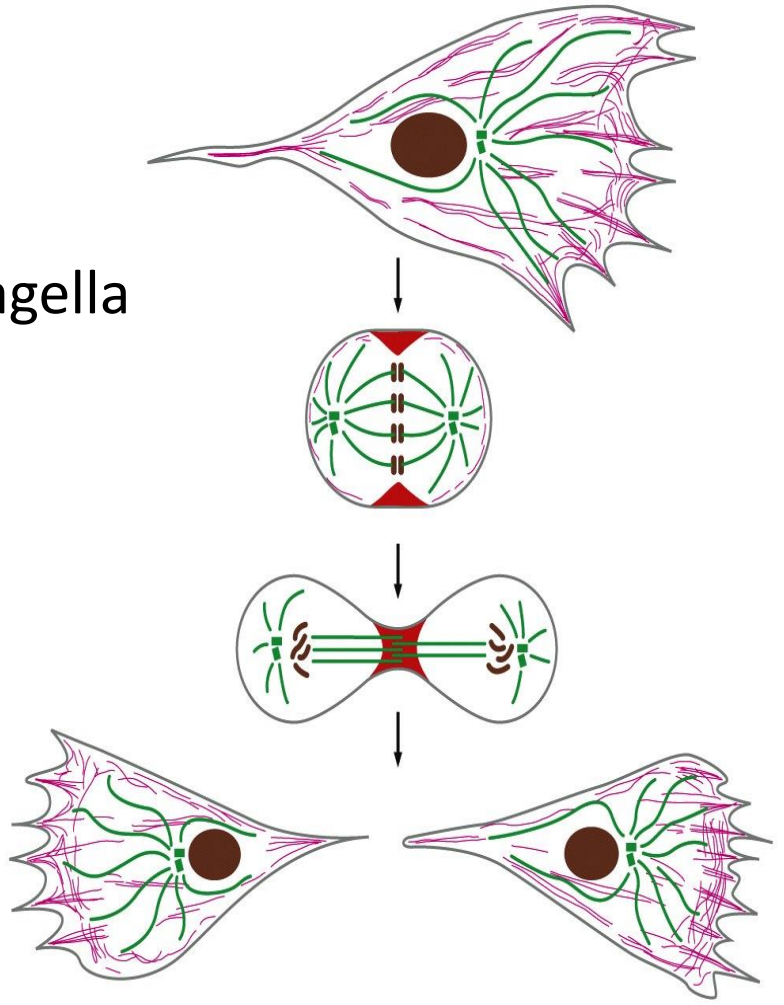
Lecture 13. Cytoskeleton and cell movement II

Outline

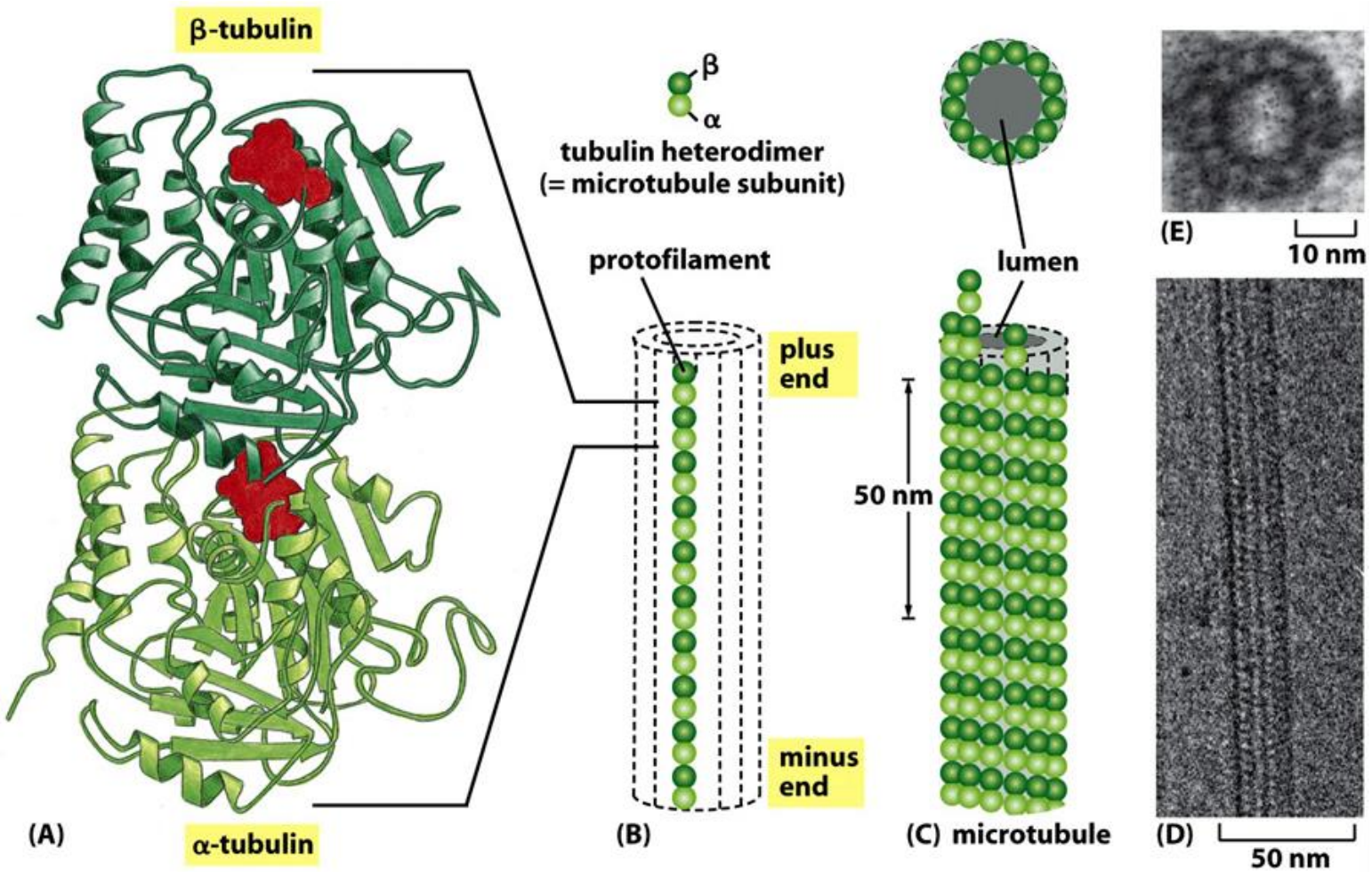
I. Microtubule structure and organization
II. Microtubule dynamics
III. Regulation of microtubule assembly
IV. Kinesins and Dyneins: microtubule-based motor proteins
V. Cilia and flagella
VI. Intermediate filaments

I. Microtubule structure and organization

- Mitotic spindle
- Structural support in axon
- Structural elements in cilia and flagella
- Centriole
- Basal bodies



Organization of microtubule



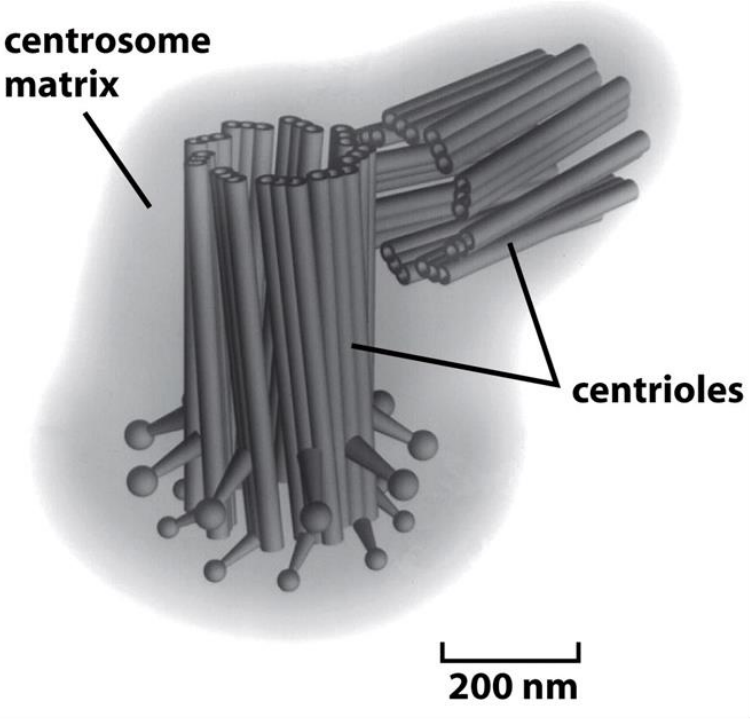
Summary about microtubule proteins

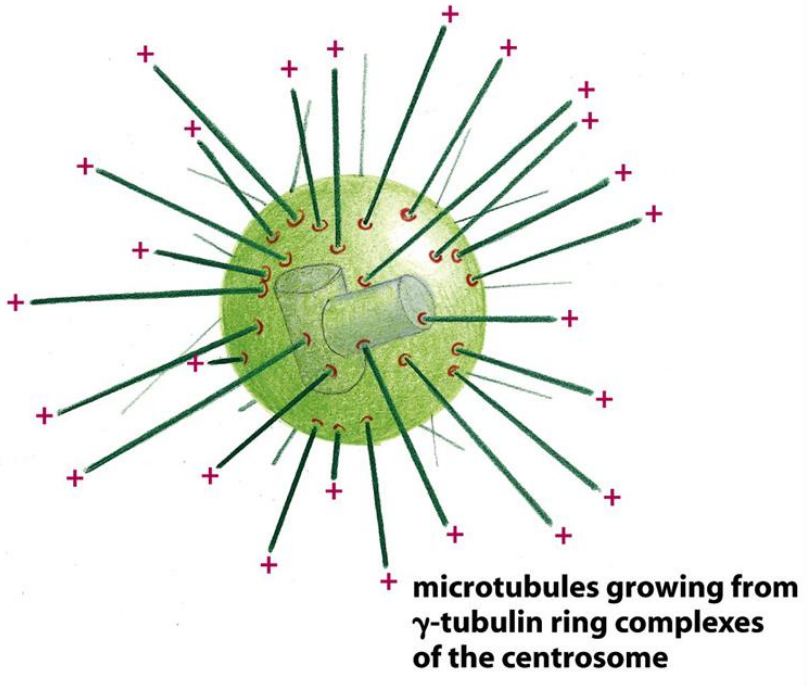
- Most microtubules are singlets, some are in doublet (cilia, flagella), some are in triplet (basal bodies and centrioles)
- Most have 13 protofilaments, some have between 11-15.
- two major types of tubulins, α-tubulin, and β-tubulin, forming heterodimer.
- All subunits are oriented in the same way, the one with exposed α-tubulin is minus end, the one with exposed β-tubulin is plus end, microtubule has polarity.
- Each α-tubulin and β-tubulin bind to one molecule of GTP, the GTP on α-tubulin is never hydrolyzed, but GTP on β-tubulin is hydrolyzed.
- γ-tubulin is important for microtubule assembly, Microtubule-associated proteins (MAP) are important in assembling and dynamics for microbutules.

