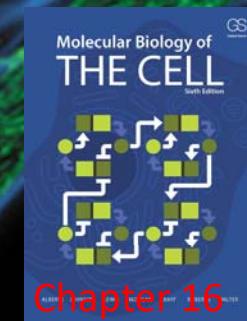


Cytoskeleton and Cell Movement I

-Lecture 13-

Outline

- I. Overview of cytoskeleton
- II. Microfilament and actin structures
- III. Dynamics of actin filaments
- IV. Mechanisms of actin filament assembly
- V. Organization of actin-based cellular structure
- VI. Myosins: actin-based motor proteins
- VII. Myosin-powered movements
- VIII. Cell migration: mechanisms, signaling, and chemotaxis



Relevance of the cytoskeleton for cellular function & viability

The cytoskeleton fulfills essential tasks:

- Determines cell shape and provides structure support
- Play roles in cell migration
- Provides anchor sites for organelles and enzymes to anchor them in specific location in cells
- Allows movement during phagocytosis
- Involved in establishment of cell polarity
- Involved in cell division/cytokinesis, etc.

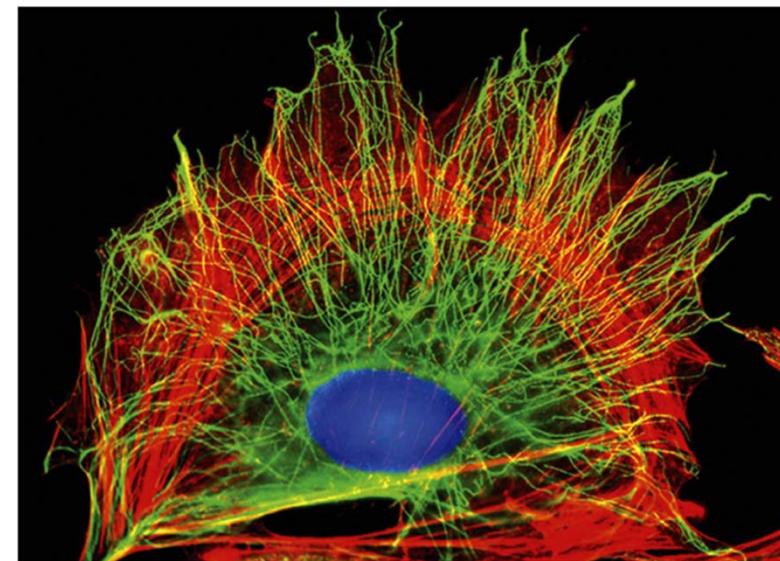
I. Overview of cytoskeleton

- 1). Components of the cytoskeleton
- 2). Functions of the cytoskeleton
- 3). Regulation of cytoskeleton

The cytoskeleton

The cytoskeleton consists of three major components:

- **Actin filaments (Microfilament)**
→ basic unit: **actin**
- **Microtubules**
→ basic unit: **tubulin**
- **Intermediate filaments**
→ basic units:
 - **keratin**
 - **vimentin**
 - **laminin...**



10 μm

Cytoskeleton of epithelial cells

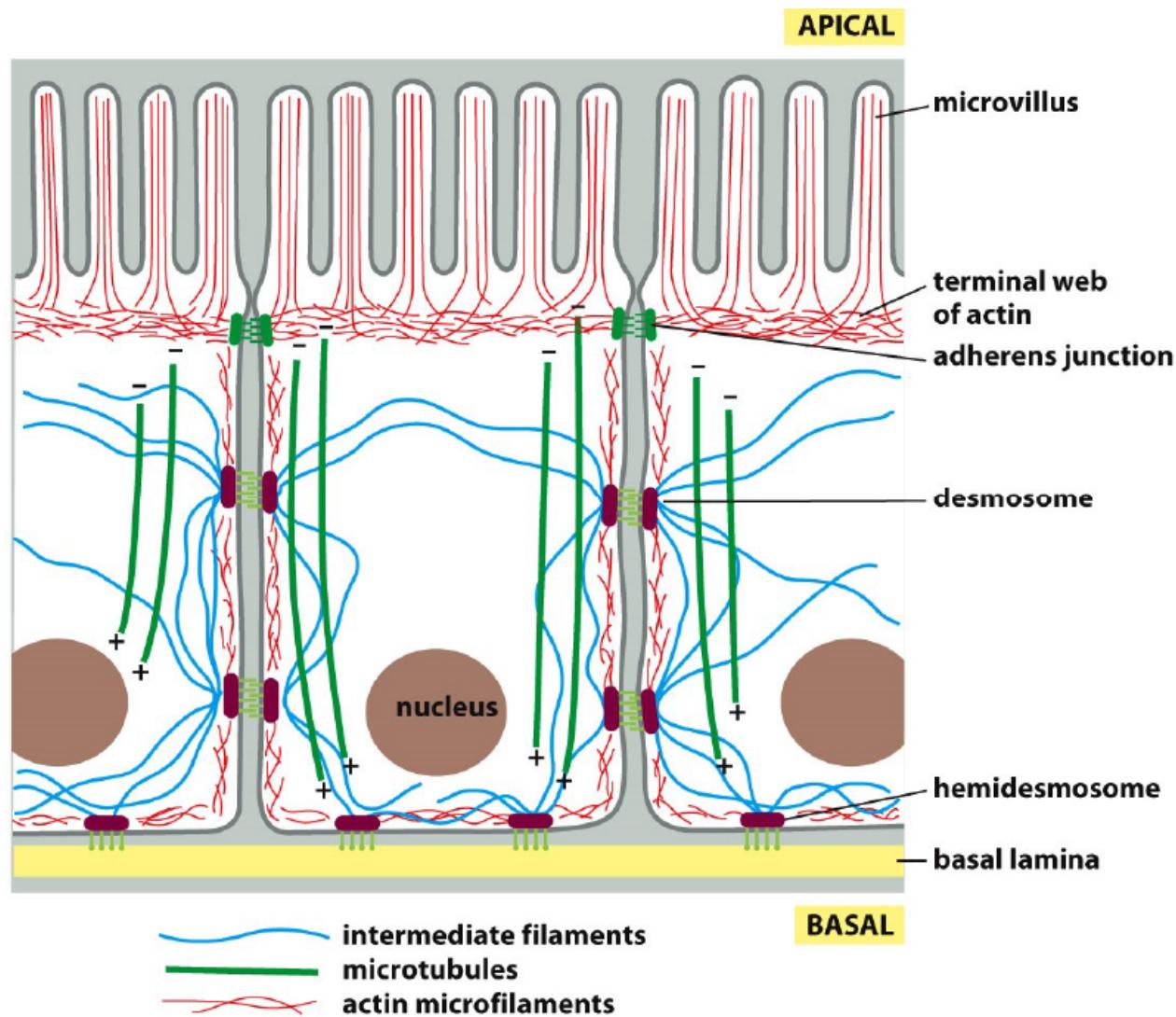
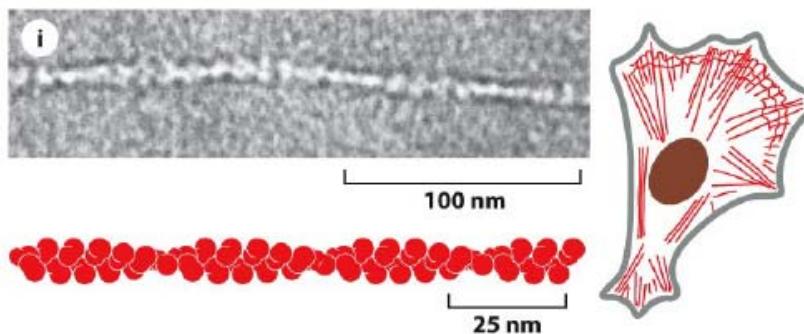


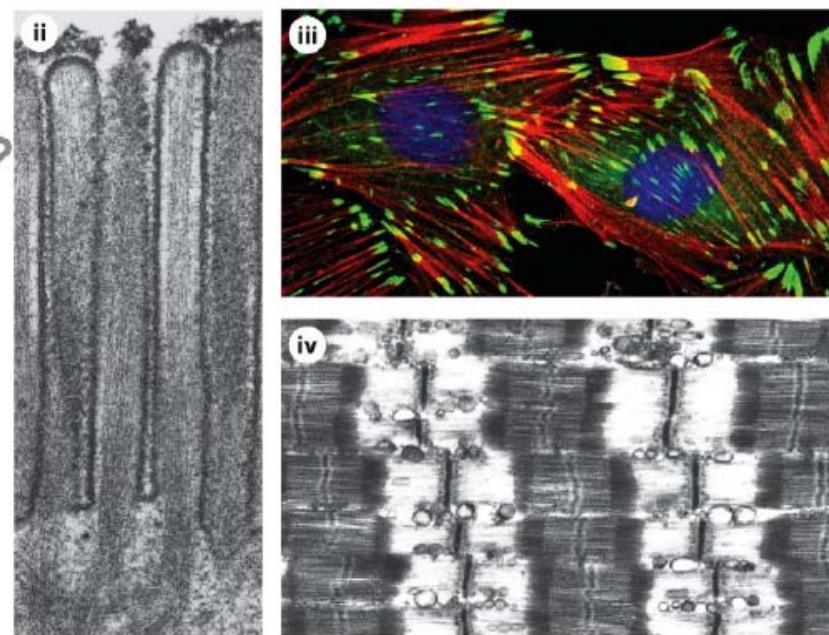
Figure 16-4 Molecular Biology of the Cell 6e (© Garland Science 2015)

Actin filaments (microfilaments)

