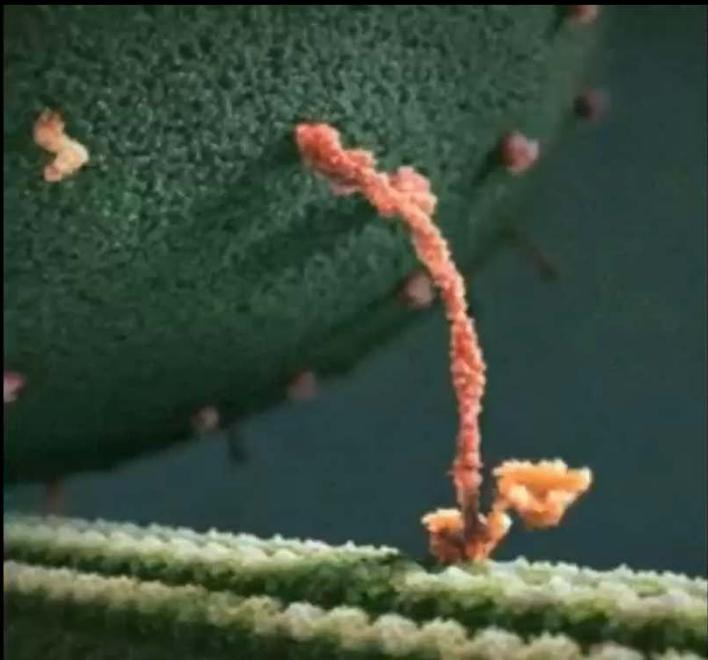


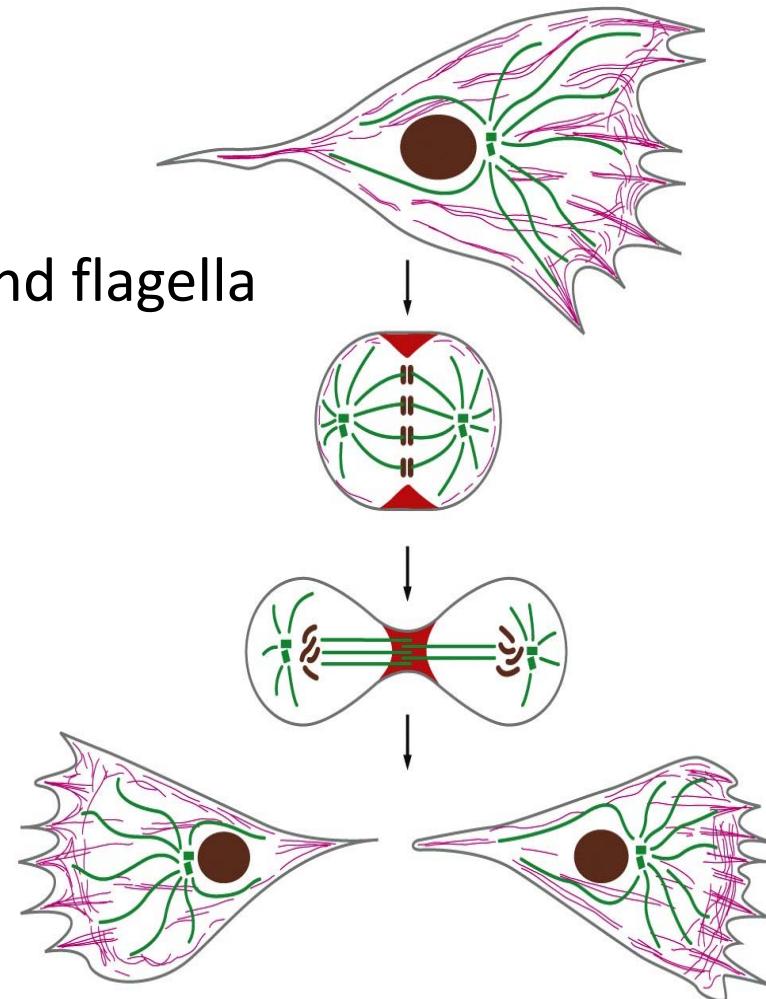
## Cytoskeleton & cell movement II



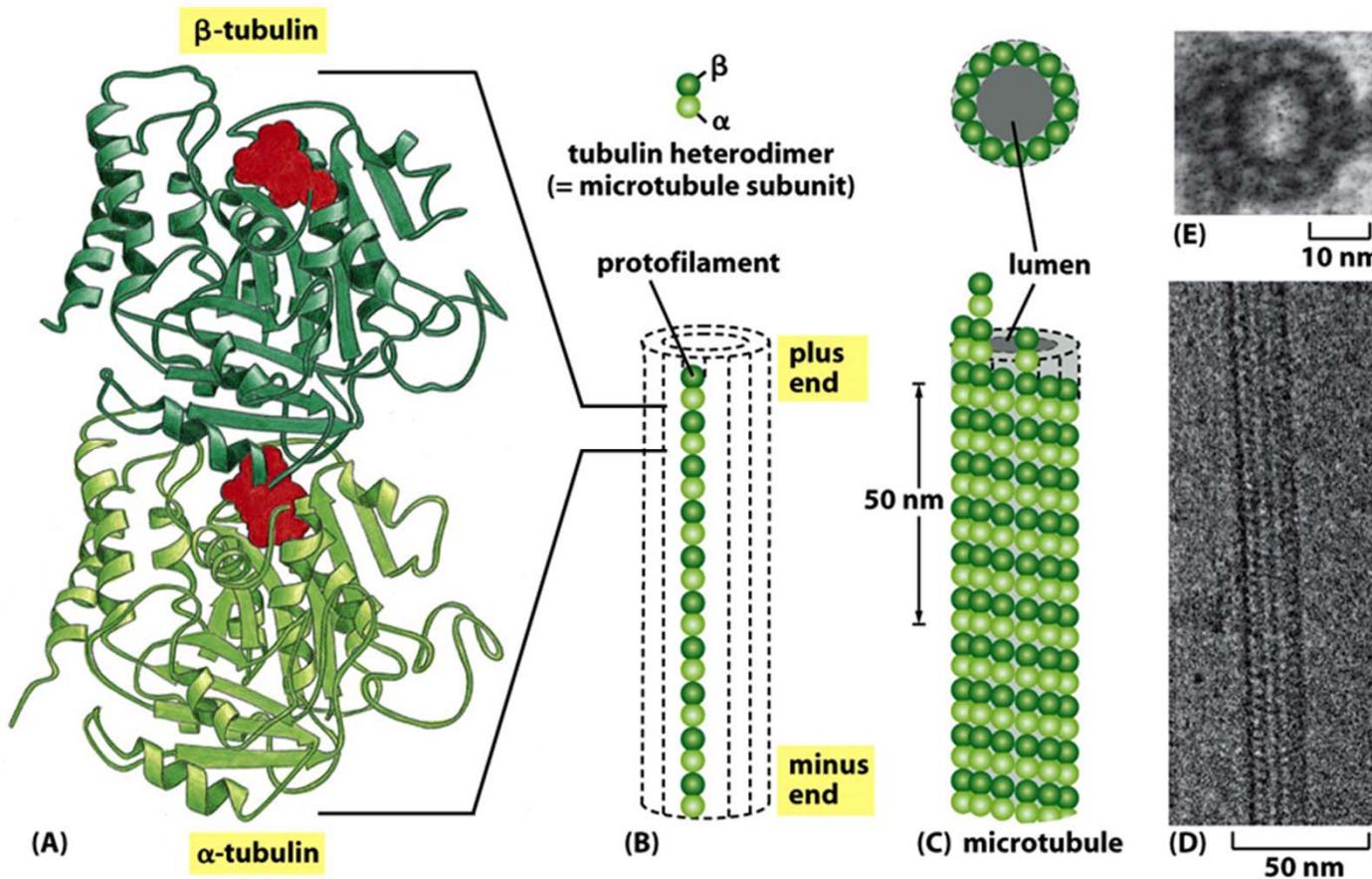
- I. Microtubule structure and organization
- II. Microtubule dynamics
- III. Regulation of microtubule assembly
- IV. Kinesins and Dyneins: microtubule-based motor proteins
- V. Cilia and flagella
- VI. Intermediate filaments

# I. Microtubule structure and organization

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



# Organization of microtubule



The microtubule is a stiff hollow tube formed from 13 protofilaments aligned in parallel

