Cytoskeleton

B. Balen

Importance and function

✓ **structural backbone of the cell**

→ supports the fragile plasma membrane

→ provides mechanical linkages that let the cell bear stresses without being ripped apart

✓ **defines cellular shape and general organization of the cytoplasm**

✓ **responsible for all cellular movements**

→ movements of the whole cell

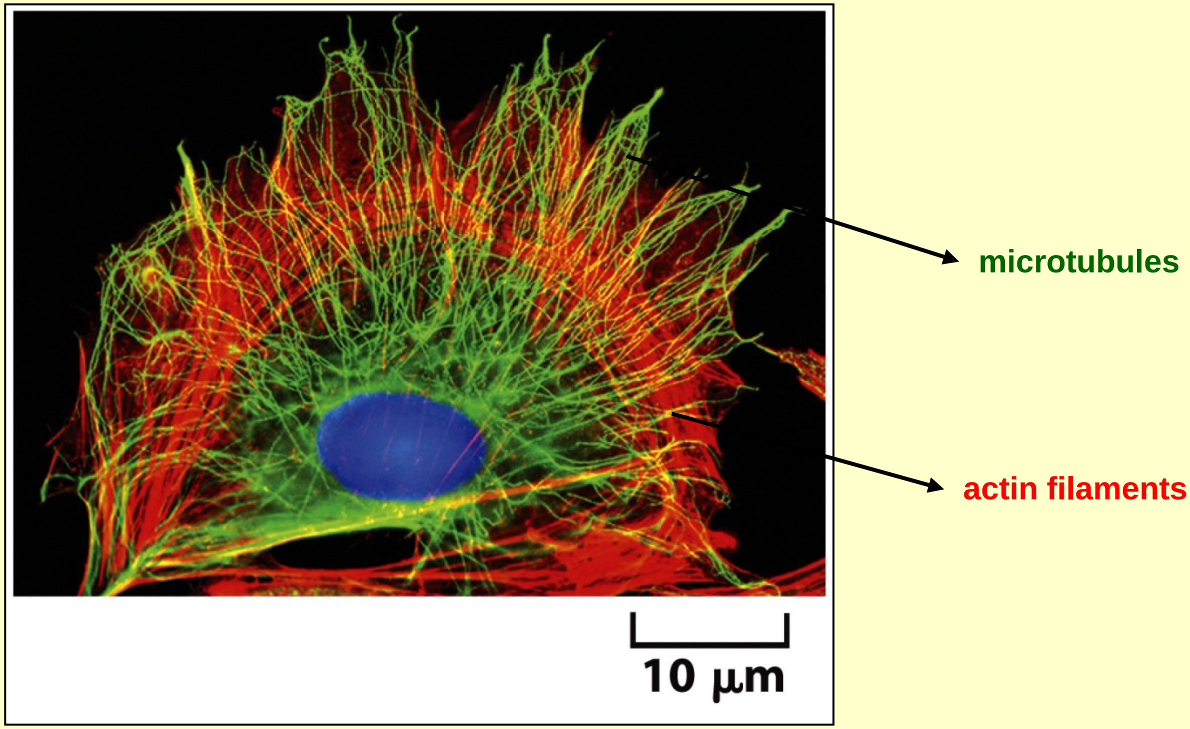
- sperm to swim
- fibroblasts and white blood cell to crawl

→ internal transport of organelles and other structures

✓ **dynamic and adaptable structure**

→ it permanently reorganizes which is dependent on cell movements and changes in cell shape

Three types of cytoskeletal filaments



• **actin filaments** (5-9 nm) – determine the shape of cell's surface and whole cell locomotion

• **intermediate filaments** (~ 10 nm) – provide mechanical strength

• **microtubules** (25 nm) – determine the positions of organelles and direct intracellular transport

Figure 16-1 *Molecular Biology of the Cell* (© Garland Science 2008)

Actin filaments (microfilaments)

✓protein **actin** → polymerization → actin filaments (thin and flexible)

✓diameter 5 - 9 nm; length up to several µm

✓Actin filaments can be organized into more complex structures:
→ linear bundles
→ 2D-networks
→ 3D-gels

✓ most highly concentrated just beneath the plasma membrane → cell cortex
→ network for mechanical support
→ cell shape
→ movements of the cell surface

Actin and actin filaments

✓ actin was isolated from the muscle cell in 1942.

✓ present in all eukaryotic cells

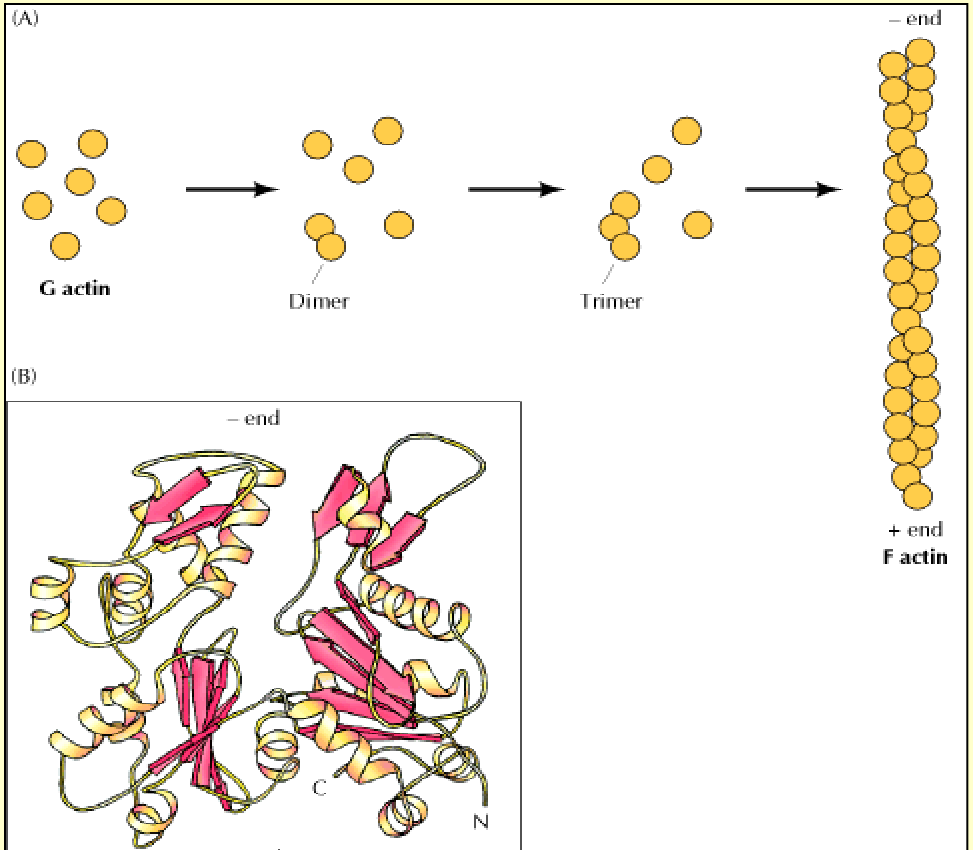
✓ yeast – only 1 actin gene

✓ other eukaryotes – actin gene family (mammals 6 genes)
→ all actins have similar amino acid sequence
→ highly conserved during the evolution

✓ individual molecules → globular proteins of 375 aa (43 kDa)

✓ polymerization of globular proteins → assembly of actin filaments

Assembly of actin filaments



2002. Bruce Alberts et al.

✓ actin monomer – **globular [G] actin**
→ has two binding sites for other 2 monomers

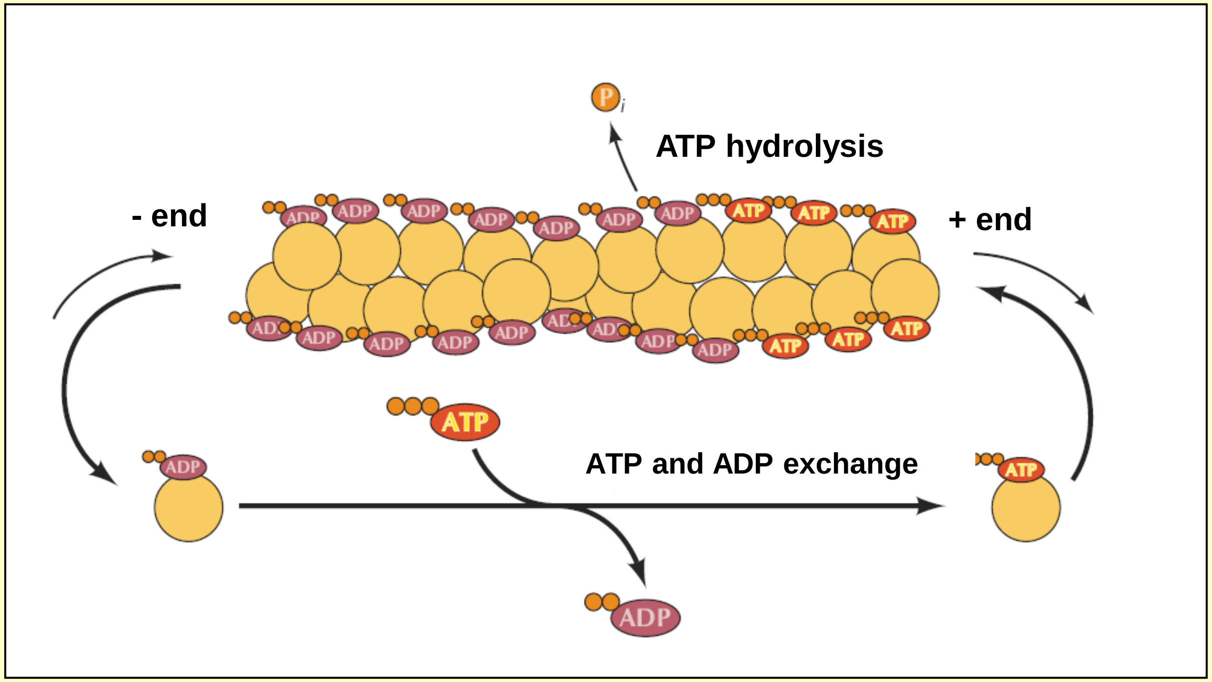
✓ after polymerization – **filament [F] actin**

✓ each monomer in filament is rotated for 166º
→ two-stranded helical polymers

✓ each monomer has the same orientation → filament polarity (**plus** and **minus** end)

✓ polarity is important for filament integration

Assembly of actin filaments



- ✓ filaments grow by reversible adding of the monomers on both ends
- ✓ plus end grows 5 -10 x faster then minus end
- ✓ actin monomers bind ATP which hydrolyze to ADP after assembly

2004. Cooper and Hausman

Actin filaments

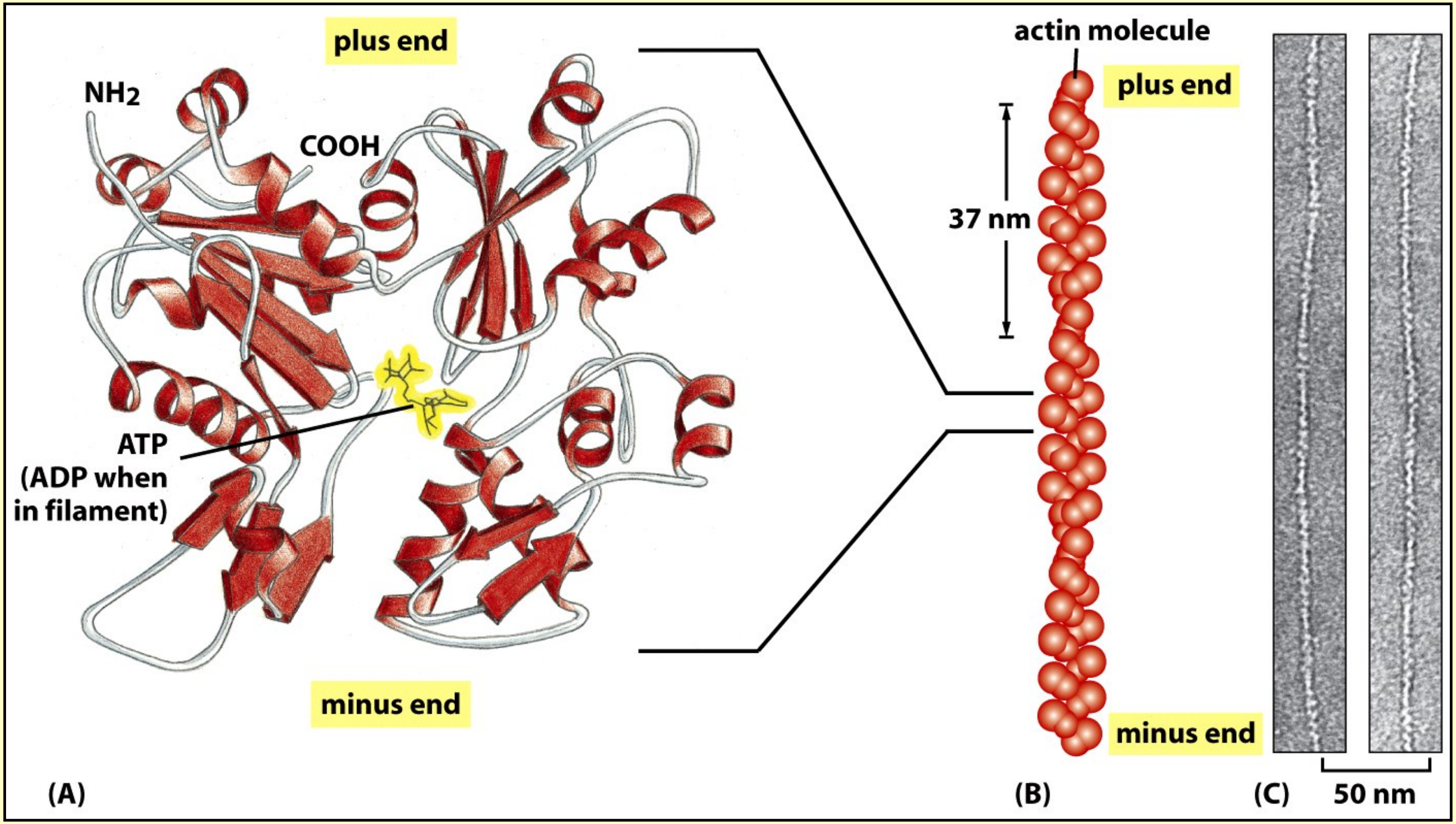
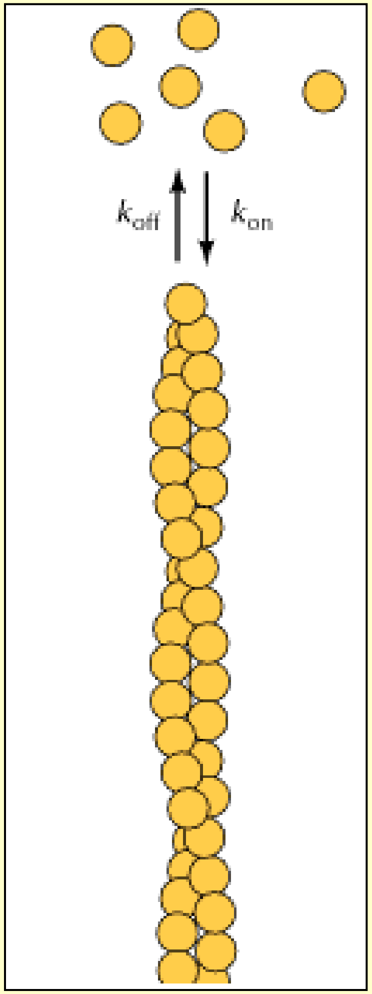


Figure 16-12 *Molecular Biology of the Cell* (© Garland Science 2008)