ACTIN FILAMENTS



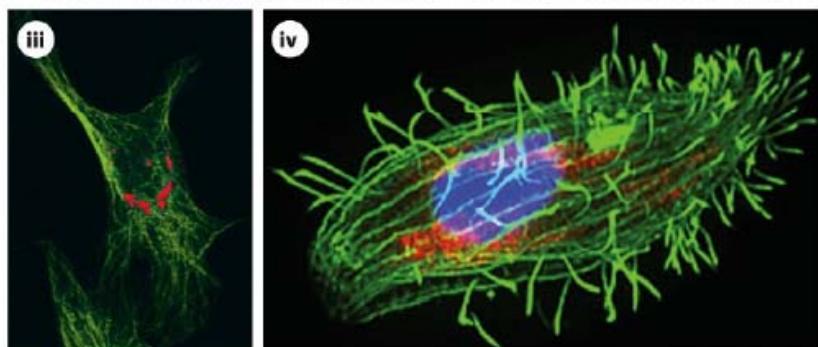
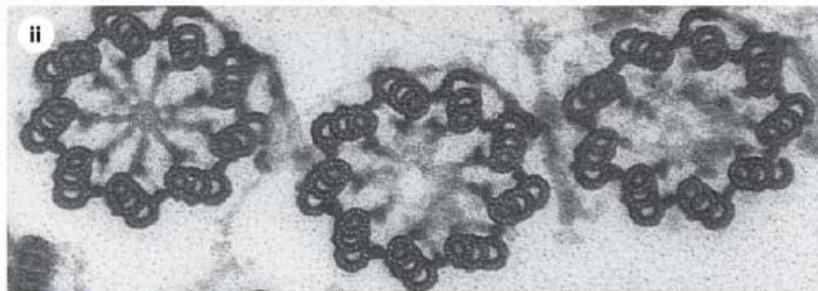
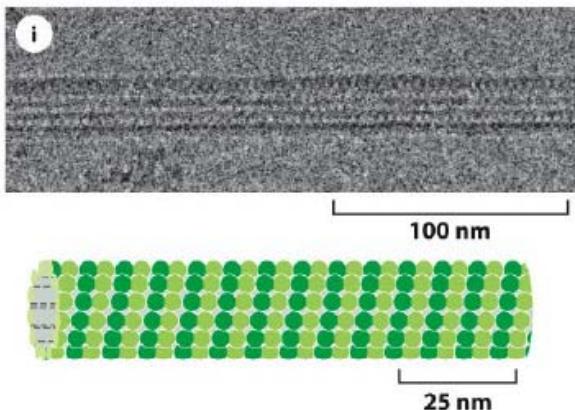
Actin filaments (also known as *microfilaments*) are helical polymers of the protein actin. They are flexible structures with a diameter of 8 nm that organize into a variety of linear bundles, two-dimensional networks, and three-dimensional gels. Although actin filaments are dispersed throughout the cell, they are most highly concentrated in the **cortex**, just beneath the plasma membrane. (i) Single actin filament; (ii) microvilli; (iii) stress fibers (red) terminating in focal adhesions (green); (iv) striated muscle.



Micrographs courtesy of R. Craig (i and iv); P.T. Matsudaira and D.R. Burgess (ii); K. Burridge (iii).

Microtubules

MICROTUBULES

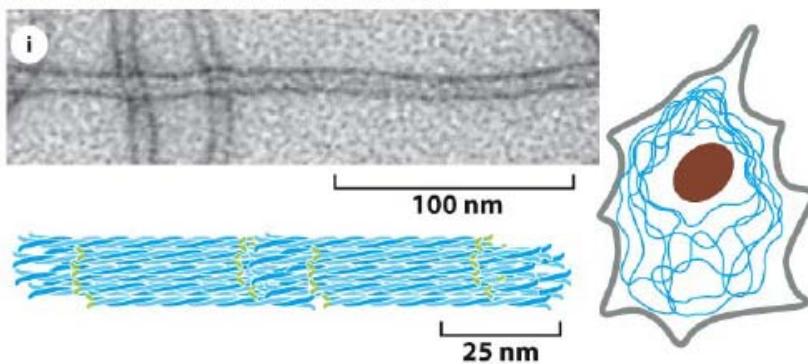


Microtubules are long, hollow cylinders made of the protein tubulin. With an outer diameter of 25 nm, they are much more rigid than actin filaments. Microtubules are long and straight and frequently have one end attached to a microtubule-organizing center (MTOC) called a centrosome. (i) Single microtubule; (ii) cross section at the base of three cilia showing triplet microtubules; (iii) interphase microtubule array (green) and organelles (red); (iv) ciliated protozoan.

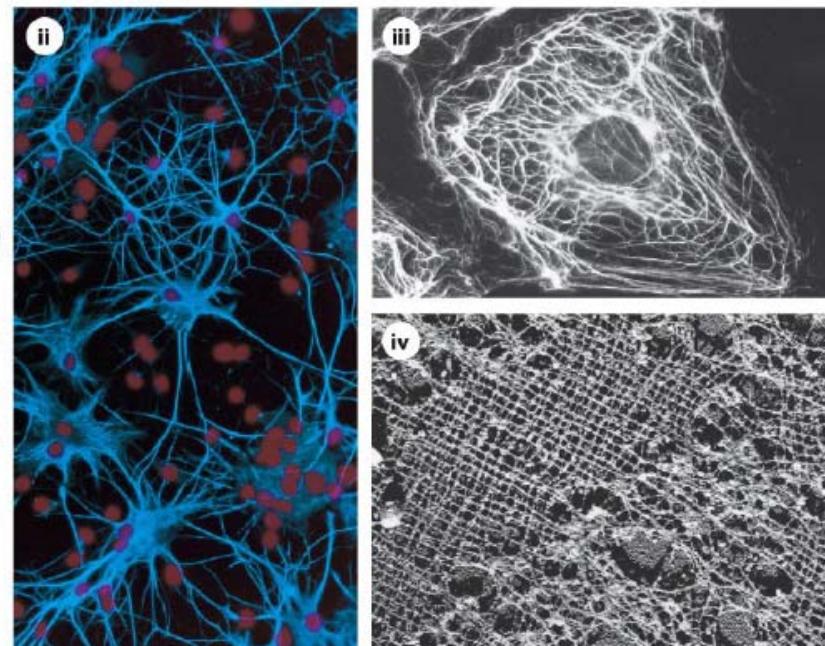
Micrographs courtesy of R. Wade (i); D.T. Woodrow and R.W. Linck (ii); D. Shima (iii); D. Burnette (iv).

Intermediate filaments

INTERMEDIATE FILAMENTS



Intermediate filaments are ropelike fibers with a diameter of about 10 nm; they are made of intermediate filament proteins, which constitute a large and heterogeneous family. One type of intermediate filament forms a meshwork called the **nuclear lamina** just beneath the inner nuclear membrane. Other types extend across the cytoplasm, giving cells mechanical strength. In an epithelial tissue, they span the cytoplasm from one cell–cell junction to another, thereby strengthening the entire epithelium. (i) Individual intermediate filaments; (ii) Intermediate filaments (blue) in neurons and (iii) epithelial cell; (iv) nuclear lamina.

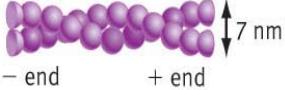
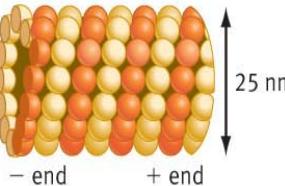


Micrographs courtesy of R. Quinlan (i); N. L. Kedersha (ii); M. Osborn (iii); U. Aebi (iv).

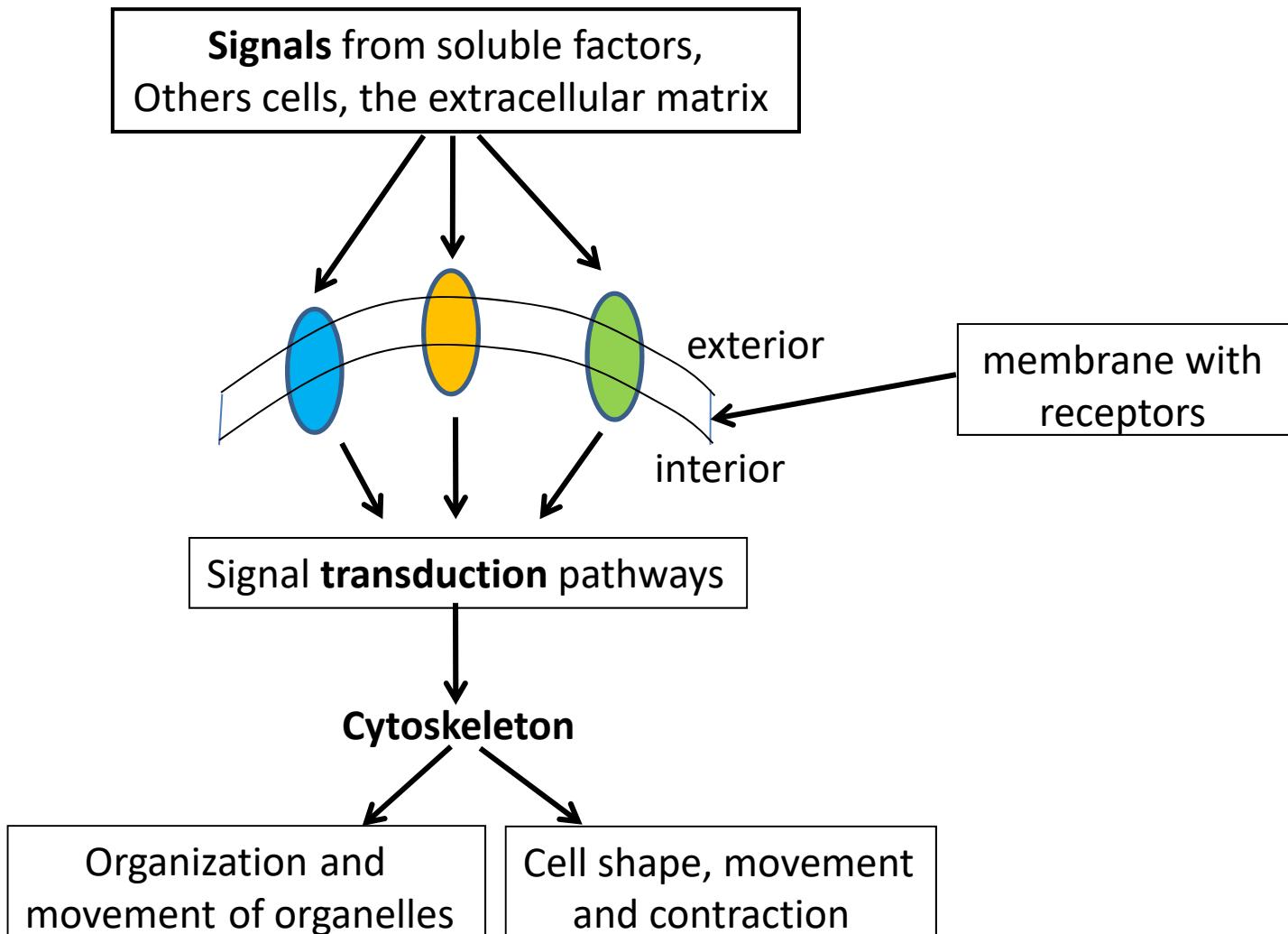
Components, structure & function of the cytoskeleton

SUMMARY TABLE 7.3 **Cytoskeletal Filaments**

The three types of filaments found in the cytoskeleton are distinguished by their size and structure, and the protein subunit of which they are made.