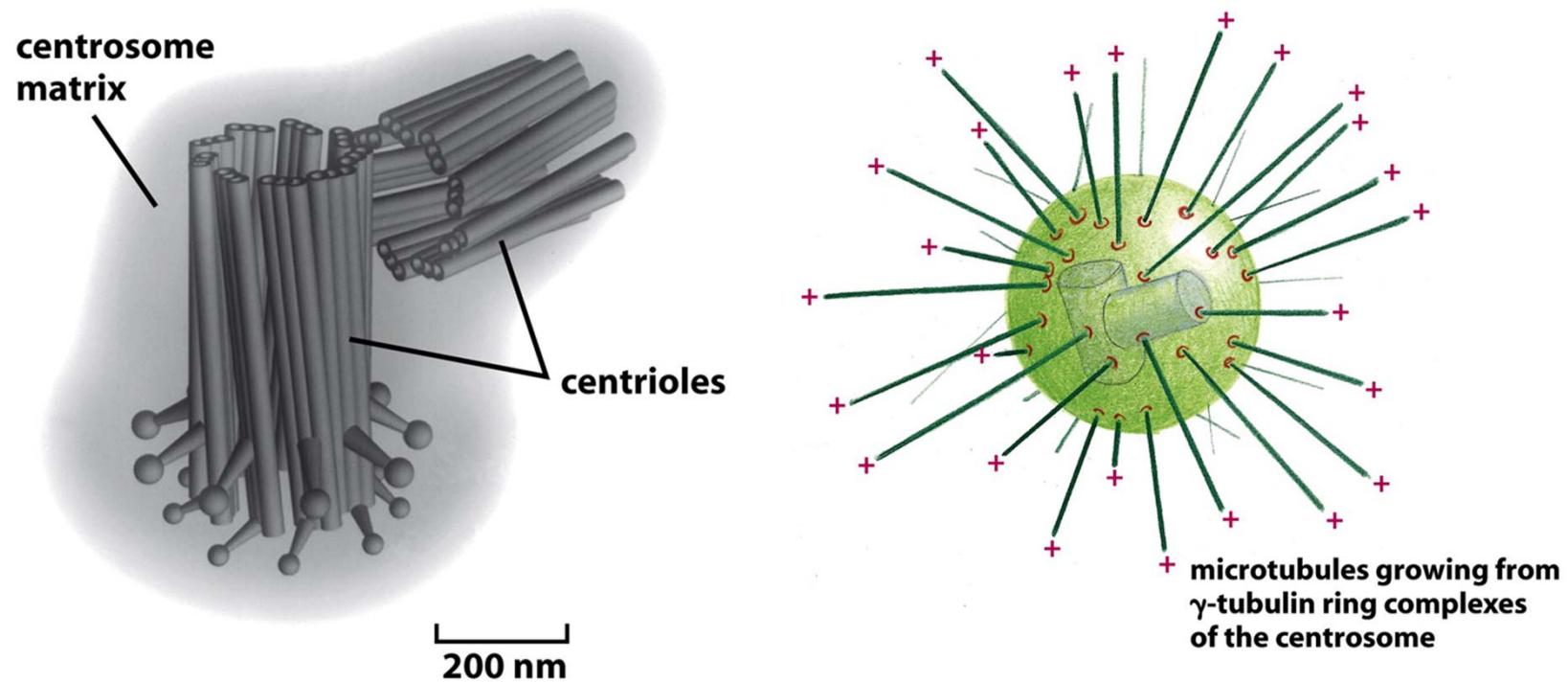
## Facts & summary about microtubules

- Microtubules are in **singlets**, in **doublets** (cilia, flagella), some are in **triplets** (basal bodies and centrioles)
- Most microtubules consists of **13 protofilaments**, others have 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**, microtubules have polarity.
- Each  $\alpha$ -tubulin and  $\beta$ -tubulin bind to **one molecule of GTP**.  
**but:** the **GTP on  $\alpha$ -tubulin is never hydrolyzed**, but GTP on  $\beta$ -tubulin is hydrolyzed.
- Another tubulin subunit,  $\gamma$ -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)**.
- **Microtubule organizing centers are:**  
**centrosomes** and **basal bodies** (cilia and flagella)
- Plants use additional/other microtubule-nucleating proteins  
e.g. SPC98 (different mechanisms to nucleate microtubules)  
and most plants do not have centrosomes

# The centrosome: $\gamma$ -tubulin ring complexes ( $\gamma$ -TuRC), pericentriolar, are critical to assemble microtubules



A centrosome with attached microtubules:

**The minus end of each microtubule is embedded in the centrosome,** having grown from a  $\gamma$ -tubulin ring complex ( $\gamma$ -TuRC), whereas **the plus end of each microtubule is free** in the cytoplasm.

## $\gamma$ -tubulin ring complex ( $\gamma$ -TuRC) nucleates microtubules

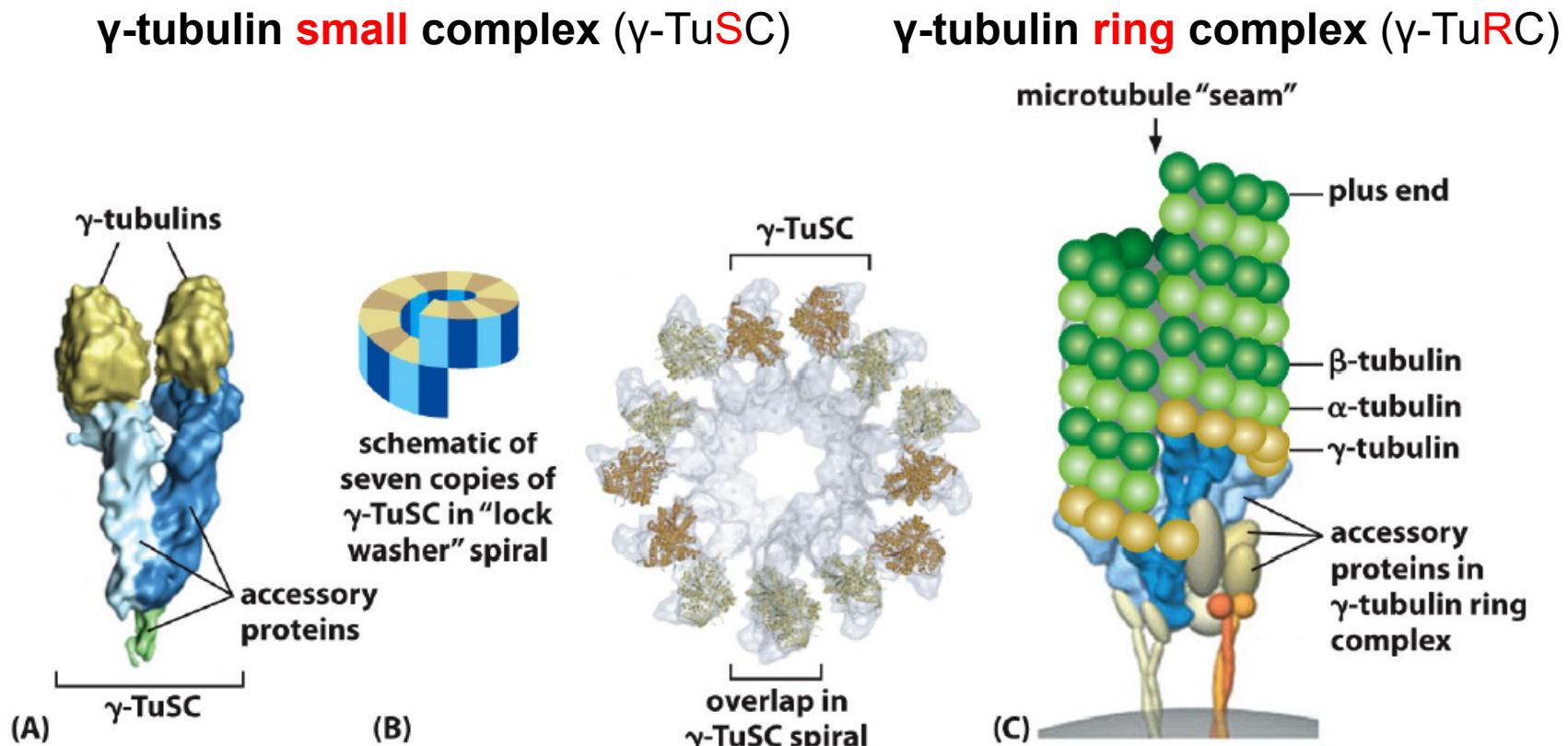
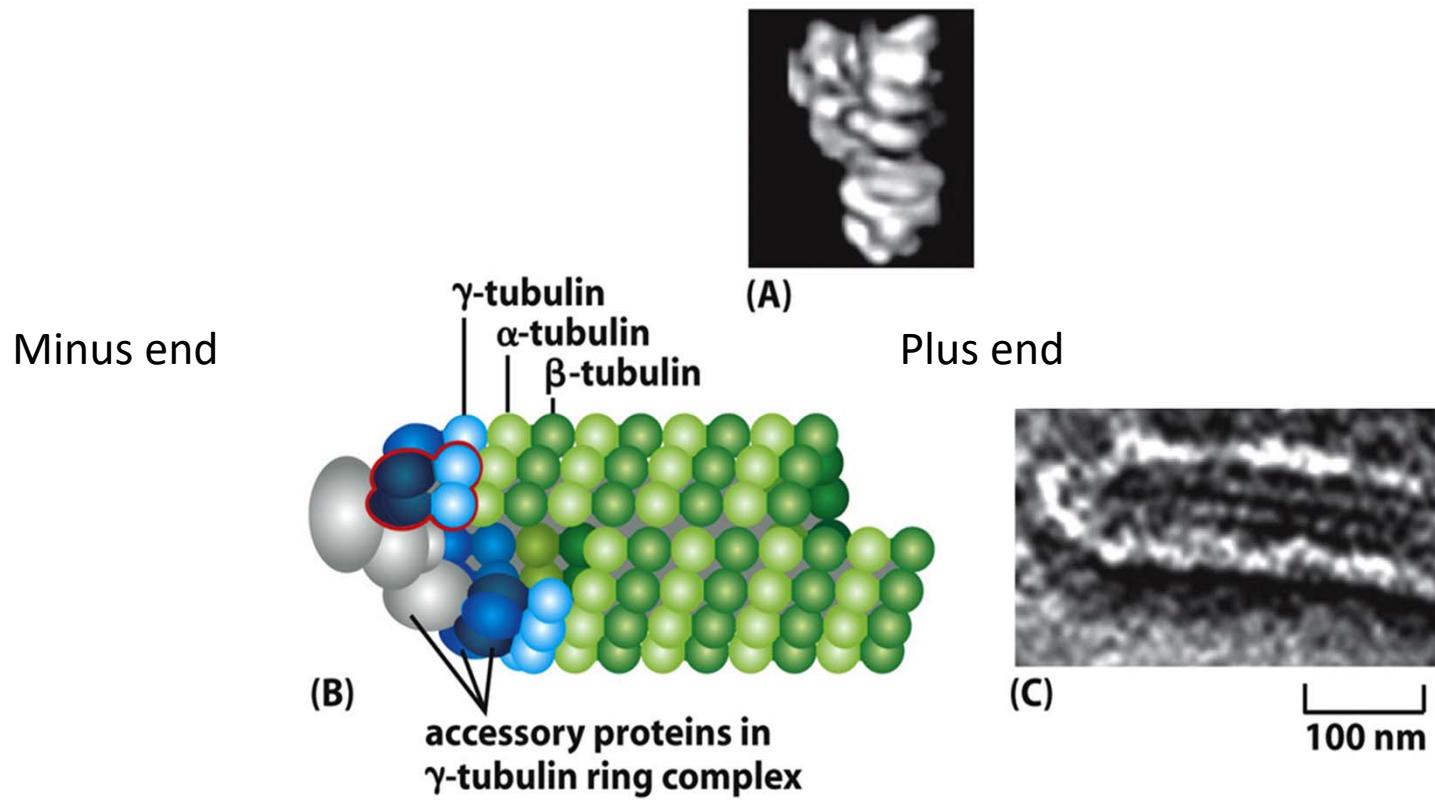


