

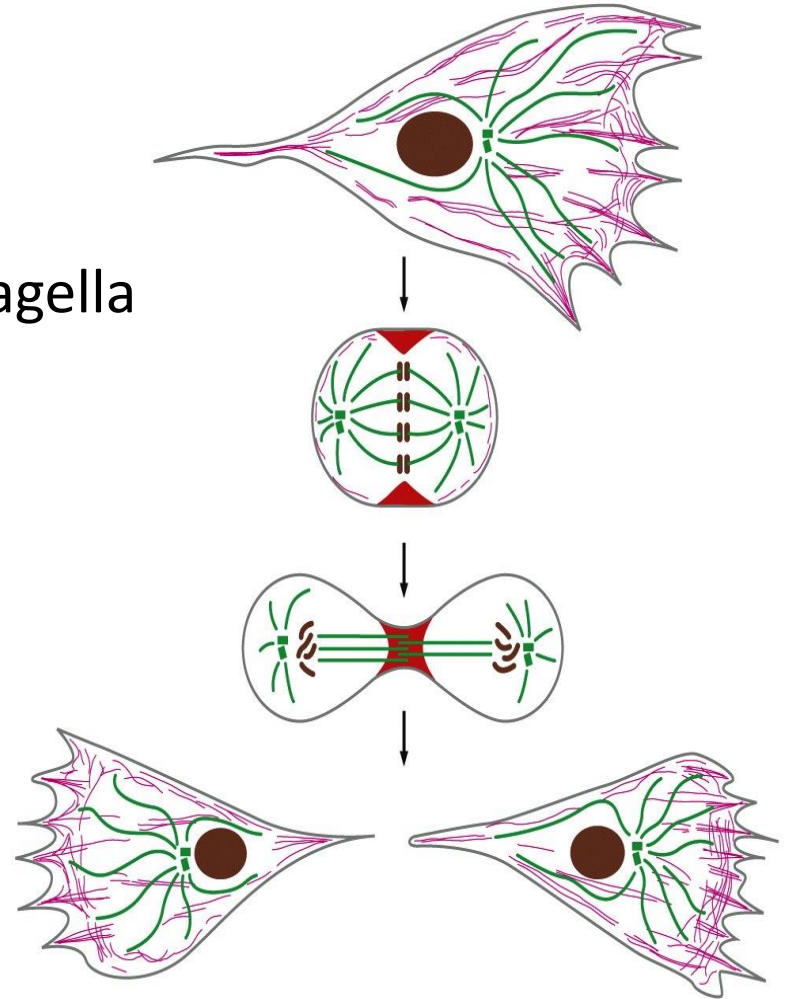
# Lecture 13. Cytoskeleton and cell movement II

## Outline

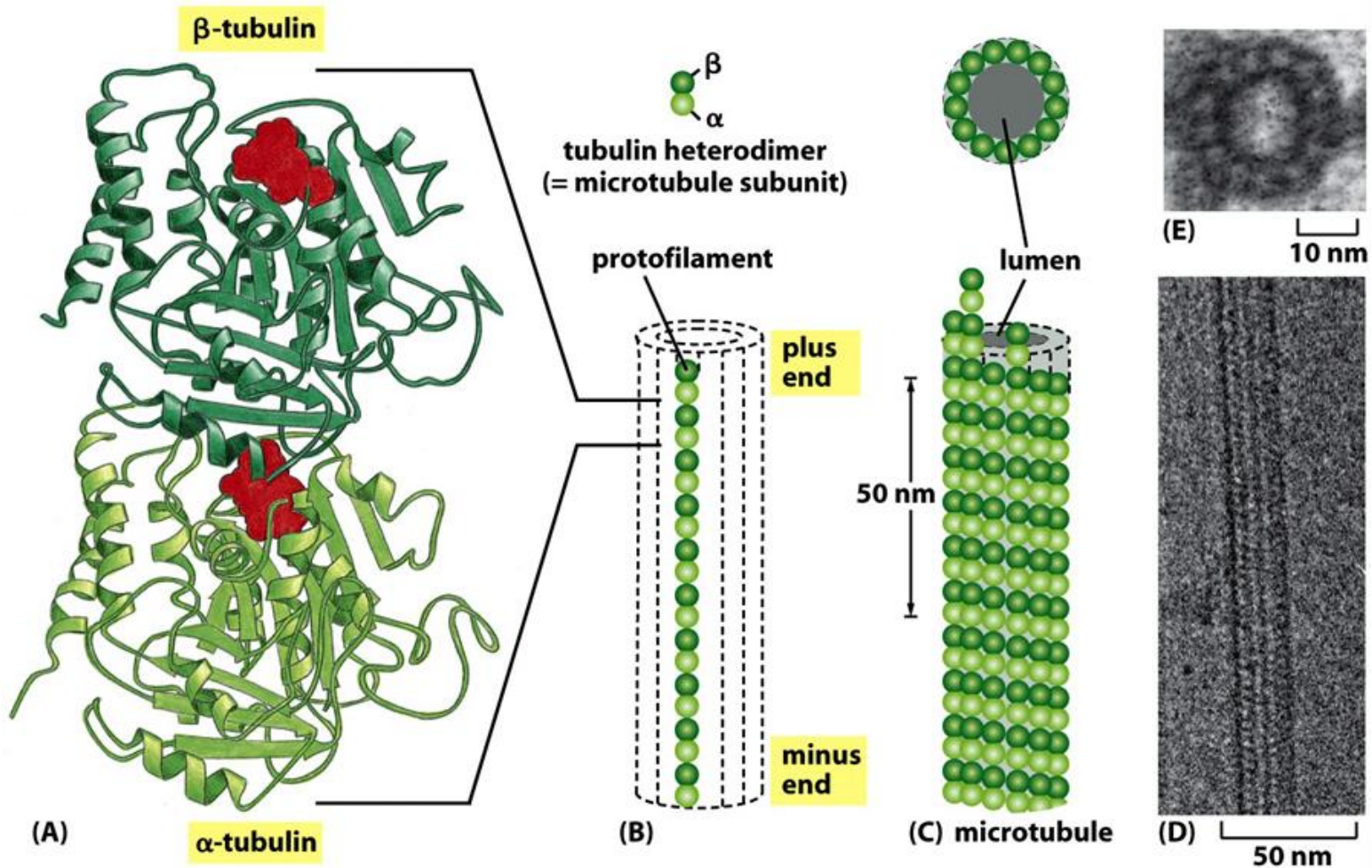
- I. Microtubule structure and organization
- II. Microtubule dynamics
- III. Regulation of microtubule assembly
- IV. Kinesins and Dyneins: microtubule-based motor proteins
- V. Cilia and flagella
- VI. Intermediate filaments

# I. Microtubule structure and organization

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



# Organization of microtubule



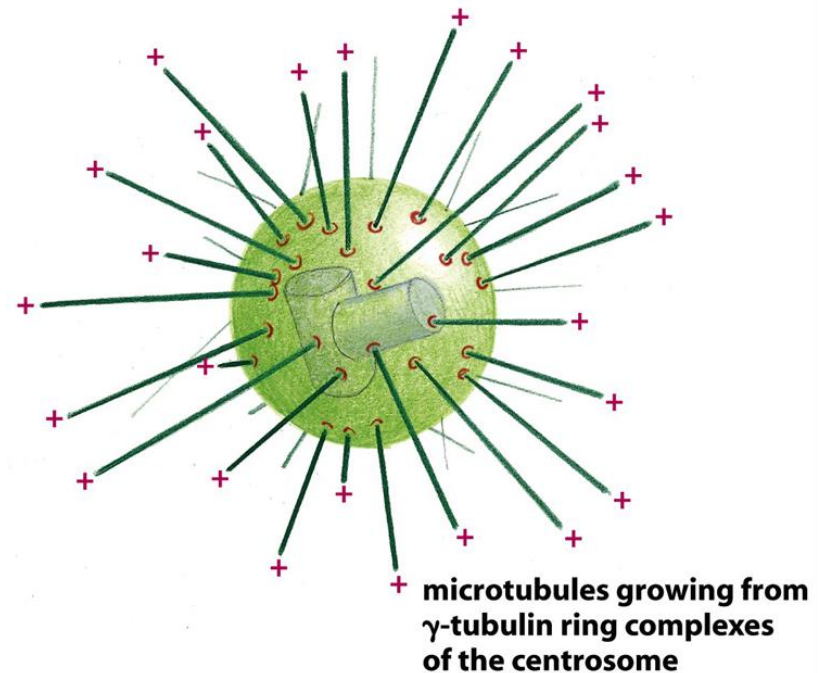
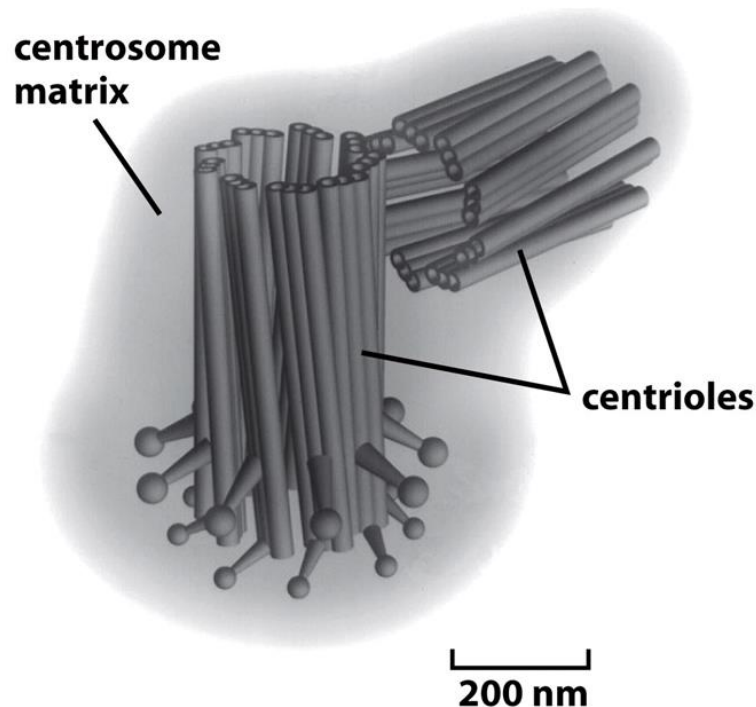
# Summary about microtubule proteins