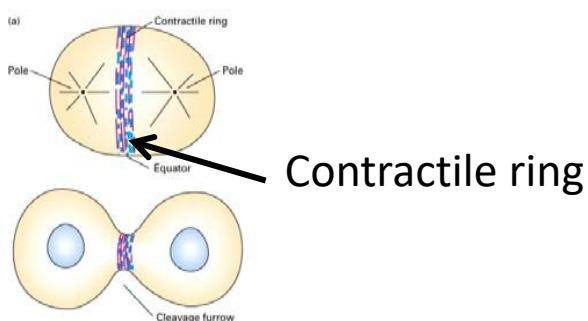
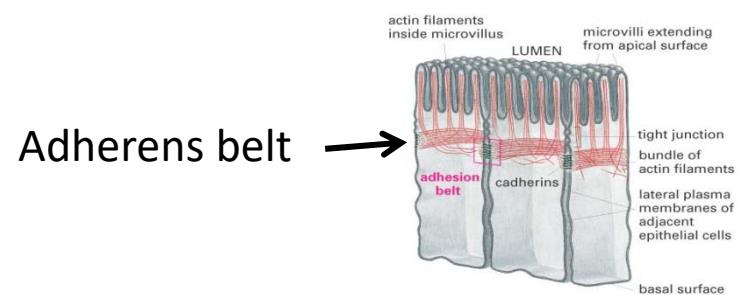
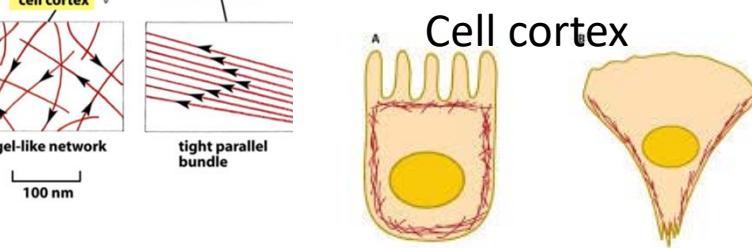
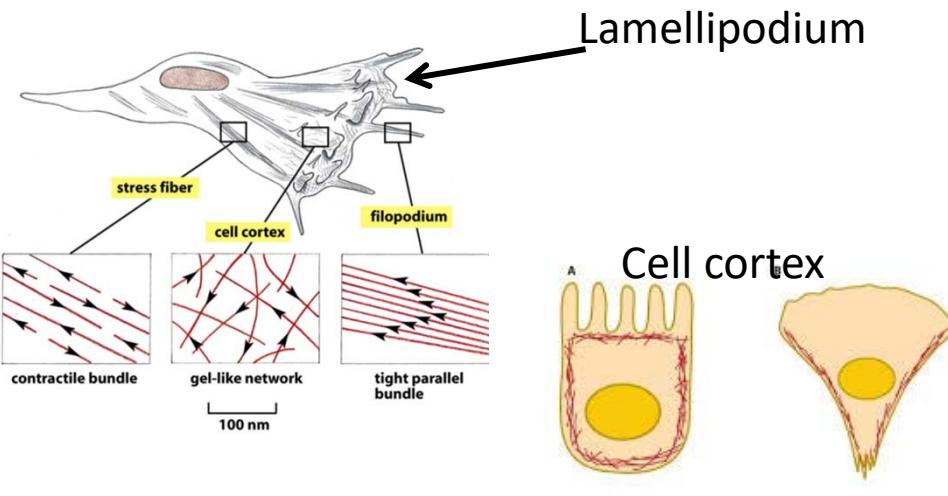
	Structure	Subunits	Functions	
Actin filaments (microfilaments)	Strands in double helix 	Actin 	<ul style="list-style-type: none">• maintain cell shape by resisting tension (pull)• move cells via muscle contraction or cell crawling• divide animal cells in two• move organelles and cytoplasm in plants, fungi, and animals	Semiflexible Motors polarized
Intermediate filaments	Fibers wound into thicker cables 	Keratin or vimentin or lamin or others 	<ul style="list-style-type: none">• maintain cell shape by resisting tension (pull)• anchor nucleus and some other organelles	Flexible No motor unpolarized
Microtubules	Hollow tube 	α - and β -tubulin dimers 	<ul style="list-style-type: none">• maintain cell shape by resisting compression (push)• move cells via flagella or cilia• move chromosomes during cell division• assist formation of cell plate during plant cell division• move organelles• provide tracks for intracellular transport	Stiff rods Motors polarized

Regulation of cytoskeleton function by cell signaling in time and space



II. Actin microfilaments and structures

- **Microvilli**
(bundled filaments)
- **Cell cortex**
- **Adherens belt**
(Adherens junctions)
- **Filopodia**
(bundled actin filaments)
- **Lamellipodium/leading edge**
- **Stress fibers**
- **Phagocytosis**
- **Moving endocytic vesicles**
- **Contractile ring**