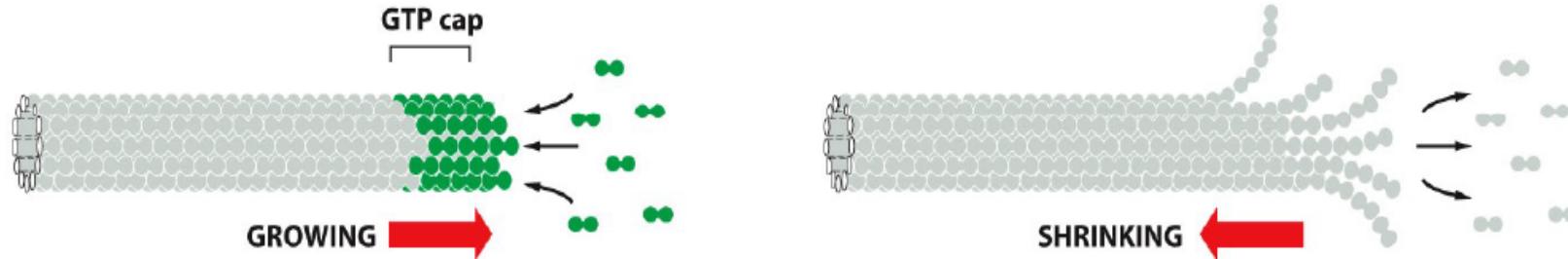
Figure 16-46 Molecular Biology of the Cell 6e (© Garland Science 2015)

**7  $\gamma$ -TuSCs associate to form a spiral structure in which the last  $\gamma$ -tubulin lies beneath the first, resulting in 13 exposed  $\gamma$ -tubulin subunits in a circular orientation that matches the orientation of the 13 protofilaments in a microtubule**

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



## Microtubules: dynamic instability

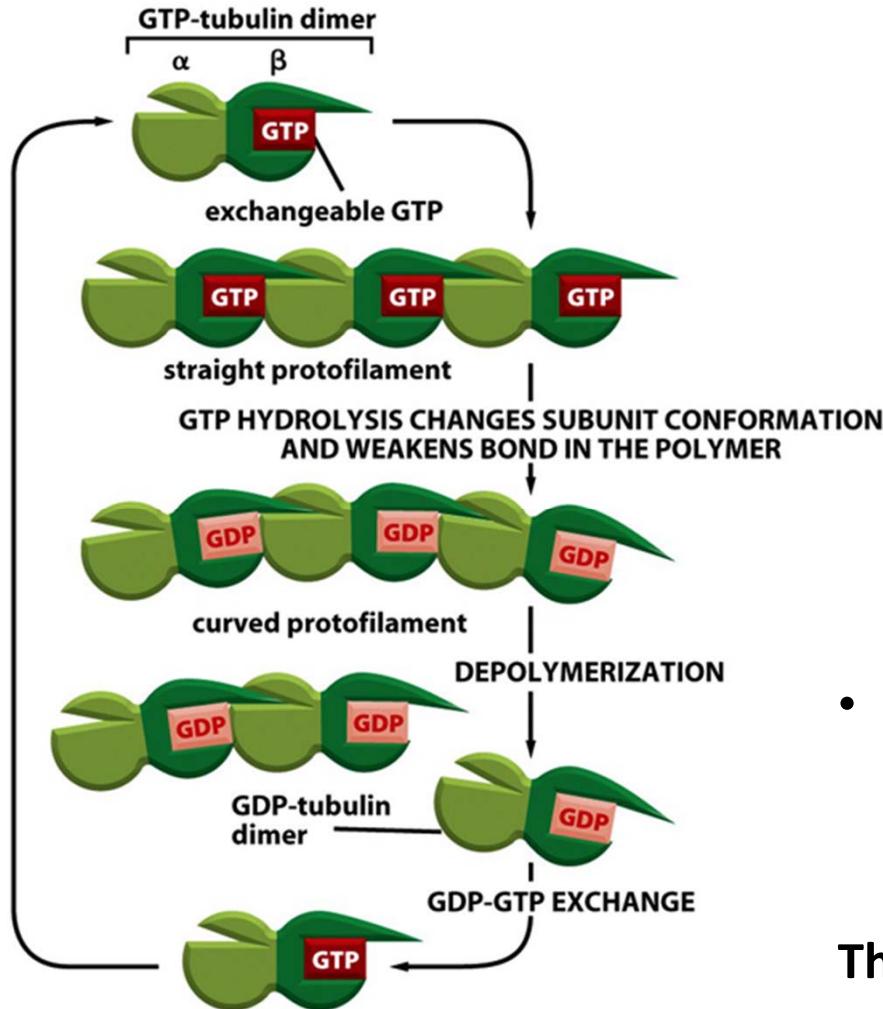


Individual microtubules can therefore alternate between a period of slow growth and a period of rapid disassembly, a phenomenon called **dynamic instability**.

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.

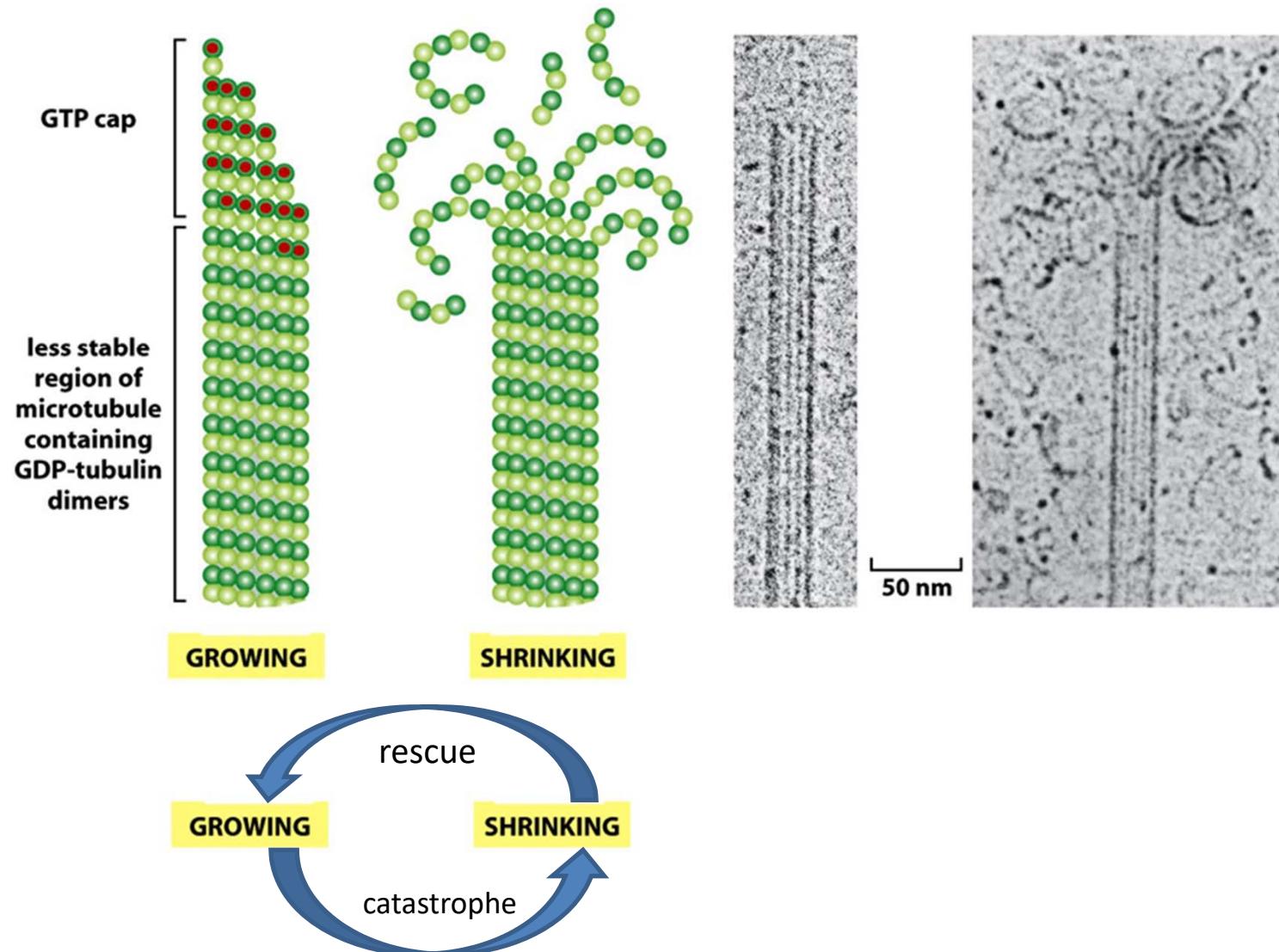
# Assembly of tubulin dimers



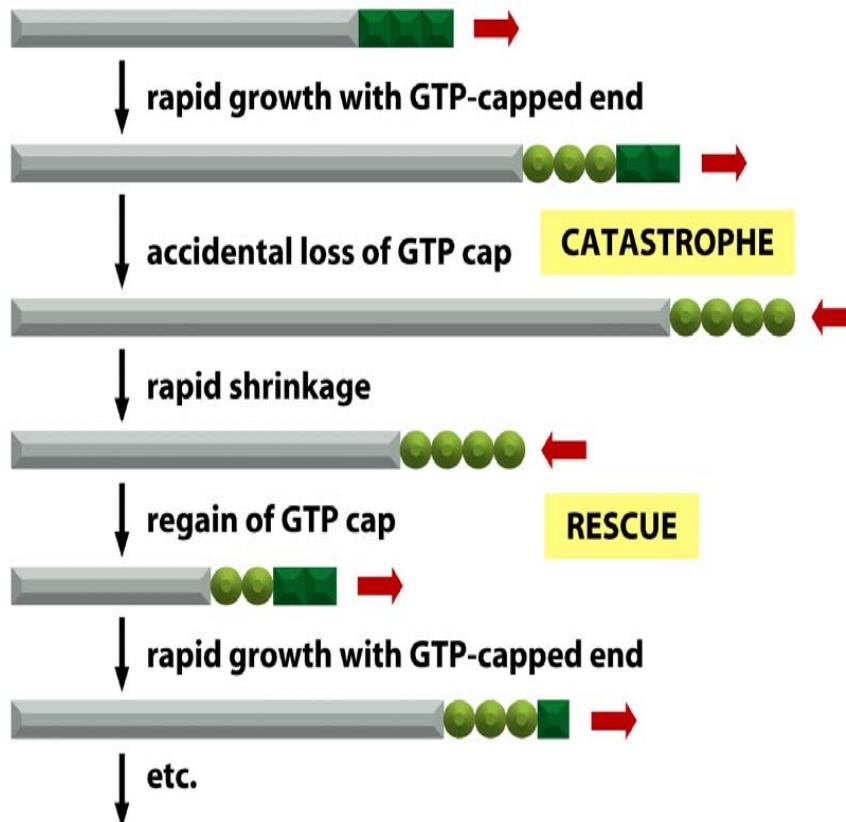
- Like actin, **assembly at plus end** is much faster faster than assembly at minus end.