- ♥ 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,  **$\alpha$ -tubulin**, and  **$\beta$ -tubulin**, forming **heterodimer**.
- ♥ All subunits are **oriented in the same way**, the one with **exposed  $\alpha$ -tubulin is minus end**, the **one with exposed  $\beta$ -tubulin is plus end**, microtubule has polarity.
- ♥ Each  $\alpha$ -tubulin and  $\beta$ -tubulin bind to one molecule of GTP, the **GTP on  $\alpha$ -tubulin is never hydrolyzed**, but GTP on  $\beta$ -tubulin is hydrolyzed.
- ♥  **$\gamma$ -tubulin** is important for microtubule assembly, Microtubule-associated proteins (**MAP**) are important in assembling and dynamics for microtubules.

# 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.

$\gamma$ -tubulin ring complex ( $\gamma$ -TuRC), pericentriolar, is critical to assemble microtubules





# $\gamma$ -tubulin ring complex nucleates microtubules

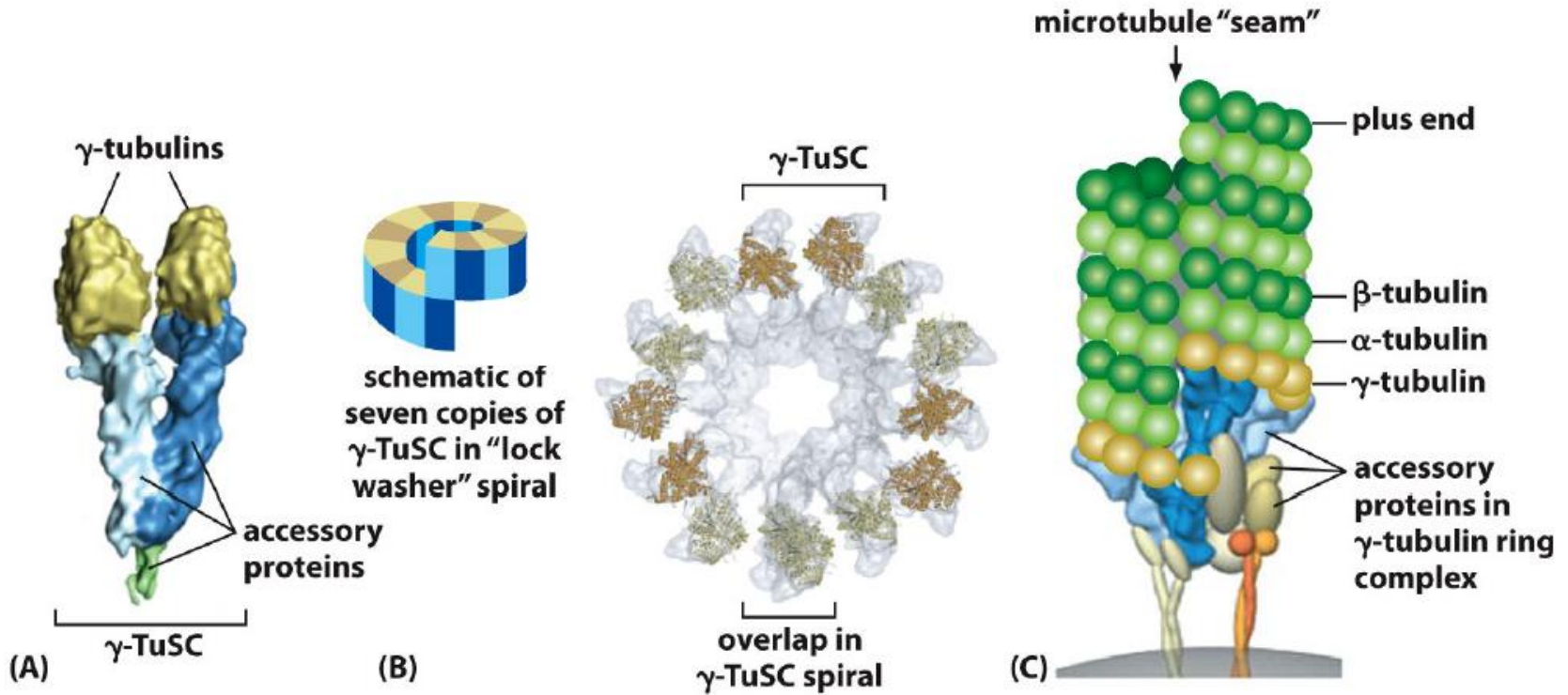
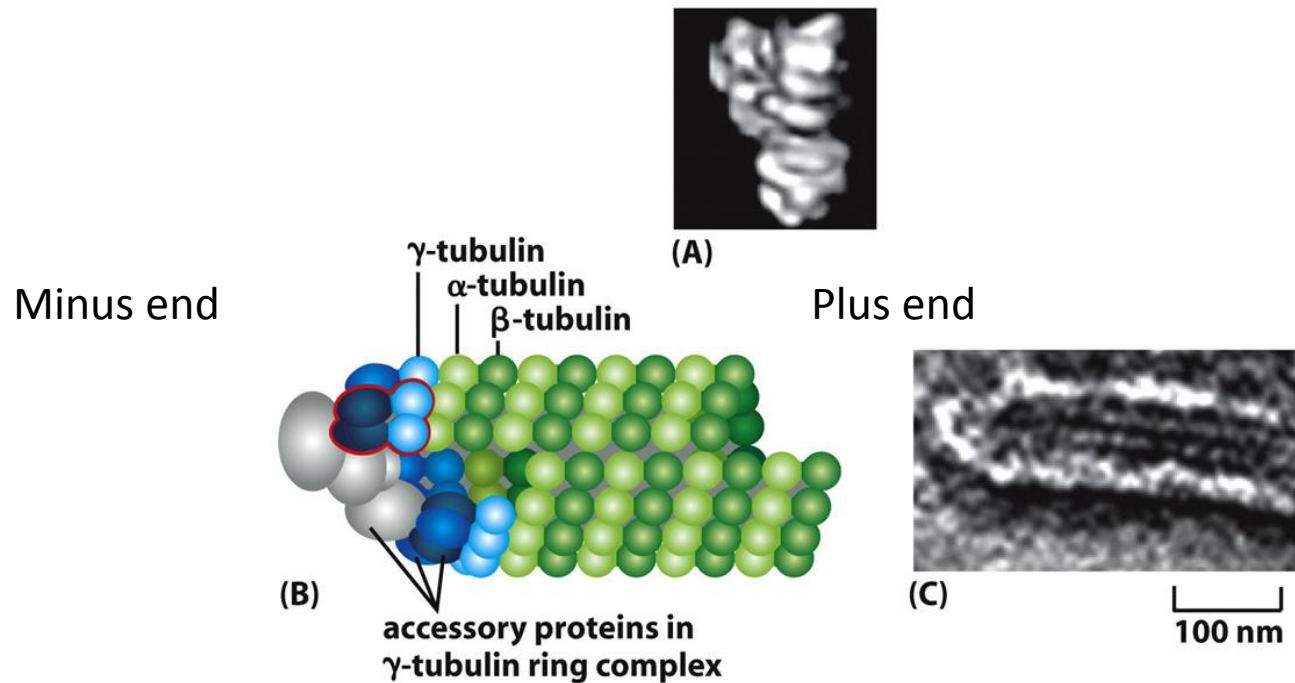


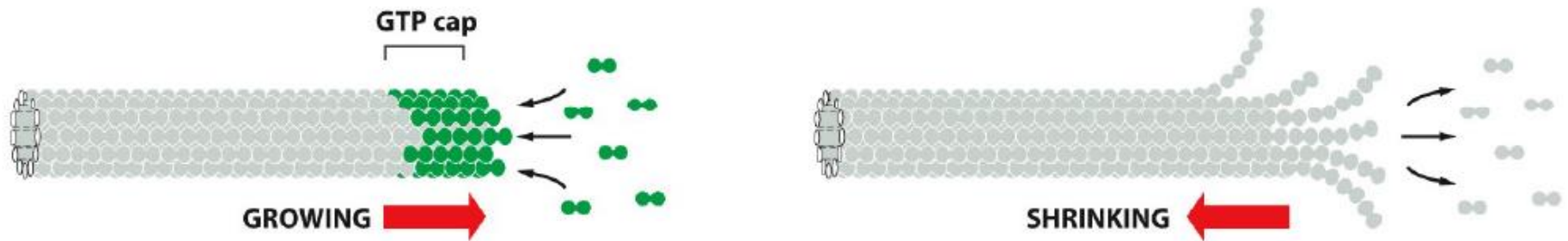
Figure 16-46 Molecular Biology of the Cell 6e (© Garland Science 2015)