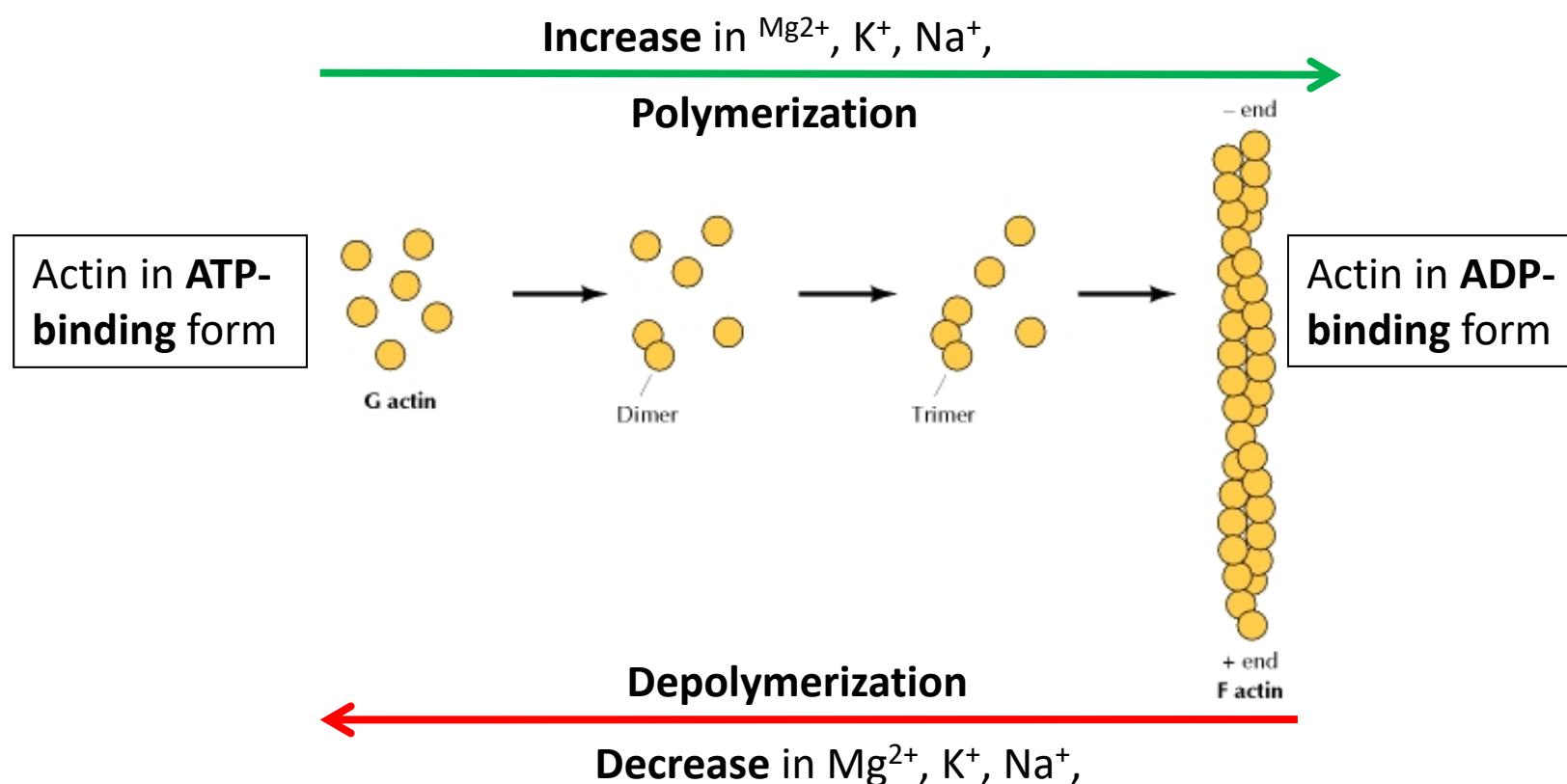


Actin

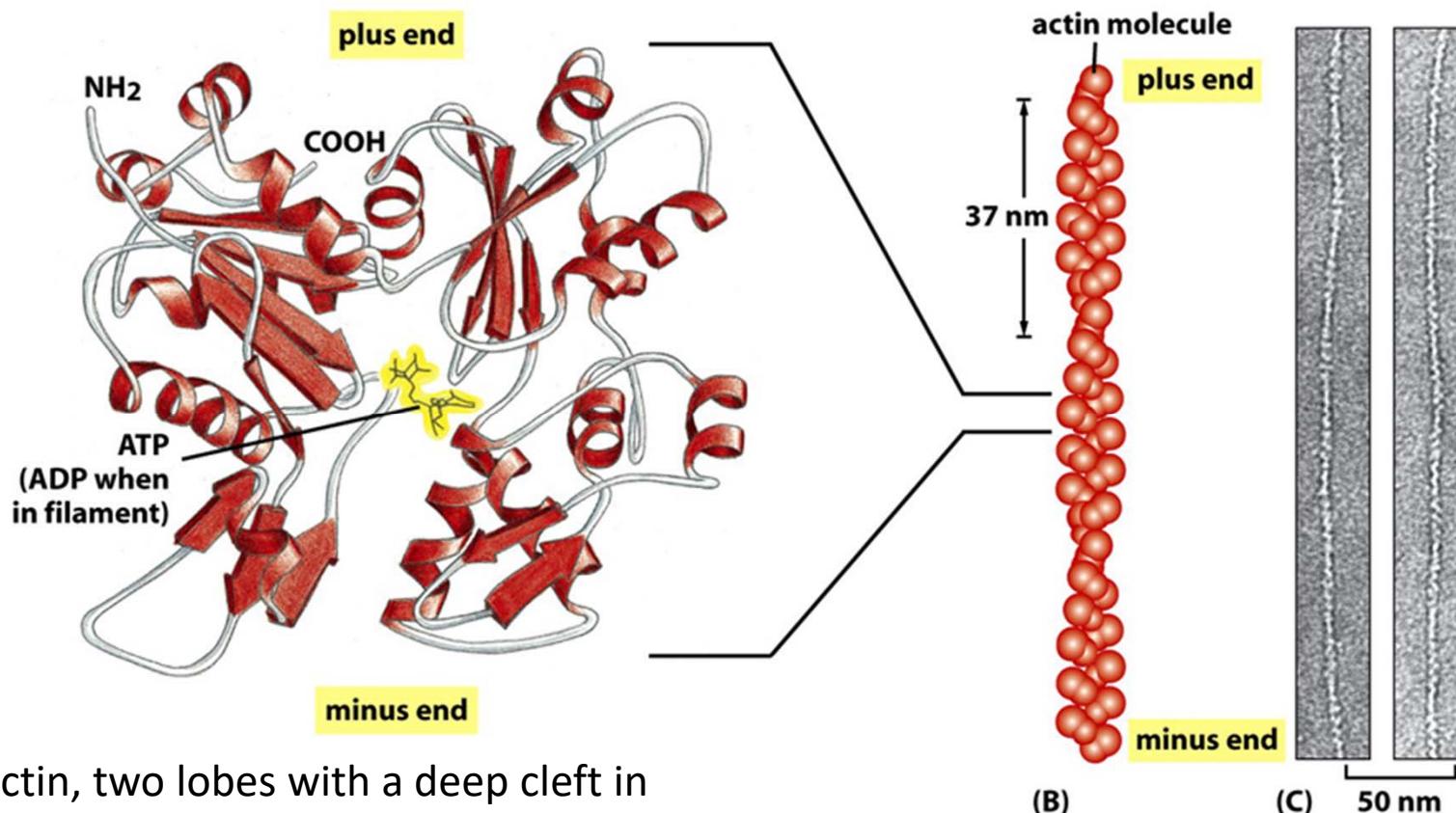
- Highly **conserved** across species,
80% homology between Amoebas and animals
- Most **abundant** protein in cells:
1-5 % cellular protein in non-muscle cells,
(10% in muscle cells)
- Exists in **three isoforms** (α -actin, β -actin, γ -actin)
 - α -actin--- contractile structure
 - β -actin--- leading edge and cell cortex
 - γ -actin---stress fibers

G-actin (globular) and F-actin (filamentous)

- G-actin: globular and monomeric actin
- F-actin: filamentous, and linear chain of G-actin



Structures of monomeric G-actin and F-actin filaments



G-actin, two lobes with a deep cleft in between, binds to ADP/ATP and Mg^{2+}

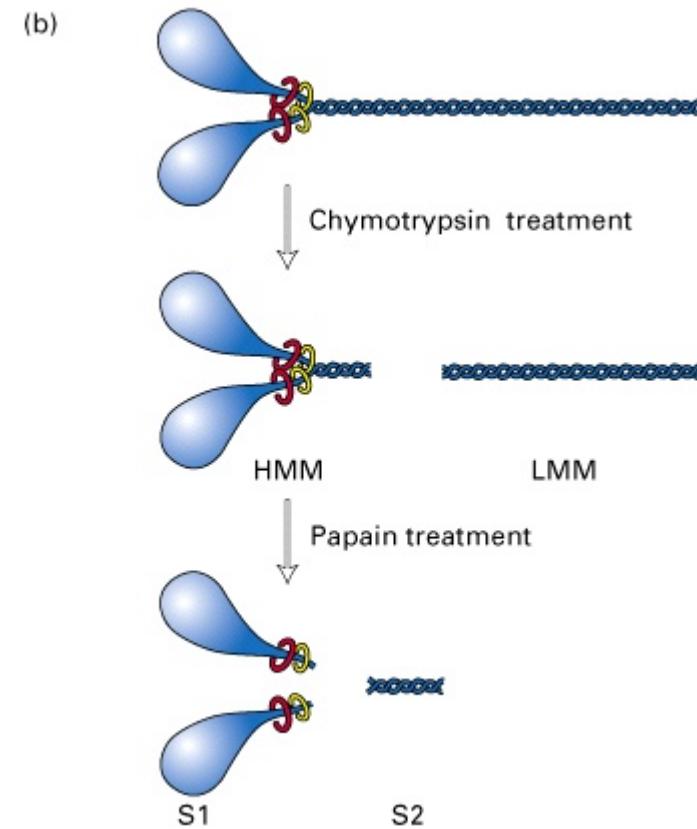
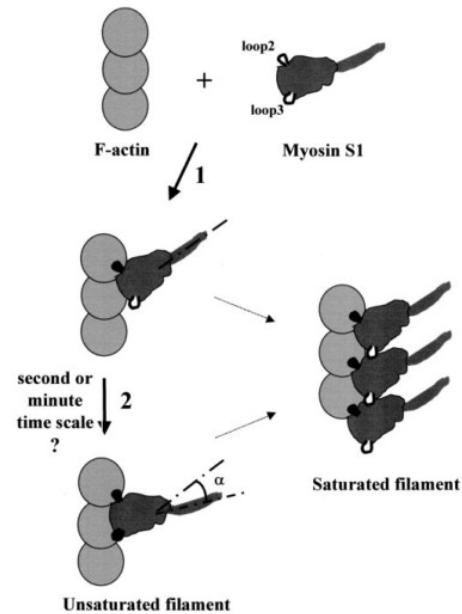
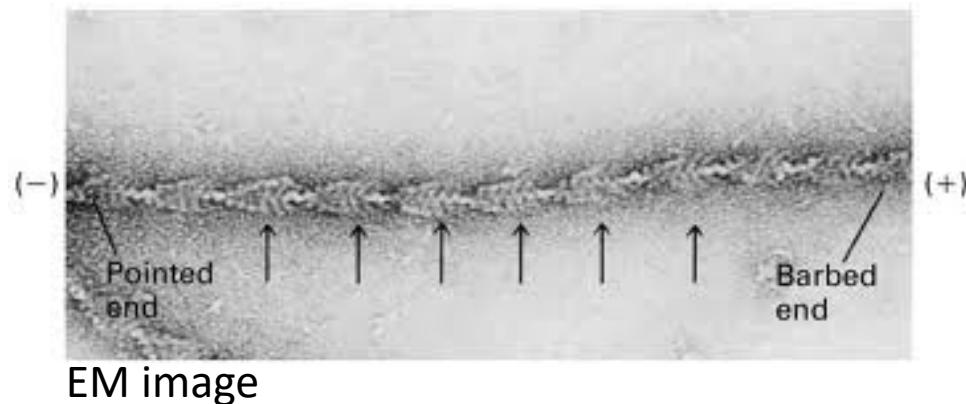
Two protofilaments form one filament
(held together by lateral contacts)

F-actin has structural and functional polarity

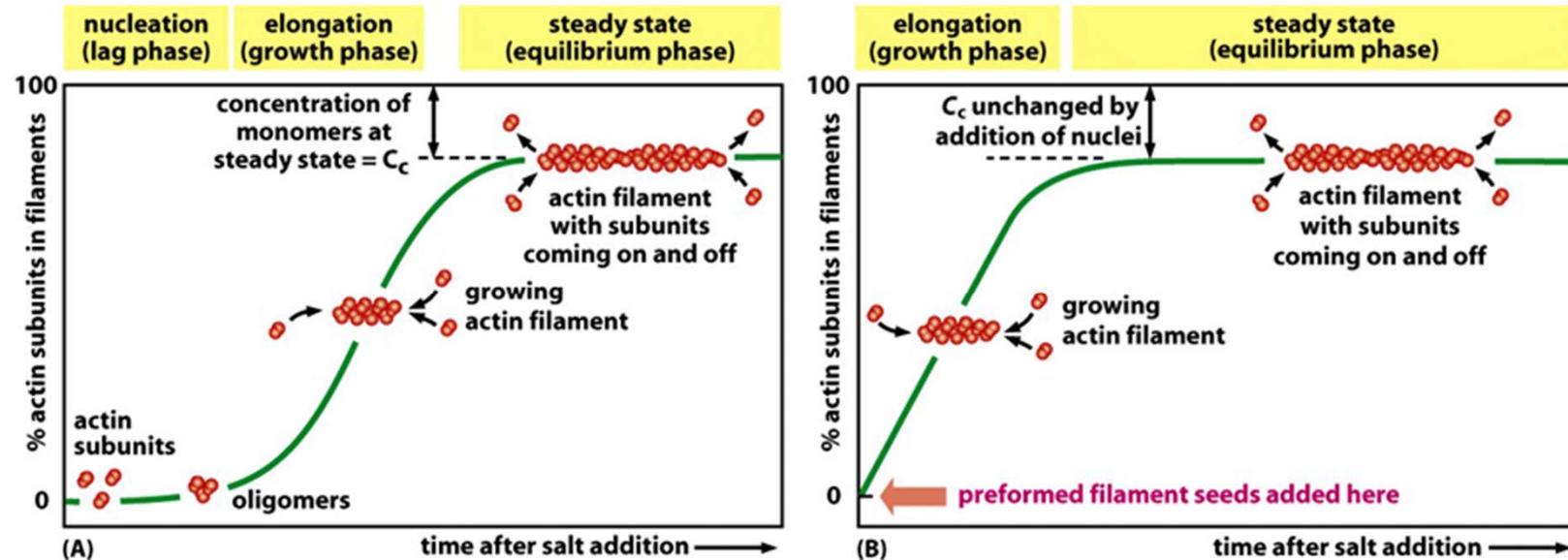
- All actin subunits are oriented the same way
- “+” end: end that is favored for **addition** of actin subunits; **ATP-binding cleft** of the terminal actin subunits **contacts the neighboring subunits**
- “-” end: end that is favored for **subunit dissociation**; **ATP-binding cleft** of the terminal actin subunits is **exposed to the solution**.

How to demonstrate the polarity of an actin filament?