**That is why they grow at the plus end!**

## II. Microtubule dynamics



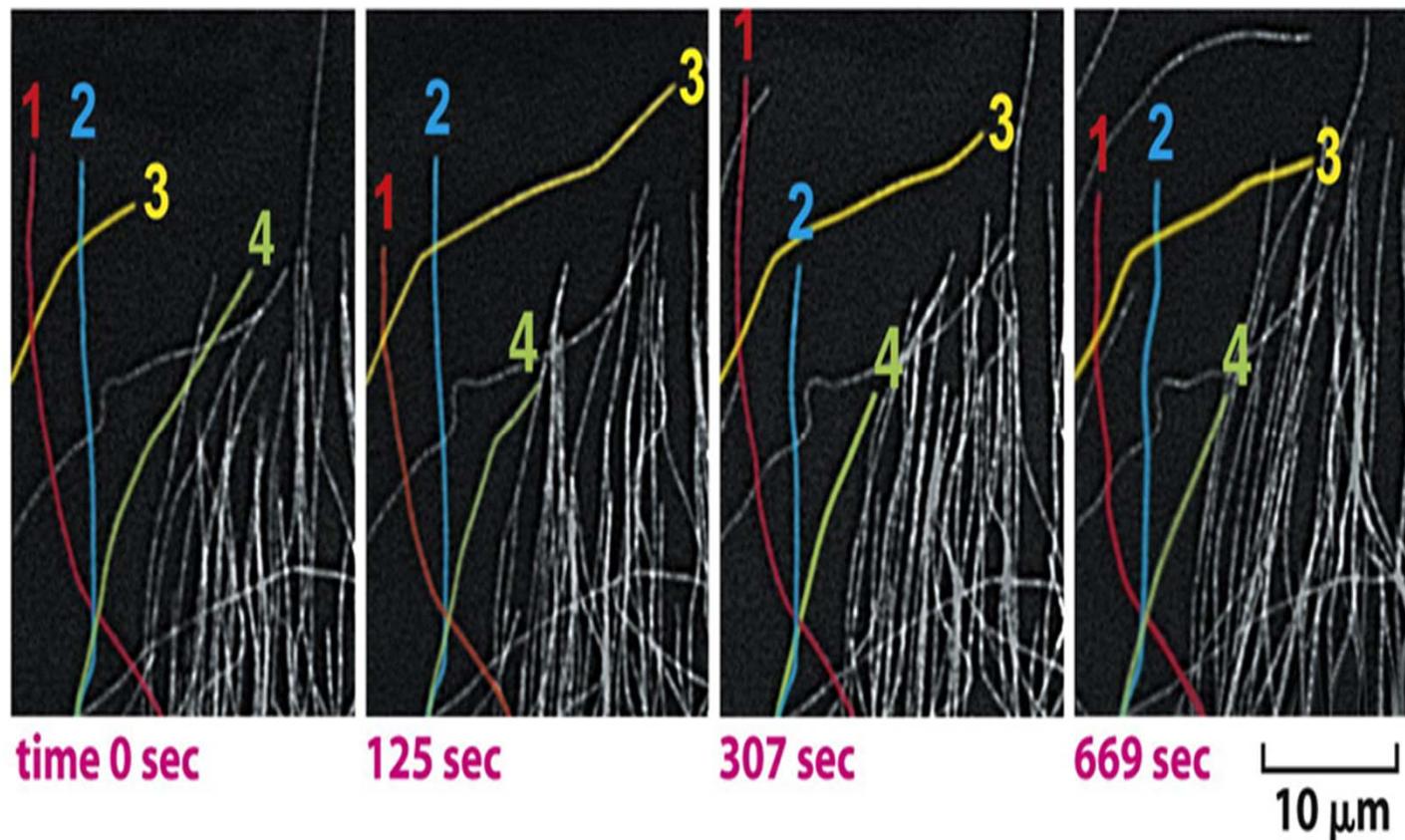
# Microtubules: dynamic instability



## On a single microtubule:

- an end might grow for a certain time in a T form, but then suddenly change to the D form and begins to shrink rapidly, even while the free subunit concentration is held constant.
- Later, it might regain a T-form end and begins to grow again.
- This rapid interconversion between a growing and shrinking state, is called **dynamic instability**

## Dynamic instability of microtubule in vivo



Rhodamine-labeled tubulin reveals the dynamic instability of microtubules at the edge of the cell: **Four** individual microtubules are highlighted for clarity; **each of these shows alternating shrinkage and growth...**

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

## Organization of microtubule bundles by MAPs (microtubule-associated proteins)

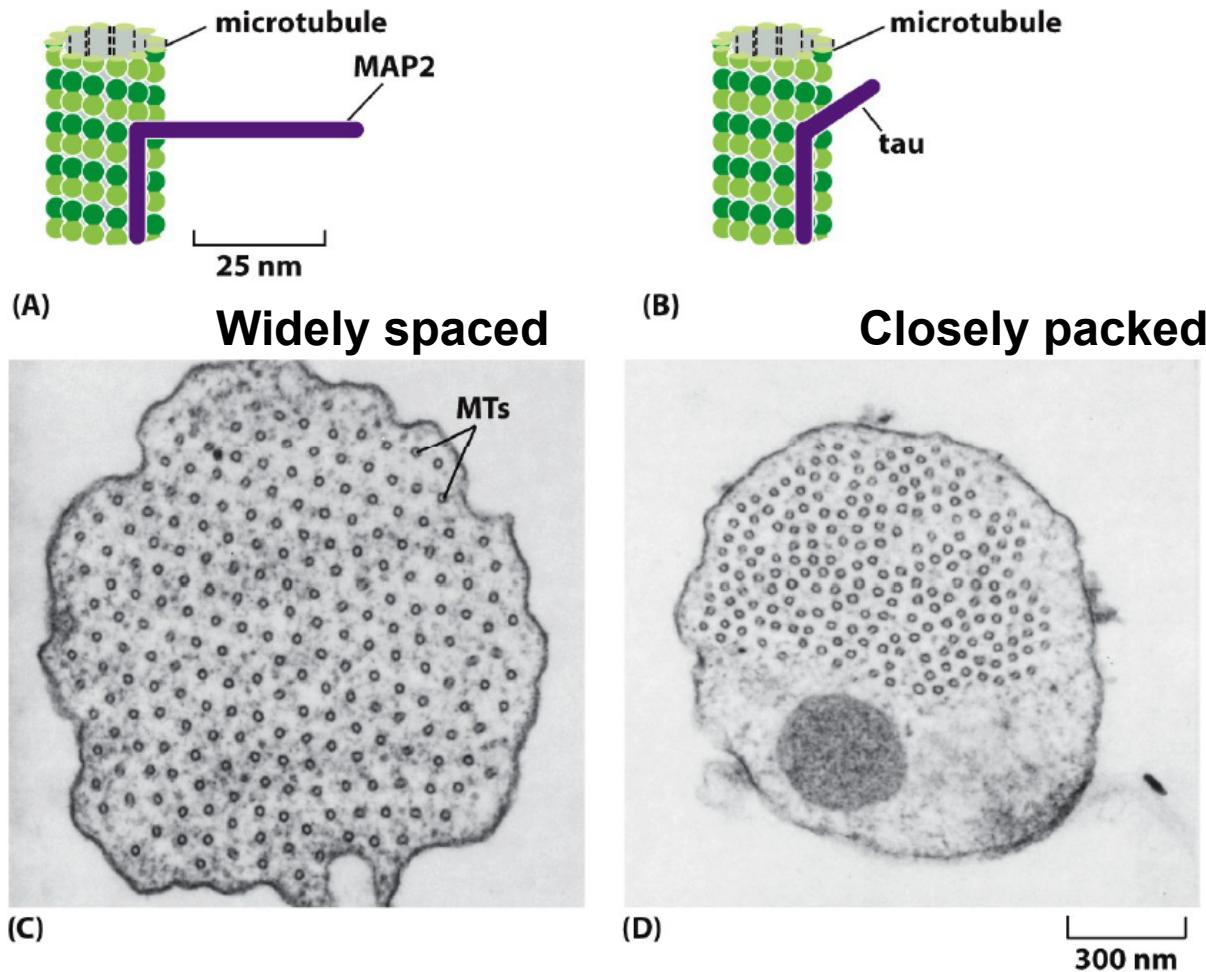


Figure 16-51 Molecular Biology of the Cell 6e (© Garland Science 2015)