Microtubule assembly

- Very rare spontaneous microtubule assembly
- All microtubules are nucleated from Microtubule-organizing centers (MTOCs), including centrosomes and basal bodies (cilia and flagella)
- Plants use different mechanisms to nucleate microtubules.

γ-tubulin ring complex (γ-TuRC), pericentriolar, is critical to assemble microtubules





γ-tubulin ring complex nucleates microtubules

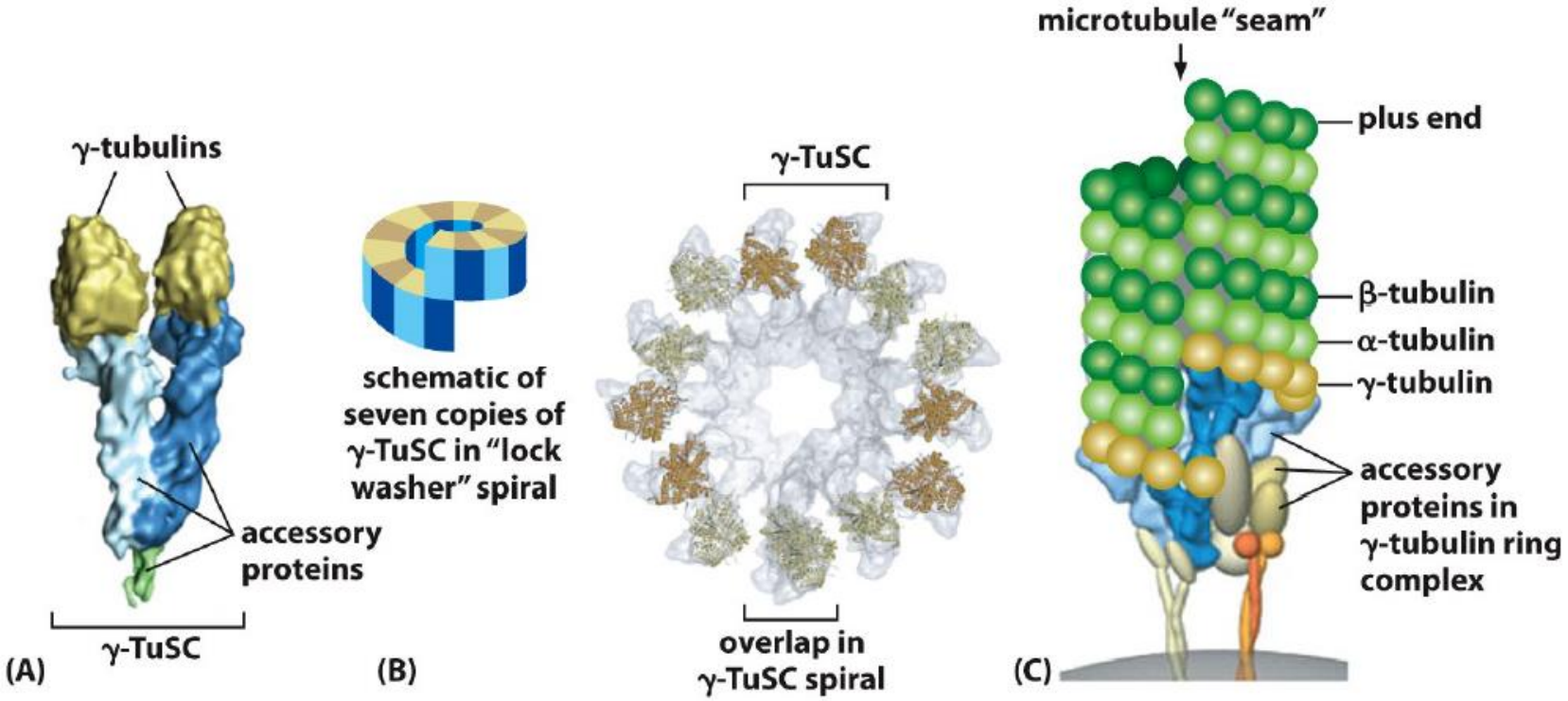
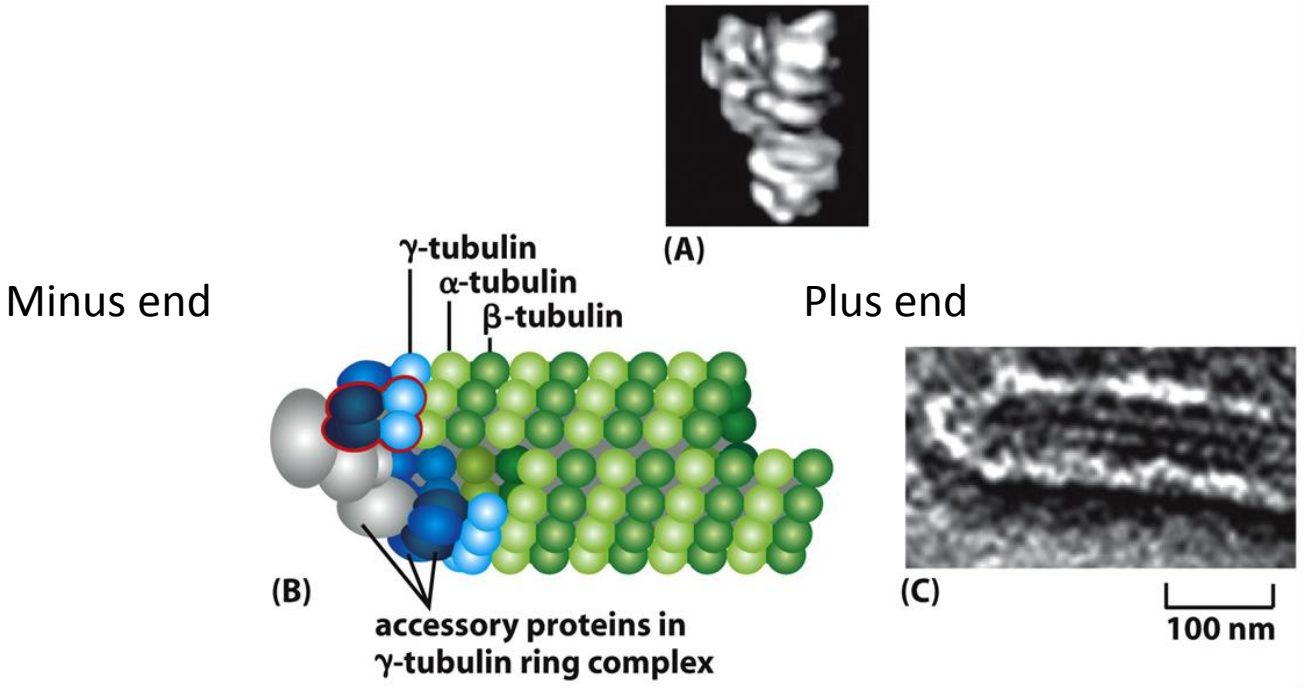
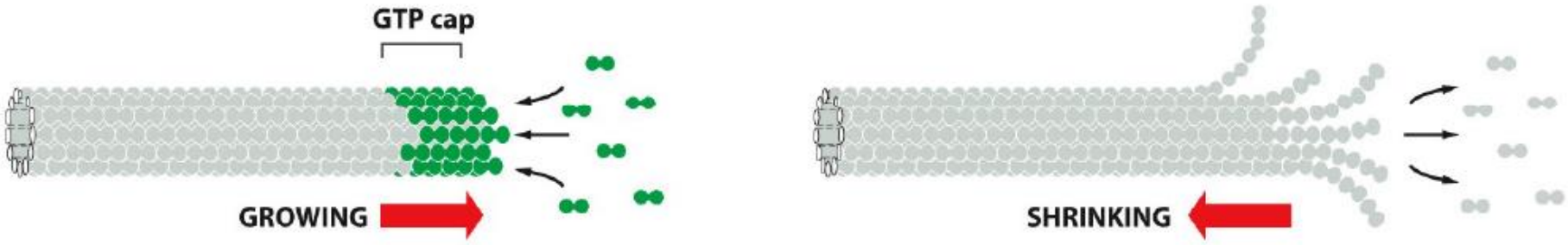


Figure 16-46 Molecular Biology of the Cell 6e (© Garland Science 2015)

γ-tubulin ring complex (γ-TuRC), pericentriolar, is critical to assemble microtubules