# $\gamma$ -tubulin ring complex ( $\gamma$ -TuRC), pericentriolar, is critical to assemble microtubules





# Microtubule dynamic instability

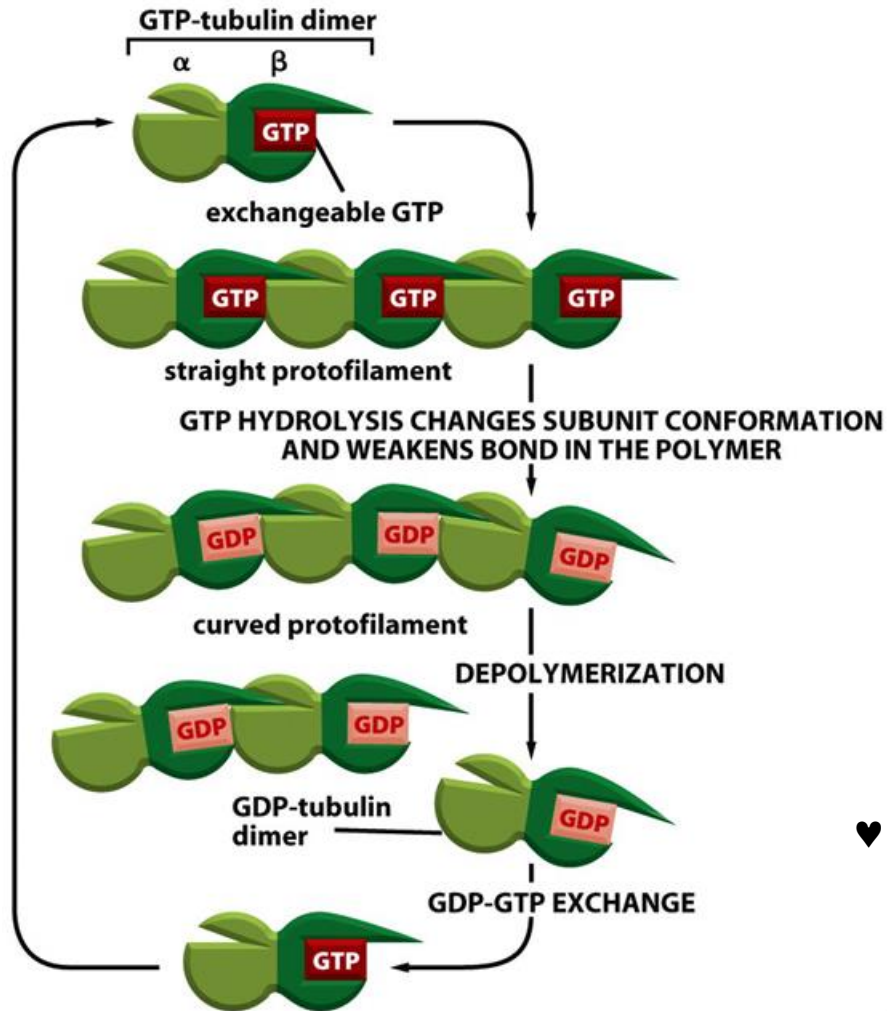


Individual microtubules can therefore alternate between a period of slow growth and a period of rapid disassembly, a phenomenon called **dynamic instability**.

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

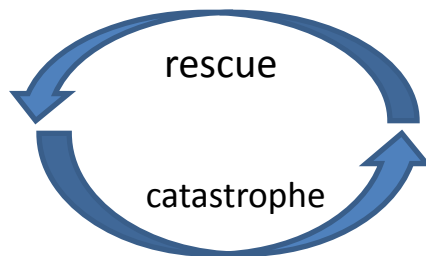
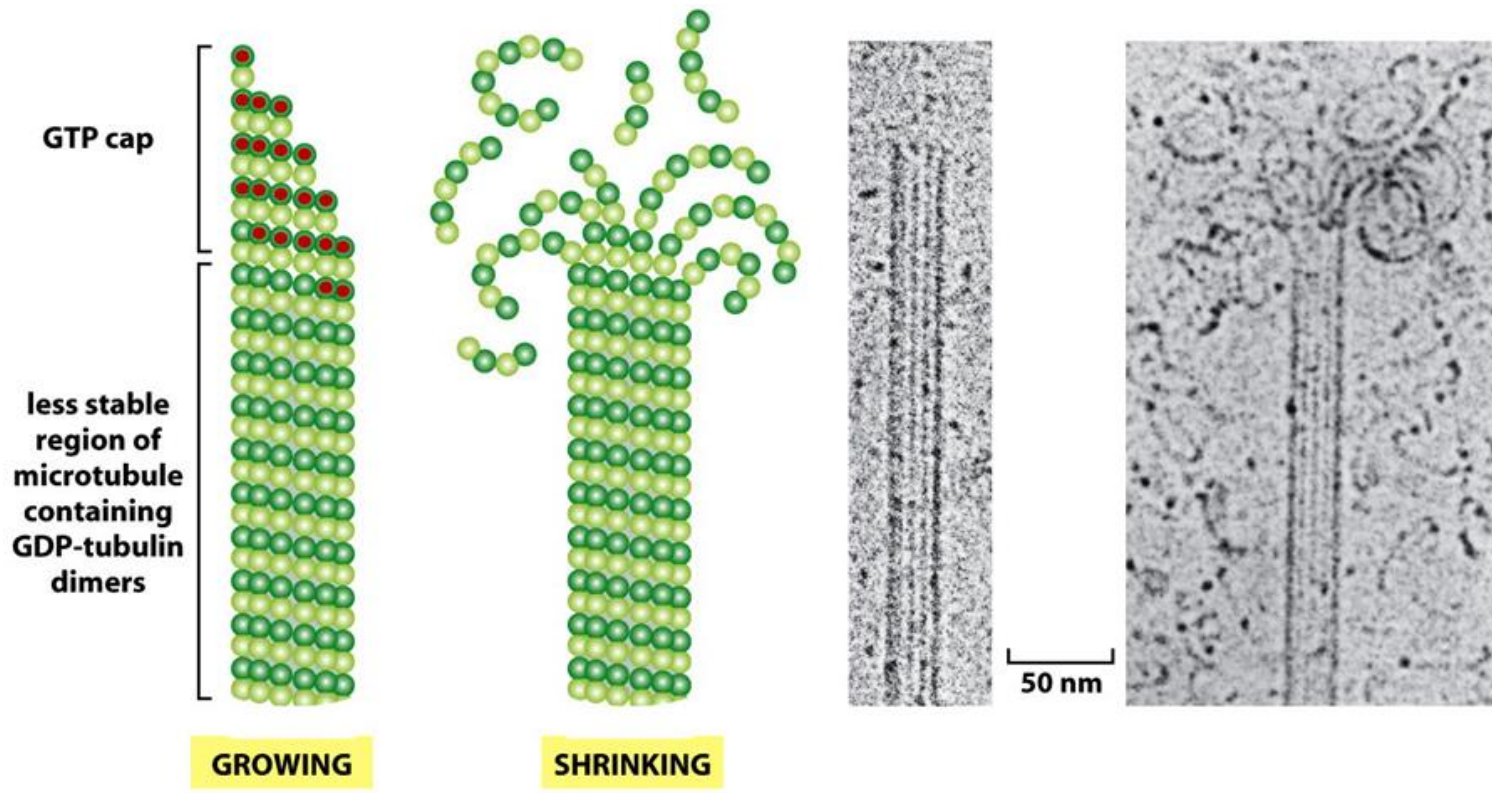
- ♥ Microtubules depolymerize about 100 times faster from an end containing GDP-tubulin than from one containing GTP-tubulin. A GTP cap favors Growth but if it is lost, then depolymerization ensues.