Myosin S1 decoration experiment



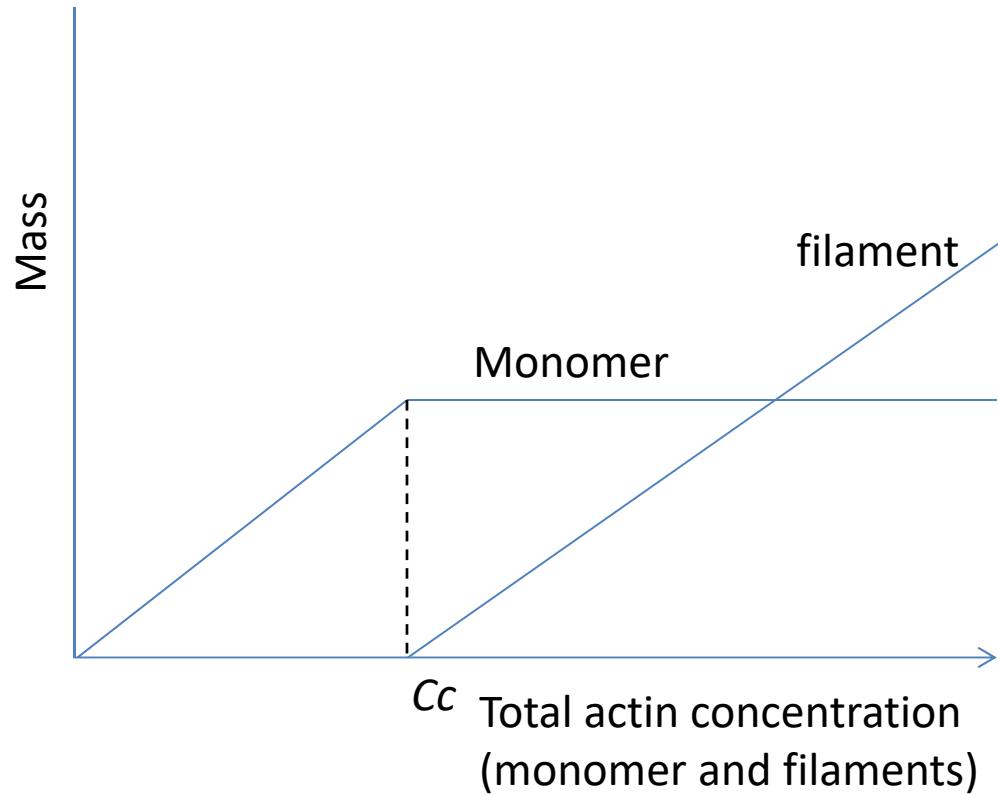
II. Dynamics of actin filaments



Three stages:

1. Nucleation--- formation of 3 subunits as seeds for polymerization, **the rating-limiting step**
2. Elongation--- rapid polymerization from the nucleated seeds
3. Steady-state---addition and removal are balanced, no net increase.

Critical actin concentrations (C_c) for the polymerization of filaments



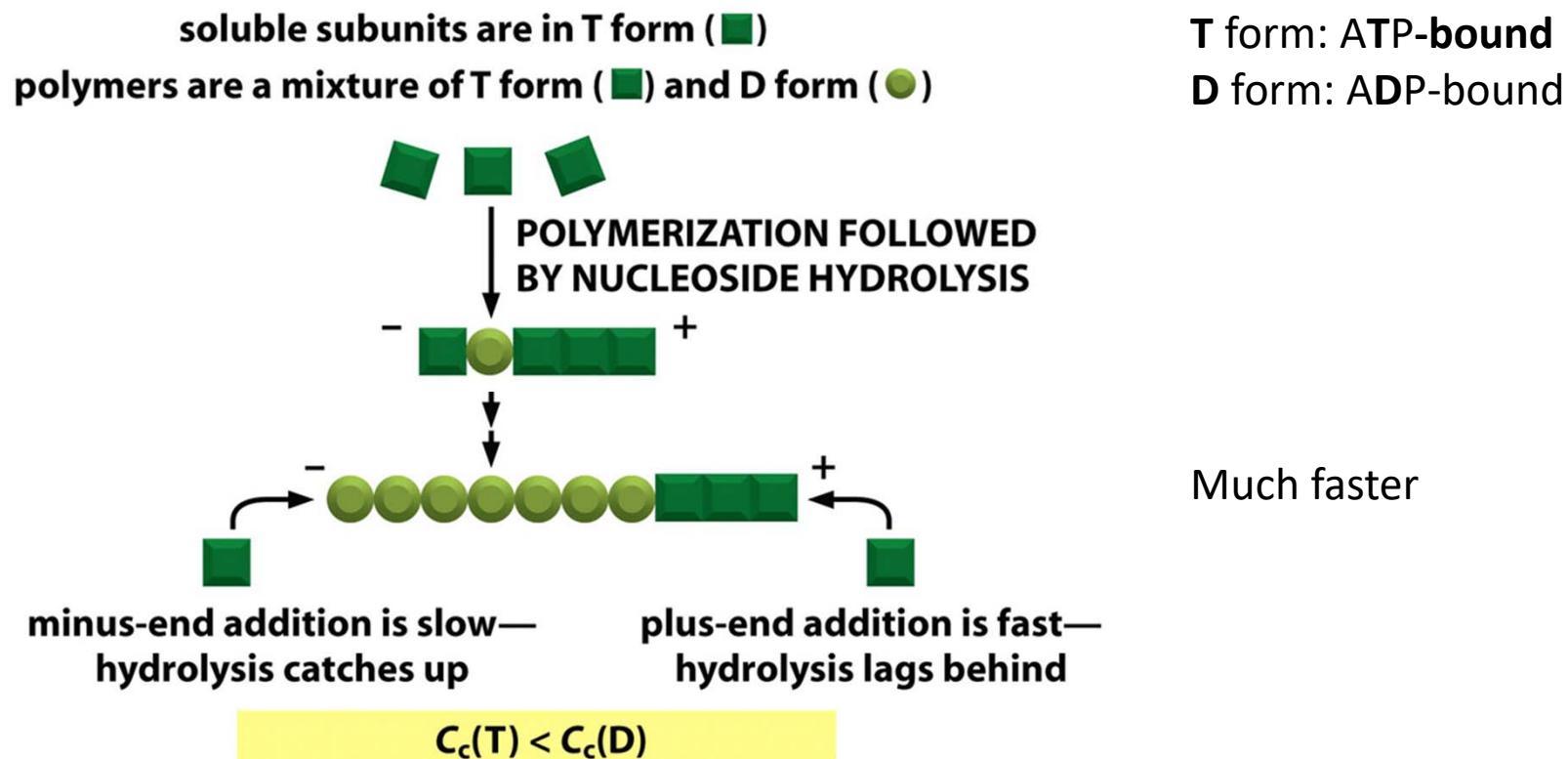
In cells, G-actin levels can be 0.1-0.4 mM, C_c is $\sim 0.2\mu\text{M}$

Definition of C_c :

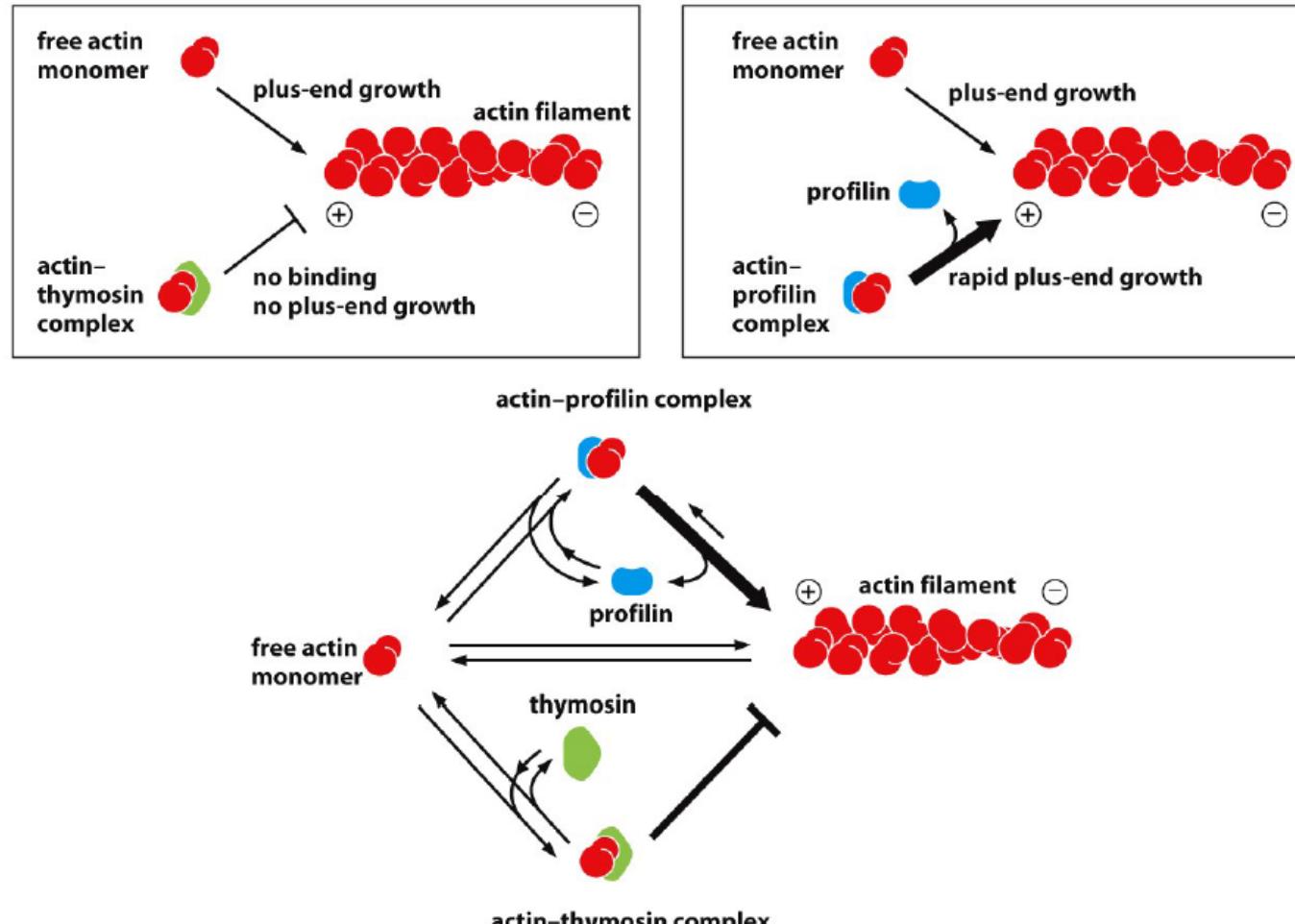
Concentration of free G-actin at which the assembly onto a filament end is balanced by loss from that end.