Microtubule dynamic instability

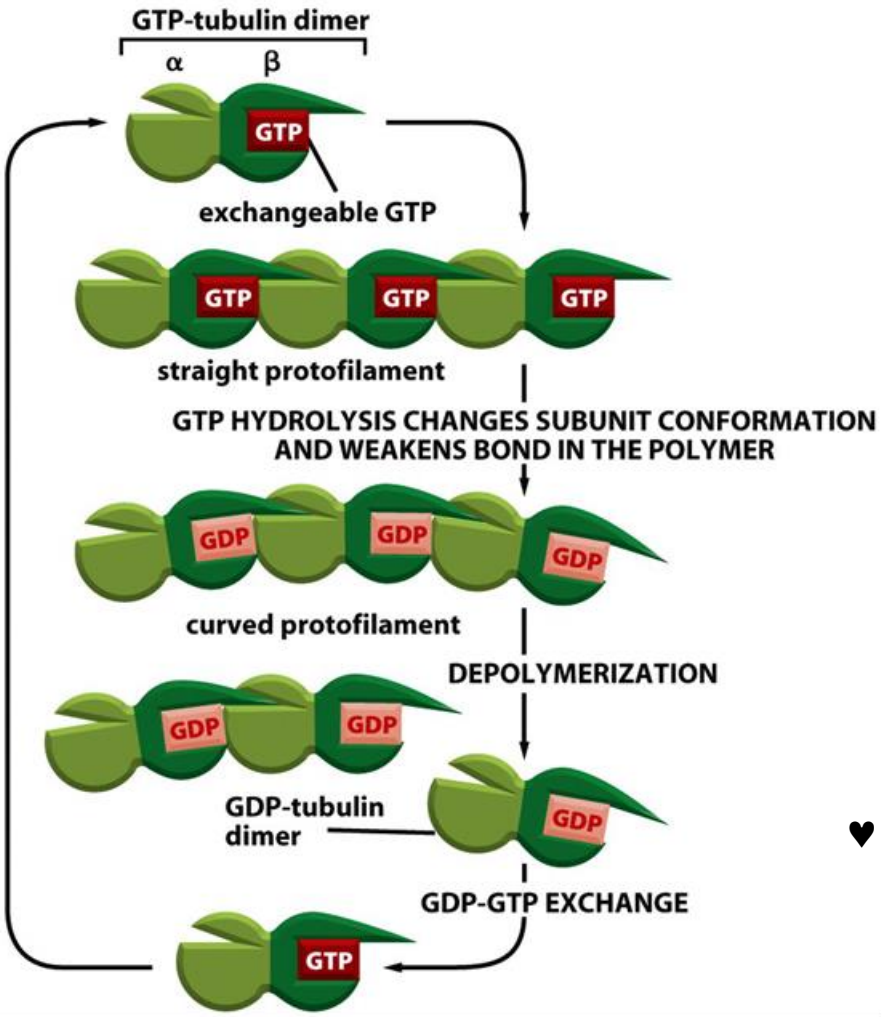


Individual microtubules can therefore alternate between a period of slow growth and a period of rapid disassembly, a phenomenon called dynamic instability.

Panel 16-2 (part 10) Molecular Biology of the Cell 6e (© Garland Science 2015)

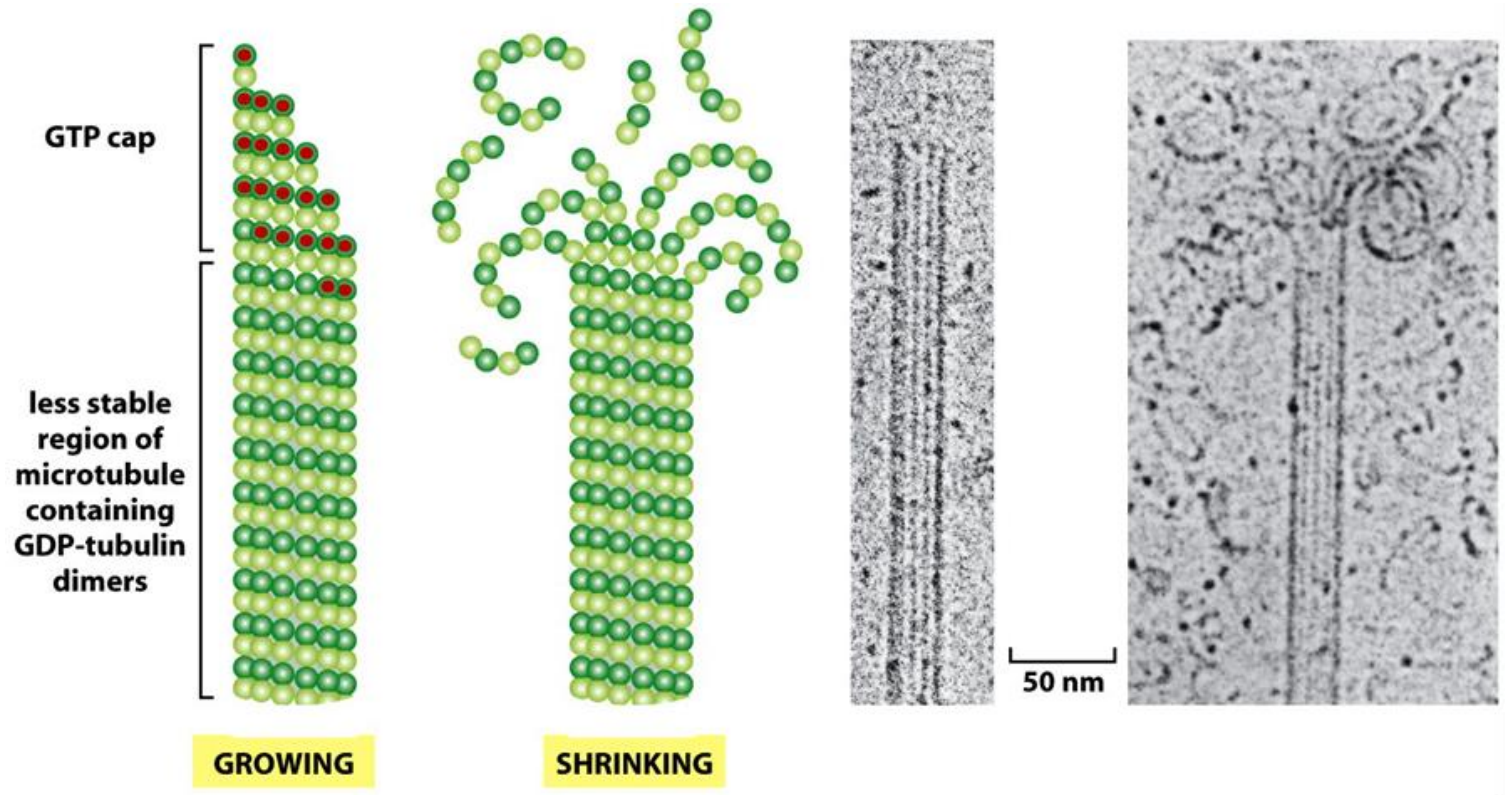
- Microtubules depolymerize about 100 times faster from an end containing GDP-tubulin than from one containing GTP-tubulin. A GTP cap favors Growth but if it is lost, then depolymerization ensues.

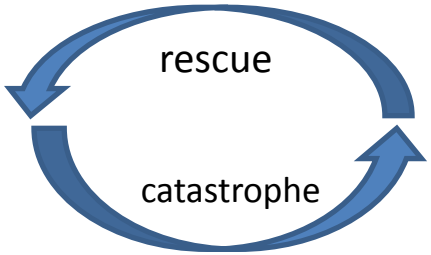
Assembly of tubulin dimers



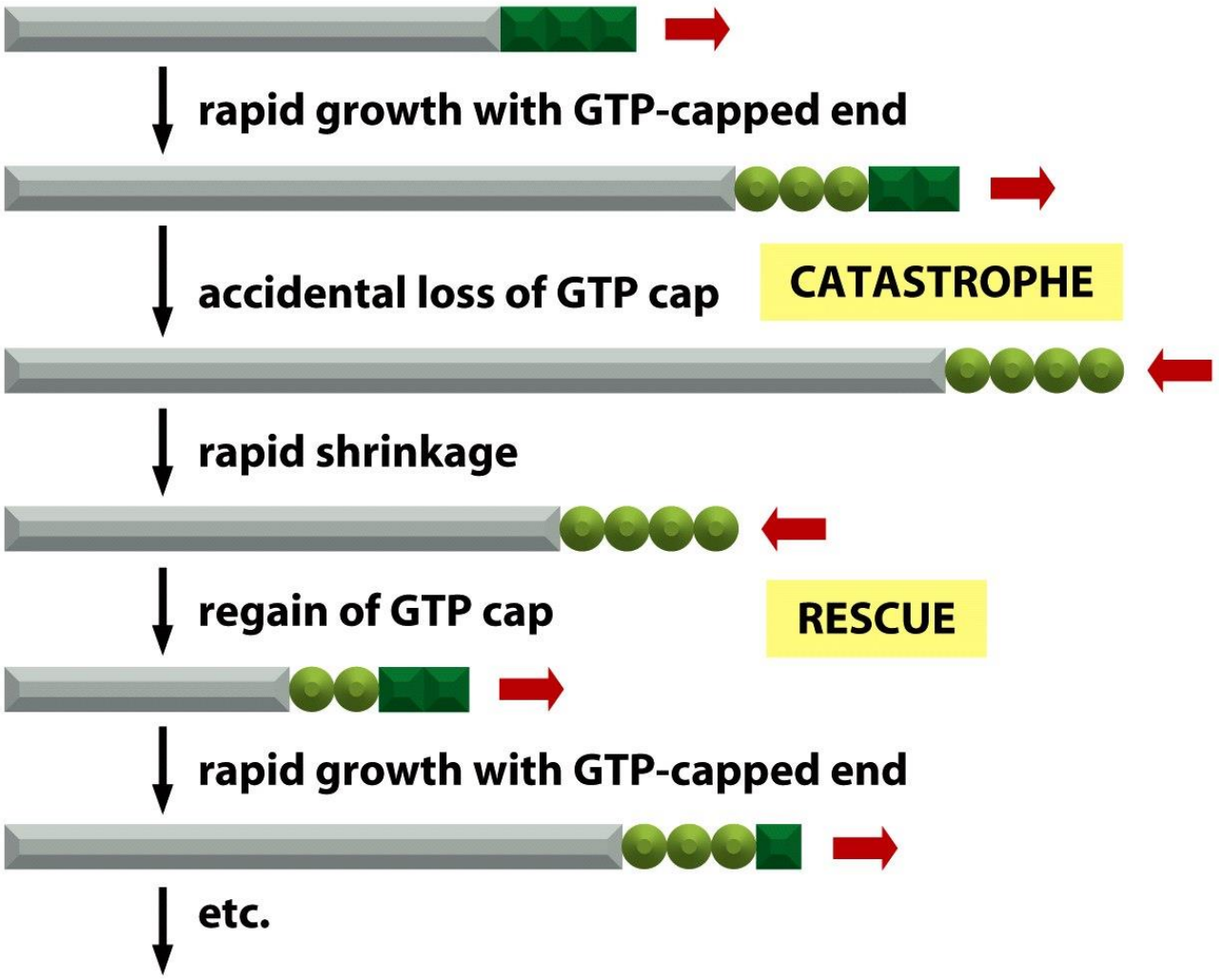
♥ Like actin, assembly at plus end is much Faster than assembly at minus end.