# Assembly of tubulin dimers

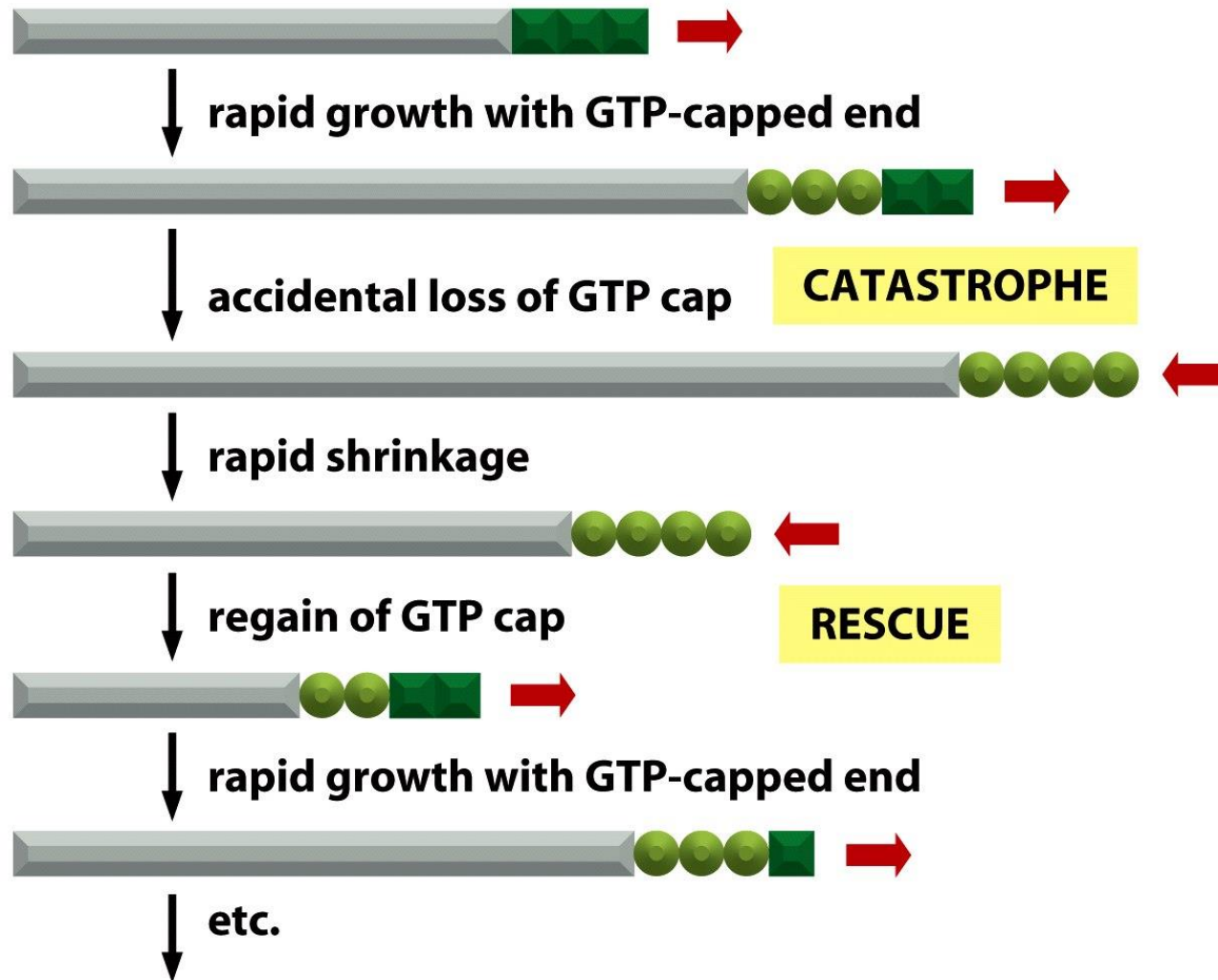


- ♥ Like actin, assembly at plus end is much Faster than assembly at minus end.

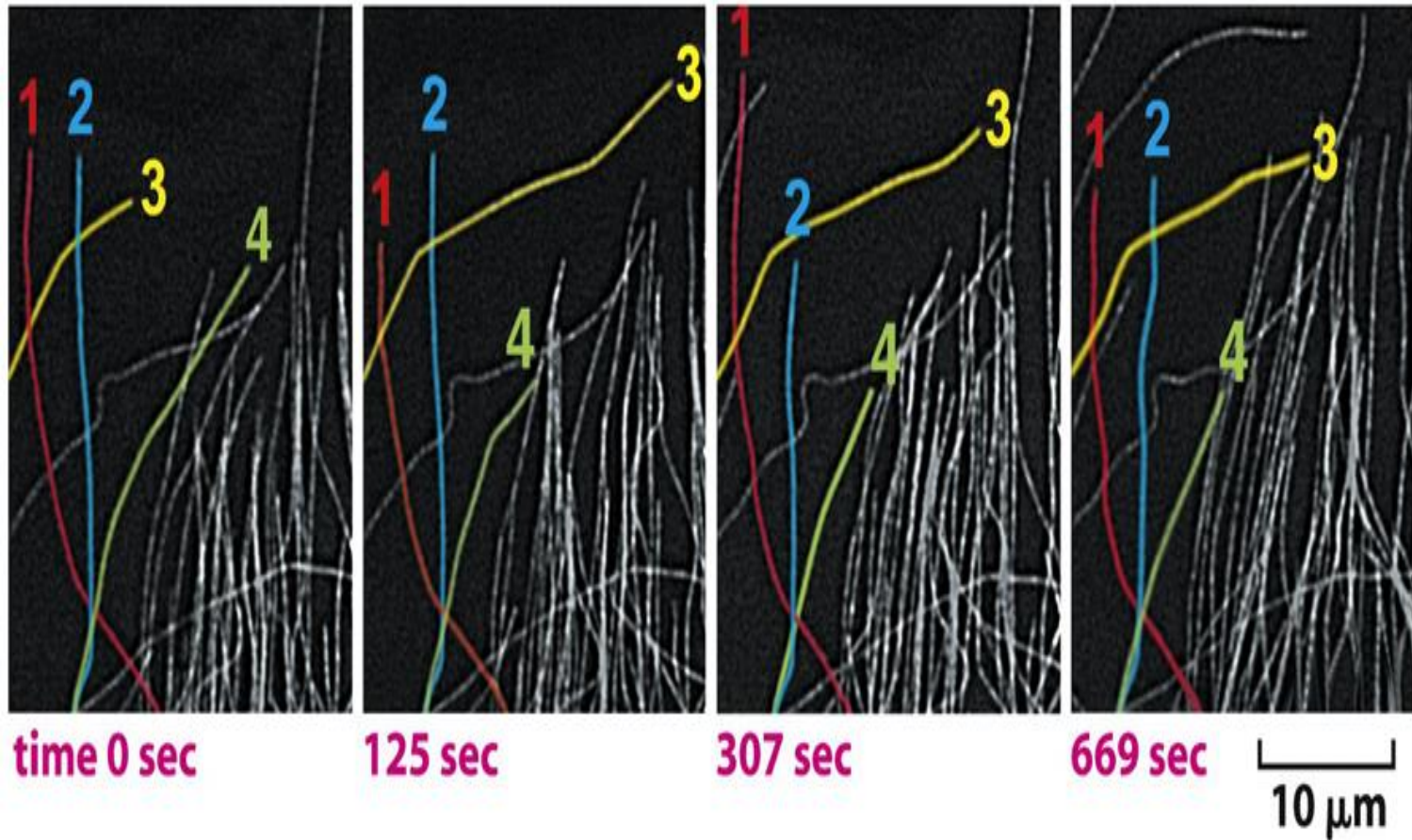
## II. Microtubule dynamics



# 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**

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



### 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

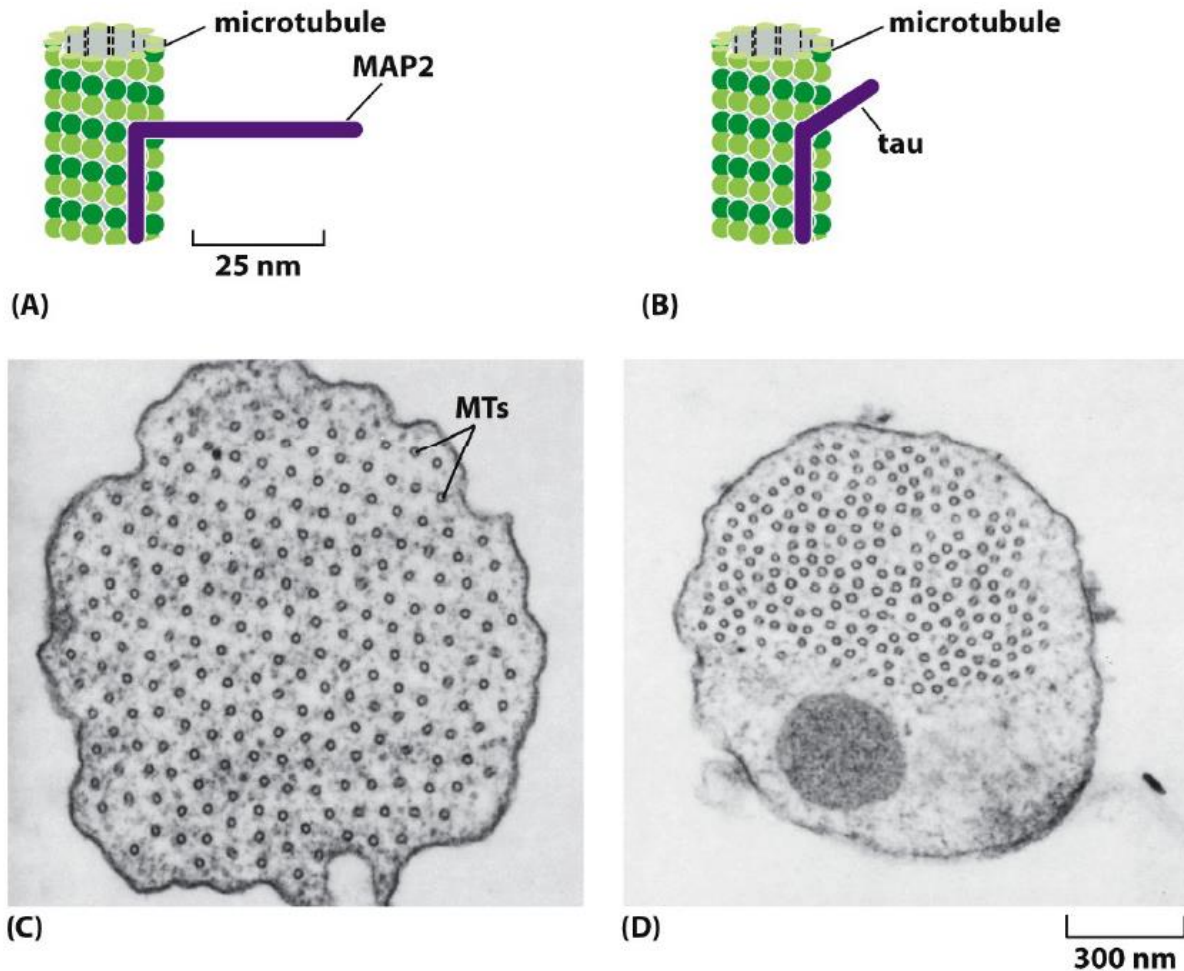


Figure 16-51 Molecular Biology of the Cell 6e (© Garland Science 2015)