# Microtubule plus end-binding proteins modulate microtubule assembly

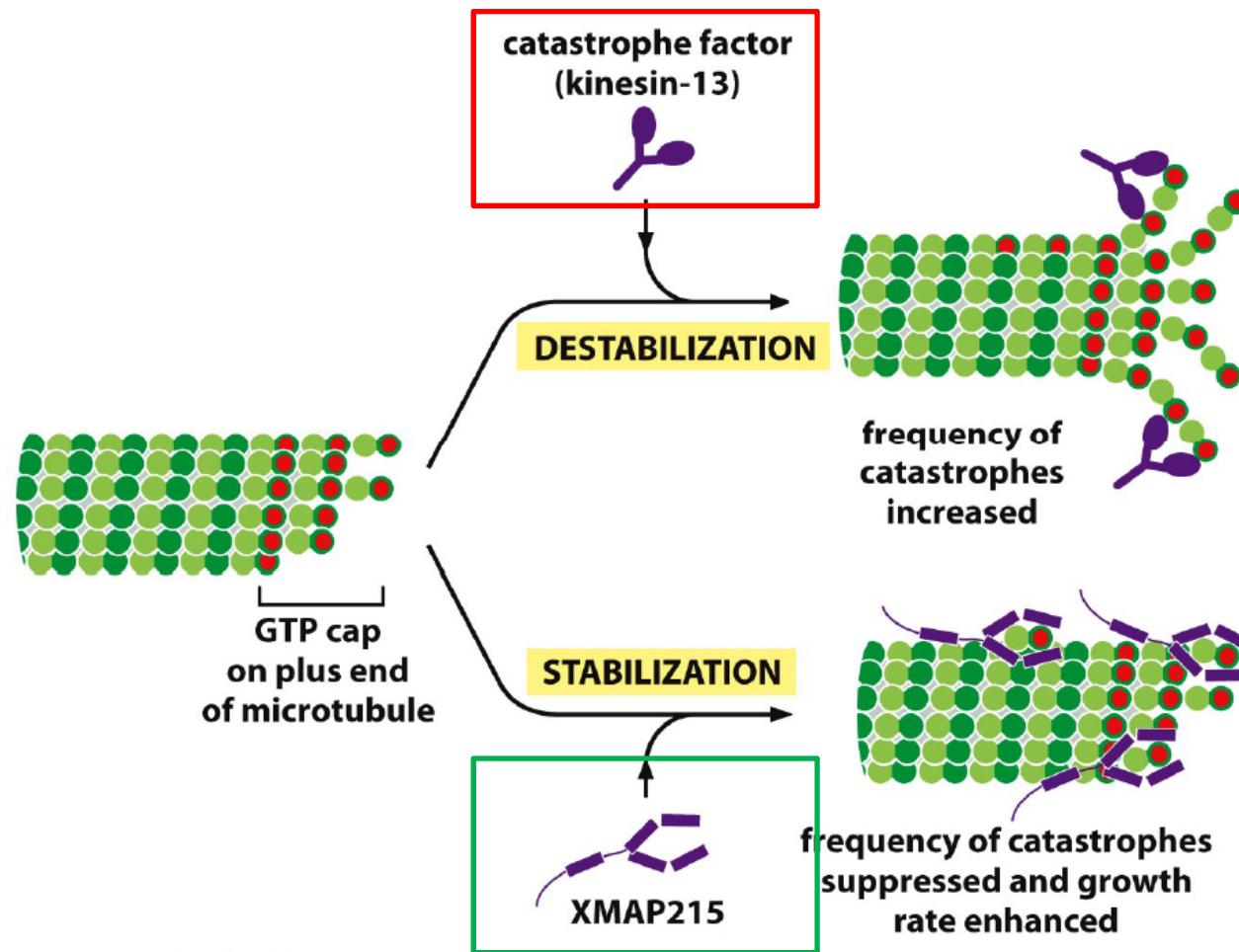
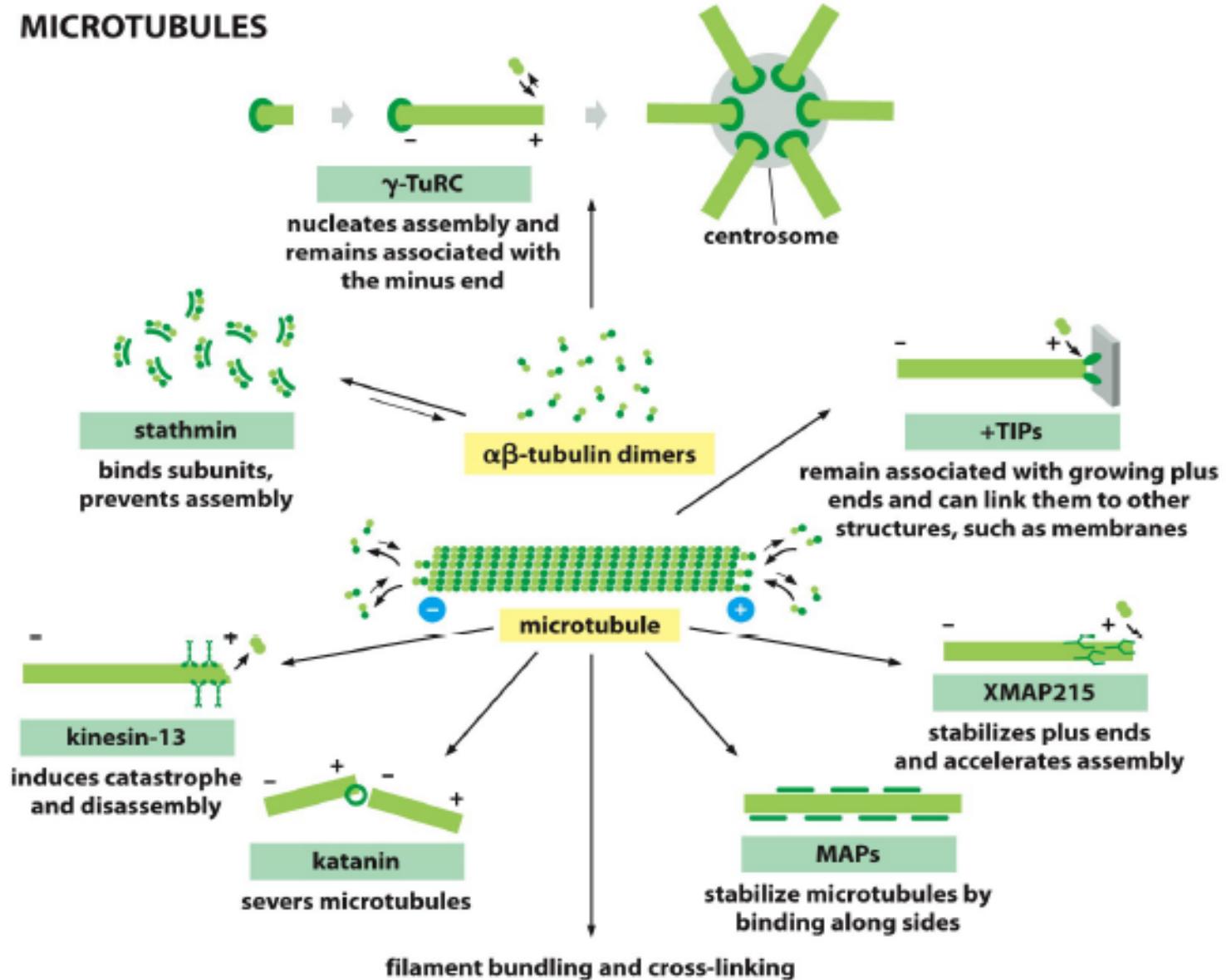
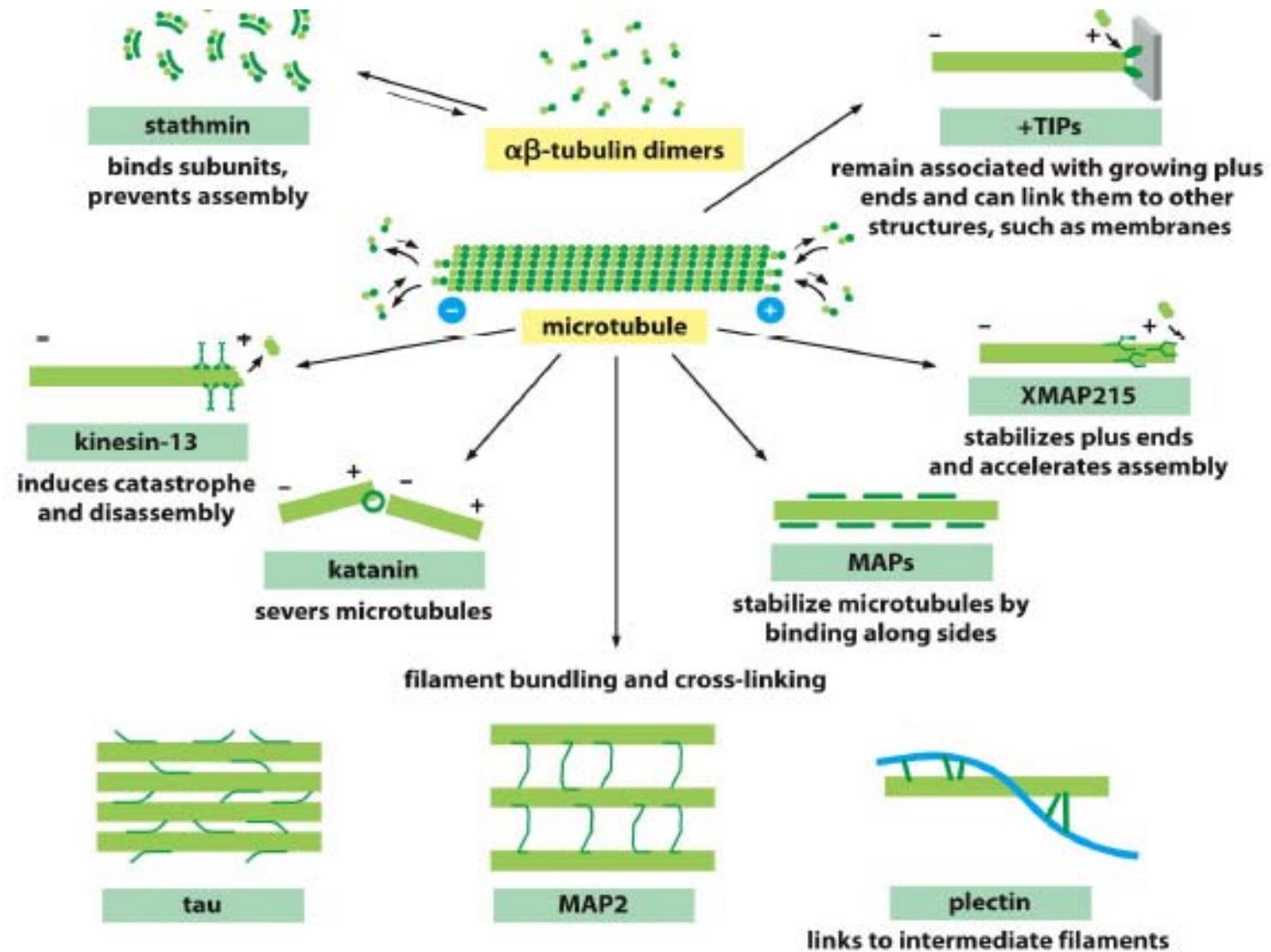


Figure 16-52 Molecular Biology of the Cell 6e (© Garland Science 2015)

## Microtubule-binding proteins modulate filament assembly and dynamics

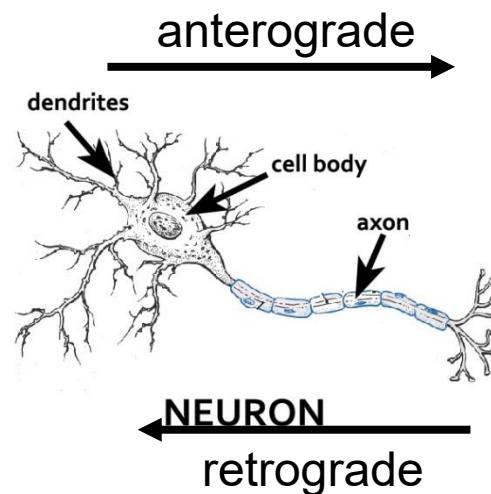


## Microtubule-binding proteins modulate filament assembly and dynamics



## 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 analyzing 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)
  - taxol-stabilized microtubule
  - purified organelle (labelled)
  - axon extract (organelle free)