Actin treadmilling

- The addition of ATP-G-actin at the “+” end with simultaneous removal of G-actin at the “-” end of F-actin, resulting in a section of filament seemingly "moving" across a stratum or the cytosol



Actin monomer availability controls actin filament assembly



PROFILIN COMPETES WITH THYMOGIN FOR BINDING TO ACTIN MONOMERS AND PROMOTES ASSEMBLY

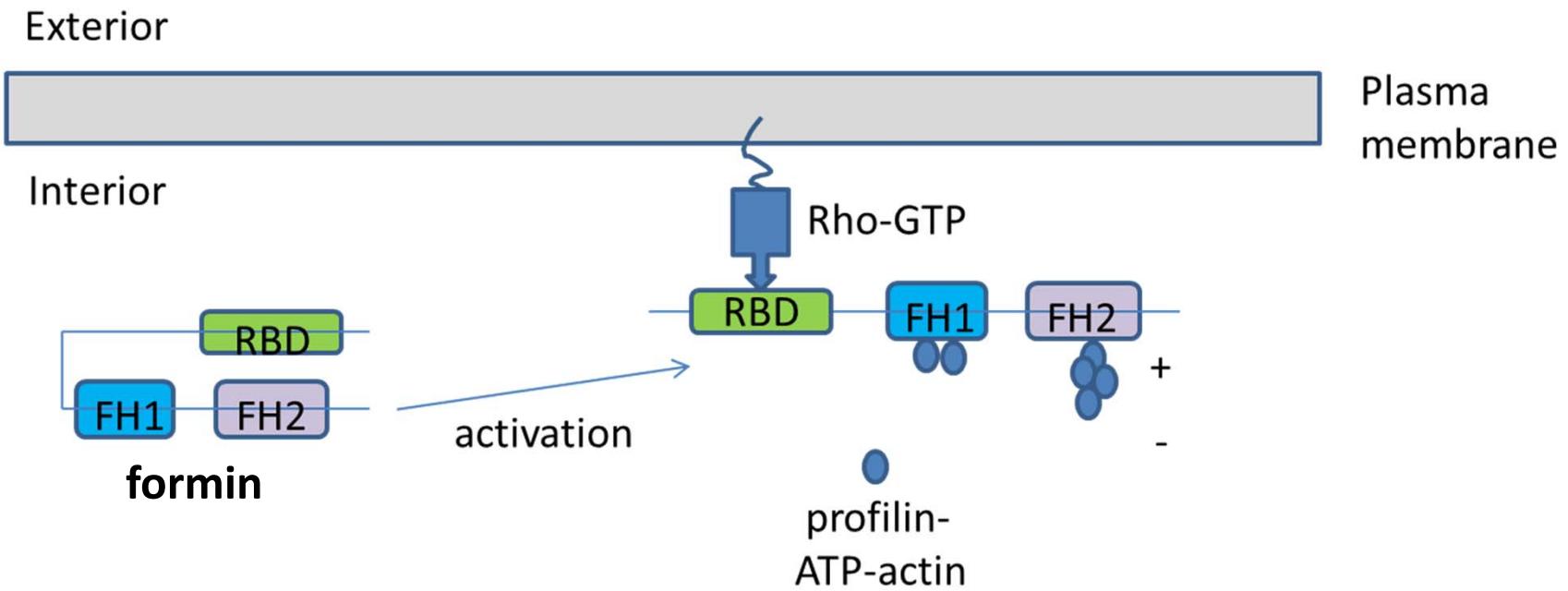
Figure 16-15 Molecular Biology of the Cell 6e (© Garland Science 2015)

IV. Mechanisms of actin filament assembly

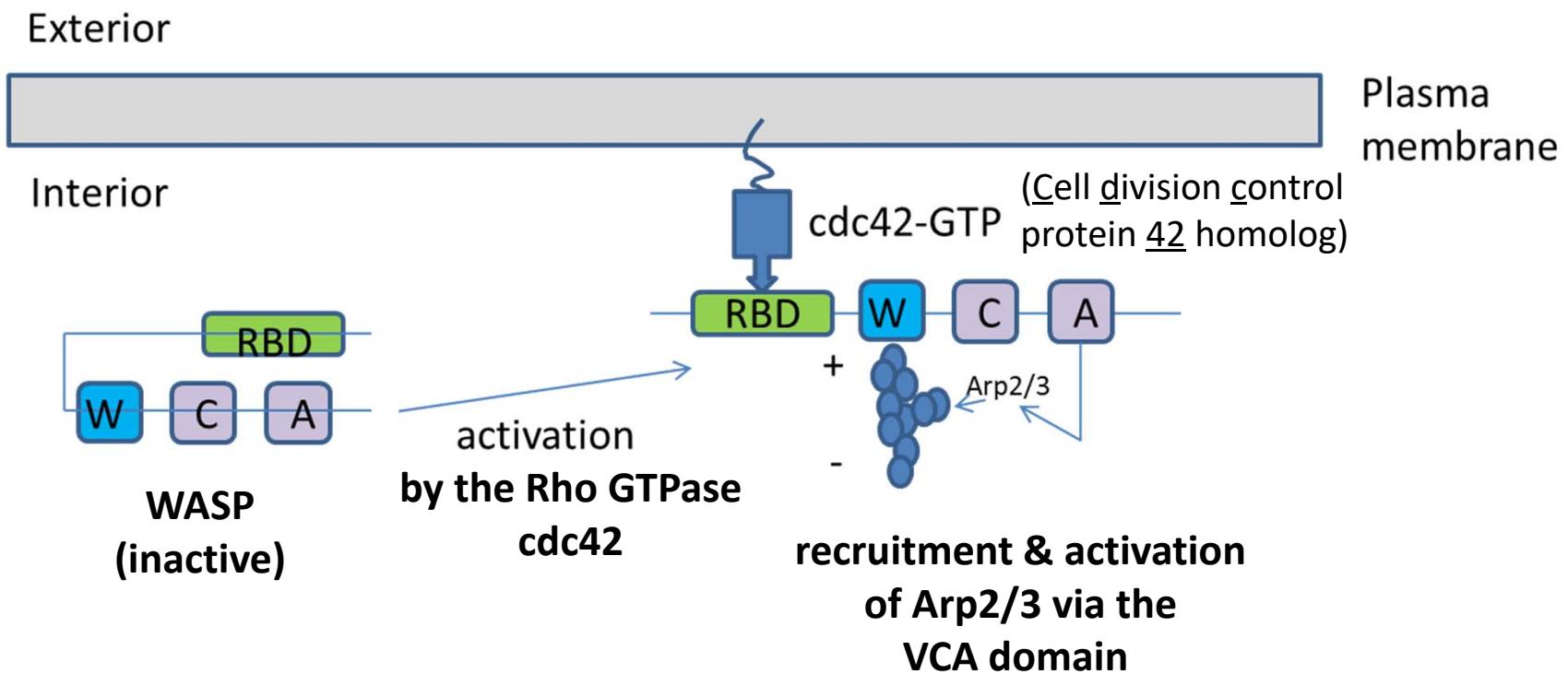
“nucleation” is the rating limiting step,
what is controlling this critical step?

- Two major classes of **actin nucleating proteins** accelerate polymerization and generate branched or straight filaments:
 - **Formin protein family**: long filament assembly
 - **Arp (“actin-related protein”) 2/3 complex**: branched filament assembly

Regulation of formins by Rho-GTPases



Regulation of the Arp2/3 complex by the nucleation promoting factor (NPF) WASP (Wiskott–Aldrich Syndrome protein)



Formin mediates straight filament assembly

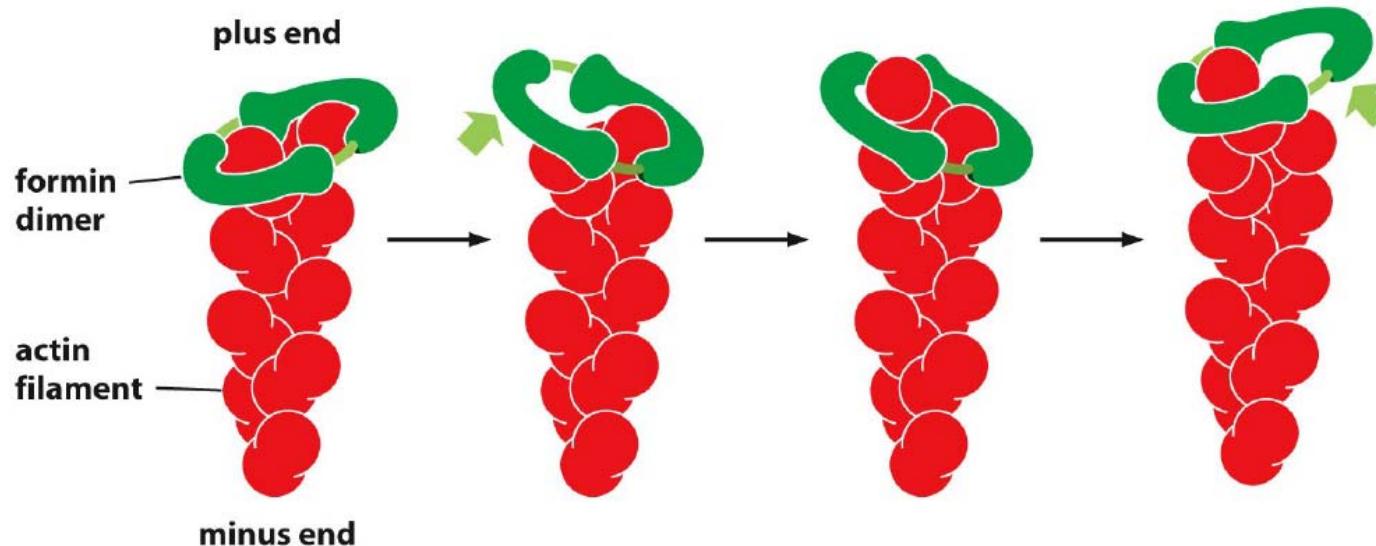
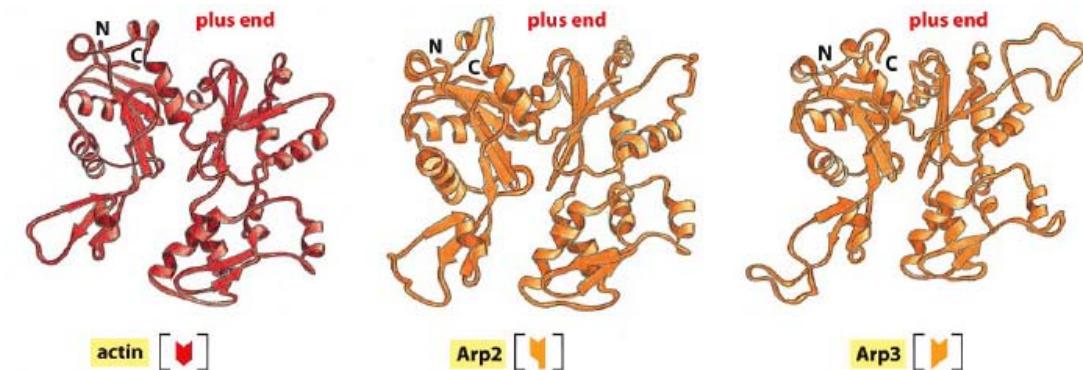