Polymerization of actin monomers



✓ **reversible process**

→ **dissociation speed** (***k*_off**) – independent of free monomer concentration

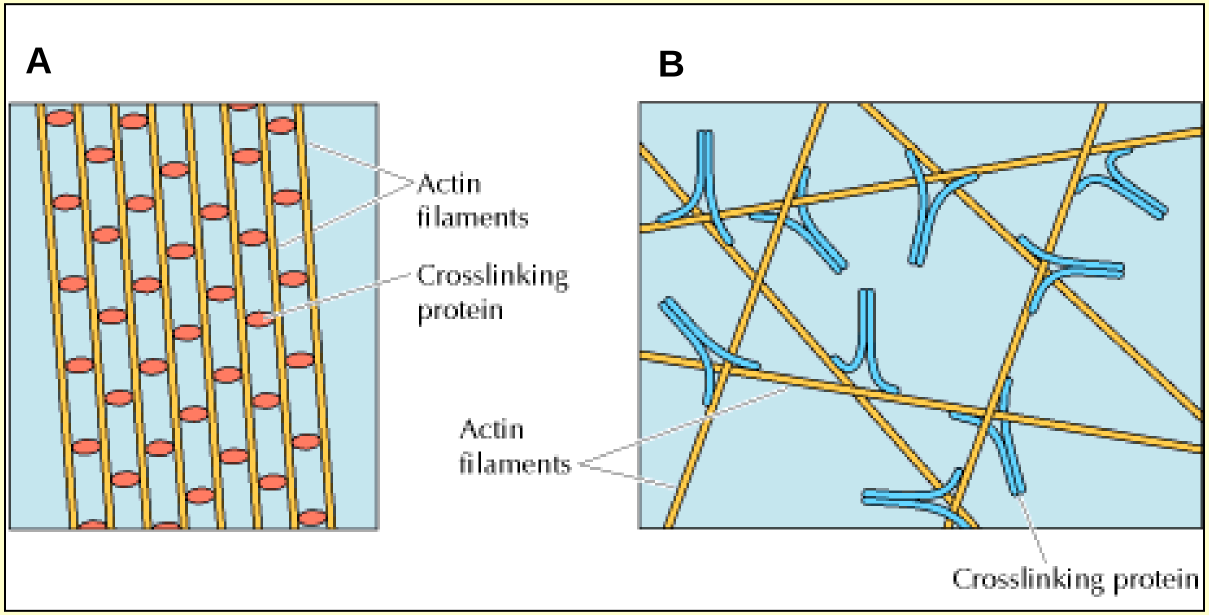
→ **association speed** (***k*_on**) – proportional to free monomer concentration

Organization of actin filaments

➢**Two major structural types:**

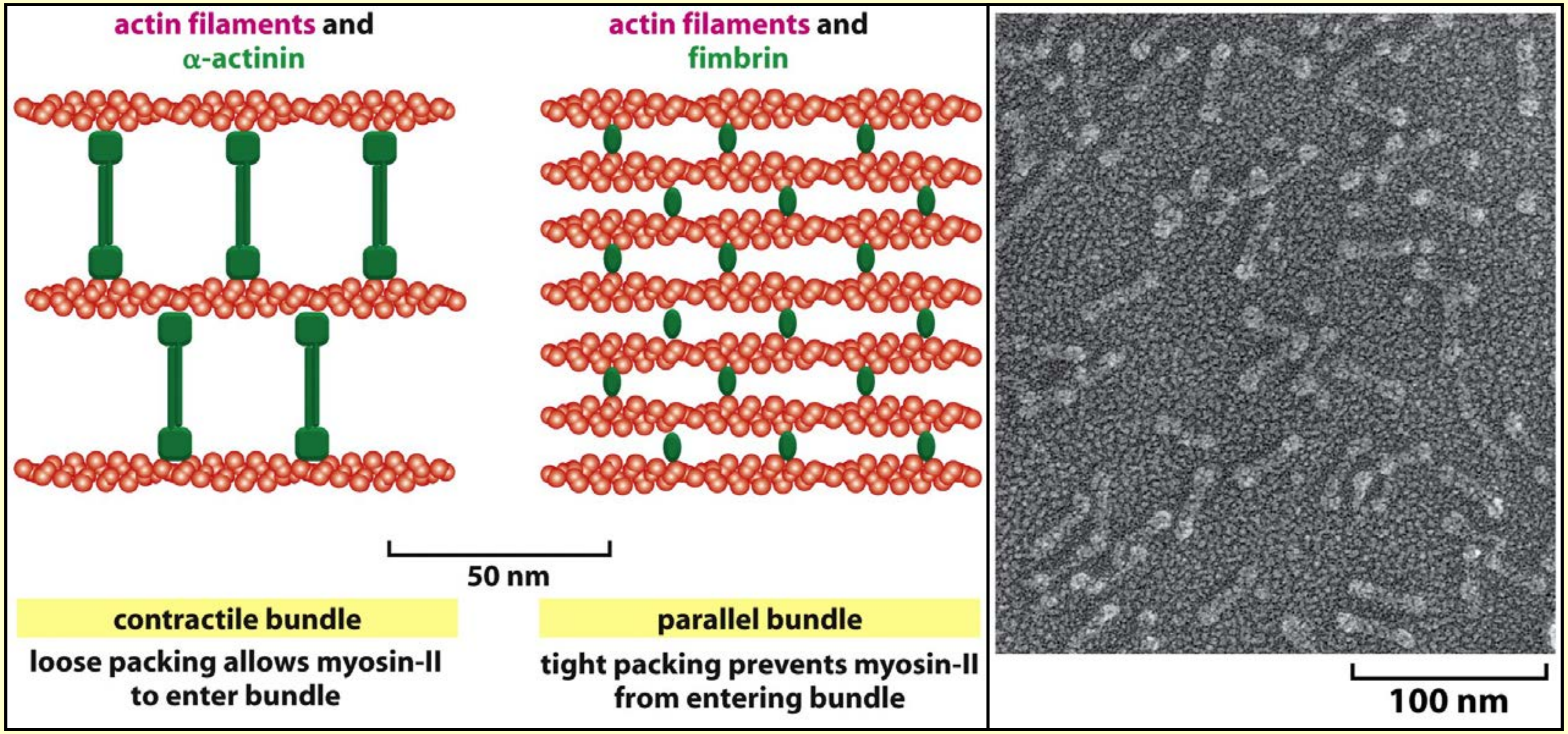
I. Bundles – actin filaments joined to form parallel arrays

II. Web-like (gel-like) networks – actin filaments cross-linked almost vertically



2004. Cooper and Hausman

Actin filament bundles

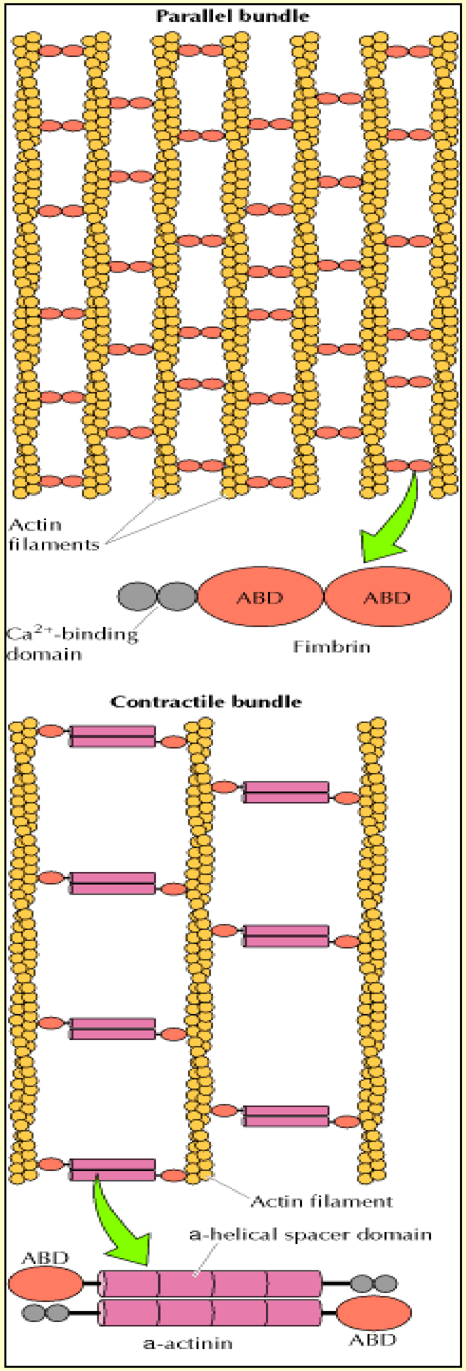


(**A**) **α-actinin** cross-links actin filaments into loose bundles

(**B**) **fimbrin** cross-links filaments into tight bundles

(**C**) EM of purified α-actinin molecules

Figure 16-49a *Molecular Biology of the Cell* (© Garland Science 2008)



❖ **Parallel bundle** – actin filaments cross-linked by **fimbrin**

✓ fimbrin - 2 neighboring domains (ABD) which bind actin

✓ tight bundles – distance between the filaments 14 nm

❖ **Contractile bundle** – actin filaments cross-linked by **α-actinin**

✓ binds to actin as a dimer; each subunit has a binding site for actin

✓ filament distance 40 nm
→ allows contractions of these bundles
→ interaction of myosin with actin

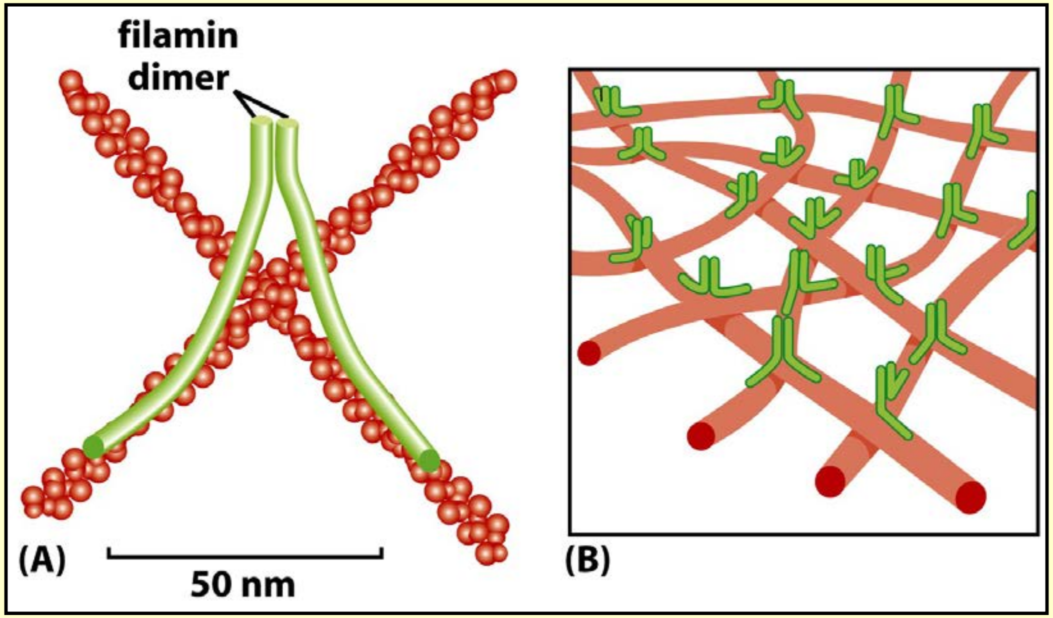
2004. Cooper and Hausman

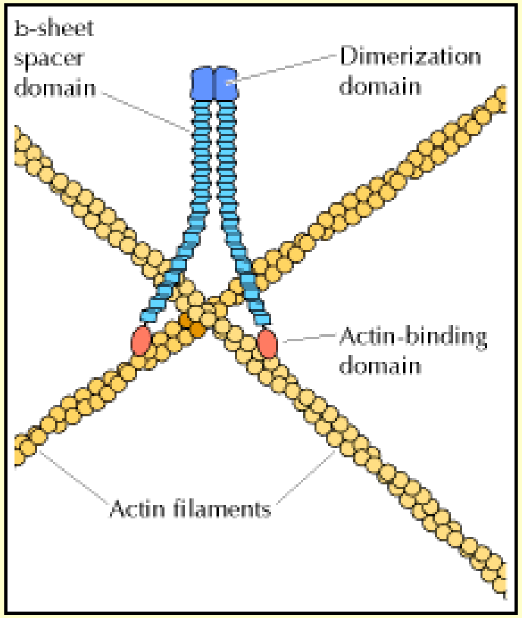
Actin filament networks

✓ actin filaments cross-linked with large proteins

✓ **filamin**
→ cross-links actin into a 3D network with the physical properties of a gel
→ homodimer
→ each dimer is about 160 nm long and forms a flexible, high-angle link between two adjacent actin filaments

→ forms mechanically strong web or a gel

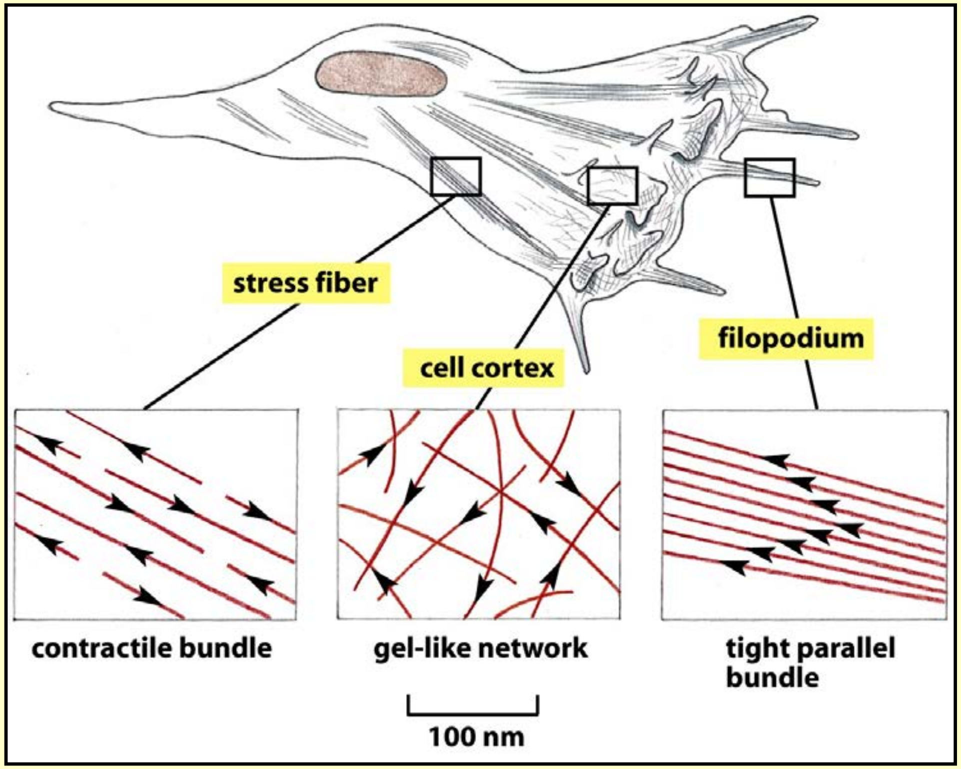




2004. Cooper and Hausman

Figure 16-5 *Molecular Biology of the Cell* (© Garland Science 2008)