II. Microtubule dynamics

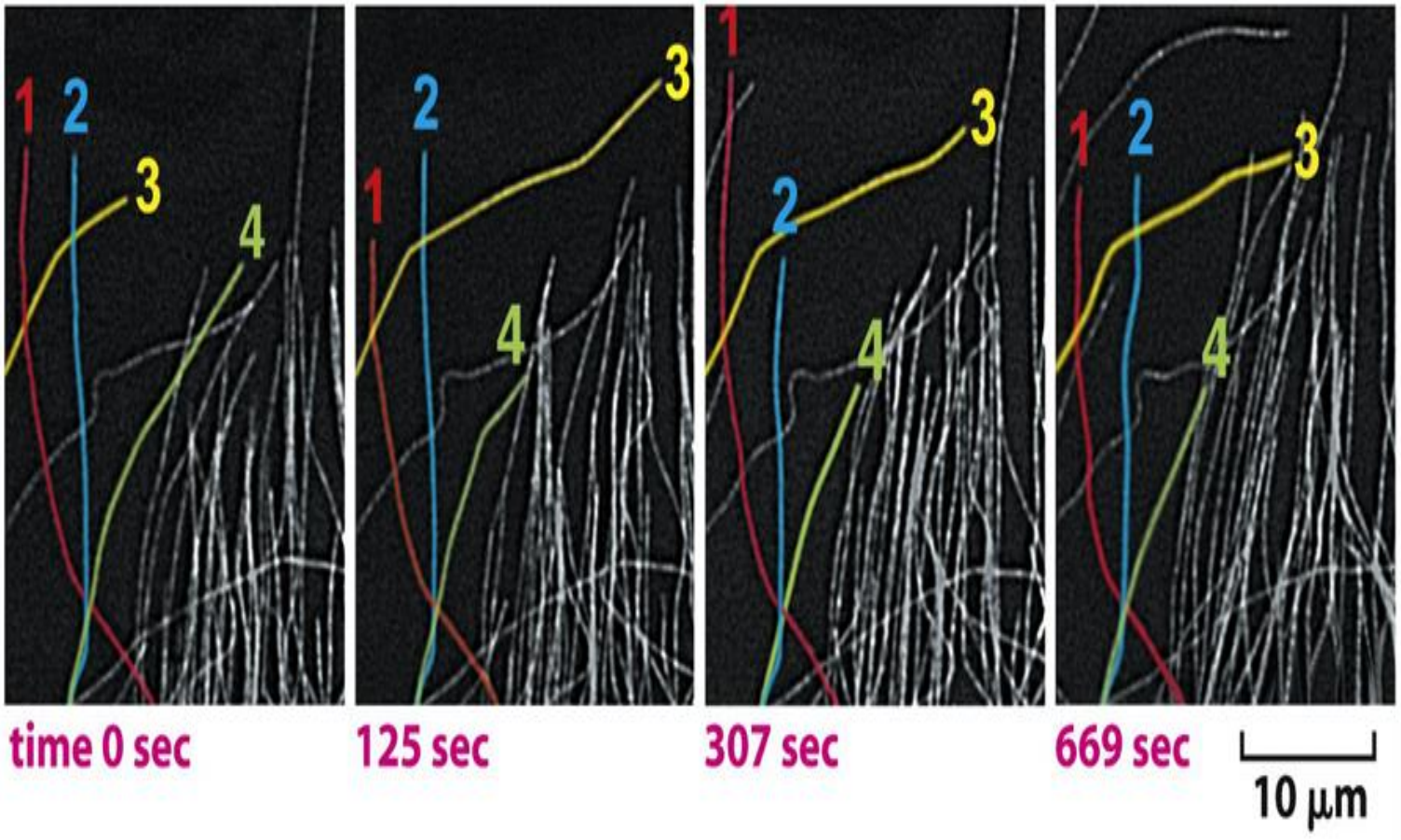




Microtubule dynamic instability



Dynamic instability of microtubule
in vivo



Some drugs to influence tubulin assembly

Table 16–2 Drugs That Affect Actin Filaments and Microtubules

ACTIN-SPECIFIC DRUGS	
Phalloidin	binds and stabilizes filaments
Cytochalasin	caps filament plus ends
Swinholide	severs filaments
Latrunculin	binds subunits and prevents their polymerization
MICROTUBULE-SPECIFIC DRUGS	
Taxol	binds and stabilizes microtubules
Colchicine, colcemid	binds subunits and prevents their polymerization
Vinblastine, vincristine	binds subunits and prevents their polymerization
Nocodazole	binds subunits and prevents their polymerization

III. Regulation of microtubule structure and dynamics

- Microtubules are stabilized by side-binding proteins: tau, MAP2, MAP4, their activity is regulated by phosphorylation

- Plus end binding proteins: +TIPs, help to grow plus end

- Microtubule destabilization proteins: kinesin-13, Op18/stathmin, their activity can also be regulated by phosphorylation.

Organization of microtubule bundles by MAPs

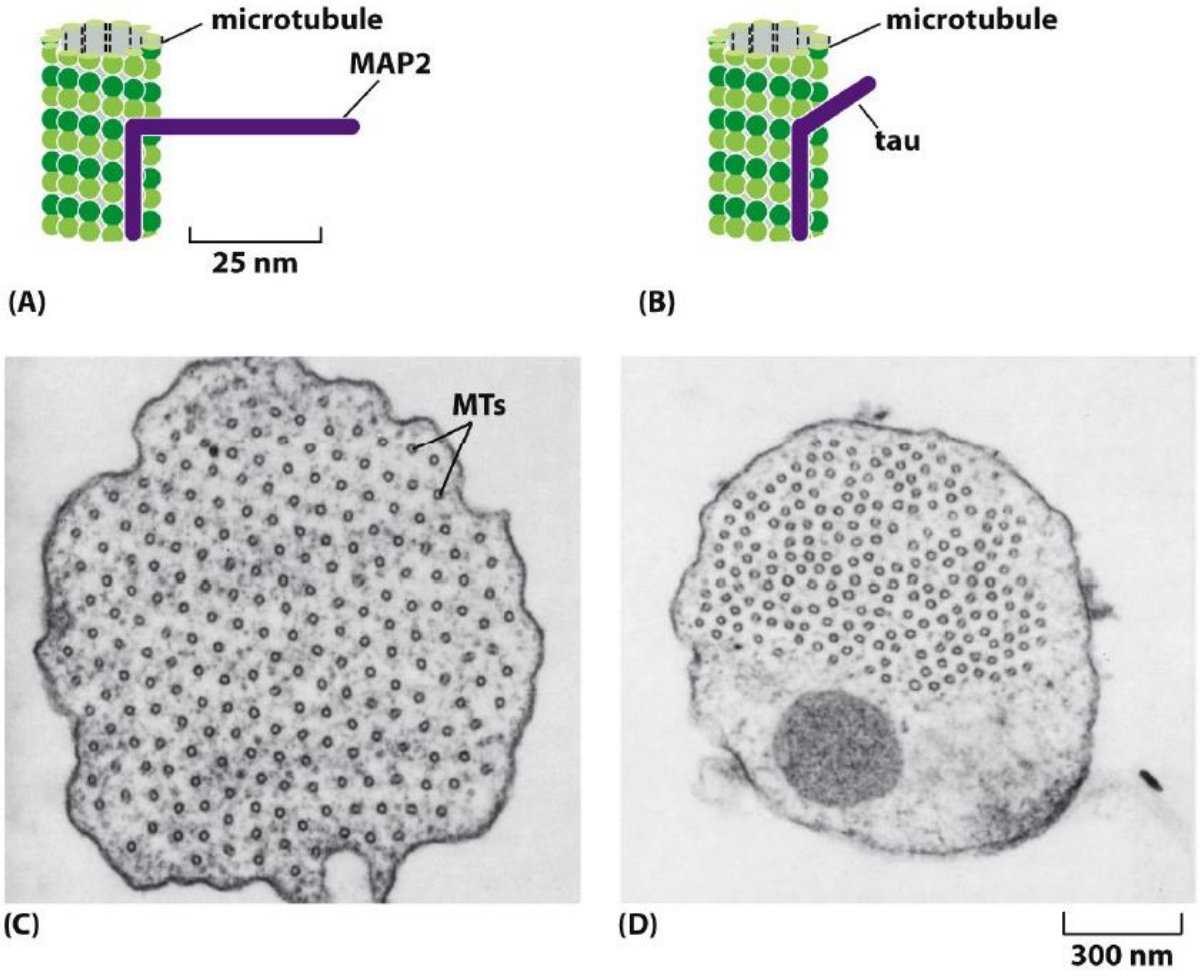


Figure 16-51 Molecular Biology of the Cell 6e (© Garland Science 2015)