Figure 16-17 Molecular Biology of the Cell 6e (© Garland Science 2015)

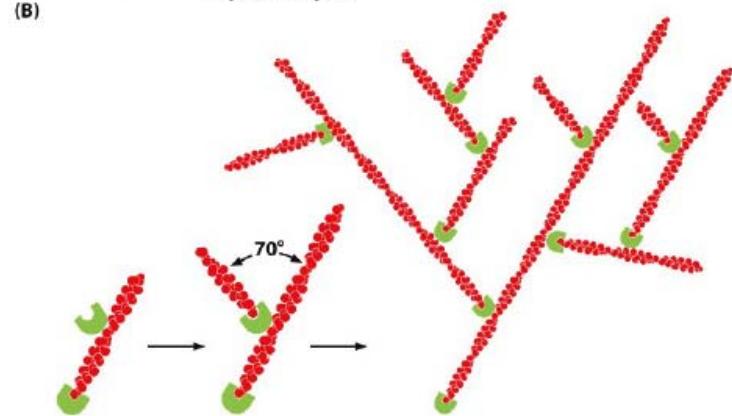
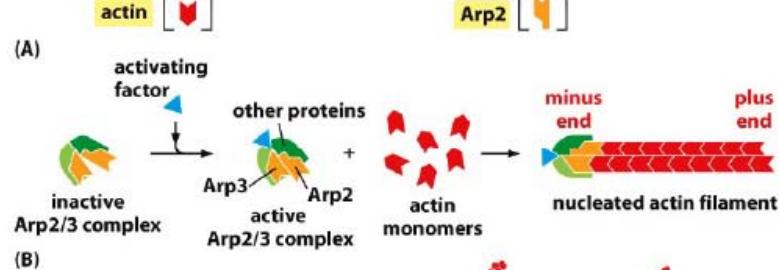
- Formins are **dimeric proteins** that **nucleate the growth of straight, unbranched filaments** that can be cross-linked by other proteins to form parallel bundles.
- Each formin subunit has a binding site for **monomeric actin**, and the **formin dimer** appears to nucleate actin filament polymerization by capturing **two monomers**.
- The newly nucleated filament grows and the formin dimer **remains associated** with the growing plus end while **still allowing the addition of new subunits at that end**

Arp2/3 mediates branched filament assembly

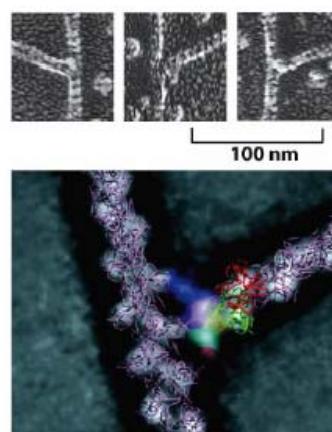
Comparison of structures: actin versus Arp2 and Arp3



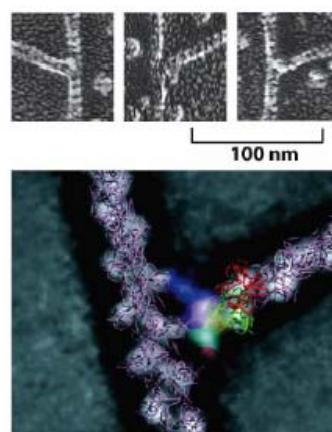
similar at (+) end but **differences** at sides and (-) end **prevent filament formation**



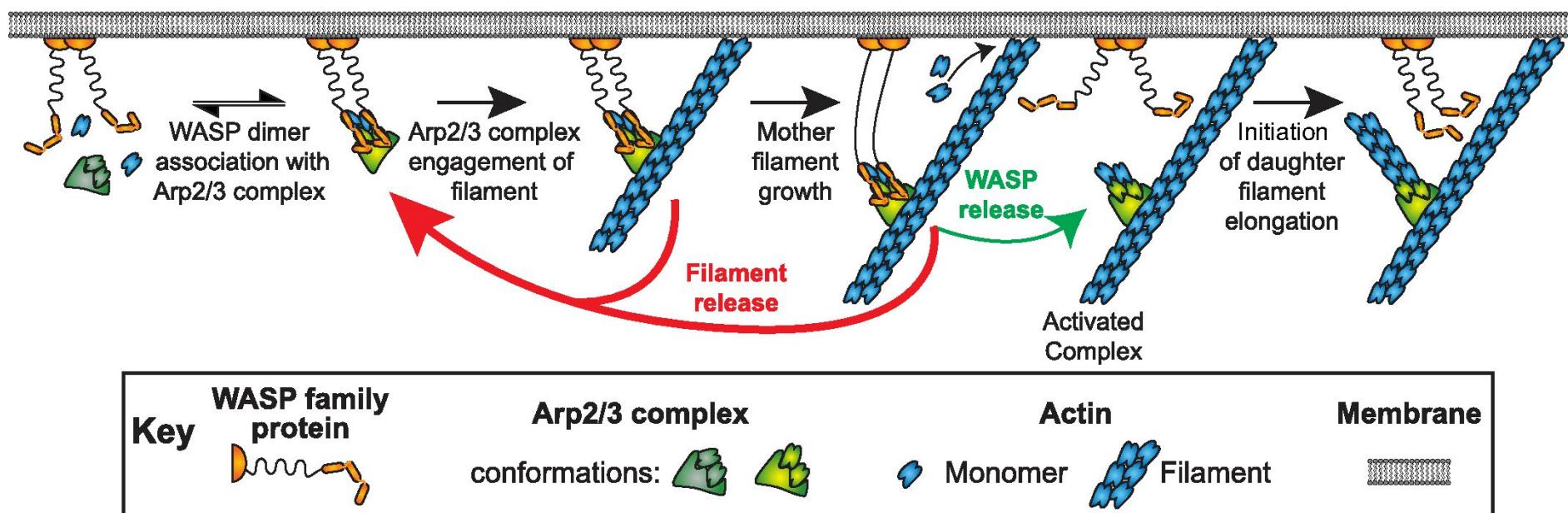
Assembly is most efficient when Arp2/3 is bound to the side of an existing filament:
resulting in branch growth at 70° angles.



Branching: actin (pink) with Arp2/3 complex fitted to the electron density.
Mother filament runs from top to bottom, daughter filament branches to the right

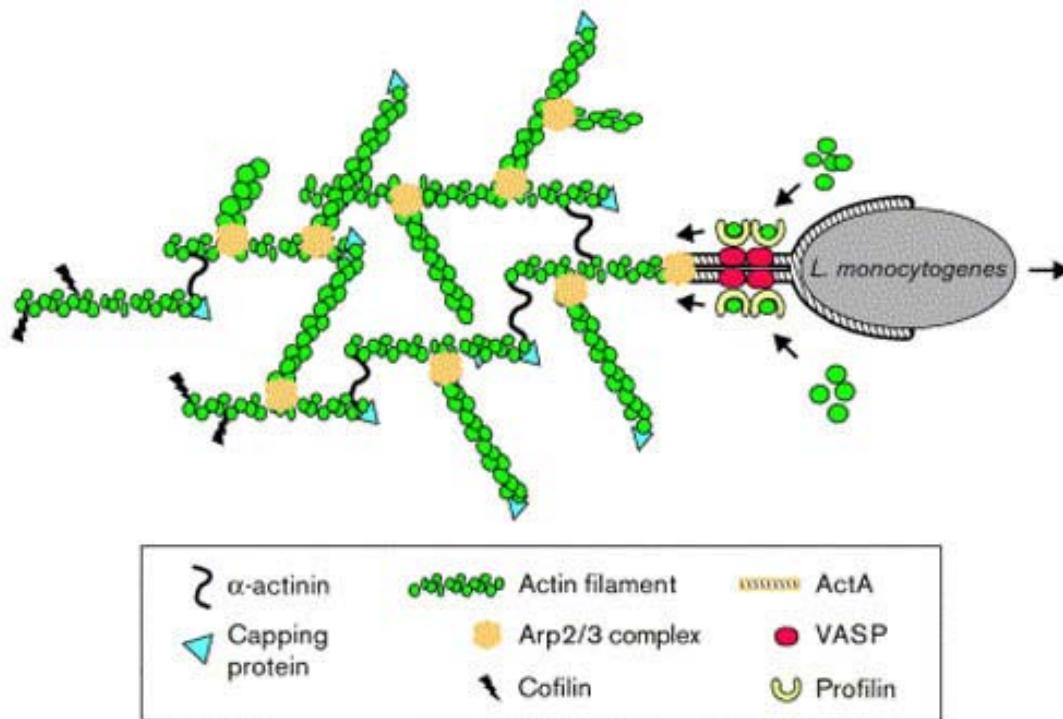


Actin nucleation by the Arp2/3 complex



new filament and old filament has an angle of 70 degree

Example 1: How does *Listeria* get around in host cells?



Listeria's cell surface protein ActA functions as a nucleation promoting factor (NPF), which interacts with VASP, Helps to recruit Arp2/3 and enhance ATP-actin assembly.