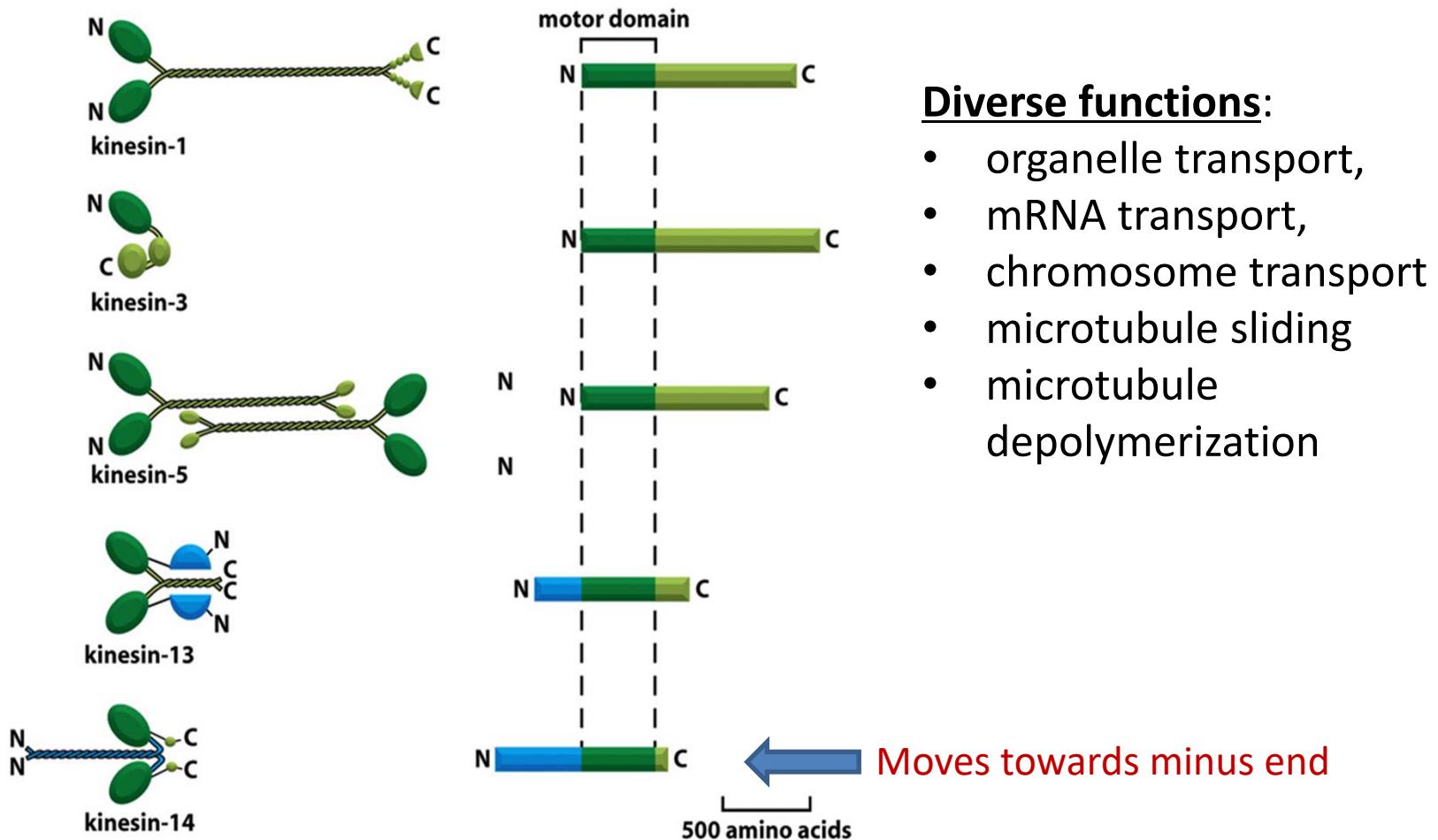
Findings:

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 organelles tightly without dissociation.



Identify kinesin 1 and other MAPs.

## 2. Kinesin family proteins for anterograde movement (toward plus end)



### 3. Action mechanism for Kinesins

- Always associate with microtubules
- “hand-over-hand” motion
- ADP-kinesin binds weakly to tubule
- ATP-kinesin binds strongly to tubule
- **Forward displacement of rear motor domain** (lagging head) is driven by **dissociation of ADP and binding of ATP in the leading head**
- **ATP binding** causes a small peptide called the **“neck linker”** to shift from a rearward-pointing to a forward-pointing conformation
- **This shift pulls the rear head forward**, once it has detached from the microtubule with ADP bound [**detachment requires ATP hydrolysis and phosphate (Pi) release**].
- The kinesin molecule is now poised for the next step, which proceeds by an exact repeat of the process shown

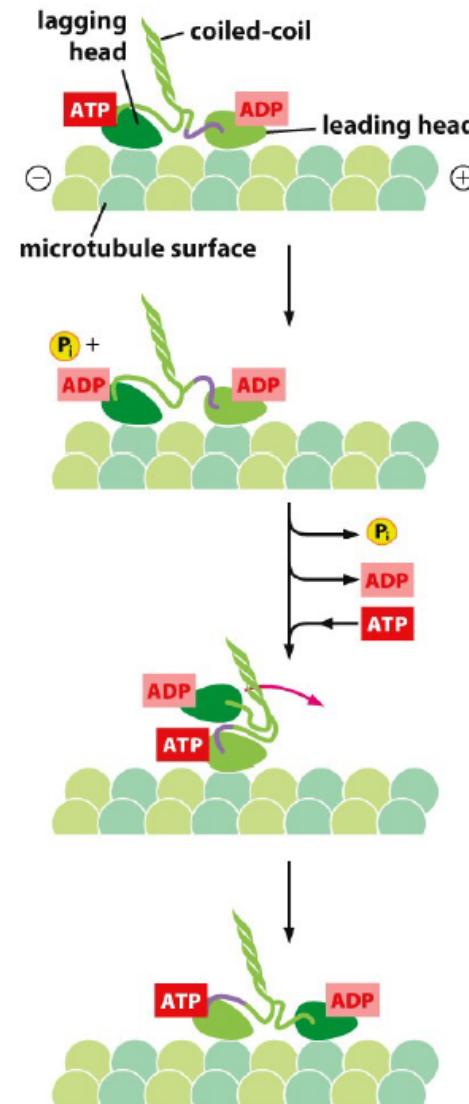
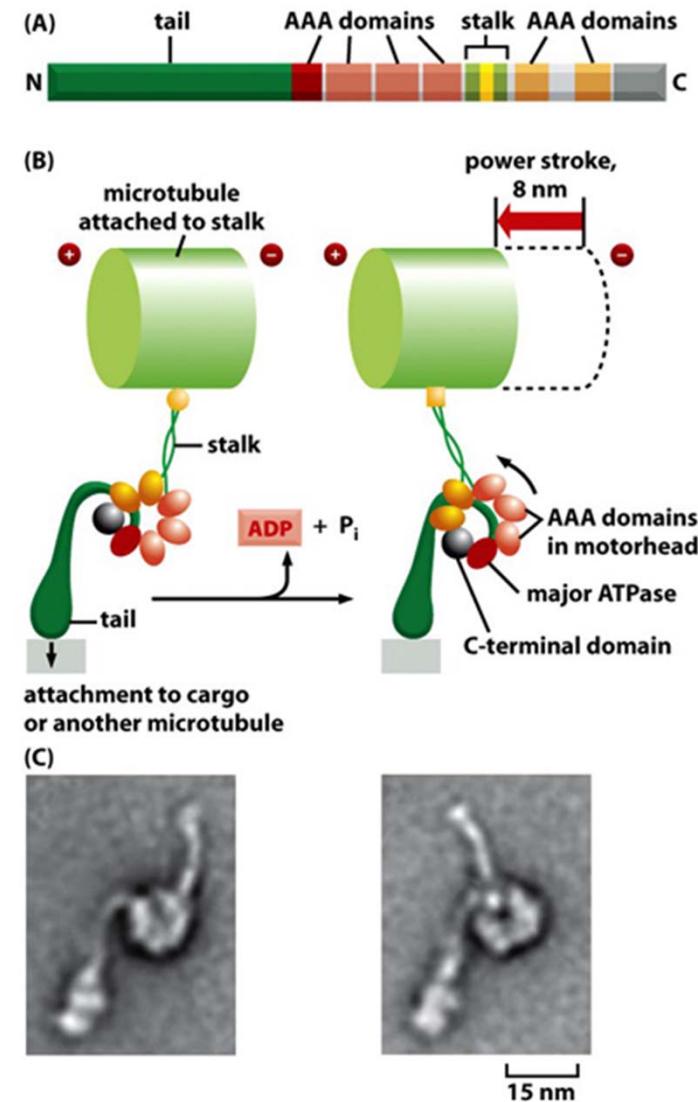


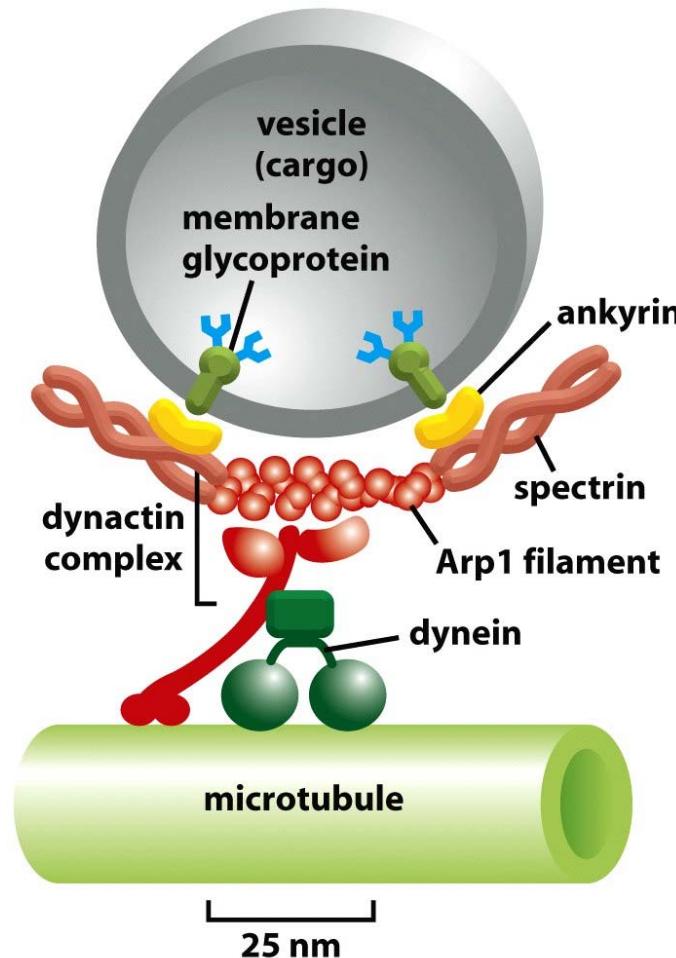
Figure 16-57 Molecular Biology of the Cell 6e (© Garland Science 2015)