Actin arrays in a cell



→ actin filaments – arrowheads pointing towards the minus end

✓ **stress fibers** → contractile bundles

✓ **cell cortex** → 3D-network with gel properties

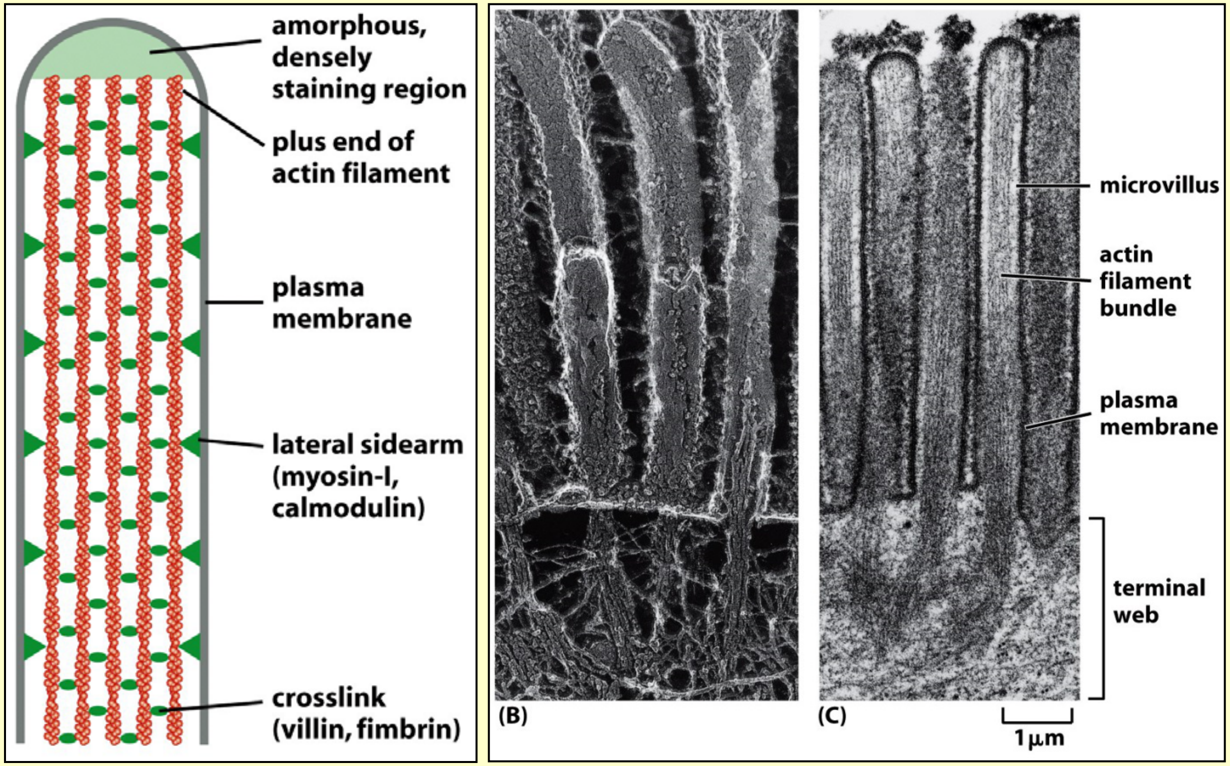
✓ **filopodia** – spike-like projections of plasma membrane → parallel bundles

Figure 16-47 *Molecular Biology of the Cell* (© Garland Science 2008)

Extensions of cell surface

- ✓ surface of the majority of cell has extensions → movements, fagocytosis,…
- ✓ most of them are based on actin filaments
- ✓ permanent structures
  - ✓ **microvilli** – finger-like extensions in plasma membrane of epithelium cells
  - ✓ **stereocillia** – in the inner ear, the mechano-sensing organelles of hair cells, which respond to fluid motion; acoustic sensors in mammals
- ✓ temporary structures - response to stimuli from the environment
  - **pseudopodia**
  - **lamellipodia**
  - **filopodia**

Microvilli



- ✓ plasma membrane extensions – increase the absorption surface of epithelial cells
- ✓ 1000 microvilli per cell of human small intestine
- ✓ intestine microvilli → parallel bundles of 20-30 actin fibers
  - **fibrin** and **villin** cross-link the actin fibers
  - **myosin-I** and **calmodulin** form sidearms

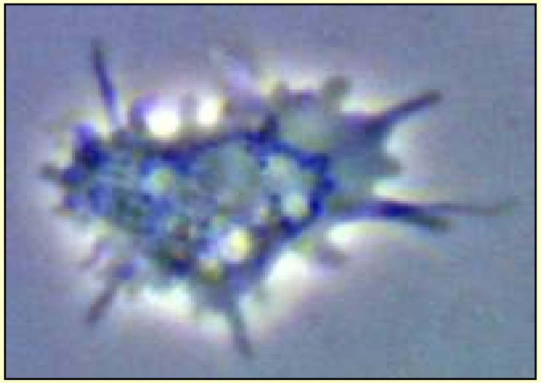
Figure 16-50a *Molecular Biology of the Cell* (© Garland Science 2008)

Temporary extensions of cell surface

❖ **Pseudopodia**

✓ flexible length

✓ phagocytosis and movements of amoebas

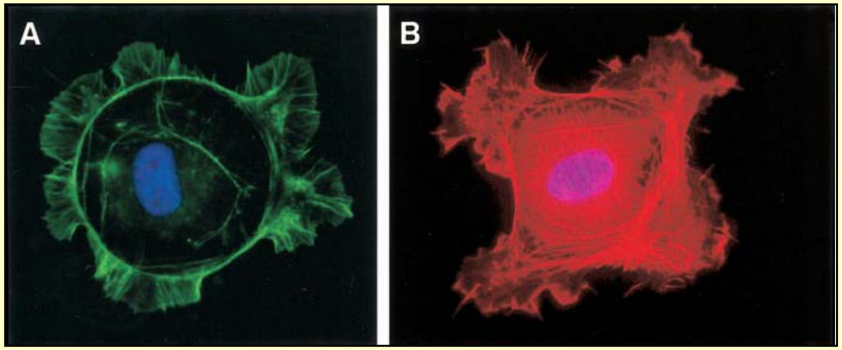


ameba *Mayorella* (*Gymnamoebae*)

http://tolweb.org/notes/

❖ **Lamellipodia**

Amoeba in motion - http://www.youtube.com/watch?v=7pR7TNzJ_pA&feature=related



✓ sheet-like membrane projections

✓ contain a network of actin filaments

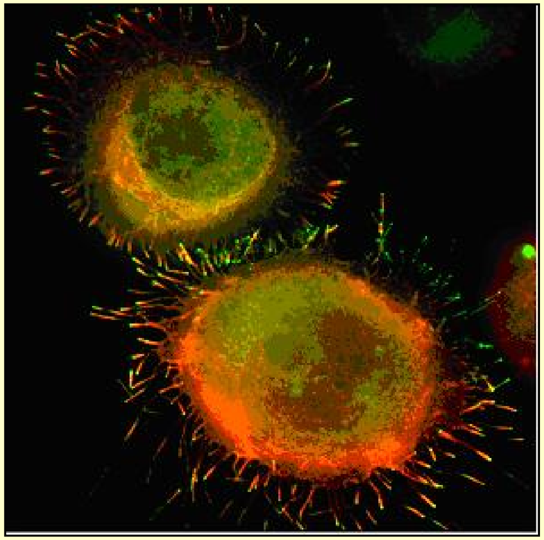
http://www.fasebj.org/cgi/reprint/

❖ **Filopodia**

✓ spike-like thin projection of cell surface supported by actin bundles

Macrophage filopodia

http://en.wikipedia.org/wiki/Filopodia



Intermediate filaments

- ✓ diameter around 10 nm
(between actin fibers ~7 nm and microtubules ~ 25 nm)
- ✓ composed out of several protein types
- ✓ nuclear lamins – all cells
- ✓ cytoplasmic intermediate filaments – only in cells of vertebrates, nematodes and mollusks
- ✓ particularly prominent in the cytoplasm of cells subjected to mechanical stress
- ✓ not involved in any movements
- ✓ basic structural role → mechanical strength

Major types of intermediate filament proteins

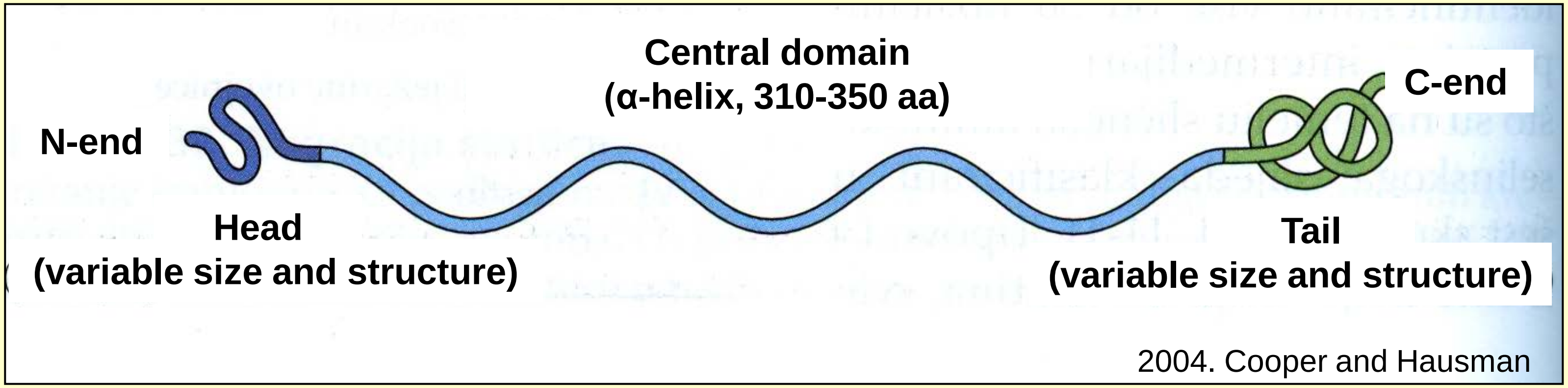
- ✓ more than 50 different proteins were identified
- ✓ classified in groups based on amino acid sequence

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)	epithelial cells and their derivatives (e.g., hair and nails)
Axonal	neurofilament proteins (NF-L, NF-M, and NF-H)	neurons

Table 16-1 *Molecular Biology of the Cell* (© Garland Science 2008)

Intermediate filament structure



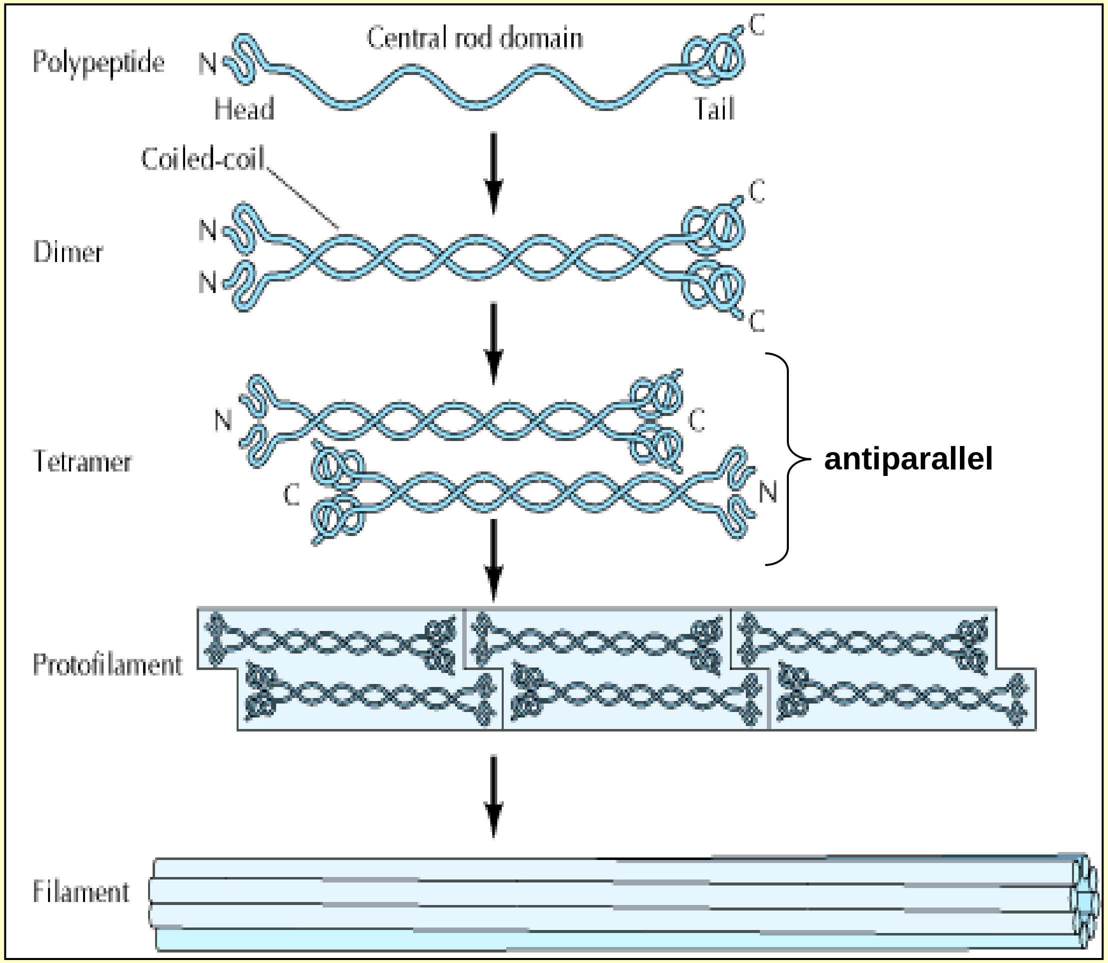
✓ **central α-helix domain** ~ 310 aa (nuclear lamin 350 aa)

→ central role in filament assembly

✓ **variable head and tail**

→ specific functions of different proteins

Intermediate filament construction



✓ monomer pairs with another monomer → **dimer formation**

✓ two dimers line up side by side – **antiparallel tetramer formation**

✓ tetramers pack together sideways – **protofilament formation**

✓ 8 tetramers pack together in a helical array – 10-nm rope-like **filament formation**

✓ built out of antiparallel tetramers → not polar!