# Microtubule plus end binding proteins modulate microtubule assembly

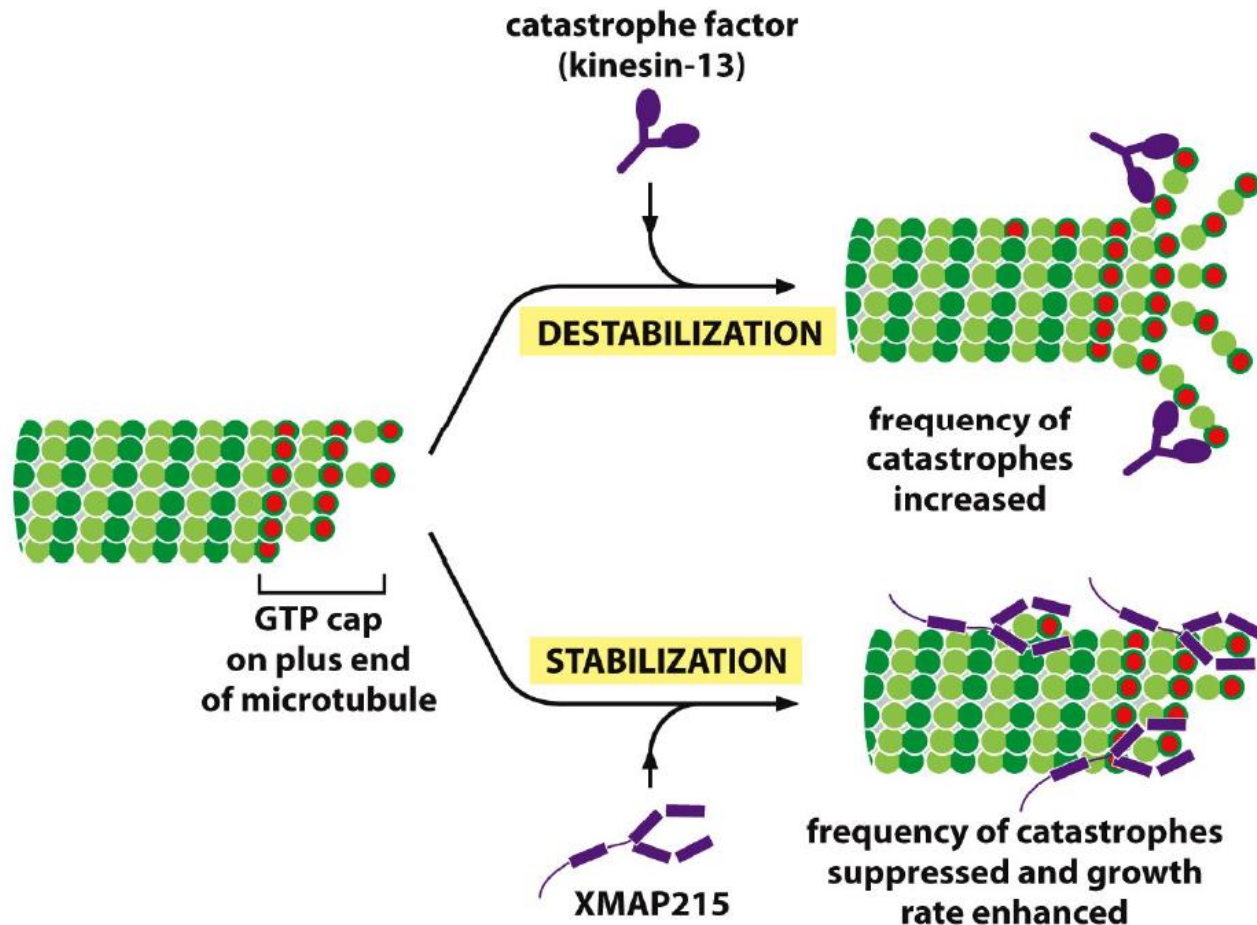
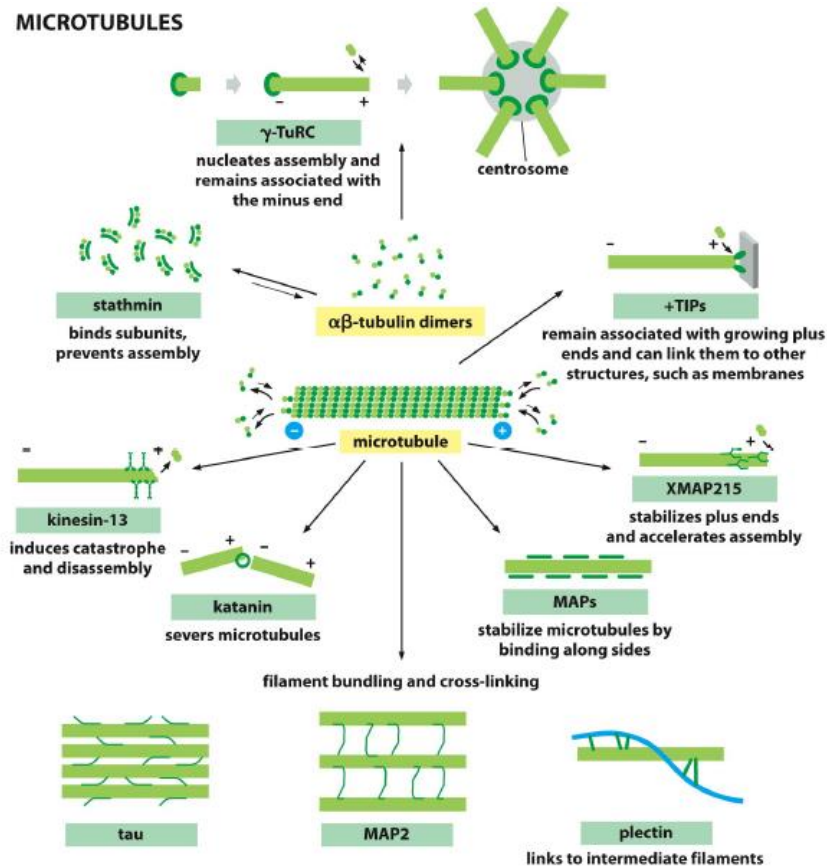


Figure 16-52 Molecular Biology of the Cell 6e (© Garland Science 2015)

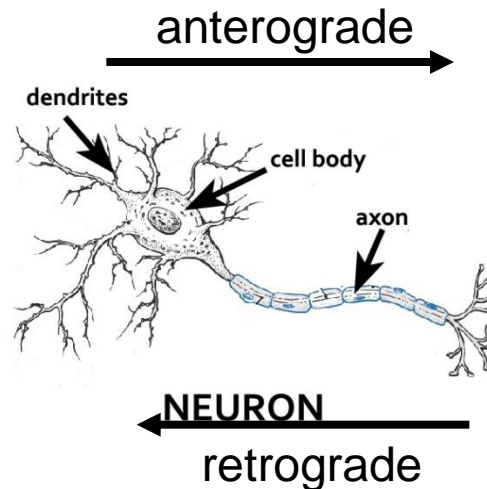
# Microtubule-binding proteins modulate filament assembly and dynamics



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) 顺行
- 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.