## 4. Dynein for **retrograde** transport: towards the **minus end**

- It is a very large multi-subunit protein
- Possesses ATPase activity and two heads
- It itself can't transport cargo, but needs dynactin to link to cargo for transport.
- Each power stroke generates a step of about ~8nm towards its minus end



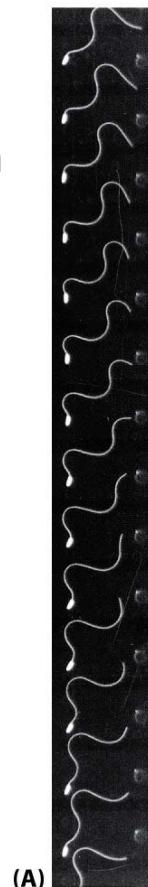
## How dynein and dynactin together transport a vesicle



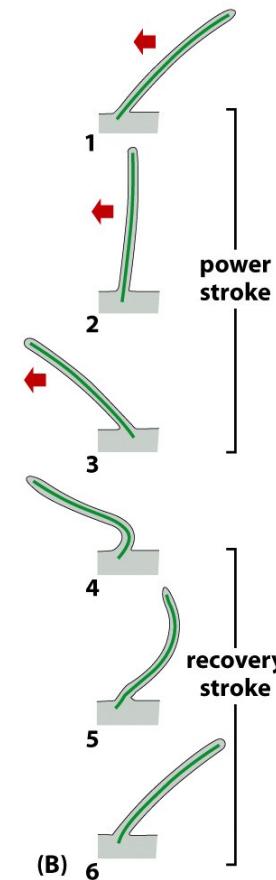
## V. Cilia and flagella: similar components, different action

Cilia and flagella are both built from microtubules and dynein but have totally different modes of motion:

Flagellum:  
undulating  
“wave-like” motion

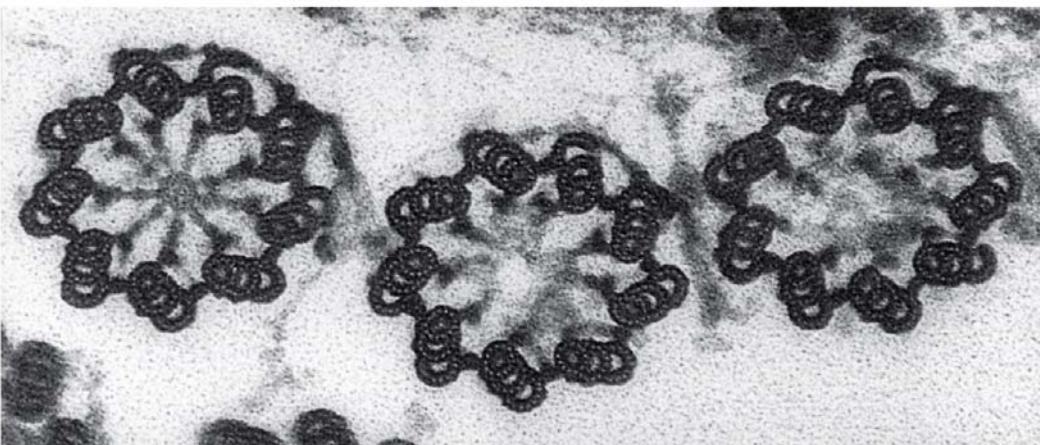


Cilium:  
beat stroke



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

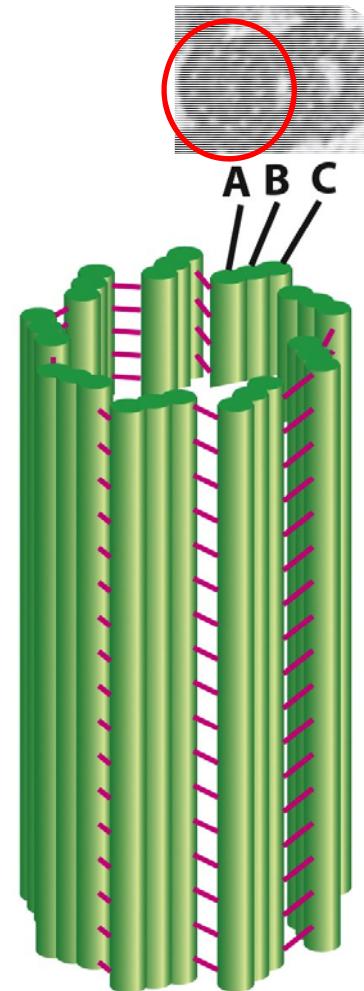
- Firmly root eukaryotic cilia and flagella at the cell surface.
- Similar to centrioles



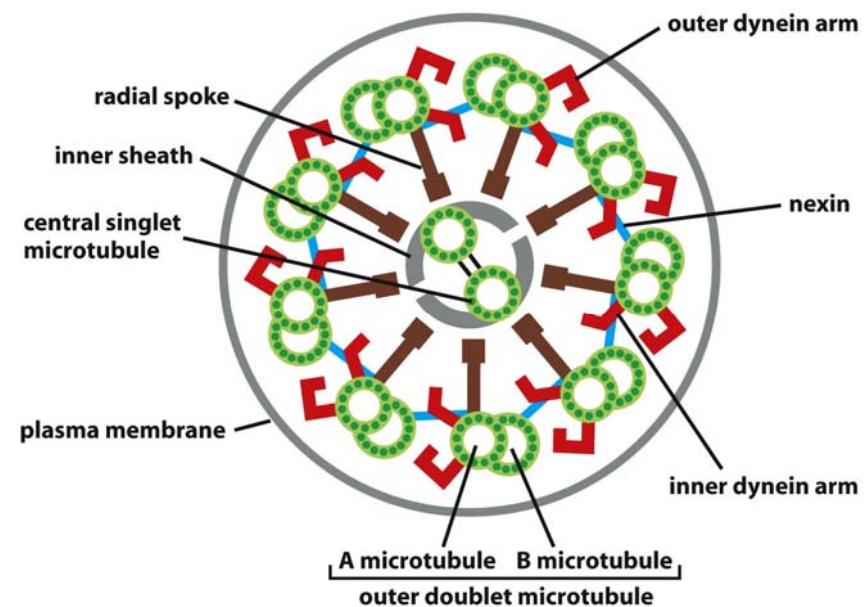
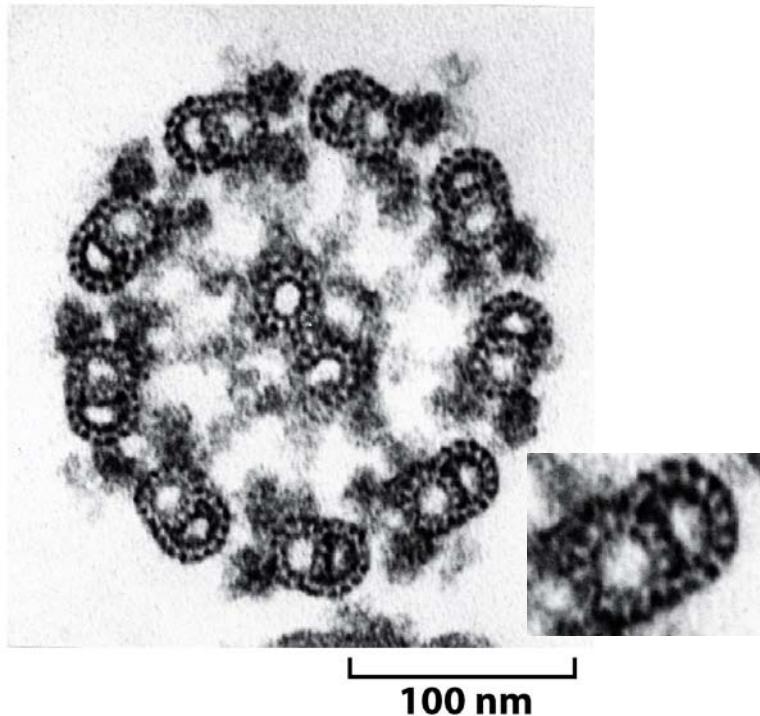
EM of a section through 3 basal bodies: 100 nm

**9 groups of fused triplet microtubules** in a cartwheel configuration.

- 1 of the 3 fused microtubules is complete, that is the **A** microtubule



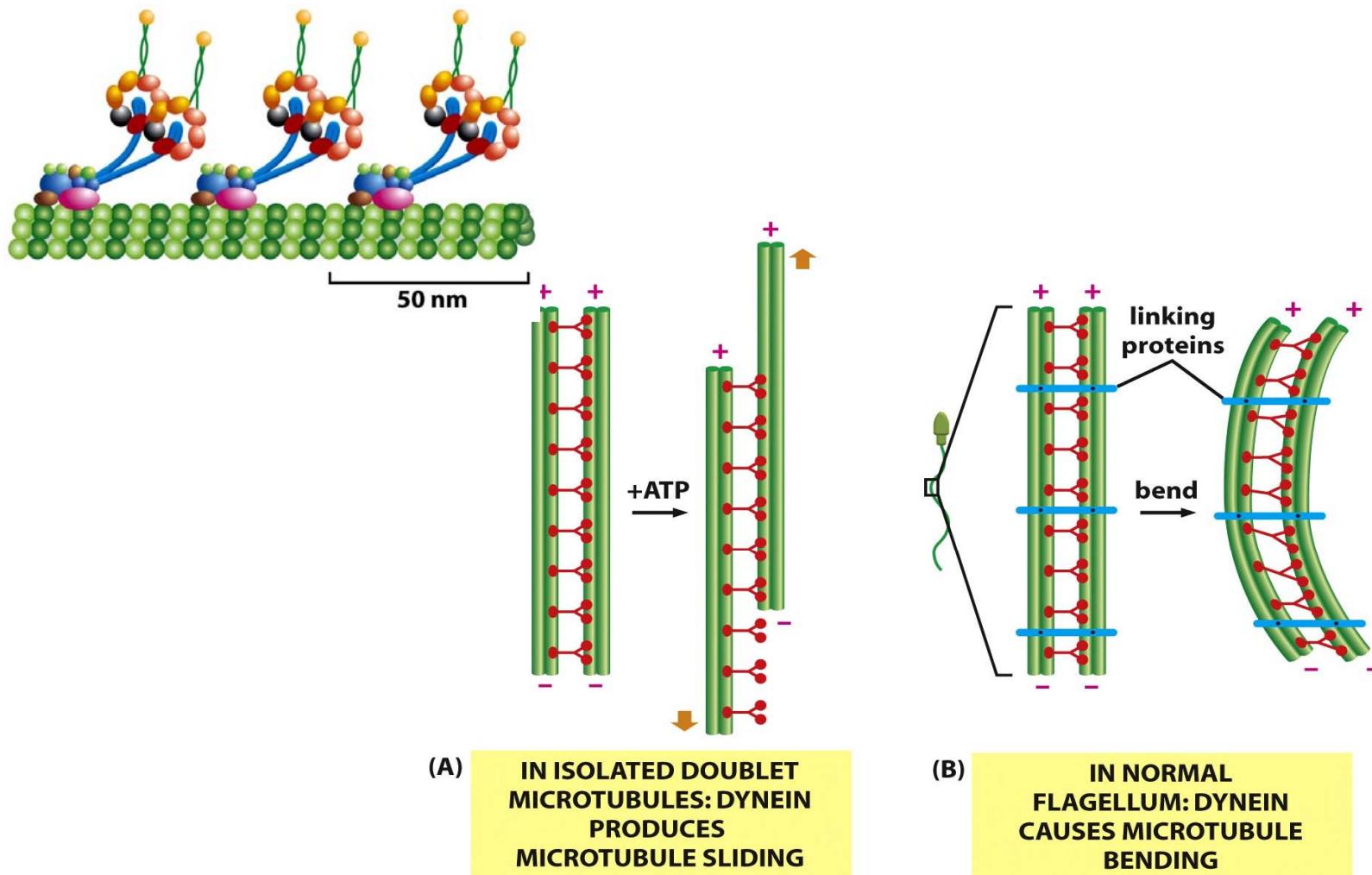
## The “9+2” arrangement for microtubules in axoneme of cilia and flagella