2004. Cooper and Hausman

Intermediate filament construction

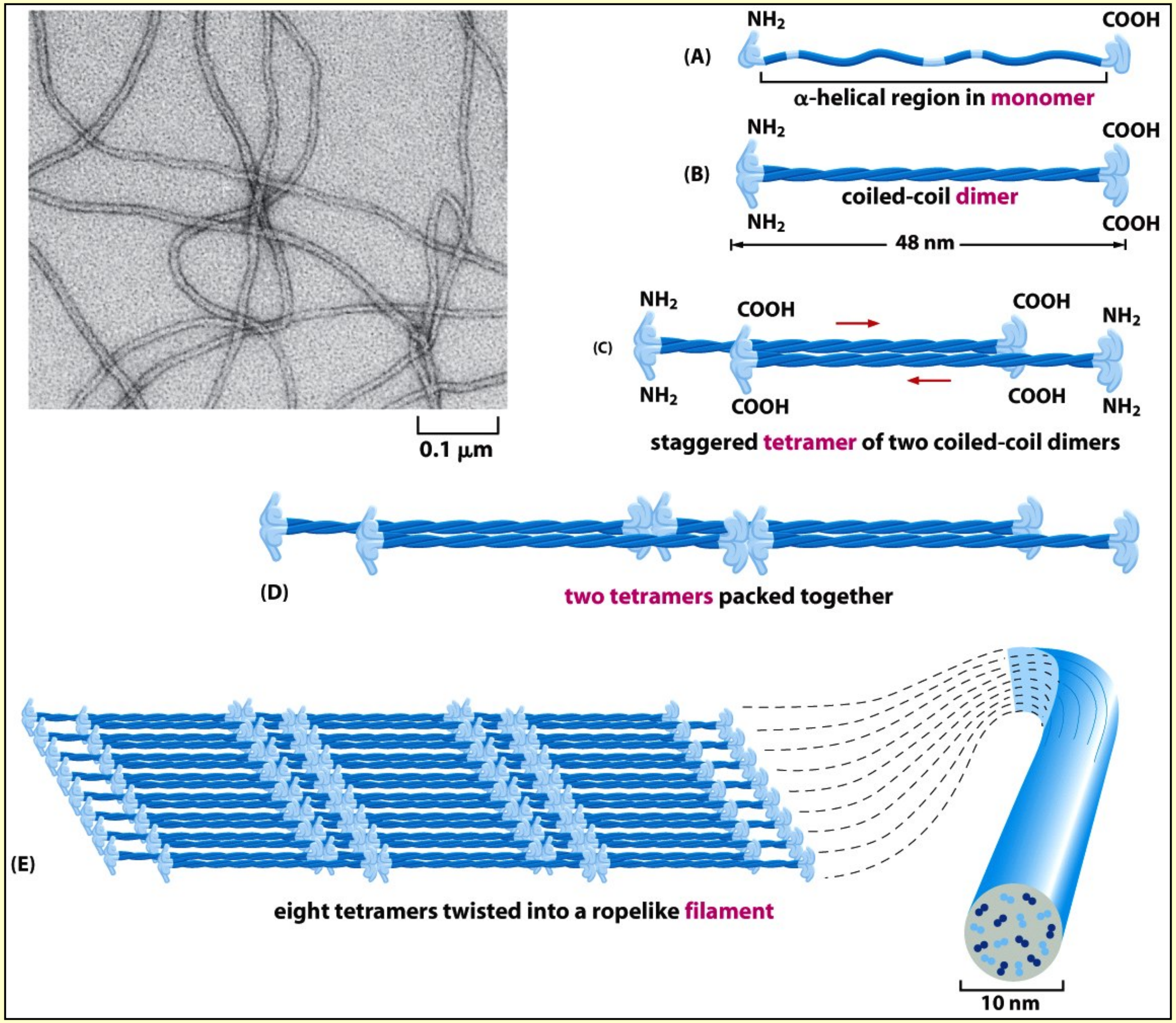
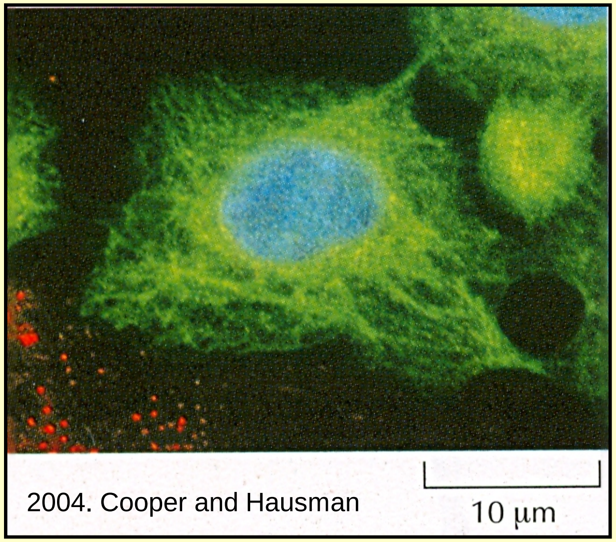
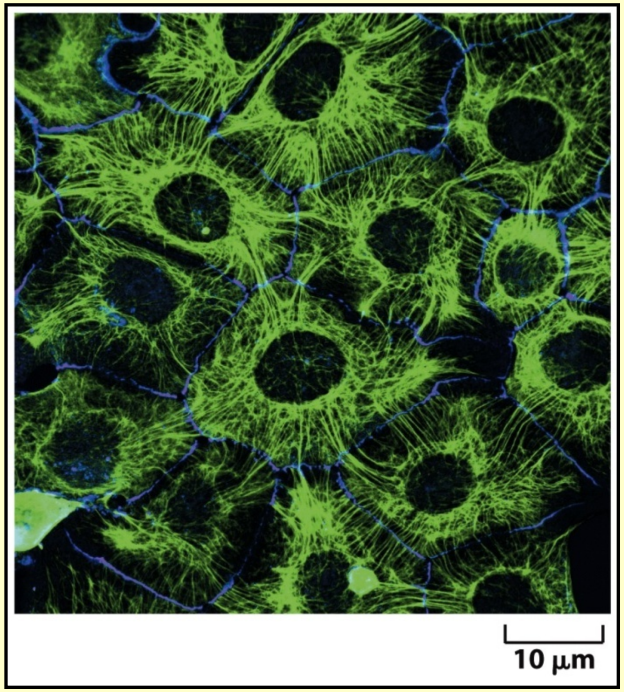


Figure 16-19 *Molecular Biology of the Cell* (© Garland Science 2008)

Intracellular organization of intermediate filaments





✓ a net in the cytoplasm forming a ring around nucleus and spreading all the way to the plasma membrane

✓ **keratin** and **vimentin** filaments bind the nuclear envelope
→ anchoring and positioning of nucleus inside cell

✓ link with actin filaments and microtubules

✓ form a support which integrates different elements of cytoskeleton and organizes internal cellular structure

Figure 16-20 *Molecular Biology of the Cell* (© Garland Science 2008)

IFs are cross-linked and bundled into strong arrays

I. many IFs further bundle themselves by self-association

- *neurofilament proteins NF-M* and *NF-H* have a C-domain that binds to a neighboring filament → robust parallel arrays

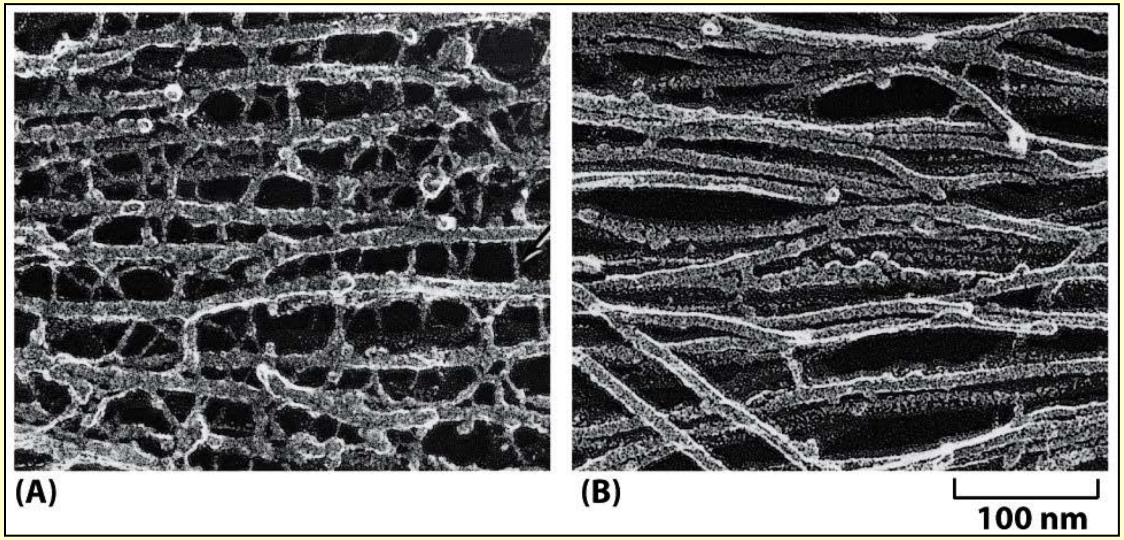


Figure 16-22 *Molecular Biology of the Cell* (© Garland Science 2008)

II. other types of IFs are held together by accessory proteins

- Plectin

→ makes bundles of *vimentin*

→ makes cross-links from **intermediate filaments** to **microtubules,** actin filament bundles and **filaments of motor proteins**

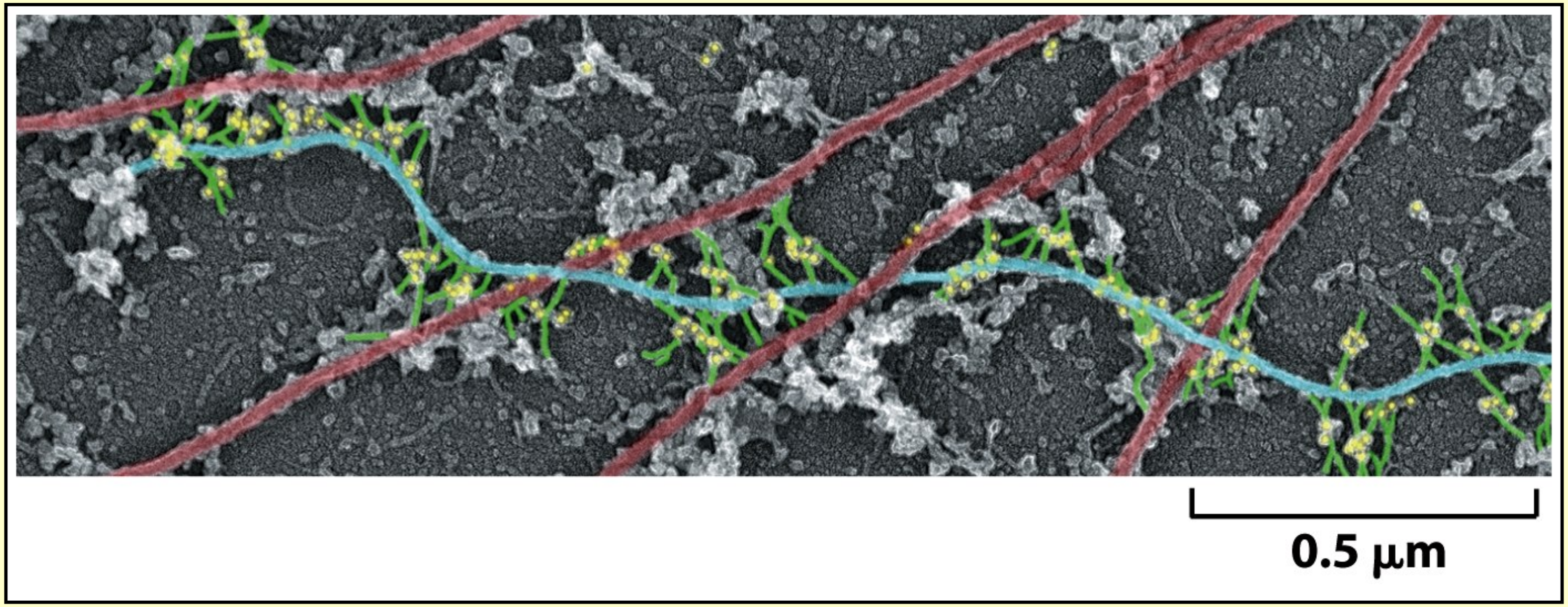


Figure 16-46 *Molecular Biology of the Cell* (© Garland Science 2008)

Microtubules

✓ diameter 25 nm

✓ dynamic structures

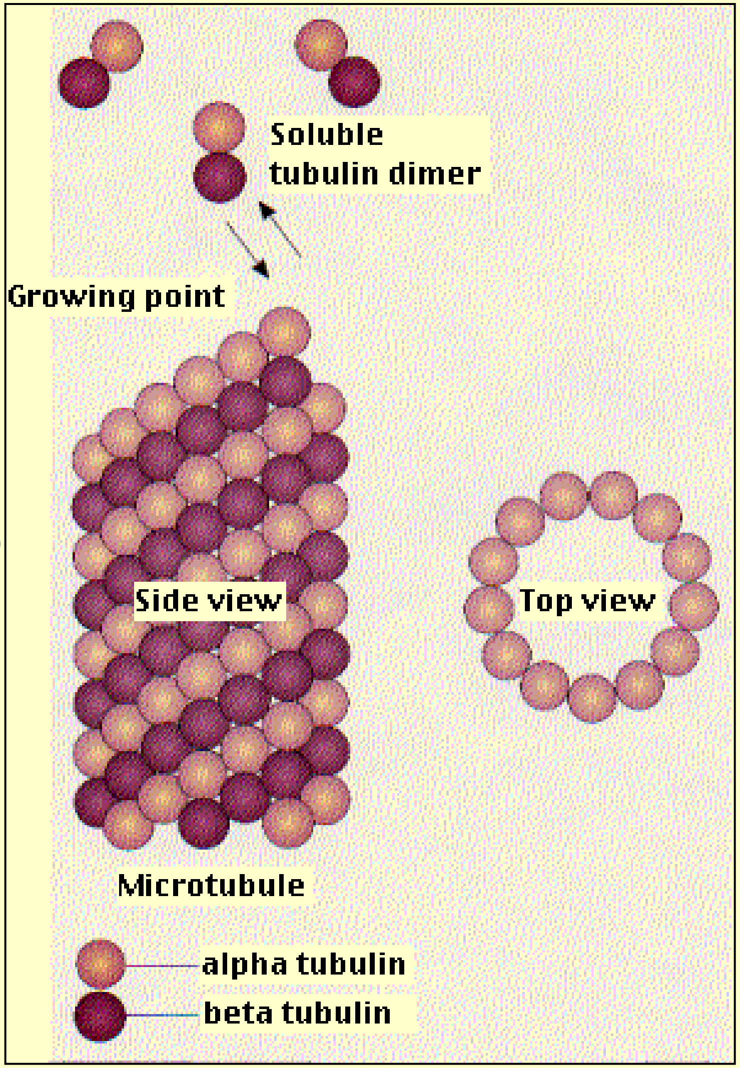
✓ participate in
- cellular shape forming
- intracellular movements
- organelle transport, mitosis
- forming of cillia and flagella

✓ **tubulin** → dimer of 2 polypeptid chains (55 kDa)

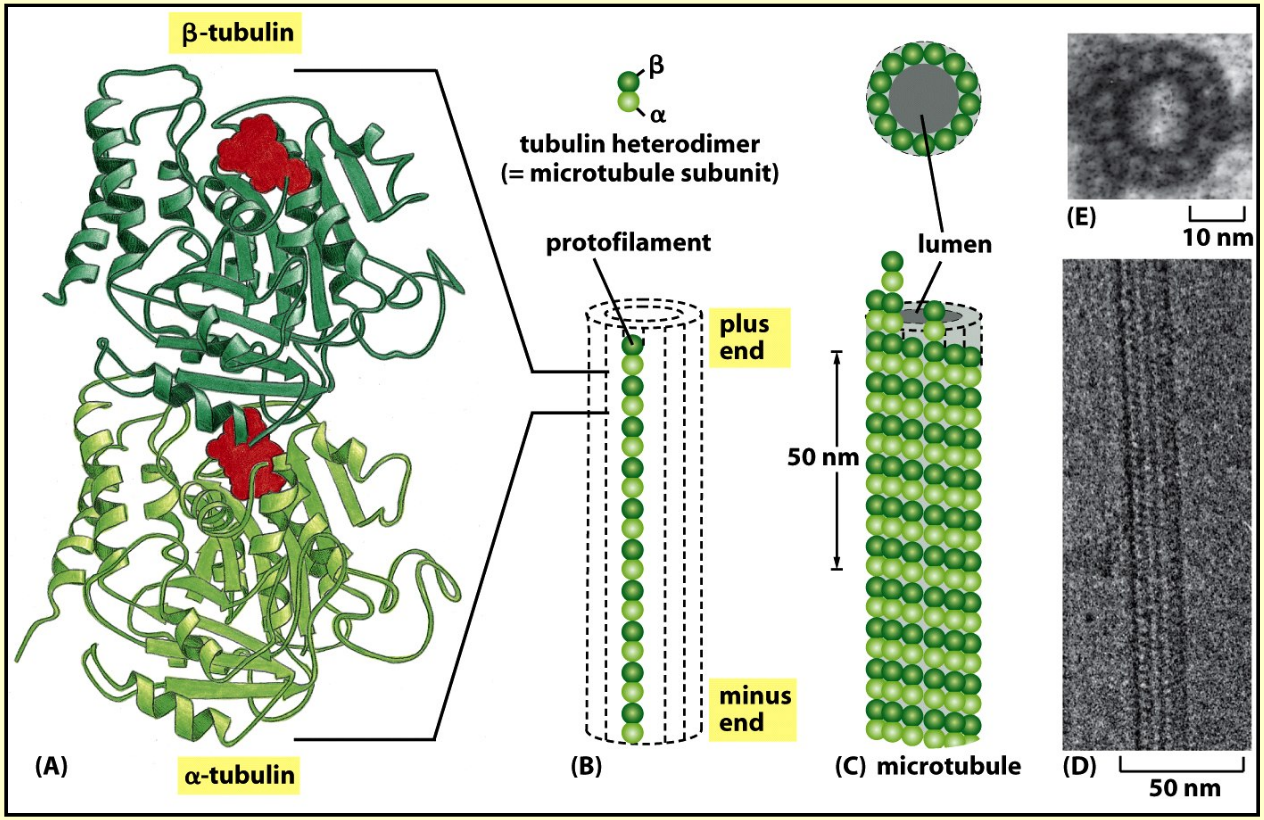
✓**α-** and **β-tubulin** → coded by a small gene family