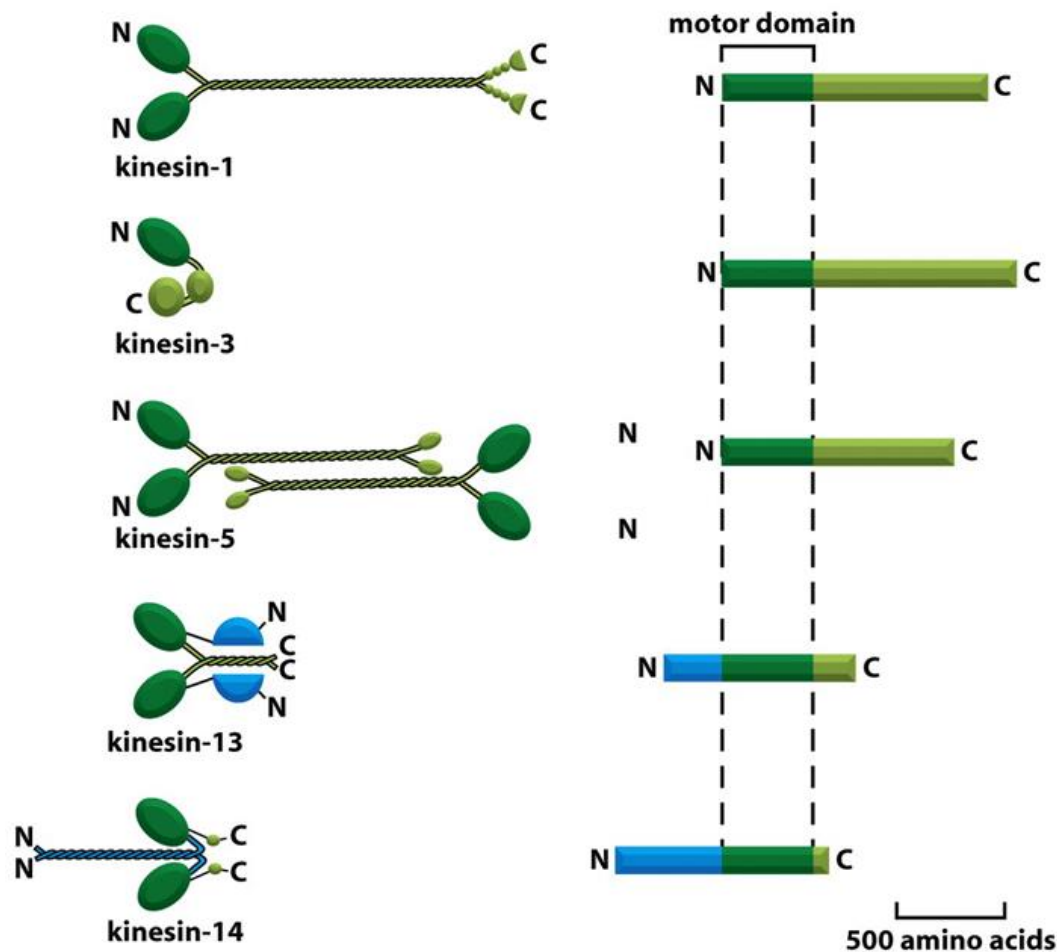


Identify kinesin 1 and other MAPs.





## 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

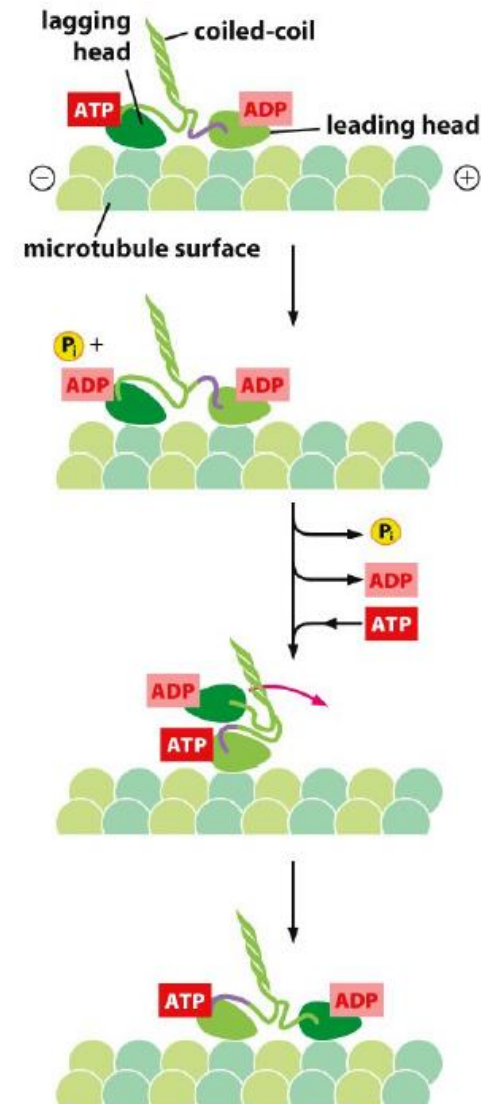


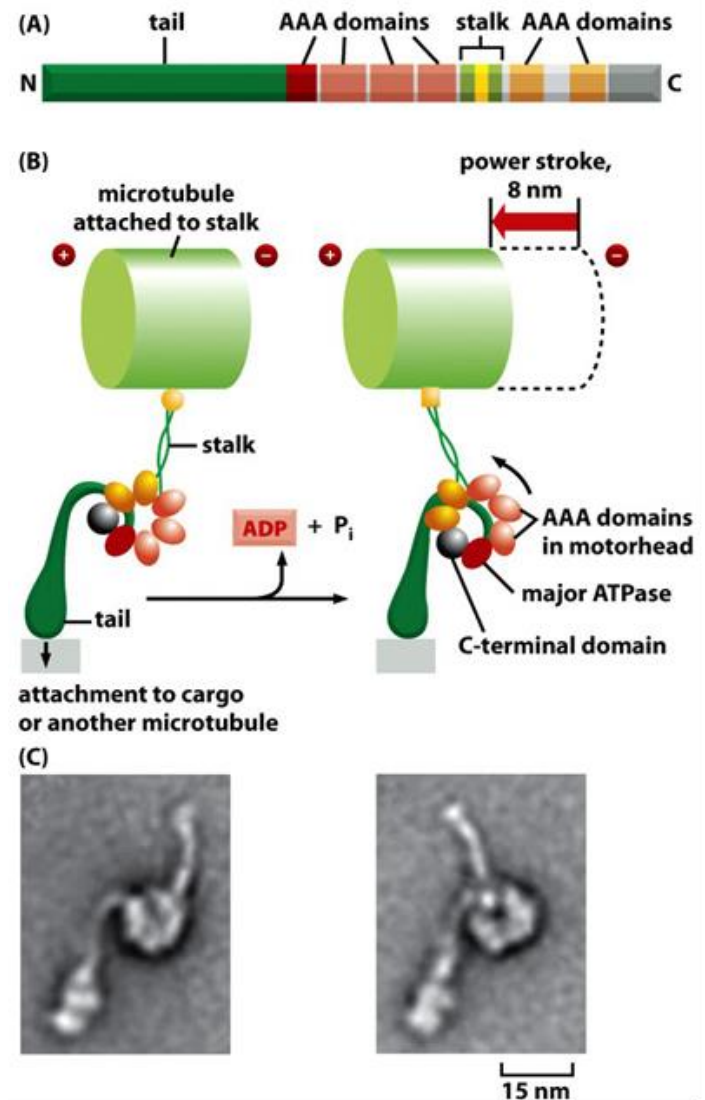
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## 4. Dynein (retrograde)---toward minus end

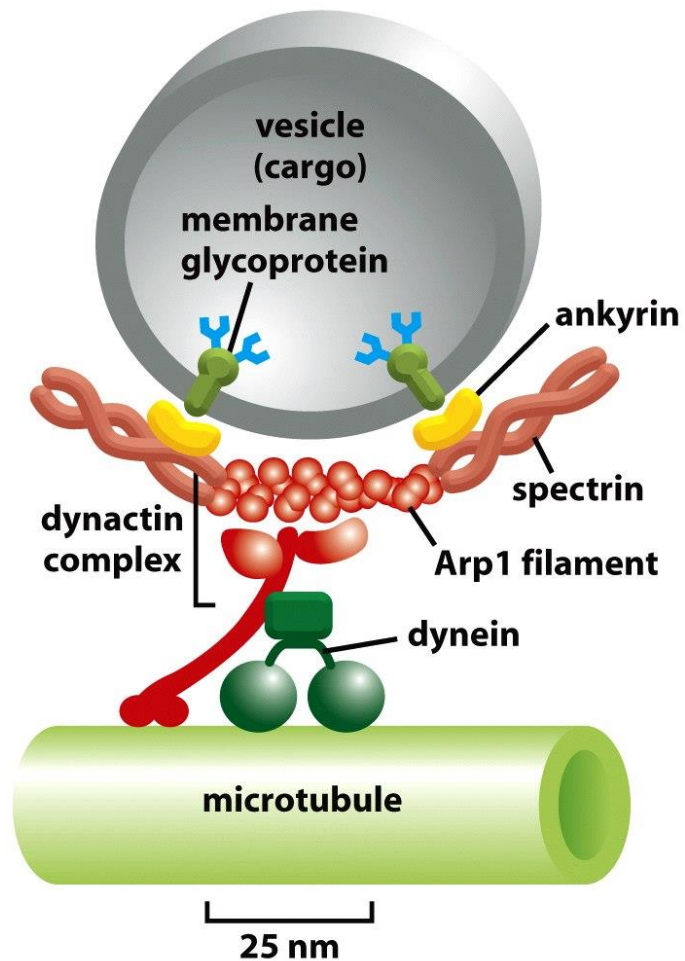
♥ It is a very large multisubunit protein with ATPase activity and two heads

♥ 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.