Microtubule plus end binding proteins modulate microtubule assembly

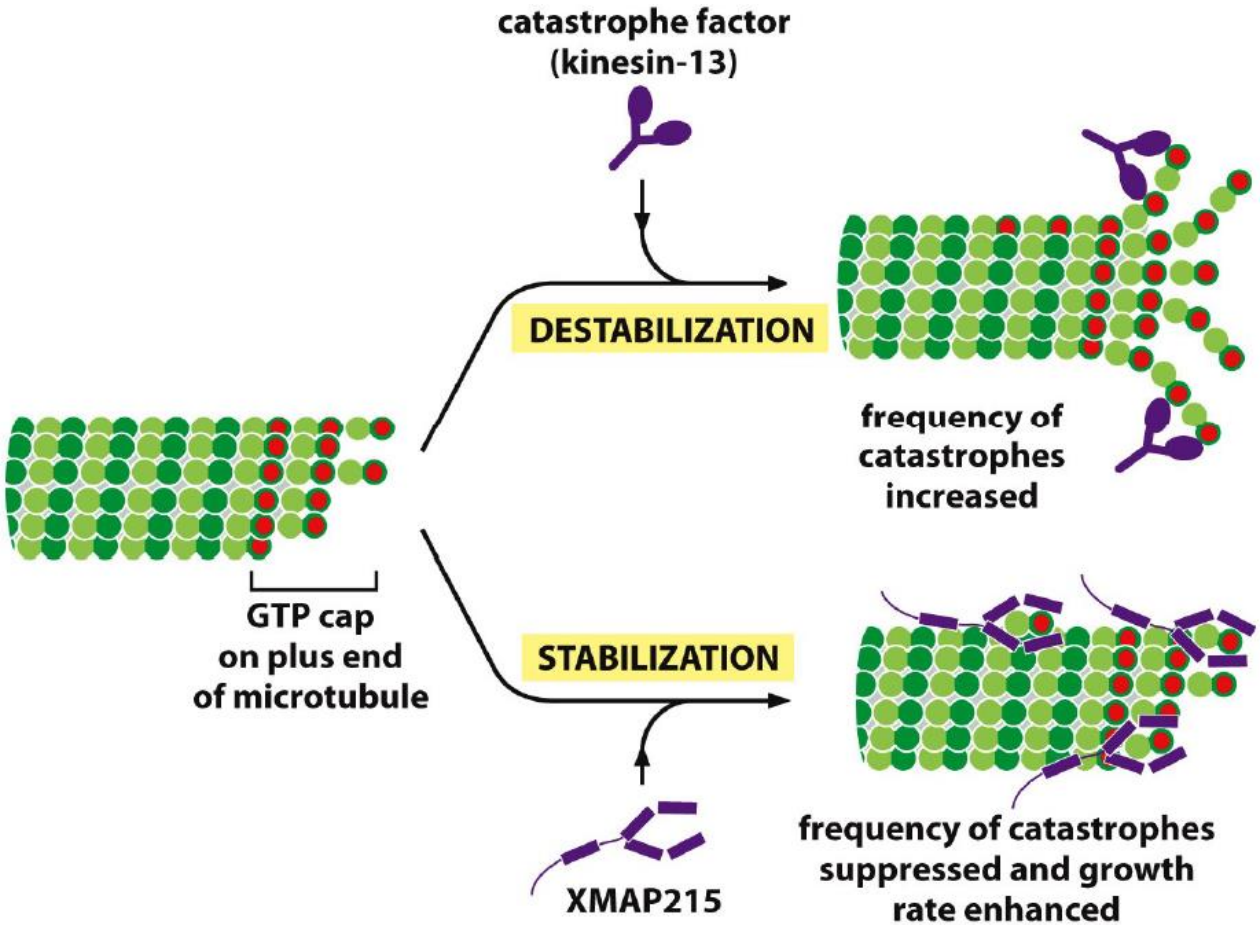
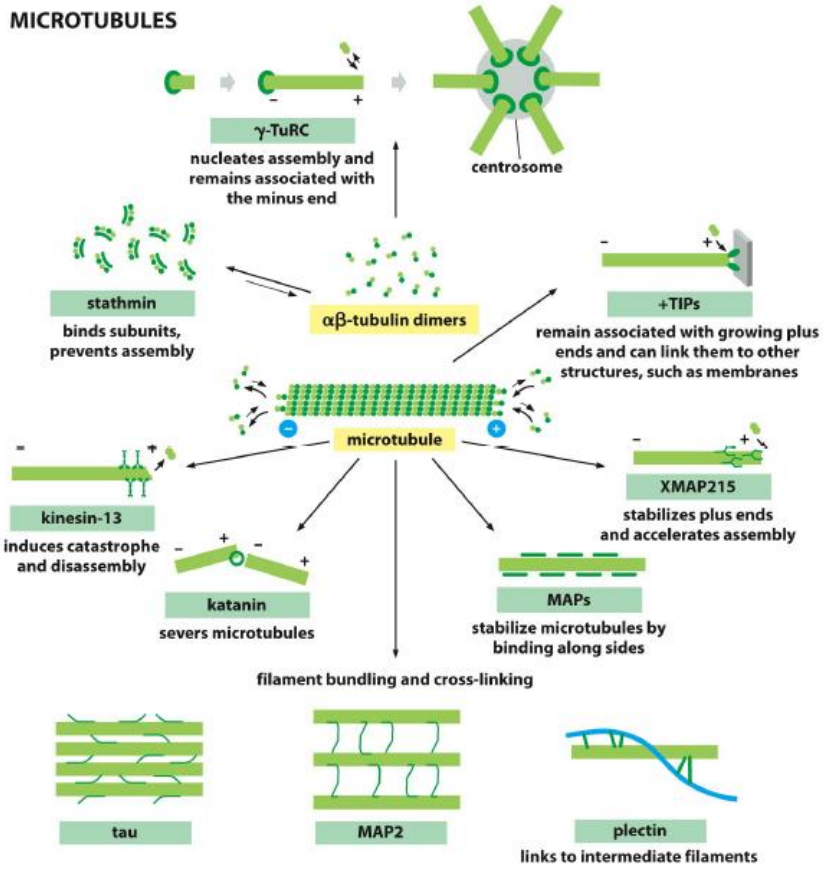


Figure 16-52 Molecular Biology of the Cell 6e (© Garland Science 2015)

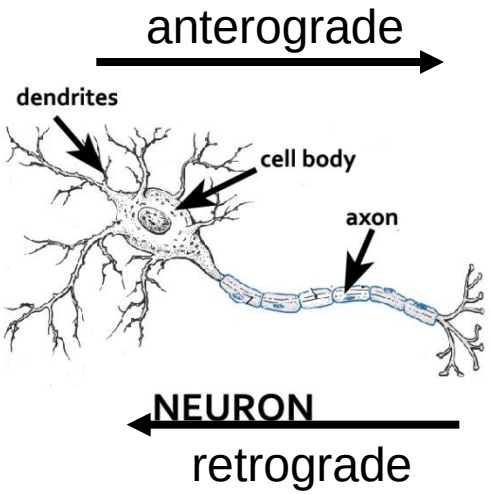
Microtubule-binding proteins modulate filament assembly and dynamics



Some of the major accessory proteins of the microtubule cytoskeleton. Except for two classes of motor proteins, an example of each major type is shown. Each of these is discussed in the text. However, most cells contain more than a hundred different microtubule-binding proteins, and — as for the actin-associated proteins — it is likely that there are important types of microtubule-associated proteins that are not yet recognized.

IV. Kinesins and dyneins: microtubule-based motor proteins

- 1. Methods to study microtubule transport
- 2. Kinesin family
- 3. Mechanism for kinesins (anterograde)
- 4. dynein (retrograde)



1. Methodology for microtubule transport

Isolate axon from giant squid, ~1mm in diameter:

1.Pulse- chase labeling:

2.Cut axons into segments- gel electrophoresis

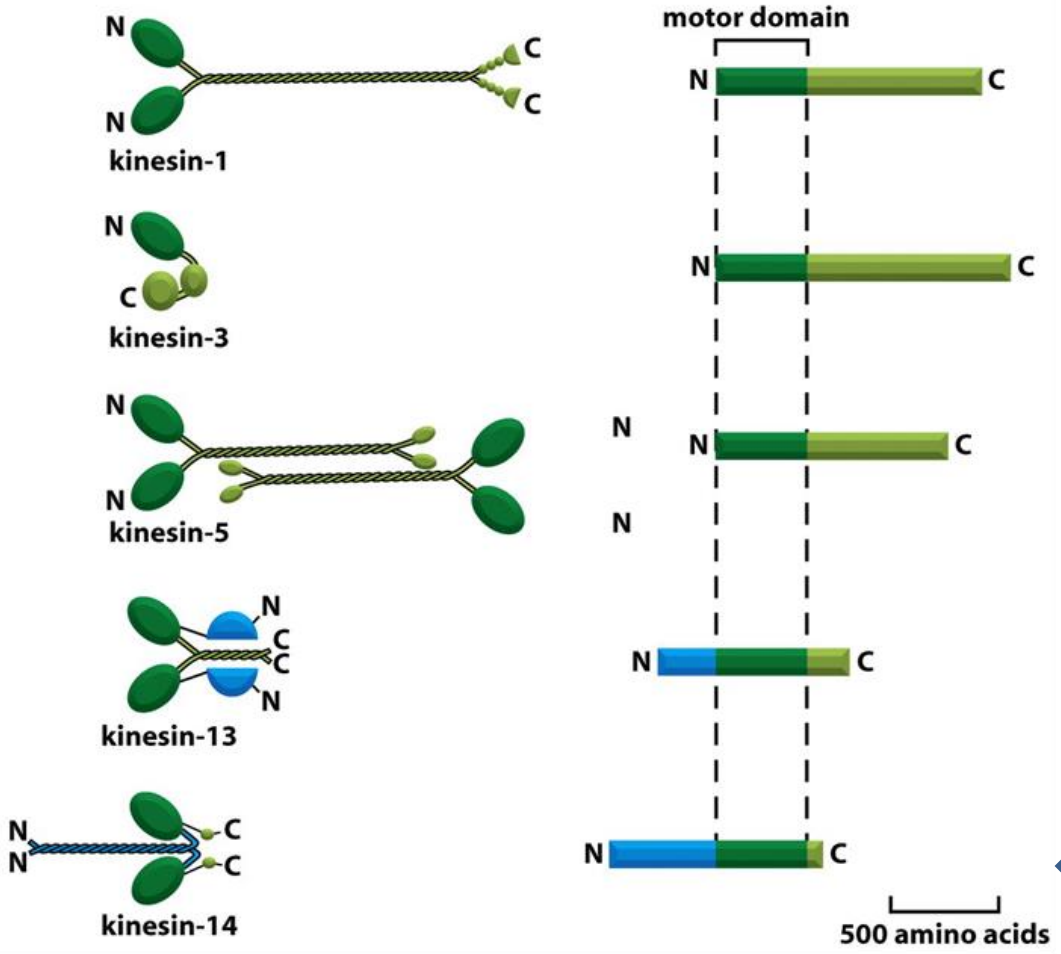
3.Cell free system:

ATP (AMP-PNP)
taxon-stabilized microtubule
purified organelle(labelled)
axon extract (organelle free)

Findings: 1. transport occurs bi-directionally
(anterograde and retrograde).
2. some are transported fast, some slow.
3. By providing AMP-PNP, motor proteins bind to microtubule and organelle tightly without dissociation.

Identify kinesin 1 and other MAPs.

2. Kinesin family(anterograde)---toward plus end



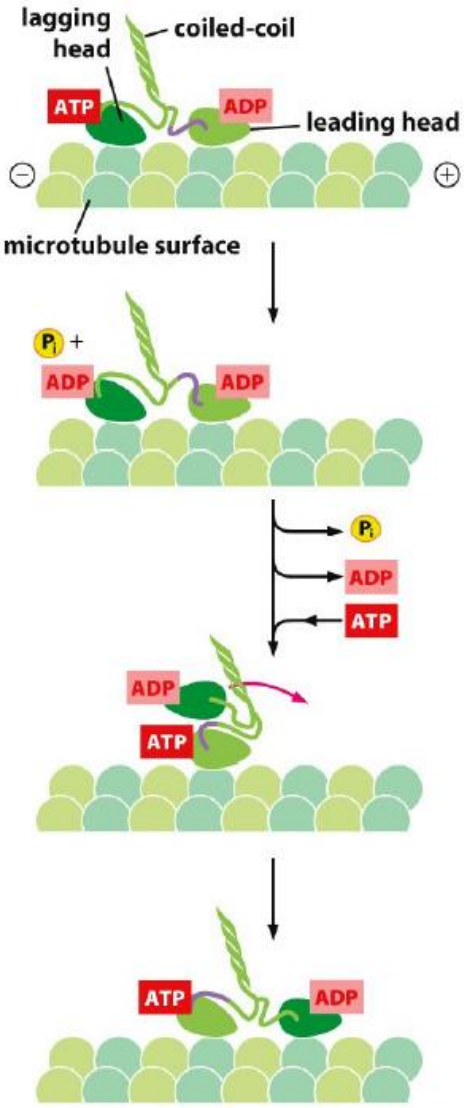
Diverse roles:

- ♠organelle transport,
- ♠mRNA transport,
- ♠chromosome transport
- ♠microtubule sliding
- ♠microtubule depolymerization

Move toward
Minus end

3. Action mechanism for Kinesin

- Always associate with microtubules
- “ hand-over-hand” motion
- ADP-kinesin binds weakly to tubule
- ATP-kinesin binds strongly to tubule



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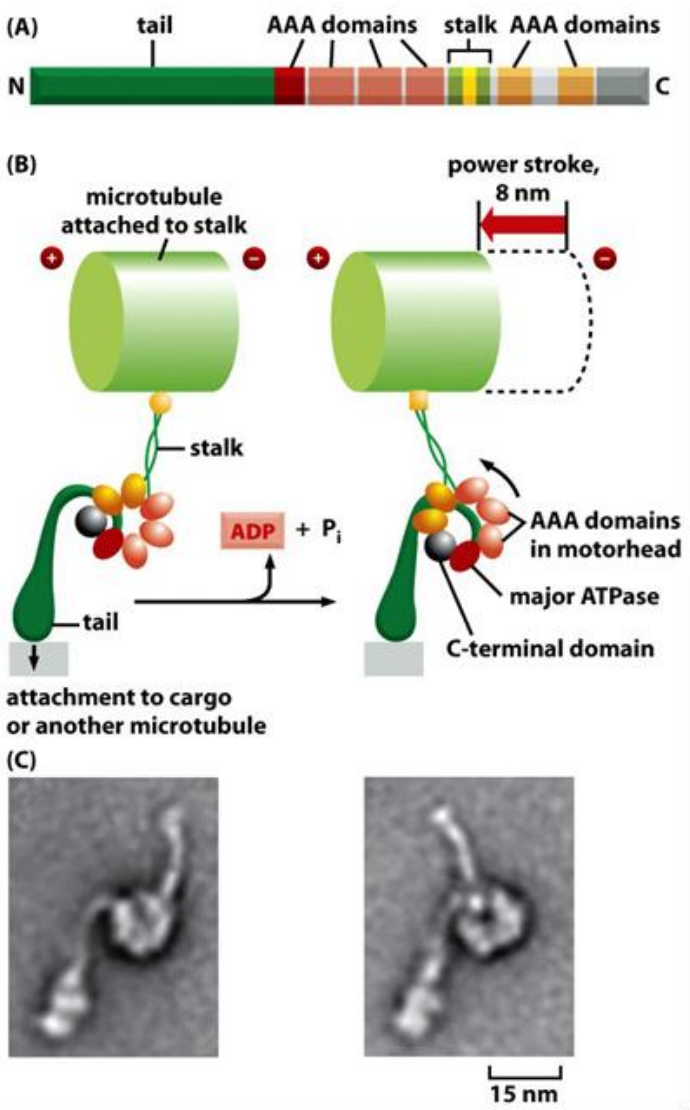
4. Dynein (retrograde)---toward minus end

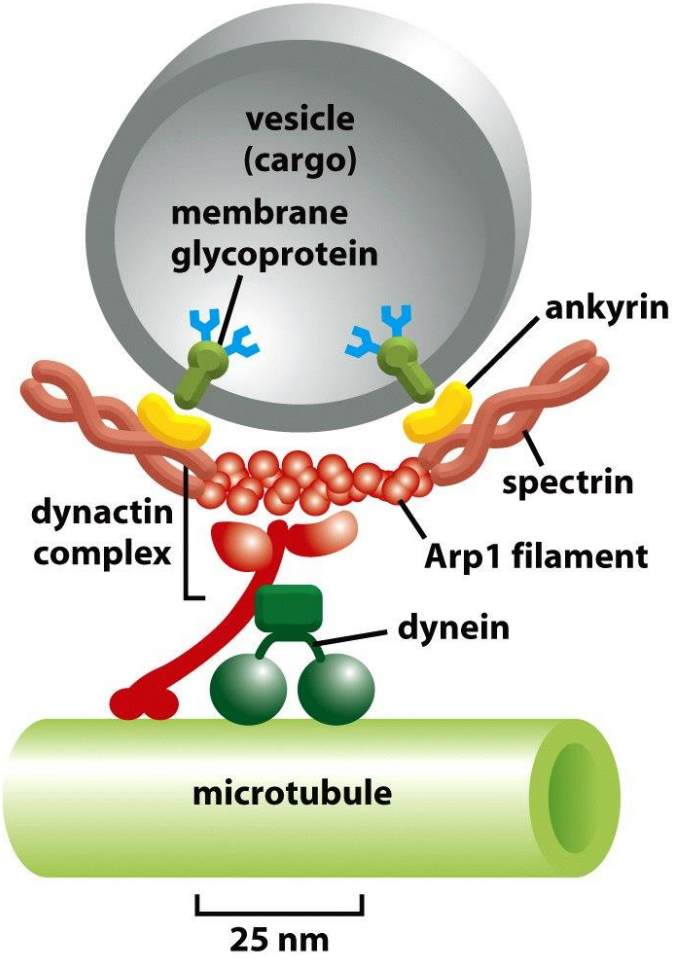
♥ It is a very large multisubunit protein with ATPase activity and two heads

♥ It itself can't transport cargo, but need dynactin to link to cargo for transport.

♥ Each power stroke generates a step ~8nm Toward its minus end.



