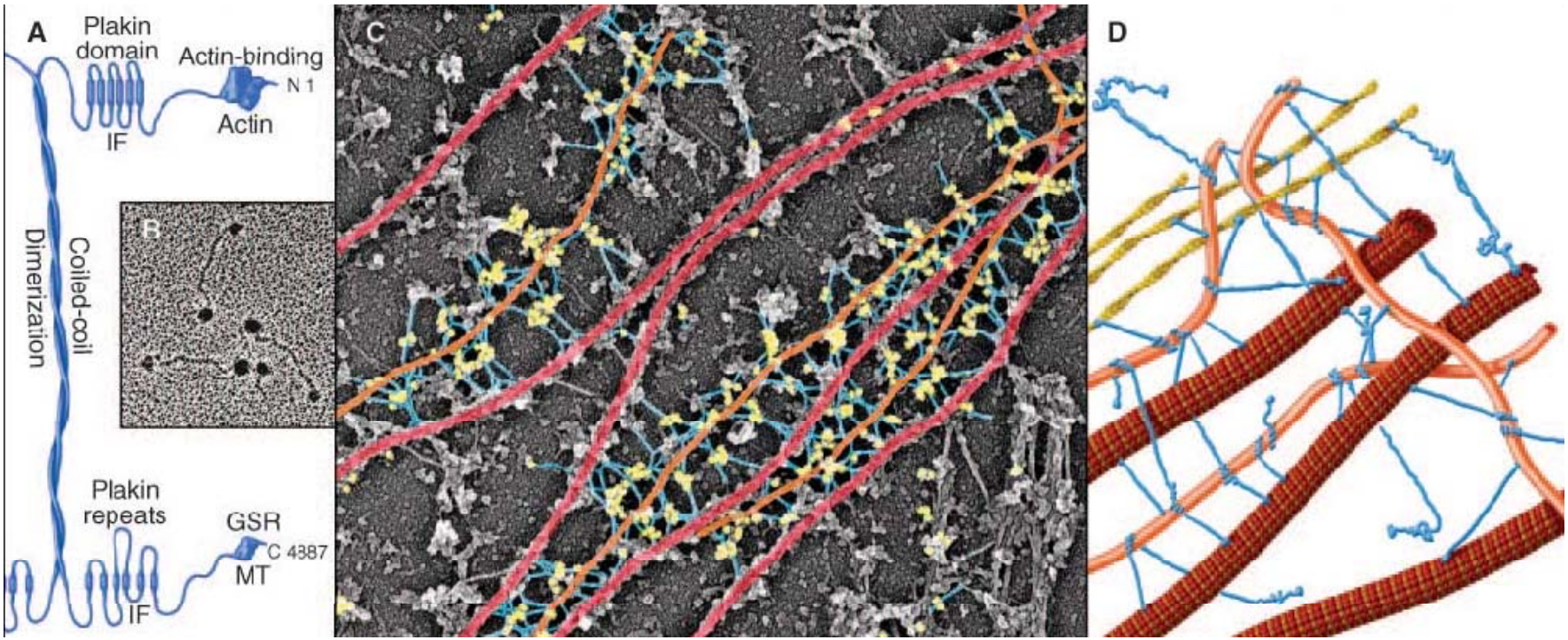
PROTEINS ASSOCIATED WITH INTERMEDIATE FILAMENTS

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



Intermediate Filaments

- Flexible but stable.
- Primary assumed function is to prevent excessive stretching.
- In general, much less is known about intermediate filaments

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

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

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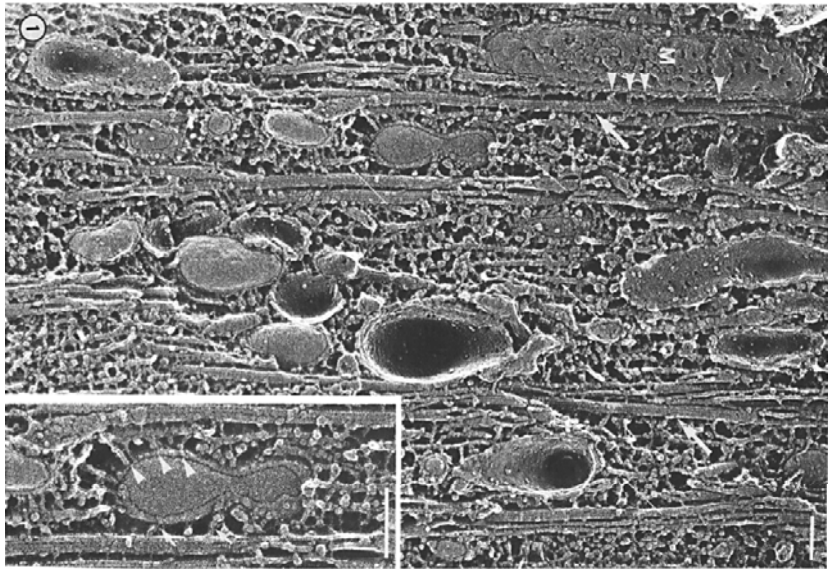

Bar: 0.1μm

Required Reading

- Chapters 2 & 16

Questions ?