✓ polymerization of tubulin → **microtubules**

✓ each microtubule consists out of **13** linear **protofilaments**



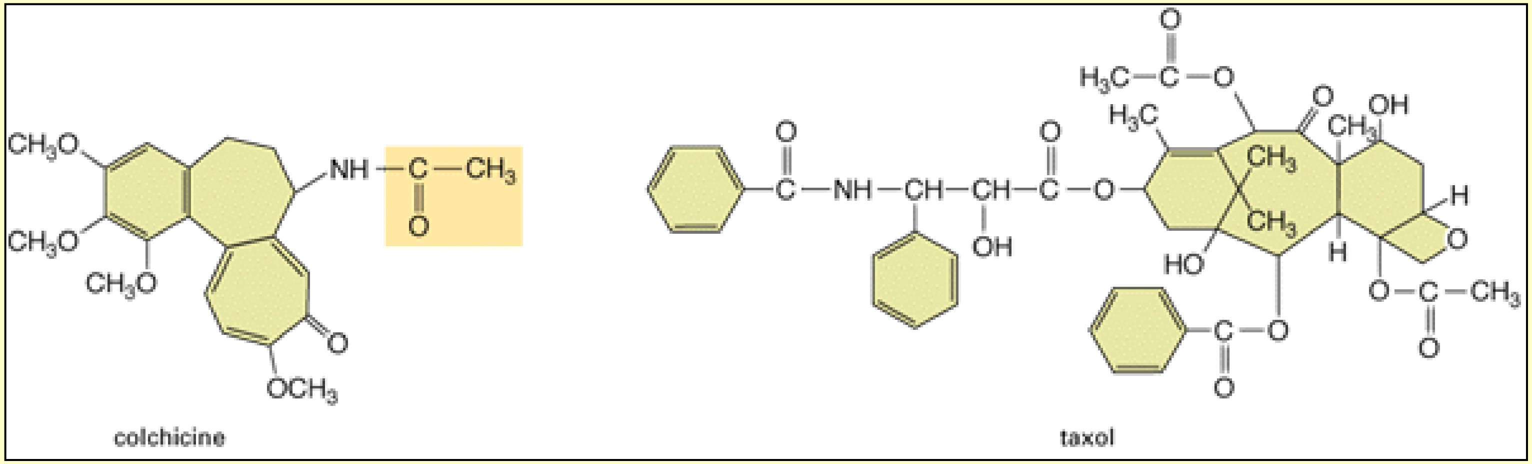
The structure of microtubule and its subunits



- ✓ protofilaments are polar structures
- ✓ fast-growing **+** and slow-growing **–** end
- ✓ α- and β-tubulin bind GTP (polymerization regulation)

Figure 16-11 *Molecular Biology of the Cell* (© Garland Science 2008)

Microtubule inhibitors



✓ ***colchicine***

→ natural toxic product and secondary metabolite

→ extracted from plants of genus *Colchicum* (*Colchicum autumnale*, meadow saffron)

✓ ***colcemid*** – related to colchicine but it is less toxic

- depolymerise microtubules and limit microtubule formation (C-mitosis)
- inactivate spindle fiber formation
- arrests cells in metaphase and allow cell harvest and karyotyping

✓ ***taxsol***

- from larch (*Taxus baccata*)
- opposite effect of colchicine and colcemid
- it stabilizes microtubules
- also prevents cell divison

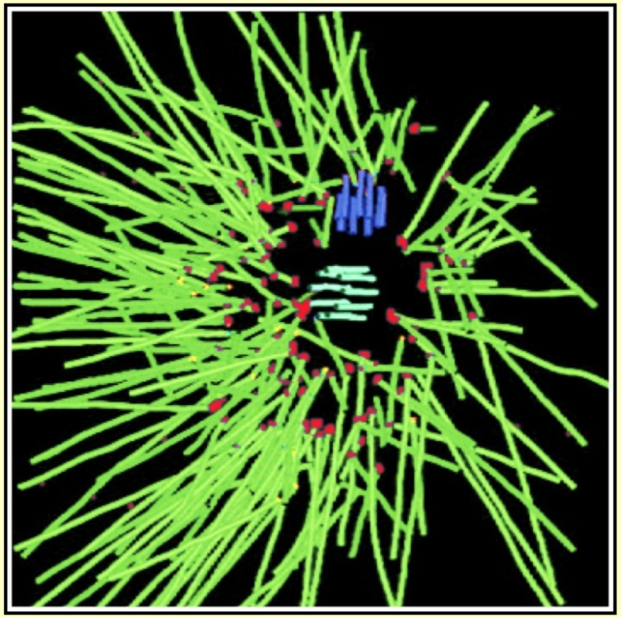
Centrosome and centriole

✓ **α-** and **β-tubulin** are regular building blocks of microtubules

✓ **γ-tubulin** has more specialized role – involved in nucleation of microtubule growth

✓ microtubules are nucleated
- from **M**icro**T**ubule-**O**rganizing **C**entre (MTOC) at their minus end
- the plus end is growing outward from each MTOC

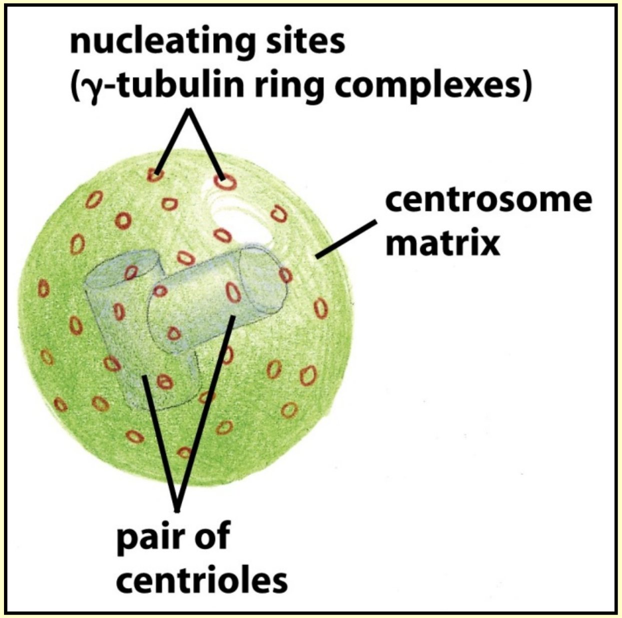
✓animal cells – a single, well-defined MTOC = **centrosome**
→ near the nucleus in interphase cells

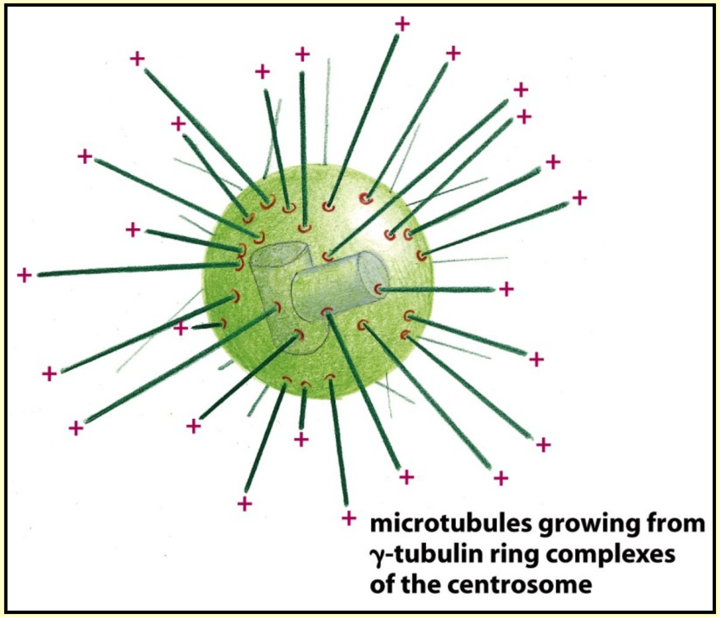


MTOC from *C. elegans* cell – dense thicket of microtubules emanating form the centrosome

Figure 16-30c *Molecular Biology of the Cell* (© Garland Science 2008)

Centrosome





- ✓ amorphous matrix of fibrous proteins to which γ-tubulin is attached
- ✓ matrix is organized by a pair of centrioles

– cylindrical structures arranged at right angles to each other
– they become basal bodies for cilia and flagella in motile cells

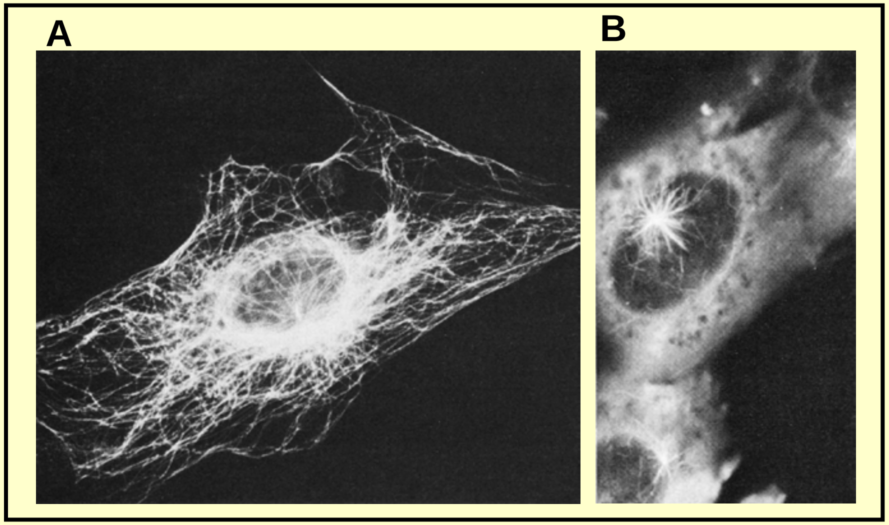
- ✓ minus end of each microtubule is embedded in centrosome
- ✓ plus end is free in the cytoplasm

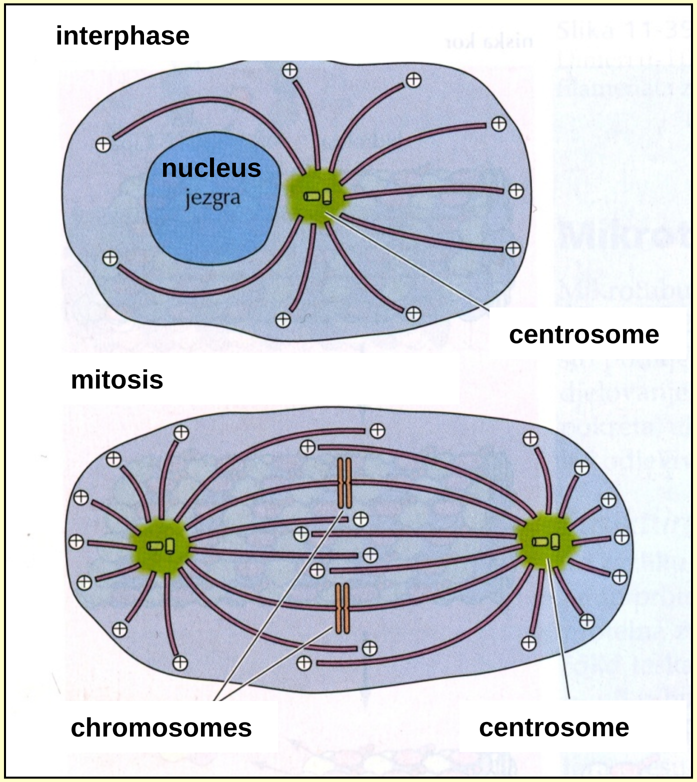
Figure 16-30a,b *Molecular Biology of the Cell* (© Garland Science 2008)

Microtubules grow from centrosome

A. interphase cell

B. cell treated with *colcemid* and recovered





➢during mitosis microtubules extend from divided centrosome to form a mitotic spindle

2004. Cooper and Hausman

Centriole

- ✓ a pair of centrioles per centrosome in animal cells
- ✓ arranged at right angles to each other
- ✓ cylindrical structures from 9 triplets of microtubules
- ✓ they become basal bodies for cillia and flagella in motile cells
- ✓ complex structures with expressed polarity