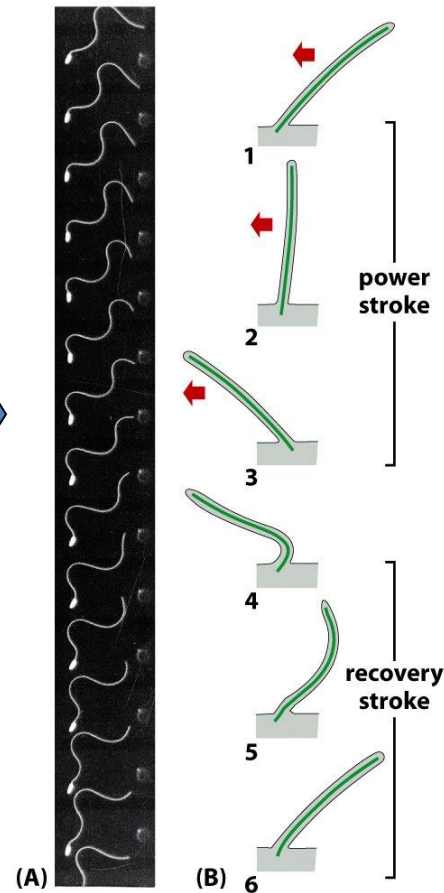
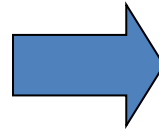
# How dynein and dynactin together transport vesicle



# V. Cilia and flagella

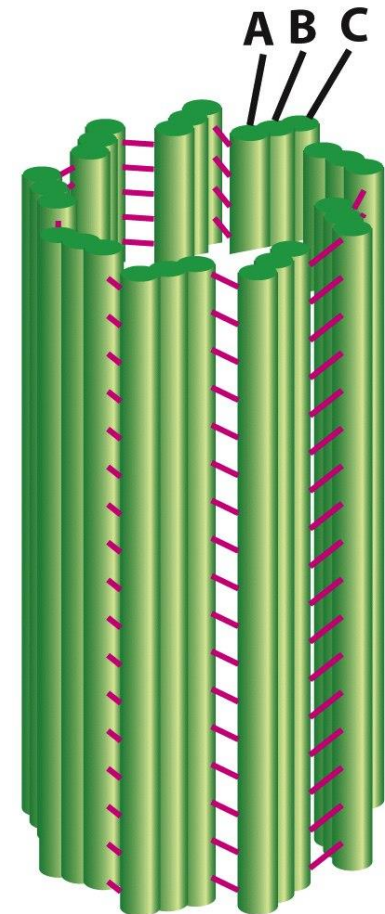
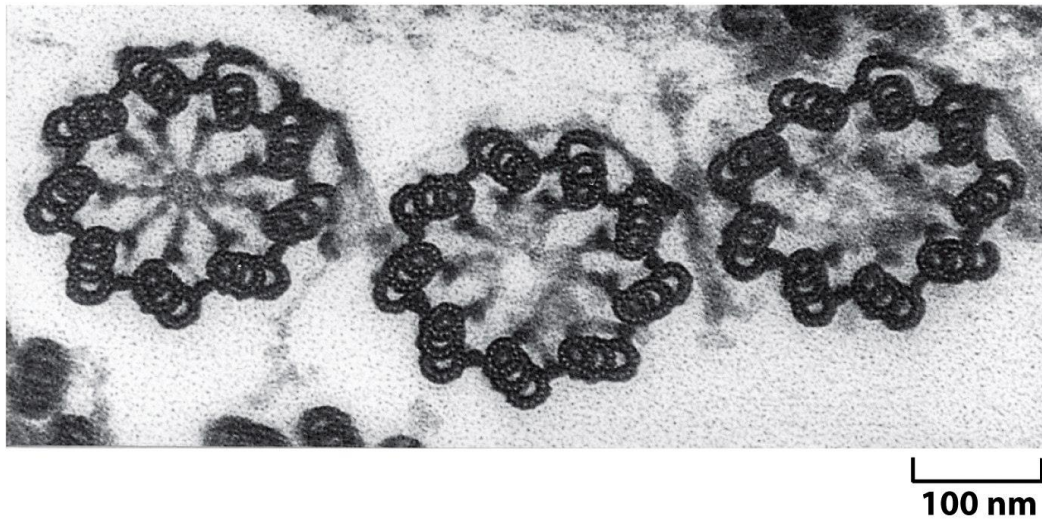
- Both are built from microtubule and dynein

Different modes of movement



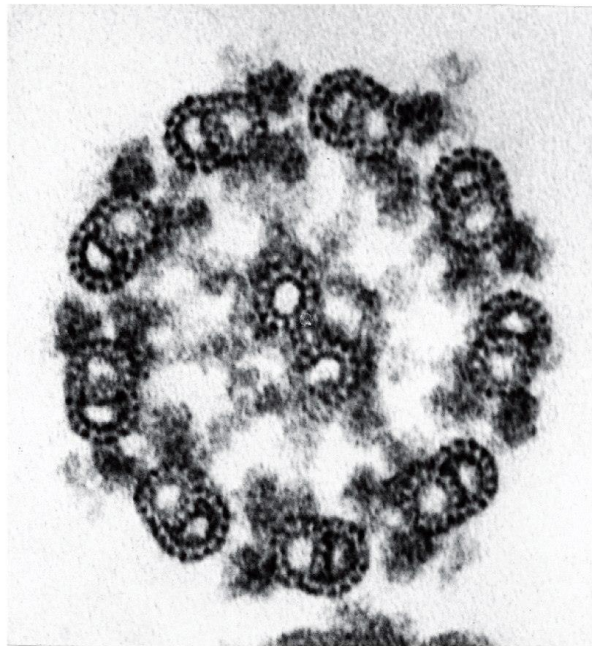
# Basal bodies---microtubule assembly sites for cilia and flagella and flagella

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

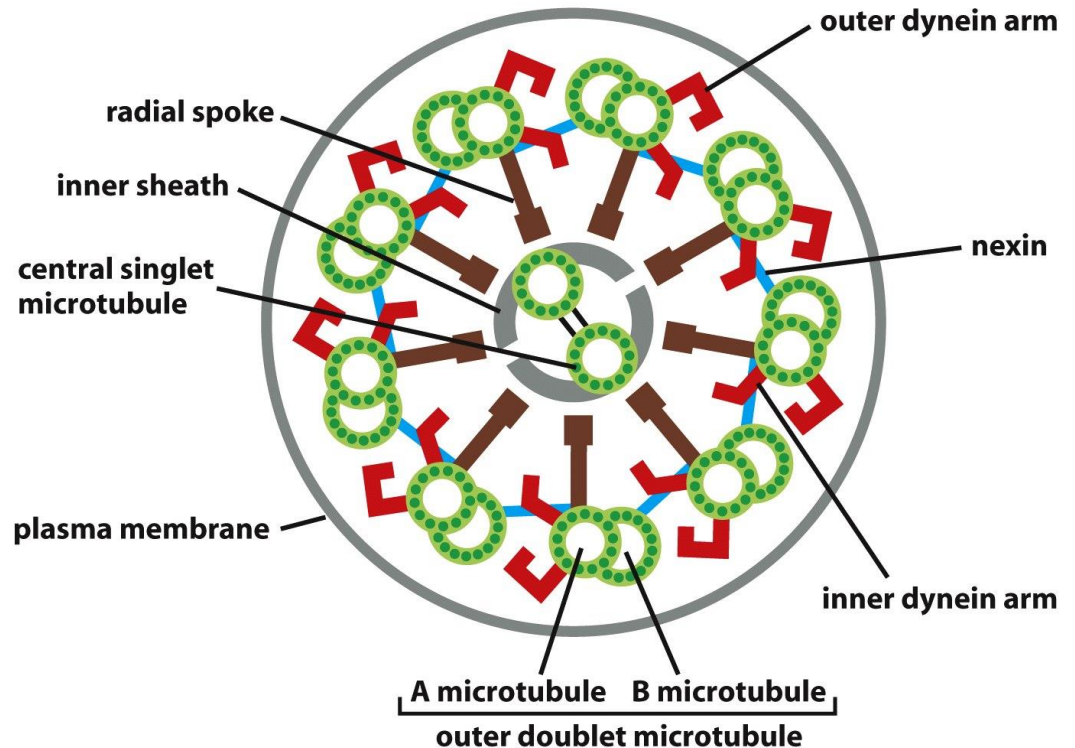




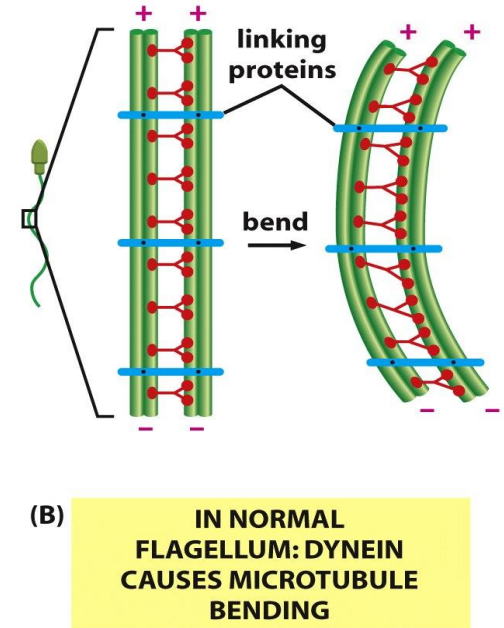
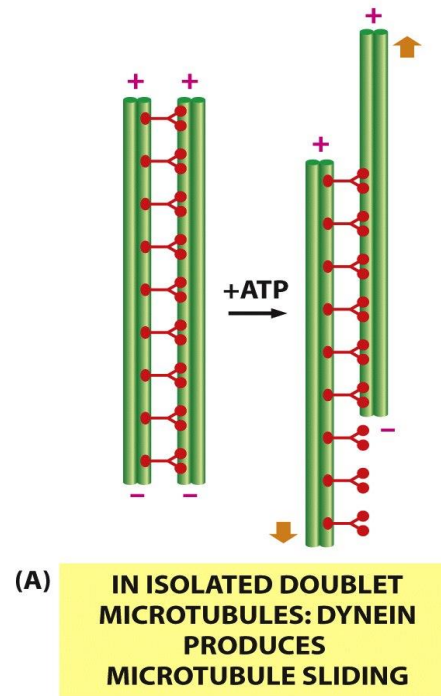
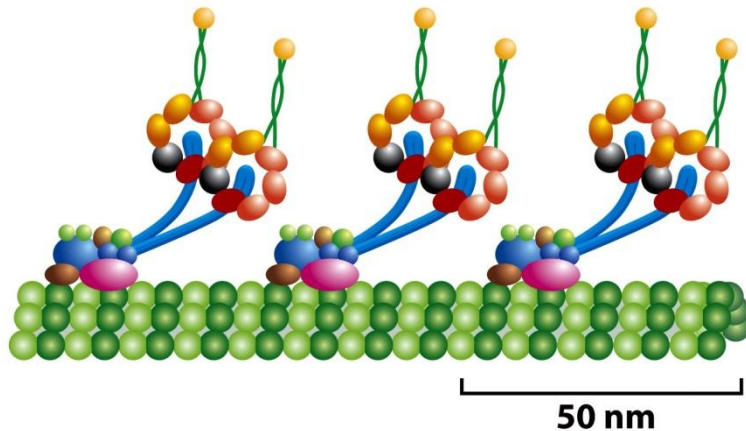
# A “9+2” arrangement for microtubules in axoneme of cilia and flagella