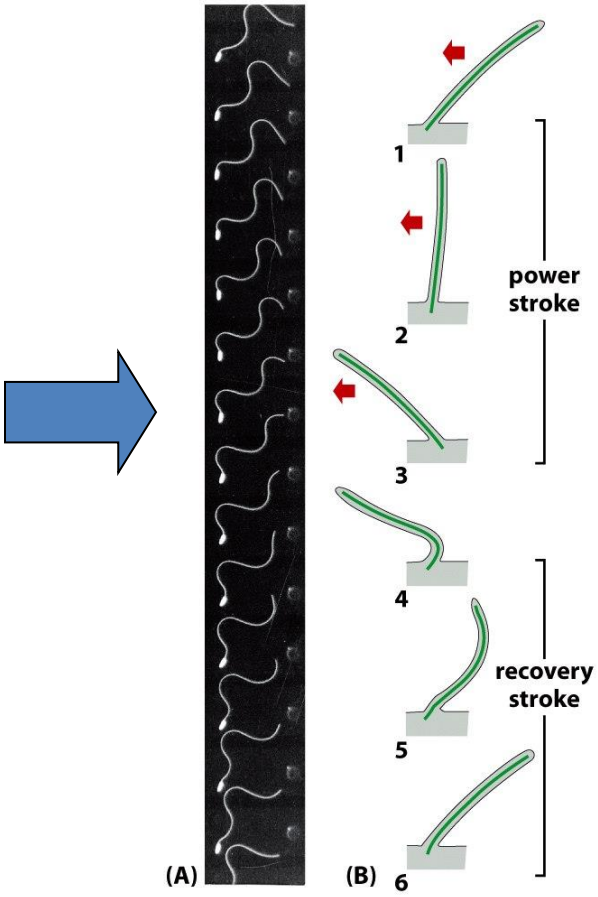
How dynein and dynactin together transport vesicle



V. Cilia and flagella

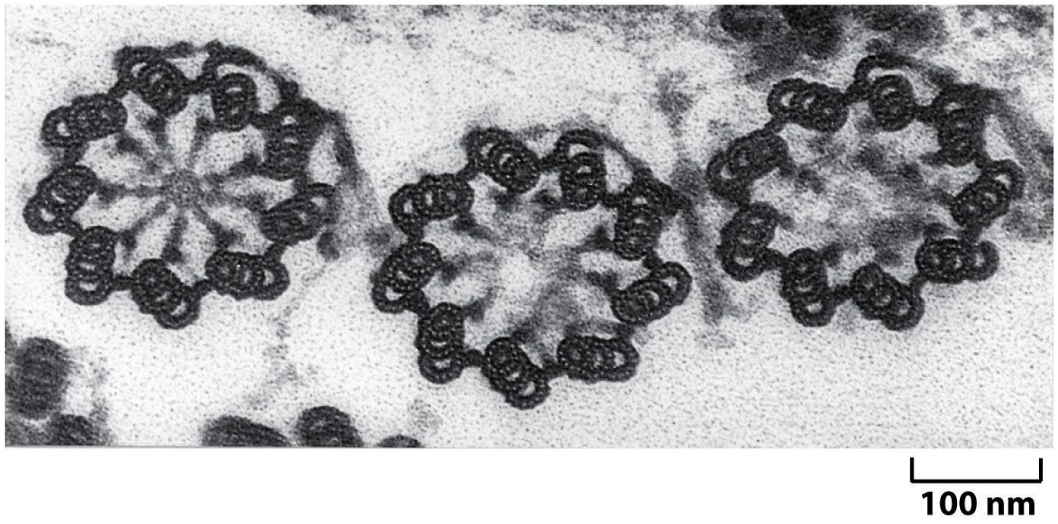
- Both are built from microtubule and dynein

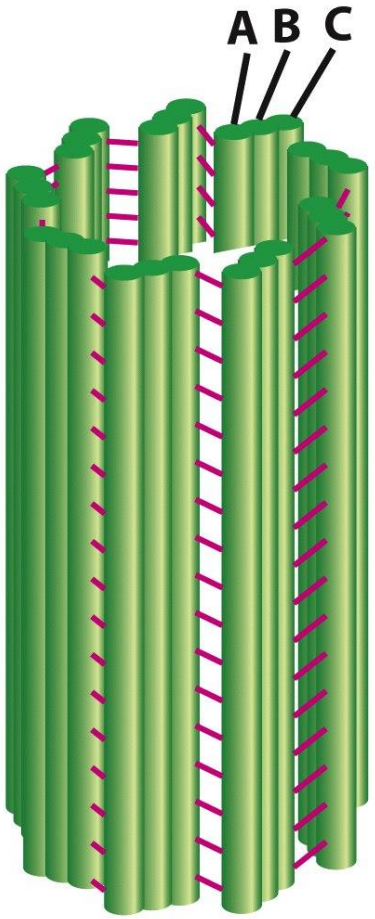
Different modes of movement



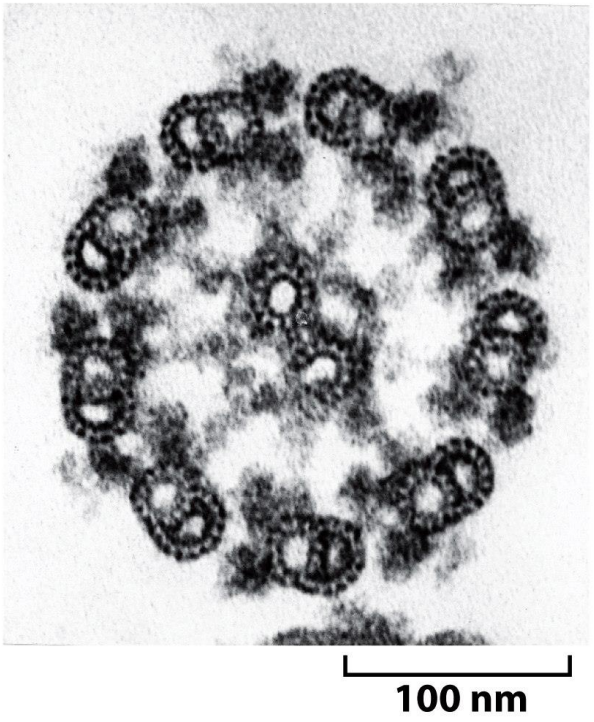
Basal bodies---microtubule assembly sites for cilia and flagella

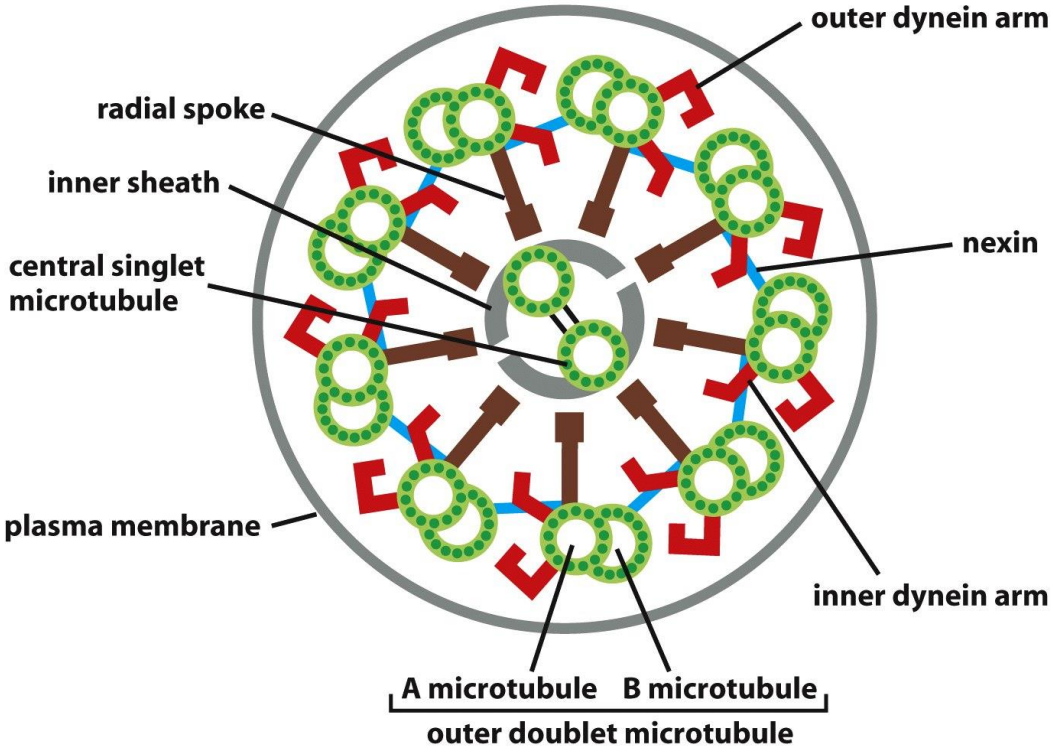
- Firmly root eukaryotic cilia and flagella at the cell surface.
- Similar to centrioles



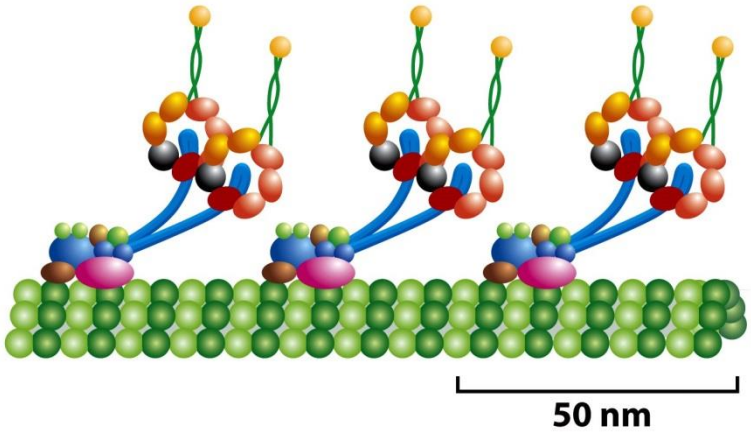


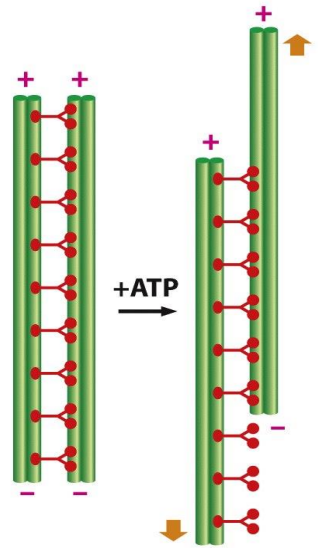
A “9+2” arrangement for microtubules in axoneme of cilia and flagella