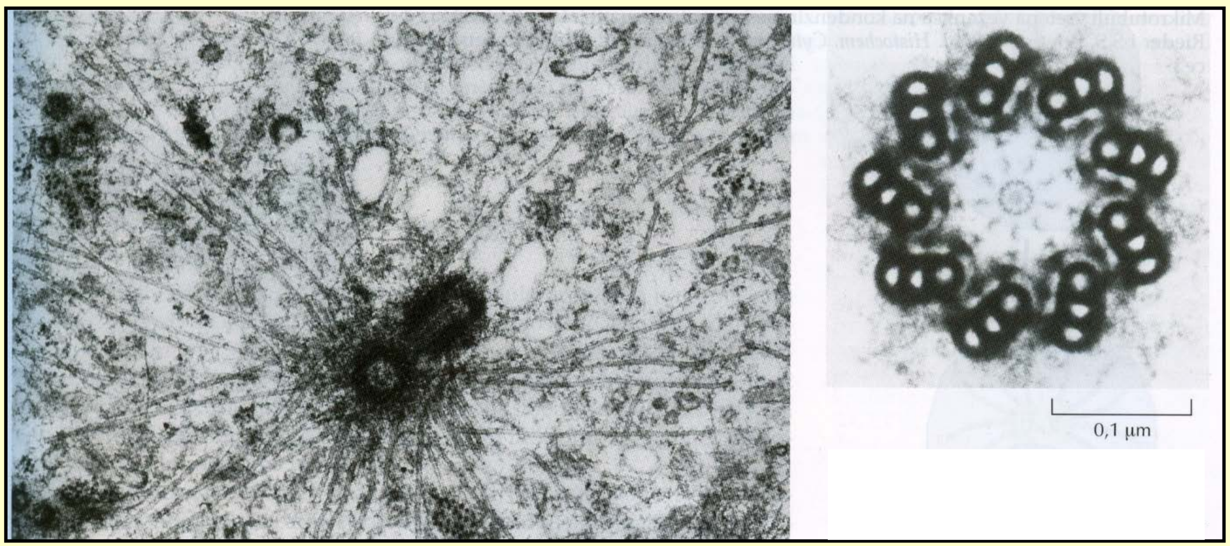


Figure 11-43. 2004. Cooper and Hausman

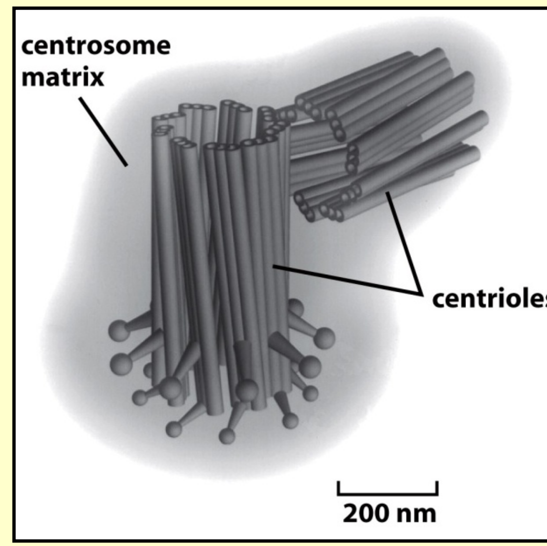


Figure 16-31b *Molecular Biology of the Cell*

Centriole replication

✓ G1 phase - centrioles separate by a few µm

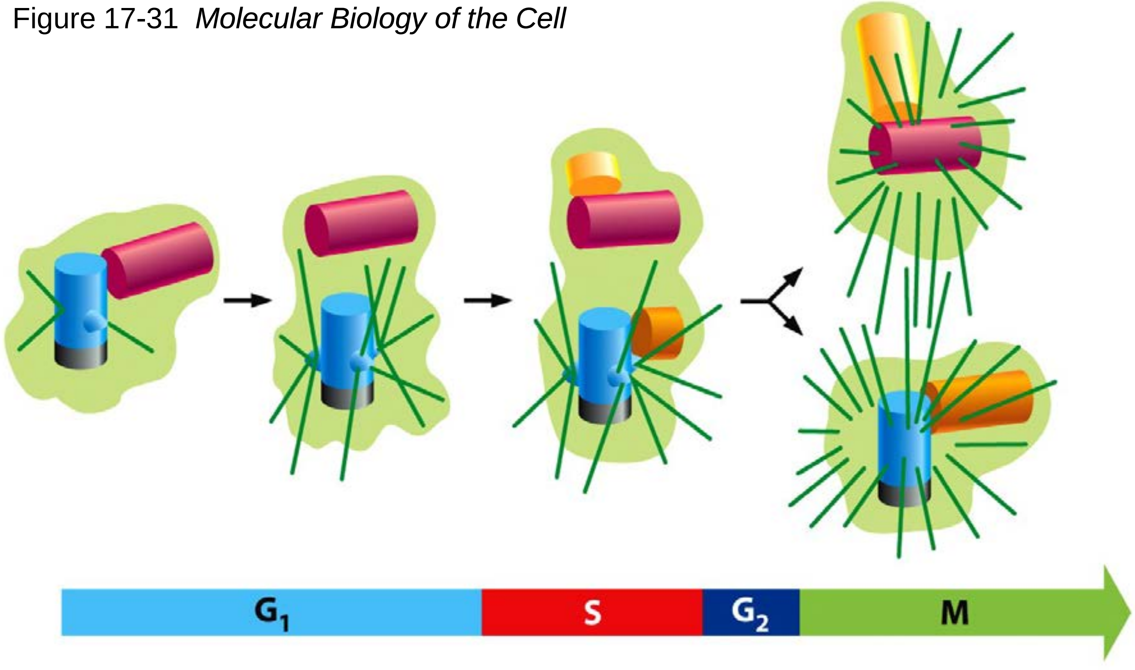
✓ S phase - daughter centriole begins to grow near the base of each mother centriole at right angle to it

✓ elongation is completed by phase G2

✓ M phase - complex splits in two

✓ each centrosome now nucleates its own radial array of microtubules

Figure 17-31 *Molecular Biology of the Cell*



Organelle transport and intracellular organization

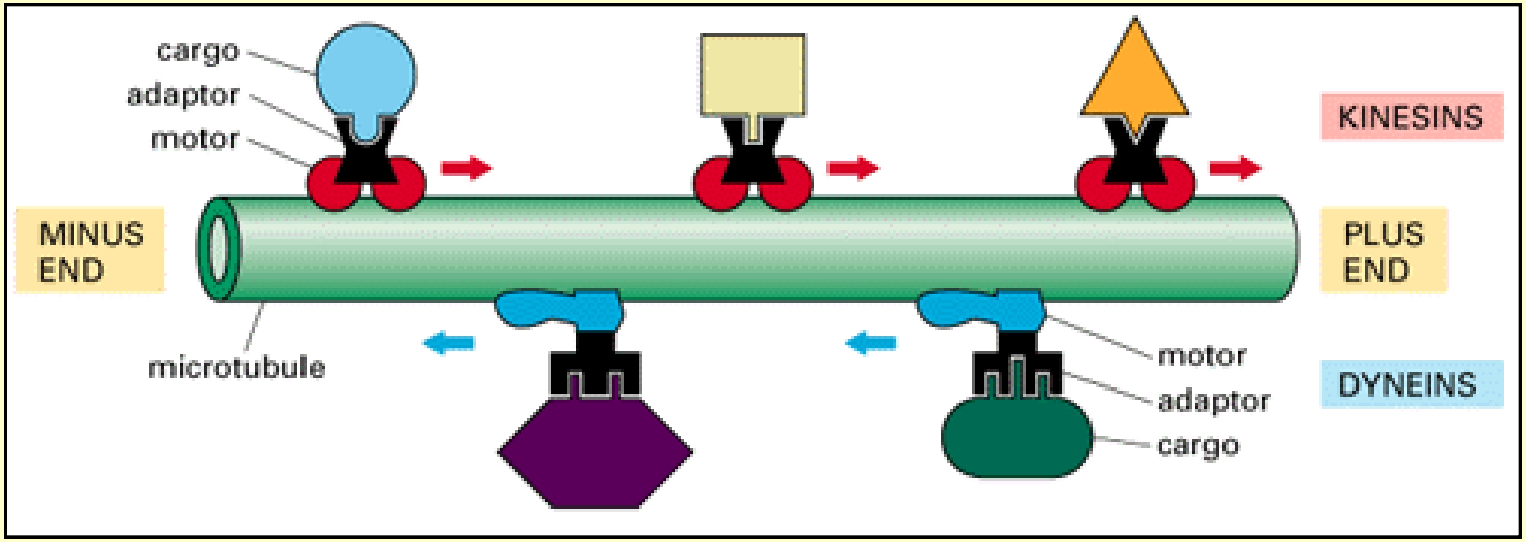
✓ one of the major roles of microtubules

✓ two motor proteins are involved – kinesins and dyneins

✓ use the energy derived form repeated cycles of ATP hydrolysis to move along the microtubules

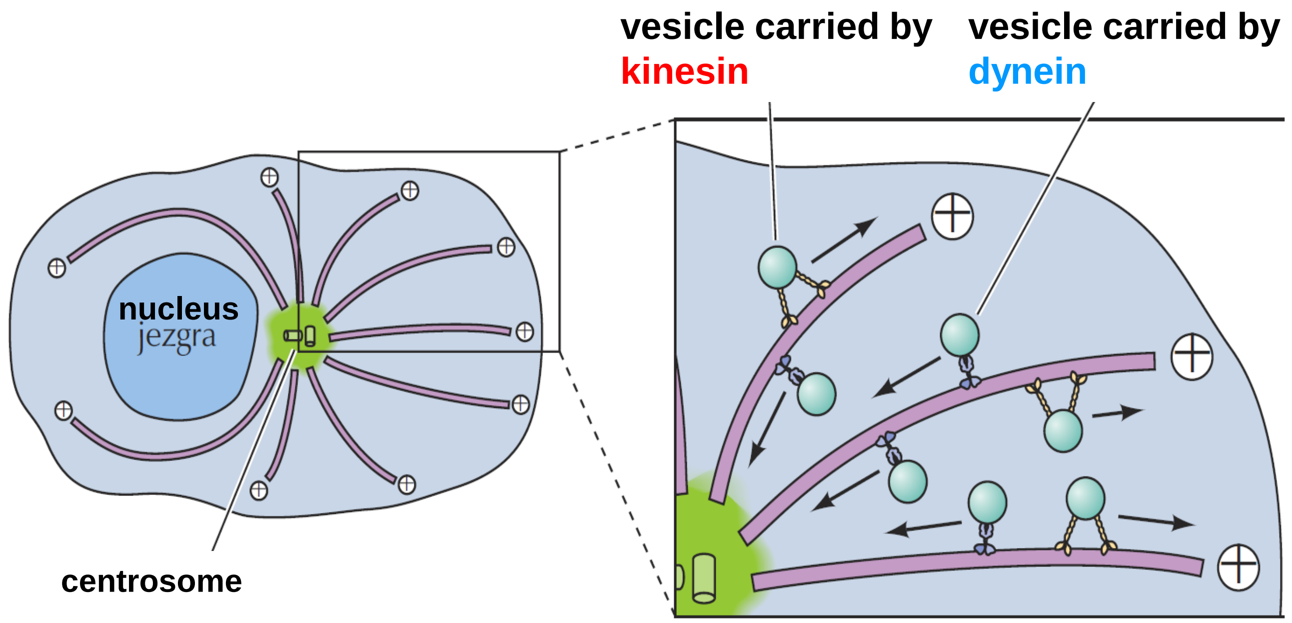
✓ carry membrane-enclosed vesicles and organelles

- **kinesins** → move towards **plus** end (plus-end-directed)
- **dyneins** → move towards **minus** end (minus-end-directed)



2002. Bruce Alberts et al.

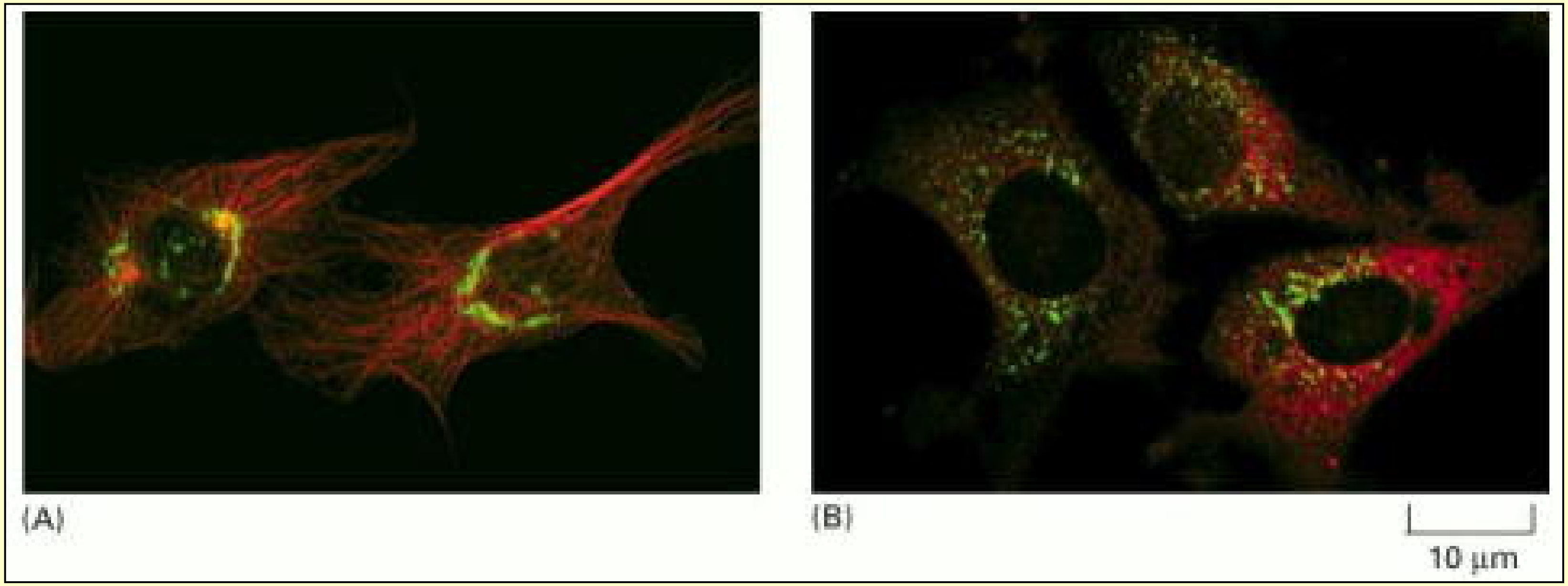
Kinesins and dyneins



Slika 11-49. 2004. Cooper and Hausman

- **kinesin** transports vesicles and organelles in plus end direction of microtubules
- **dynein** transports ”cargo” in minus end direction

The effect of depolarization of microtubules on Golgi complex



(A) endotel cells - **microtubules** and **Golgi complex**; while microtubules are intact, Golgi complex is positioned near the nucleus in cell centre

(B) after treatment with *nocodazol*, which induces depolymerization of **microtubules**, **Golgi complex** is fragmentized and dispersed throughout the cell

2002. Bruce Alberts et al.

Cillia and flagella

✓ cytoplasm extensions based on microtubules

✓ organelles for movements → enable cell to swim through liquid medium

✓ cillia and flagellum
→ two versions of the same structures
→ distinctive 9 + 2 arrangement of microtubules

✓ diameter ~ 0,25 µm

✓ cillia length ~ 10 µm, numerous per cell

✓ flagellum length ~ 200 µm, 1-2 per cell

✓ undulating motion – wave-like motion
→ waves of constant amplitude move continuously from base to tip

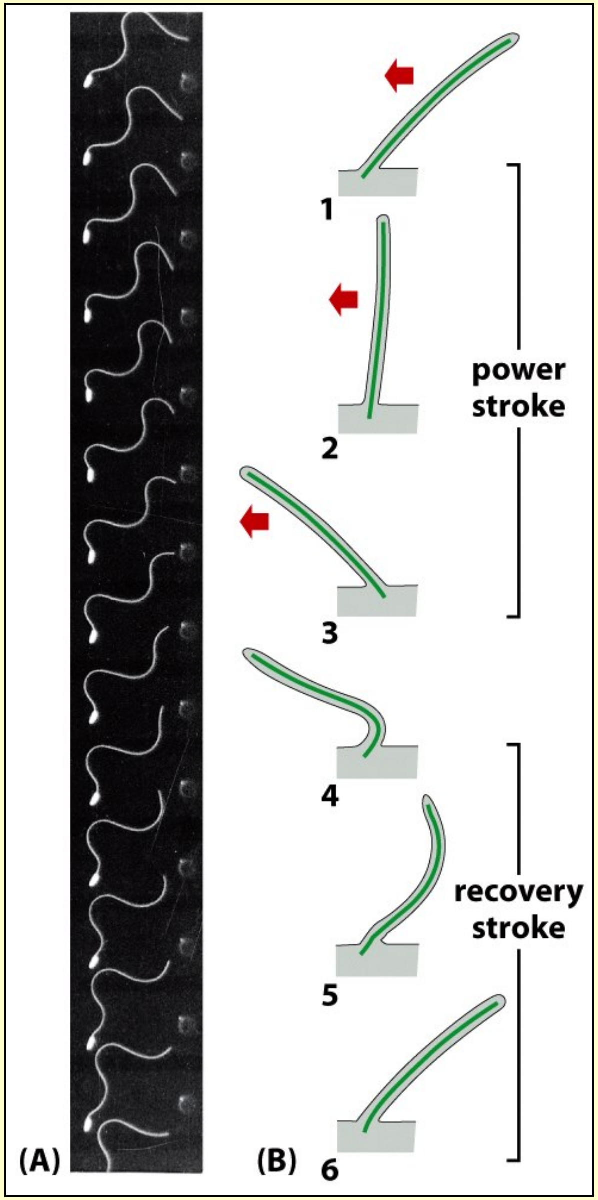
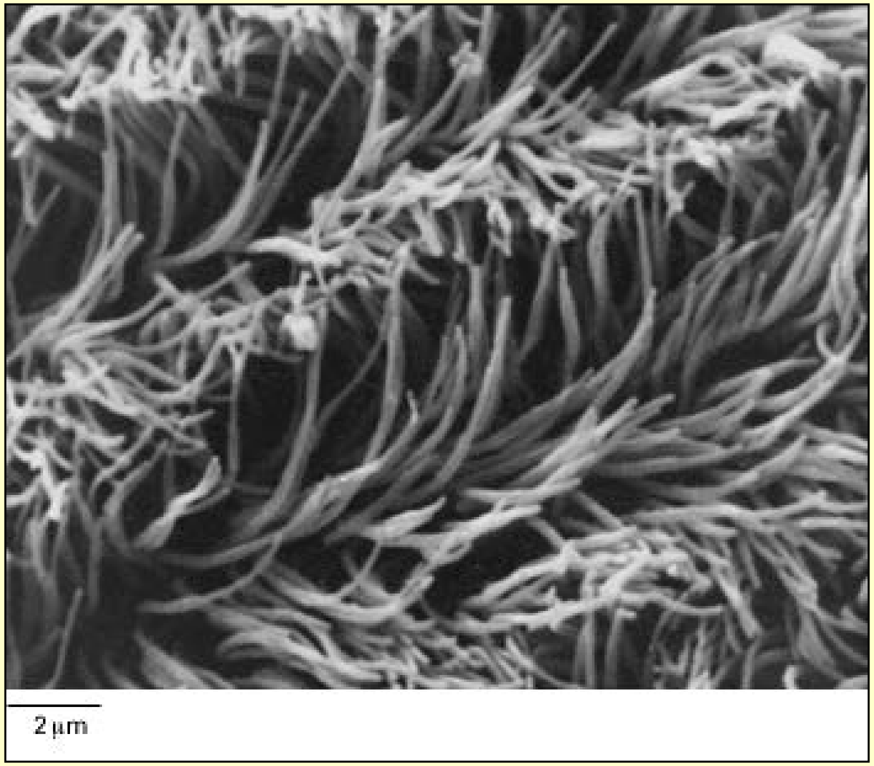