100 nm



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



## Kartagener's syndrome due to genetic defects in dynein

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

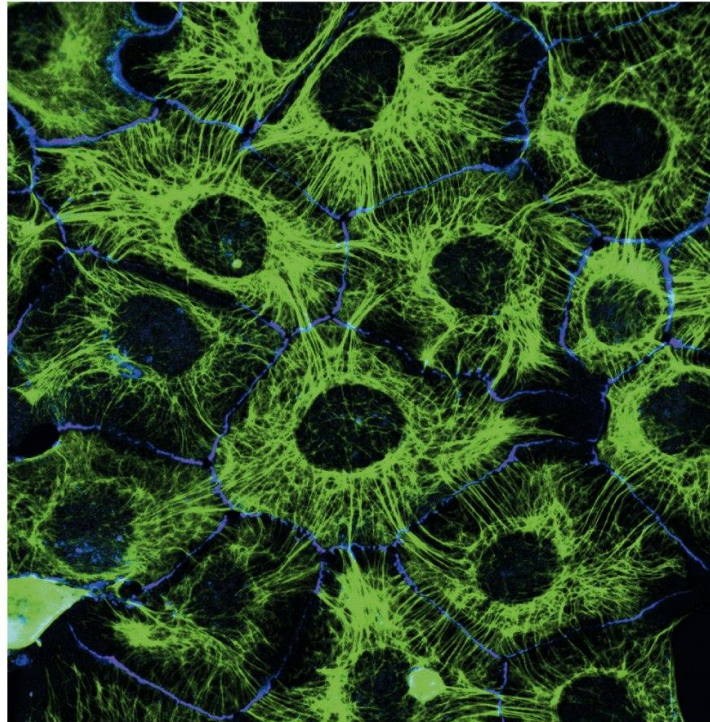
# Charcot-Marie-Tooth disease

- 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 多相的
7. Defects in genes for intermediate filaments are associated with ~50 clinical disorders. 临床



10  $\mu\text{m}$

Keratin in epithelia



# Major types of intermediate filament proteins

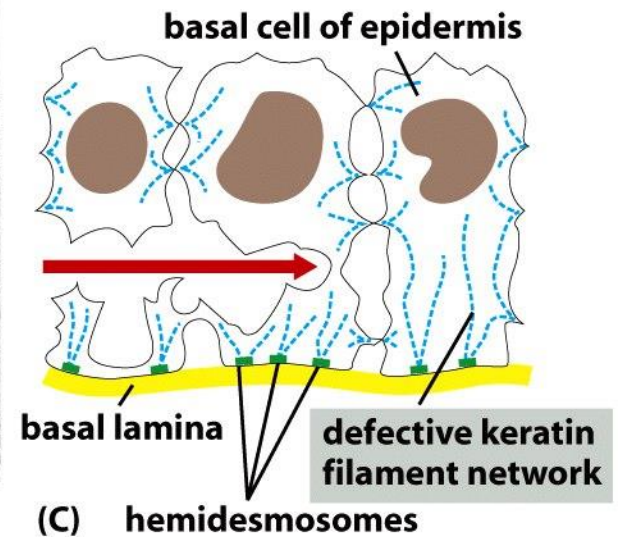
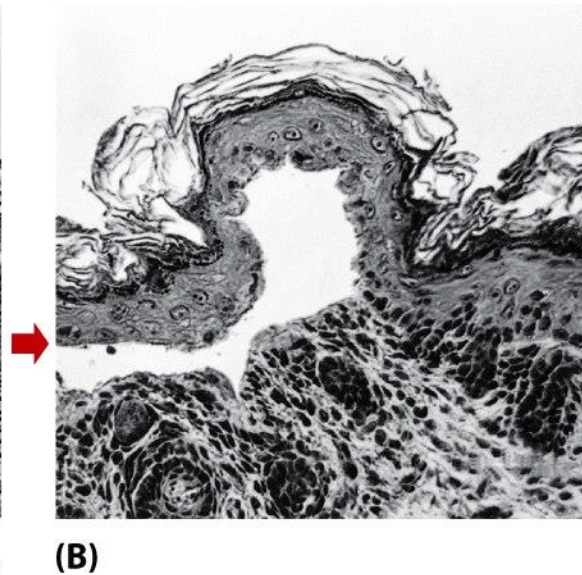
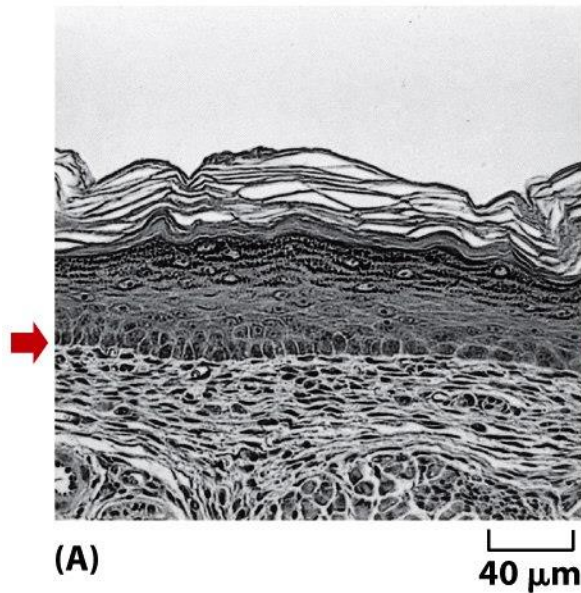
**Table 16–1 Major Types of Intermediate Filament Proteins in Vertebrate Cells**

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

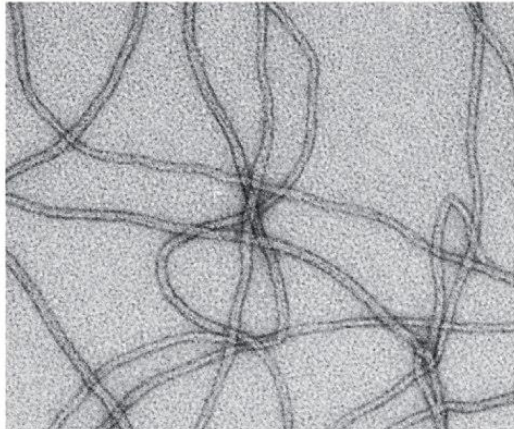


# Defects in keratin results in skin blistering

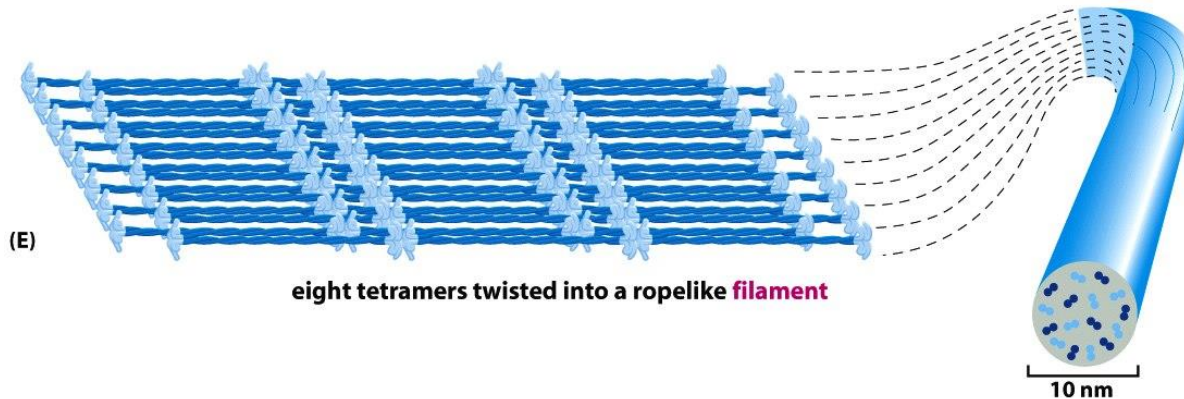
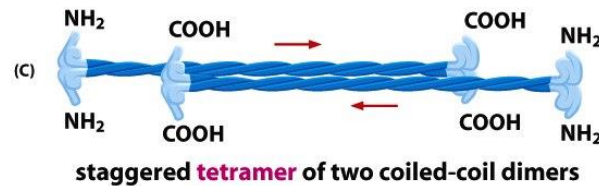
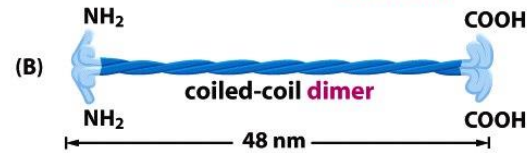
水泡



# How intermediate filament is assembled?



0.1 μm



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.