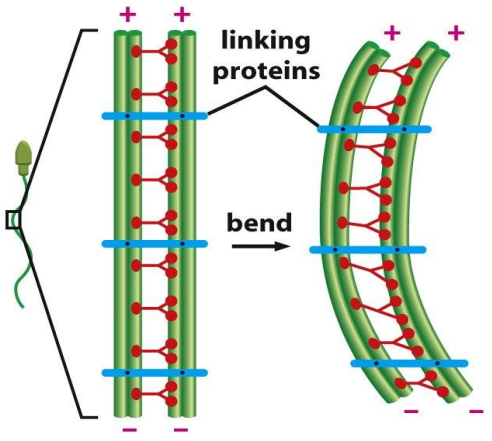


The bending of axoneme due to **fixed links** between microtubule doublets





(A) IN ISOLATED DOUBLET MICROTUBULES: DYNEIN PRODUCES MICROTUBULE SLIDING



(B) IN NORMAL FLAGELLUM: DYNEIN CAUSES MICROTUBULE BENDING

Kartagener’s syndrome due to genetic defects in dynein

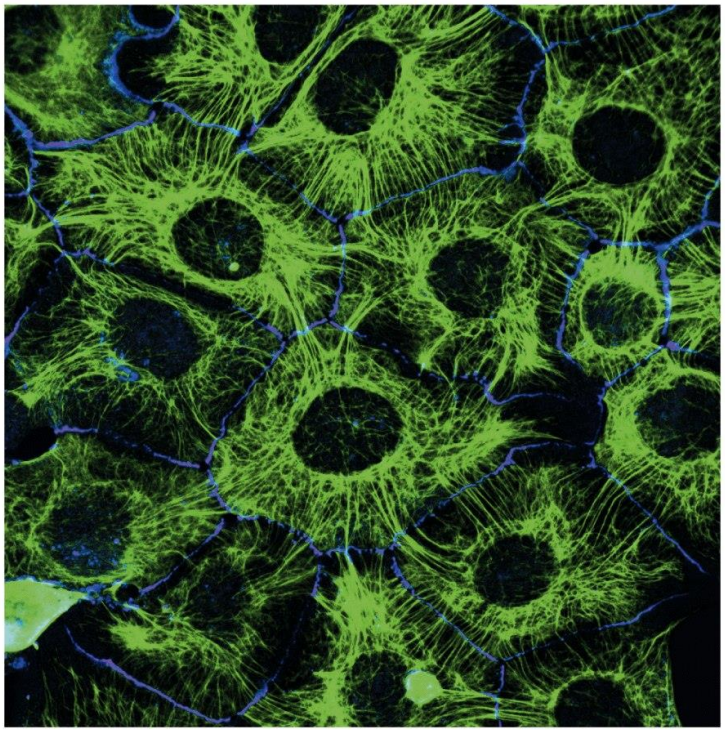
- Male sterility
- High susceptibility to lung infection
- Defects in left-right axis determination in development.

Charcot-Marie-Tooth disease

- A neurological disease, due to point mutation in a kinesin family member that transports synaptic vesicle precursors down the axon

VI. Intermediate filament

1. No polarity,
2. no motor activity,
3. Tensile and stable
4. hard to be solubilized
5. Not all eukaryotic cells have this, fungi and plants don’t have so far.
6. Very heterogeneous
7. Defects in genes for intermediate filaments are associated with ~50 clinical disorders.



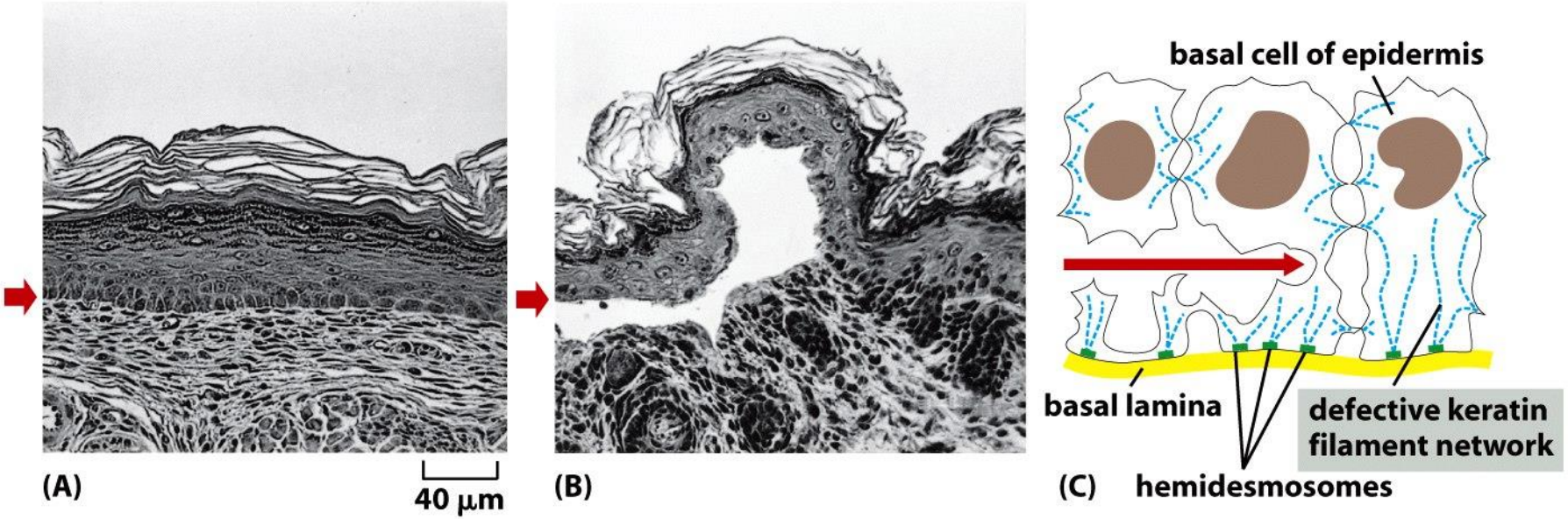
Keratin in epithelia

Major types of intermediate filament proteins

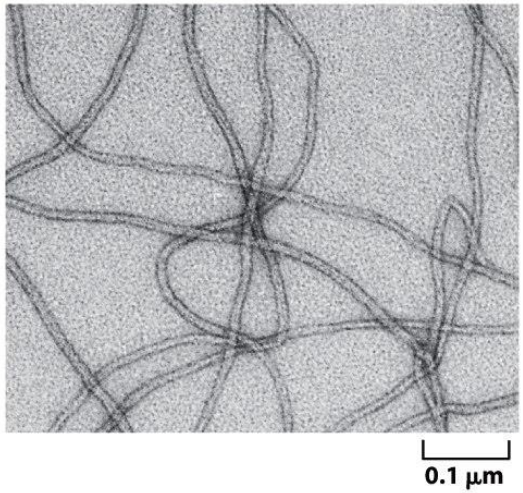
Table 16–1 Major Types of Intermediate Filament Proteins in Vertebrate Cells

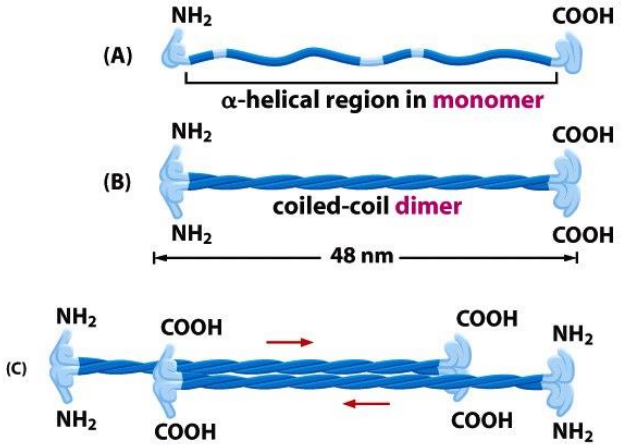
TYPES OF IF	COMPONENT POLYPEPTIDES	LOCATION
Nuclear	lamins A, B, and C	nuclear lamina (inner lining of nuclear envelope)
Vimentin-like	vimentin	many cells of mesenchymal origin
	desmin	muscle
	glial fibrillary acidic protein	glial cells (astrocytes and some Schwann cells)
	peripherin	some neurons
Epithelial	type I keratins (acidic)	epithelial cells and their derivatives (e.g., hair and nails)
	type II keratins (basic)	
Axonal	neurofilament proteins (NF-L, NF-M, and NF-H)	neurons

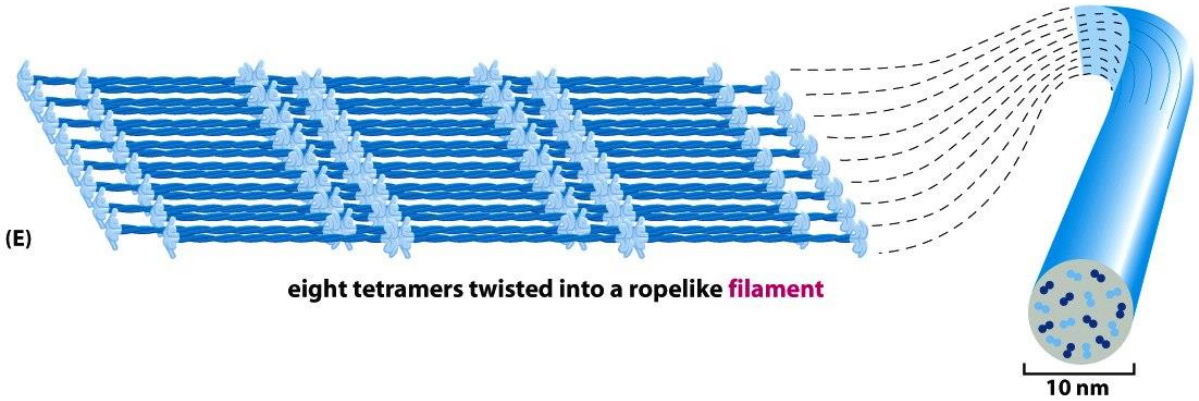
Defects in keratin results in skin blistering



How intermediate filament is assembled?







Symmetrical, no polarity

Intermediate filaments are crosslinked and bundled into strong arrays

- Through lateral contacts
- Through proteins such as filaggrin- keratin filaments, plectin—crosslinks intermediate filaments. Mutation in plectin results in serious human disease characterized by epidermolysis bullosa, muscular dystrophy and neurodegeneration.
- Keratins are further crosslinked by disulfide bonds.