Figure 16-80 *Molecular Biology of the Cell* (© Garland Science 2008)



Cilia. SEM photo of cillia from intestine of sea worm

Examples of cillia and flagellum

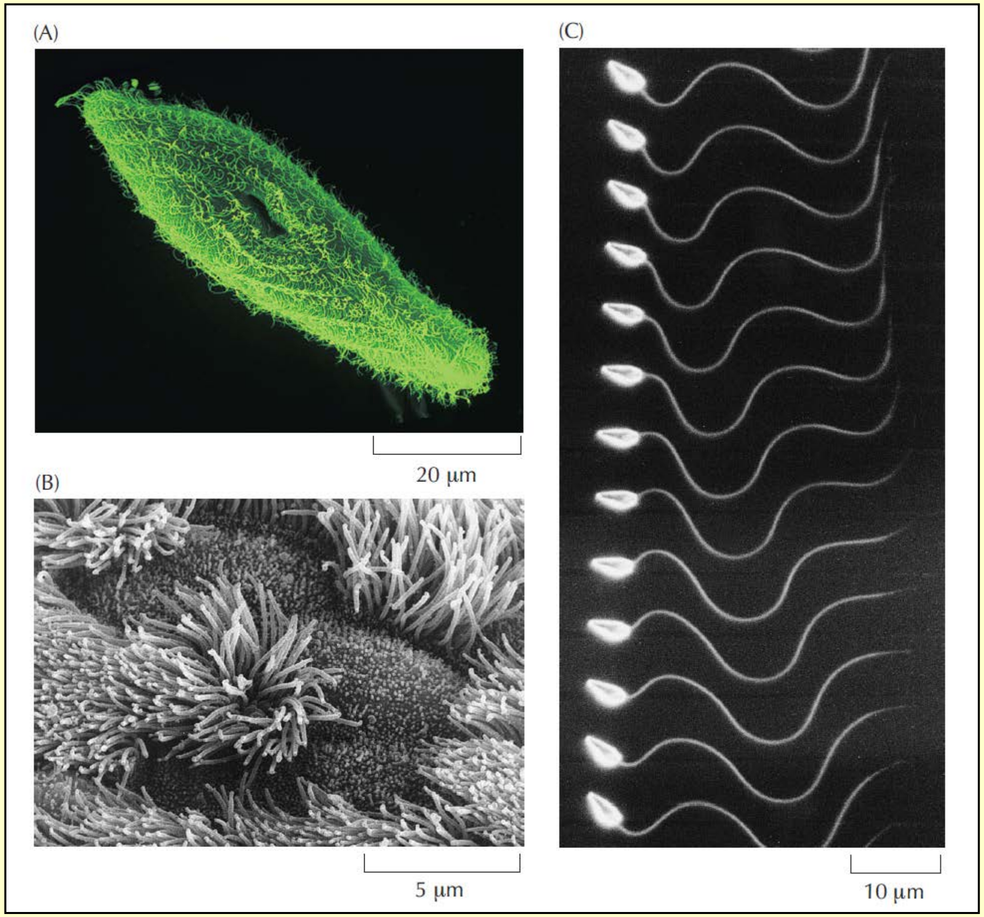


Figure 11-53. 2004. Cooper and Hausman

(A) SEM photo of cillia covering *Paramecium*

(B) SEM photo of cillia of epithelium cells covering trachea surface

(C) Wave-like motions of sperm flagellum

Axoneme

✓ core structure of cillia and flagella

✓ microtubules and associated proteins

✓ microtubules arranged in a distinctive and regular pattern **(9+2)**

✓**9** doublet microtubules arranged in a ring around a **pair of single** microtubules

✓ microtubules of the doublet are different:

• A-tubule
→ complete microtubule of 13 protofilaments

• B-tubule
→ incomplete; 10 or 11 protofilaments
→ fused with A-tubule

✓ movement of cillia and flagella is produced by bending of axoneme

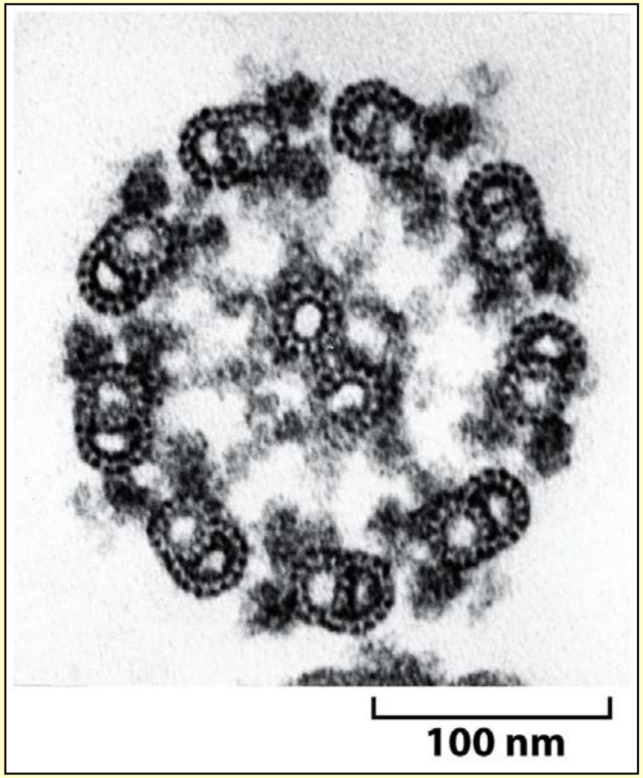
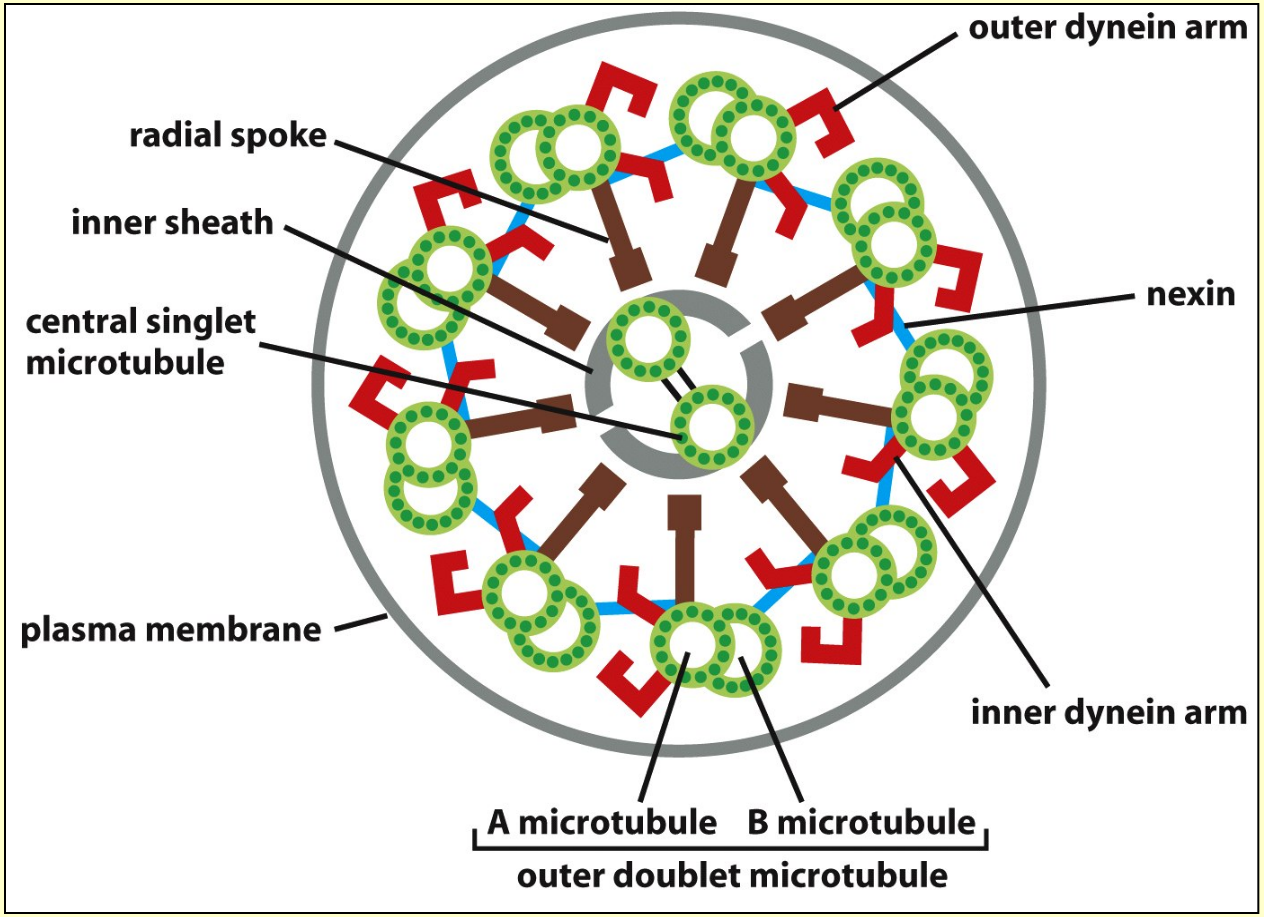


Figure 16-81a *Molecular Biology of the Cell* (© Garland Science 2008)

The arrangement of microtubules and accessory proteins in axoneme



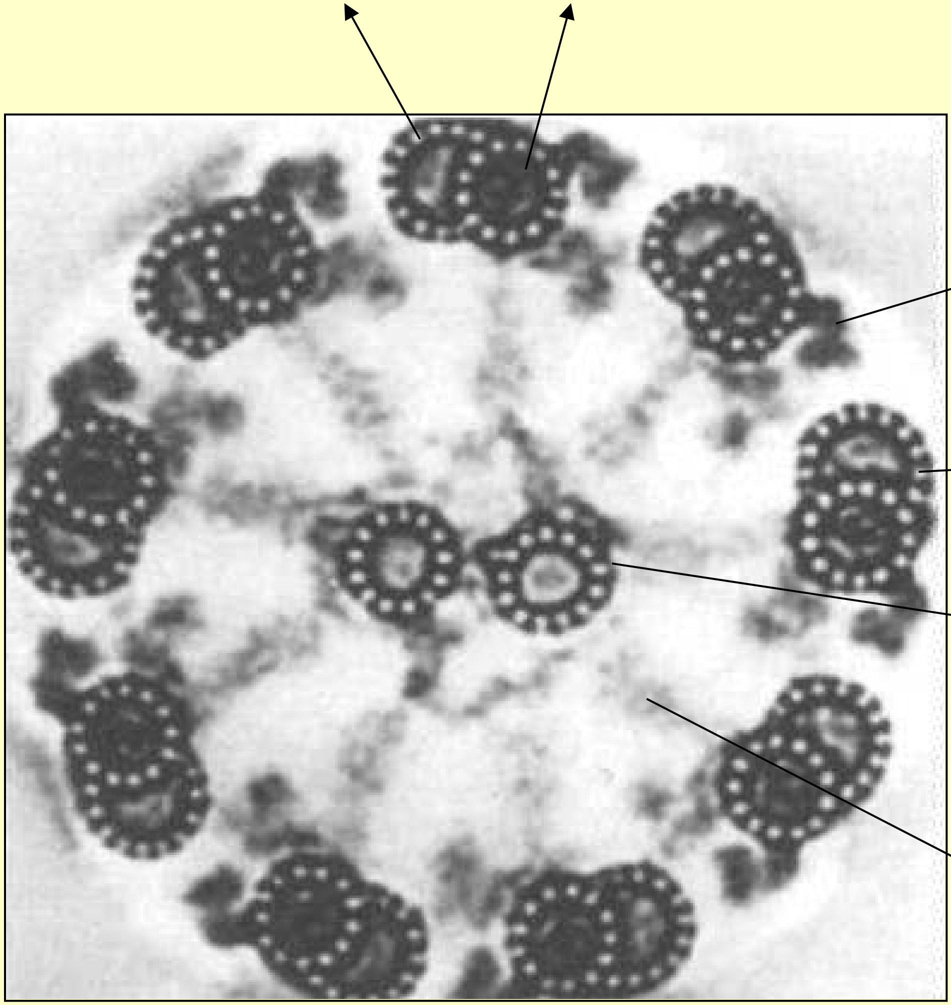
microtubules – 9+2

Accessory proteins:

dynein – form bridges between neighboring doublets

nexin – prevents the sliding of doublets and converts dynein force into a bending motion