EM of a section through flagellum/cilium:

**9 doublet microtubules** (1 of the 2 is complete) are arranged around  
**a pair (two) of single microtubules (9+2 configuration)**

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



## Genetic defects in dynein cause severe diseases

### **Kartagener's syndrome (genetic defect in dynein)**

- defective movement of cilia, leading to recurrent chest infections, ear/nose/throat symptoms, and infertility.
- Male sterility
- High susceptibility to lung infection
- Defects in left-right axis determination in development.

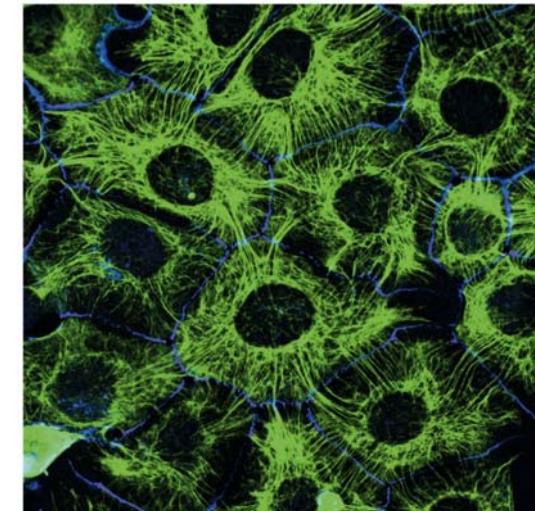
### **Charcot-Marie-Tooth disease (point mutation in a kinesin)**

- point mutation in a kinesin family member that transports synaptic vesicle precursors down the axon
- The neuropathy of CMT affects both motor and sensory nerves. (Motor nerves cause muscles to contract and control voluntary muscle activity such as speaking, walking, breathing, and swallowing.)
- A typical feature: weakness of the foot and lower leg muscles, which may result in foot drop and a high-stepped gait with frequent tripping or falls. Foot deformities, such as high arches and hammertoes (a condition in which the middle joint of a toe bends upwards)

## VI. Intermediate filaments

### **Facts about intermediate filaments:**

- **No** polarity,
- **No** motor activity,
- **Tensile** and stable
- **Difficult** to solubilize
- Very **heterogeneous** substances
- Defects in genes for intermediate filaments are associated with ~50 clinical disorders.
- **Not in all eukaryotic cells:**  
**fungi and plants don't have IFs**



Keratin in epithelia

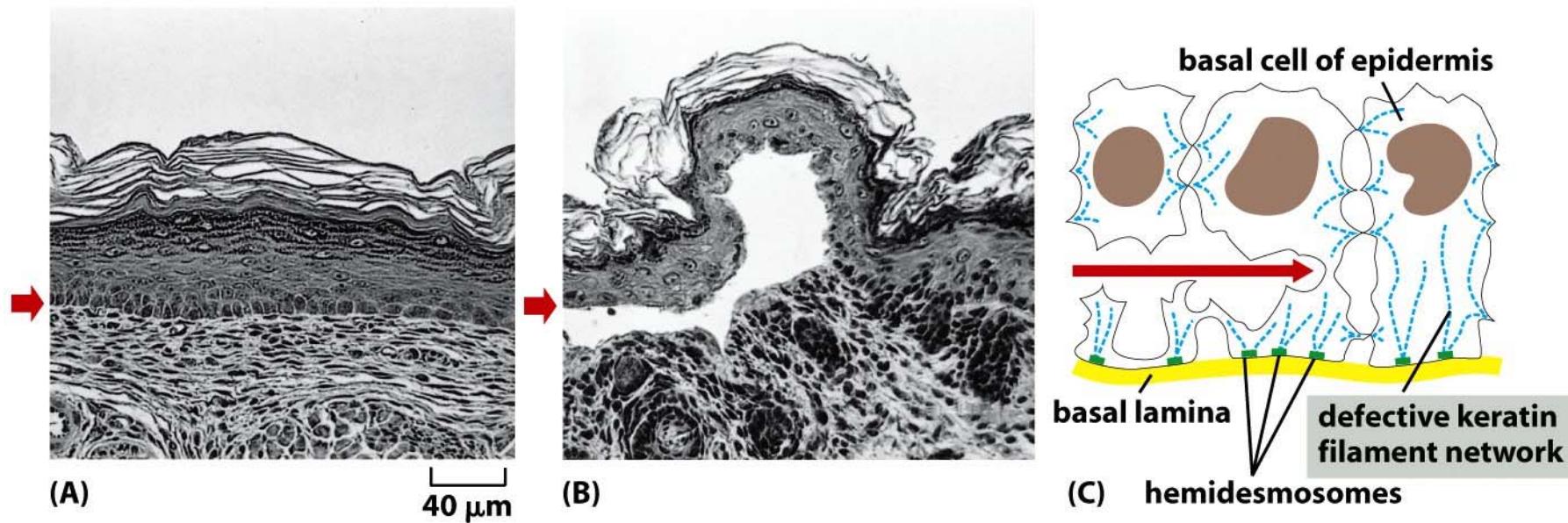
10  $\mu\text{m}$

## Major types of intermediate filament proteins

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

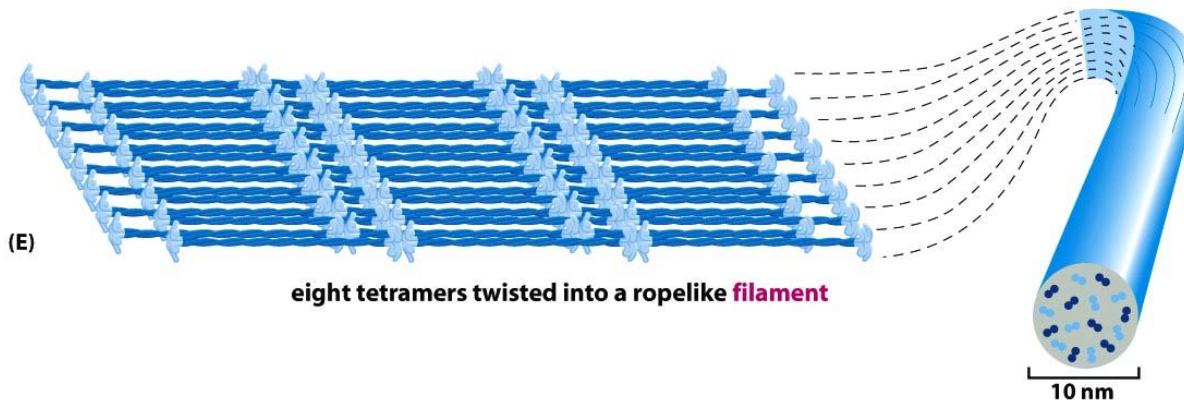
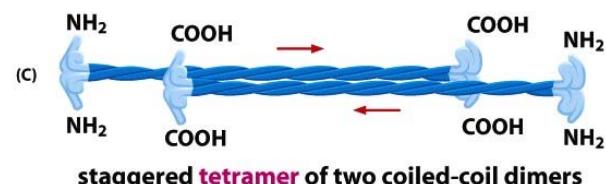
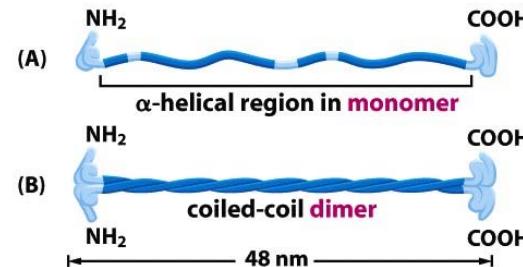
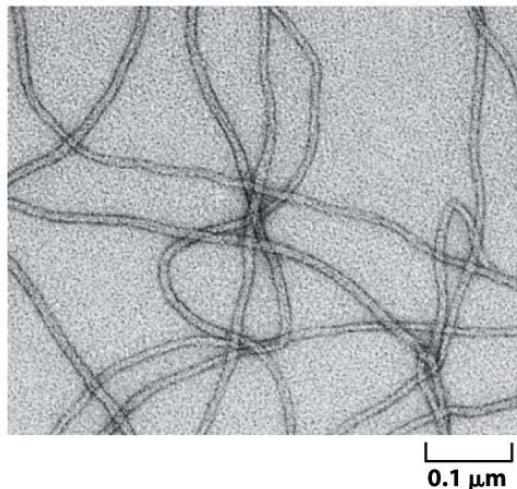
TYPES OF IF	COMPONENT POLYPEPTIDES	LOCATION
Nuclear	lamins A, B, and C	nuclear lamina (inner lining of nuclear envelope)
Vimentin-like	vimentin	many cells of mesenchymal origin
	desmin	muscle
	glial fibrillary acidic protein	glial cells (astrocytes and some Schwann cells)
	peripherin	some neurons
Epithelial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

## Defects in keratin results in skin blistering



# How do intermediate filaments assemble?

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.