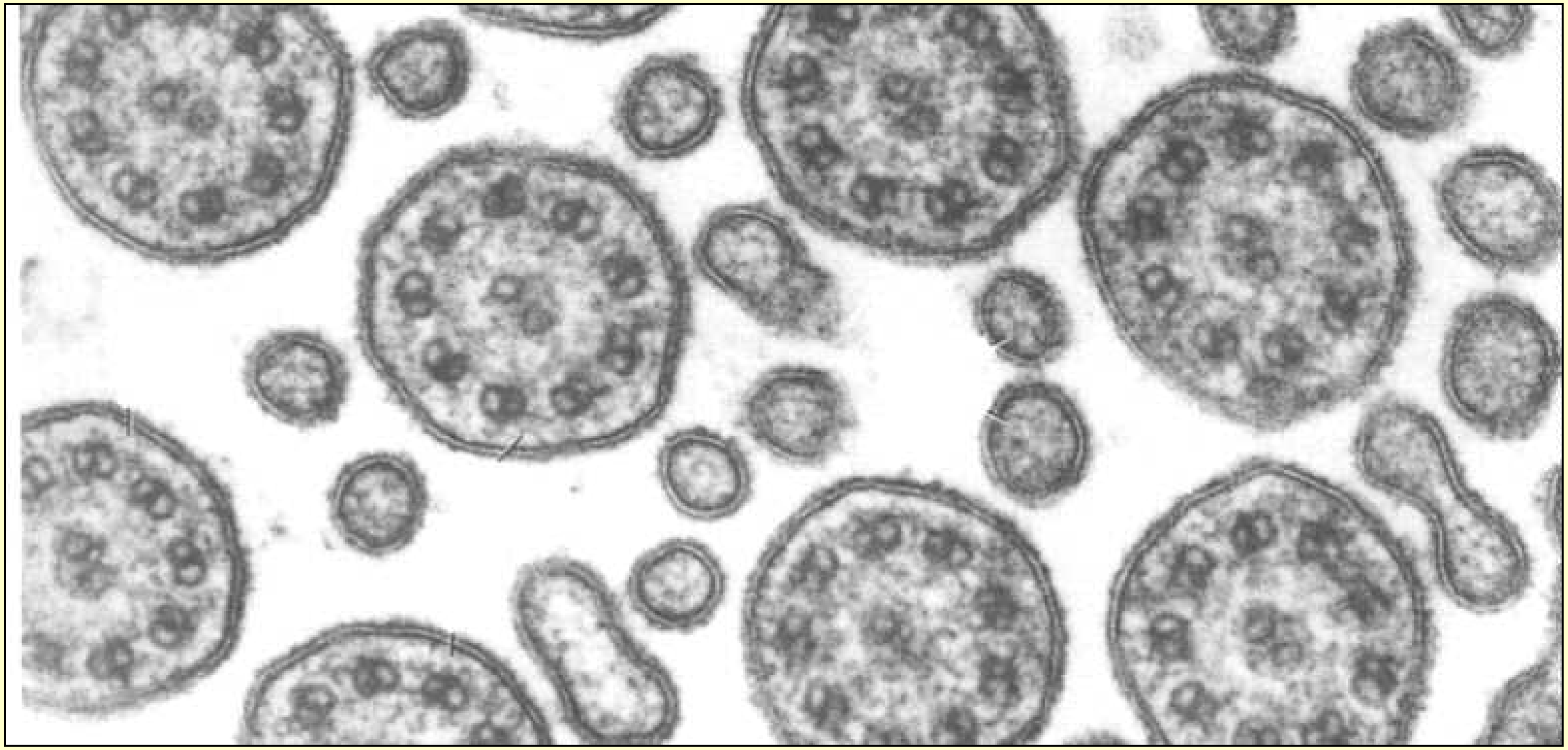
Figure 16-81b *Molecular Biology of the Cell* (© Garland Science 2008)



Microtubule arrangement: 9 + 2

2004. Cooper and Hausman

Cross section of cillia



2004. Cooper and Hausman

The bending of an axoneme

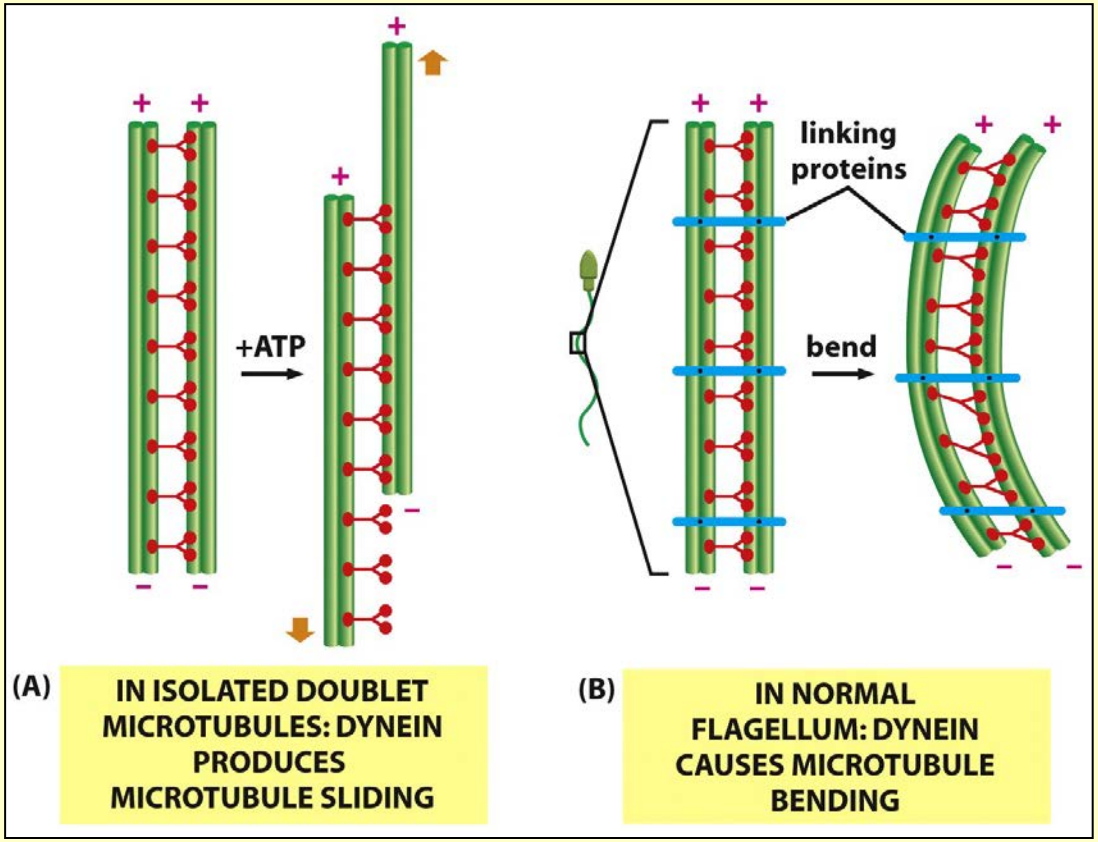


Figure 16-83 *Molecular Biology of the Cell* (© Garland Science 2008)

(A) – **without nexin**
→ sliding of microtubules

(B) – **intact axoneme**
→ nexin prevents sliding of doublets
→ motor action causes a bending motion creating waves or beating motions

Animation - http://www.youtube.com/watch?v=vQ3CdSiVzUk

Basal body

→ microtubule ends embedded in basal body

→ structure similar to centriole

→ **9 triplets of microtubules arranged in a cartwheel**

→ root cilia and flagella at the cell surface

→ each of the outer microtubule doublets in axoneme starts to elongate from two microtubules in basal body triplets

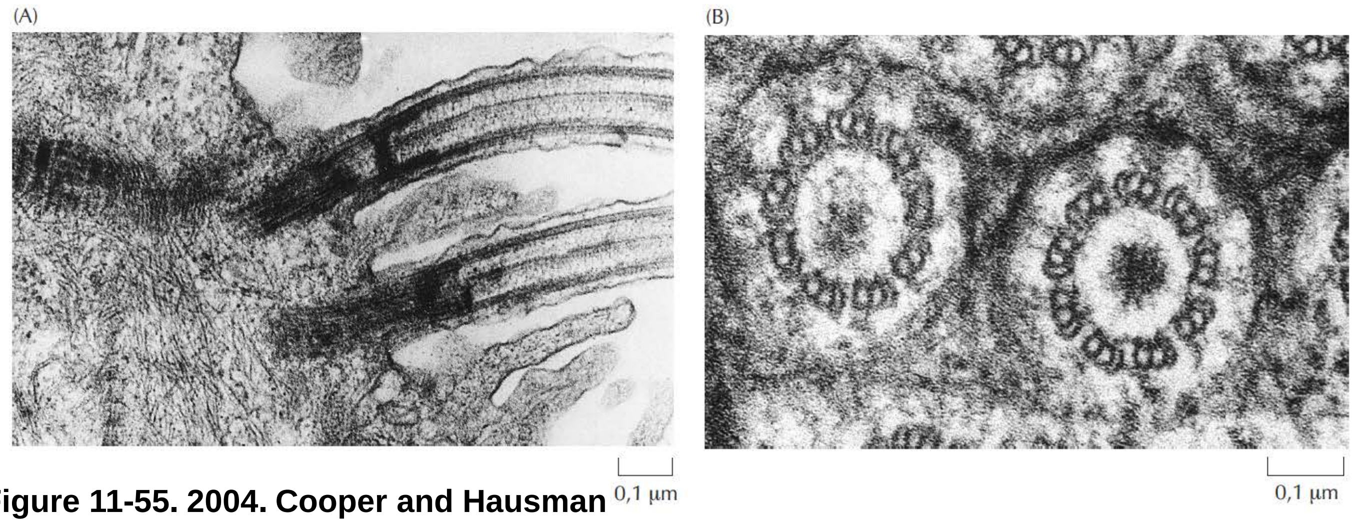
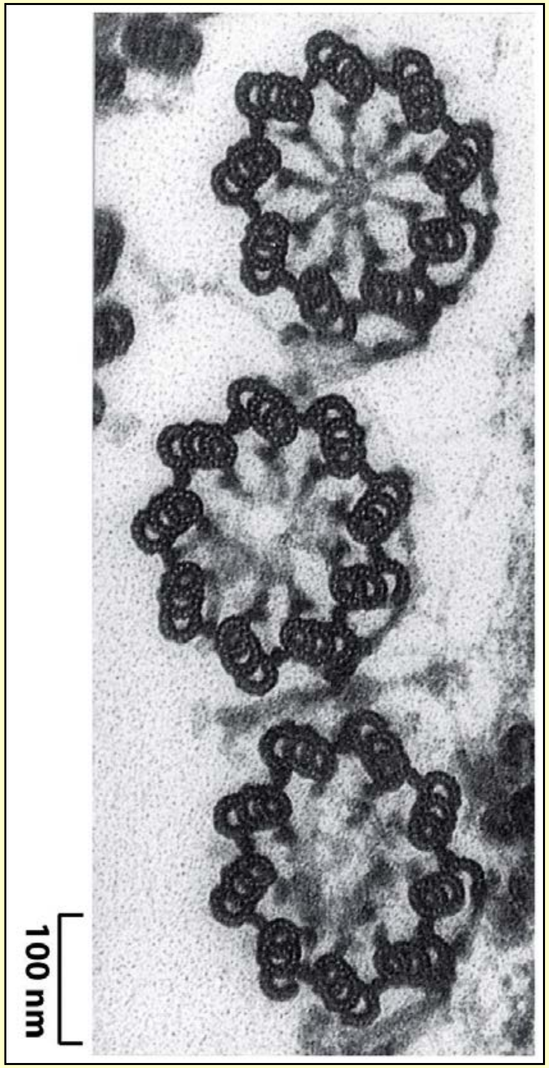


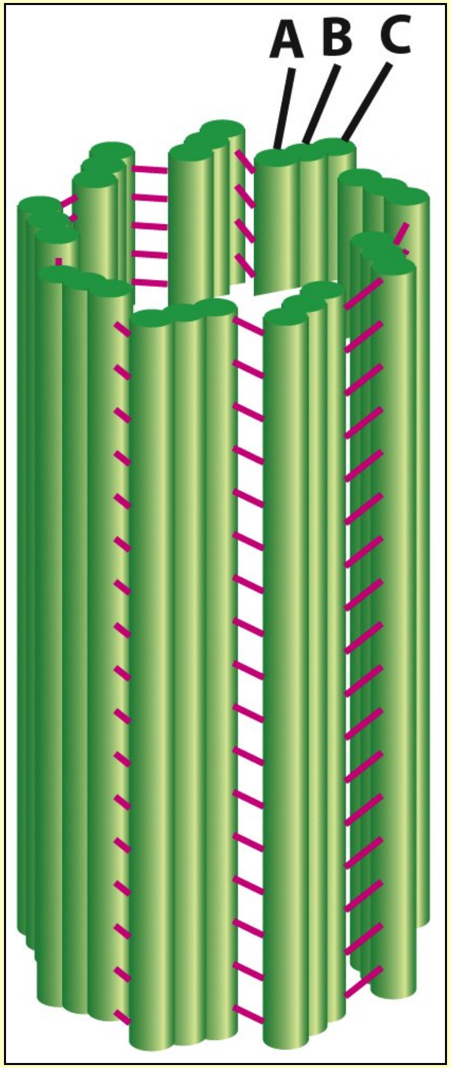
Figure 11-55. 2004. Cooper and Hausman

(**A**) Cilia rooted to basal body

(**B**) Cross section of basal body

Basal bodies





✓ EM of a cross section through 3 basal bodies in the cortex of a protozoan

✓Diagram of a basal body viewed from the side

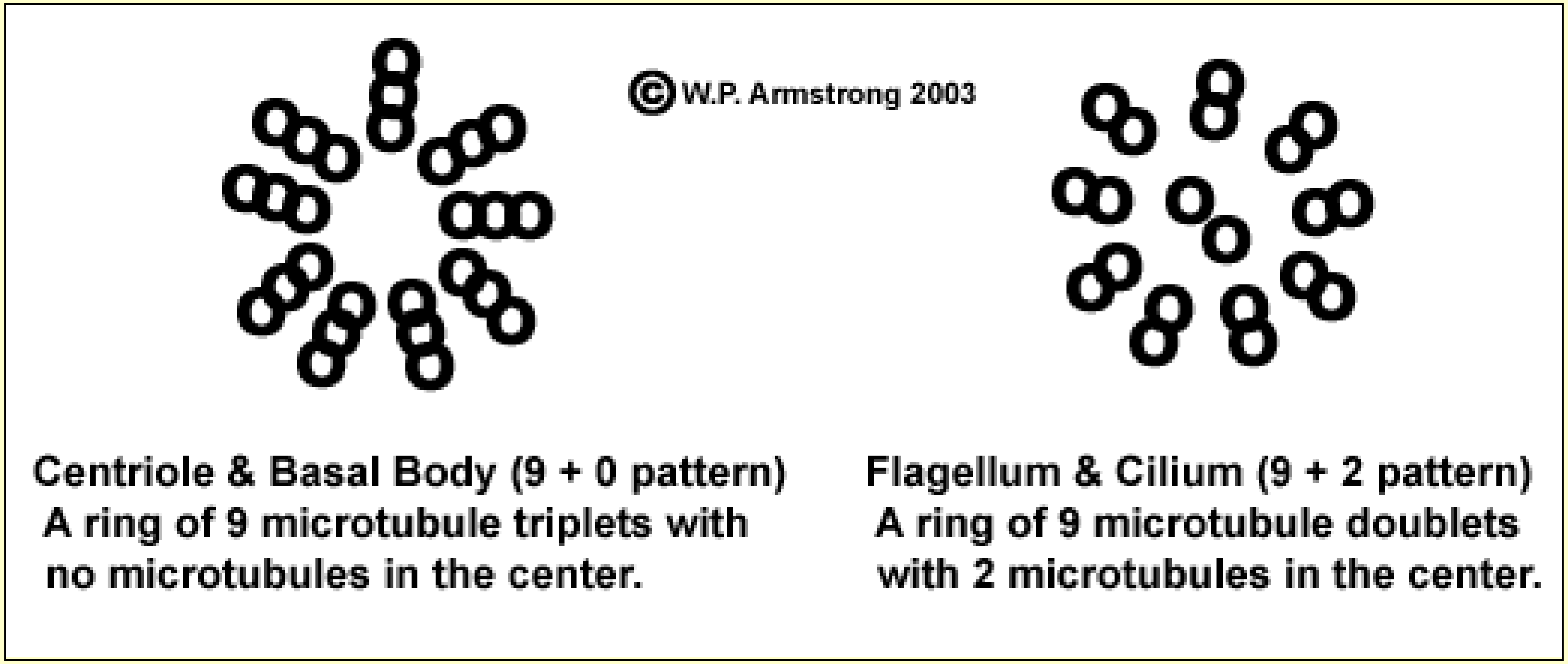
- 9 sets of triplet microtubules

- each triplet contains:
• one complete microtubule – A
• fused to two incomplete microtubules – B and C

➢Link proteins – hold cylindrical microtubule structure together

Figure 16-84 *Molecular Biology of the Cell* (© Garland Science 2008)

Comparison of basal body and axoneme structure



http://www.cellsalive.com/cells/cell_model.htm

http://waynesword.palomar.edu/lmexer1a.htm