The recruited Arp2/3 complex nucleates the assembly of actin filaments that generate a substantial force and push the bacterium through the cytoplasm of the cell, at rates of up to 1 $\mu\text{m/sec}$, leaving behind a long actin "comet tail"

Toxins that perturb actin dynamics

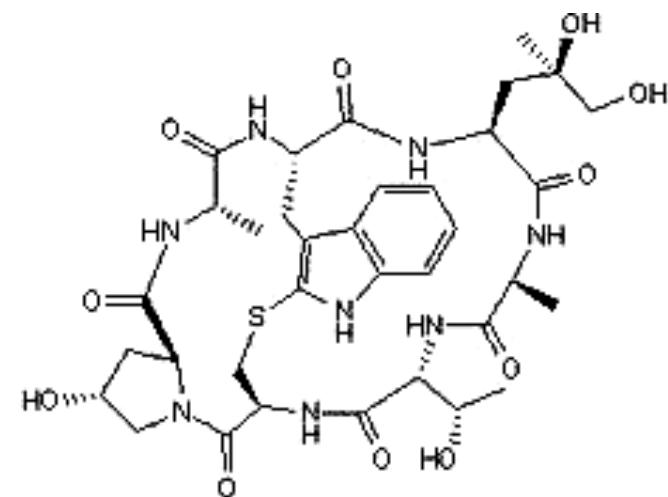
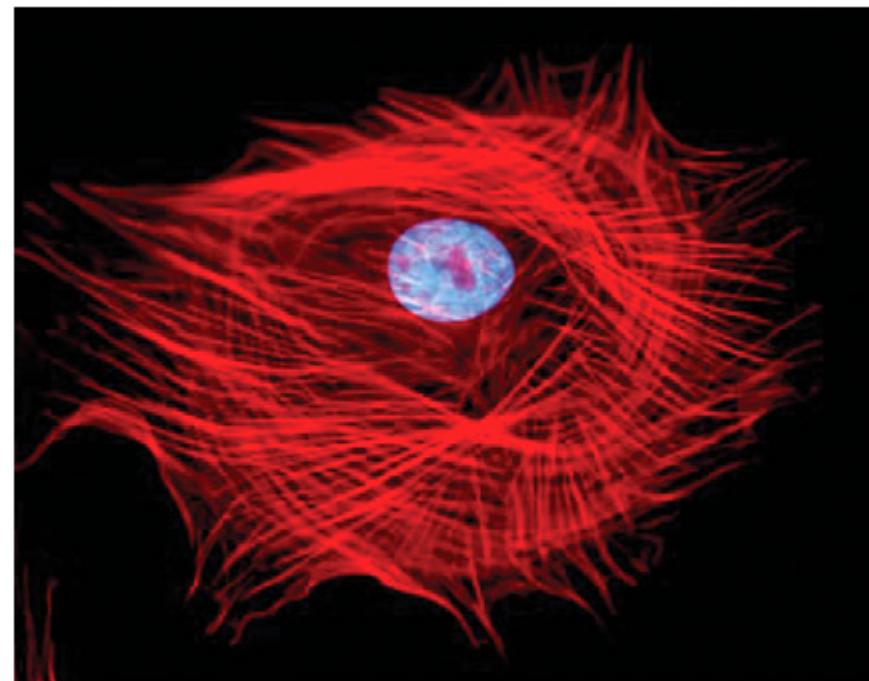
- Microfilament depolymerization drugs:

1. **Cytochalasin D**: a fungal alkaloid binds to “+” end of F-actin, blocks addition of subunits.
2. **Latrunculin**: binds to and sequesters G-actin, inhibiting its addition into a filament end.

- Microfilament polymerization drugs:

1. **Jasplakinolide**: enhances nucleation by binding and stabilizing actin dimers and lowering the Cc.
2. **Phalloidin** : binds at the interface between subunits in F-actin, locking adjacent subunits together and preventing actin filaments from depolymerizing.

Phalloidin has been used extensively in research for fluorescence-labelling F-actin



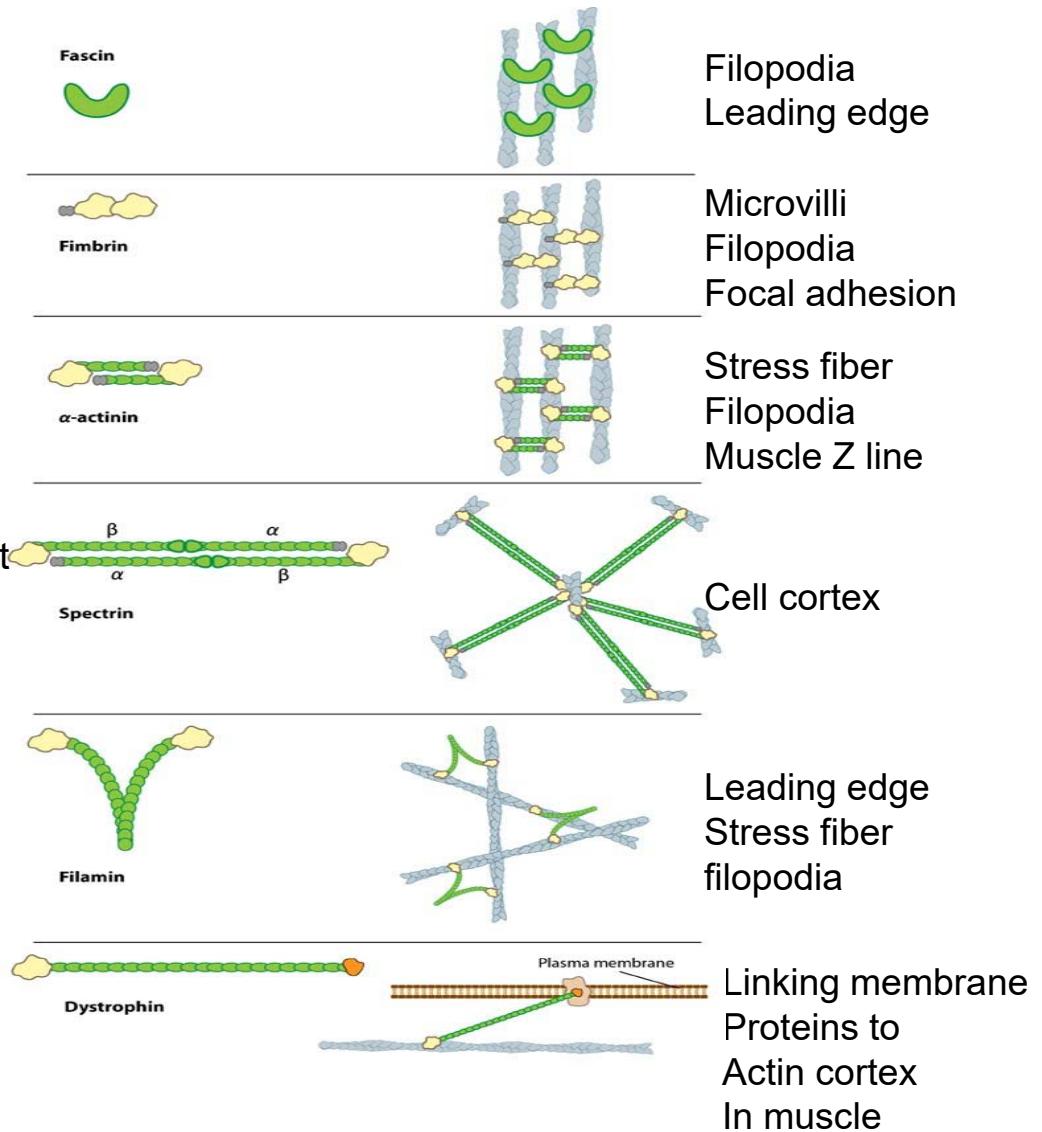
V. Organization of actin-based cellular structures

Various actin filament crosslinking proteins:

- fascin
- Fimbrin
- α -actinin
- Spectrin
- Filamin
- Dystrophin

Farther apart

Even farther apart



Different actin network in cells

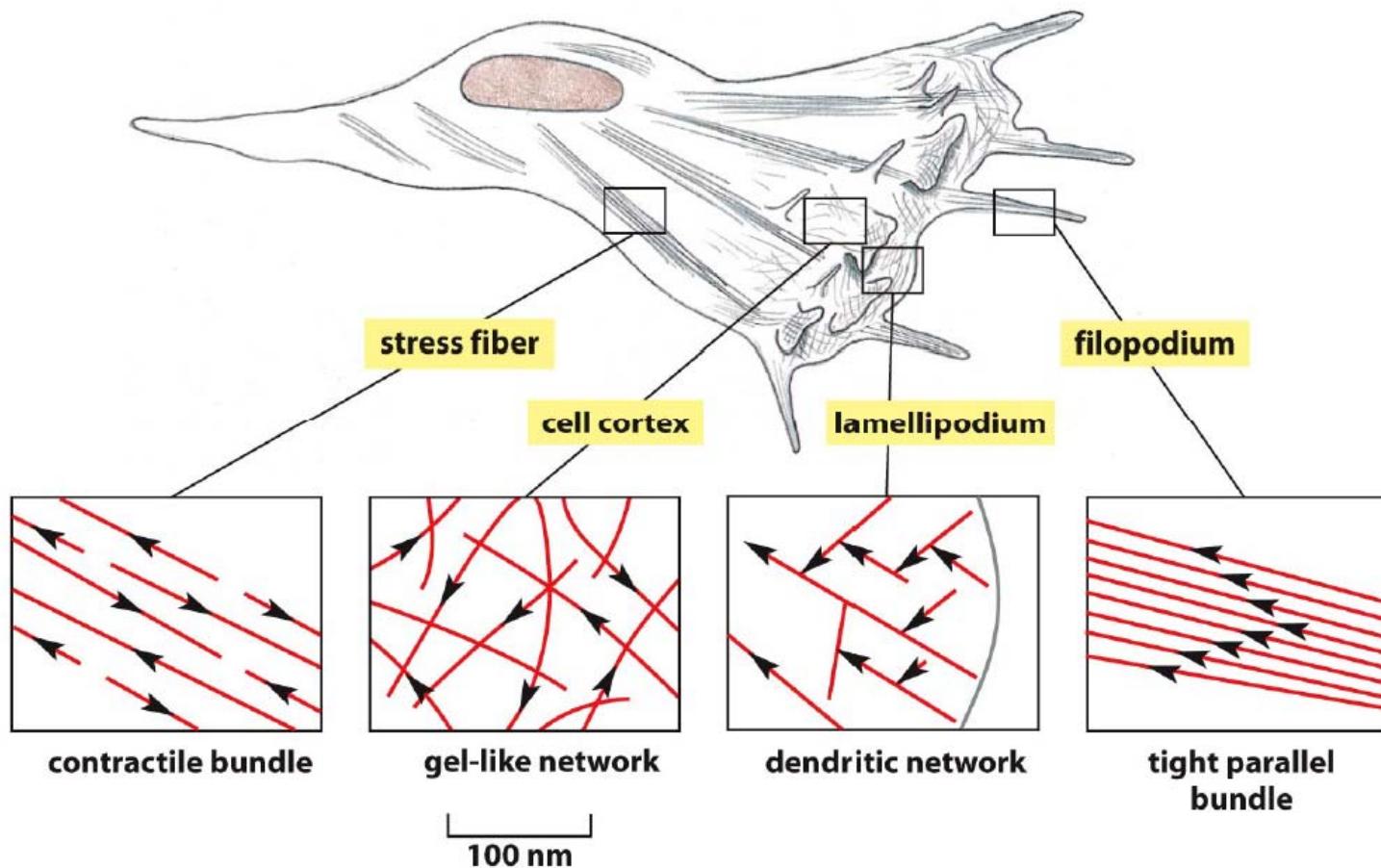


Figure 16-21 Molecular Biology of the Cell 6e (© Garland Science 2015)

A fibroblast crawling in a tissue-culture dish with four areas enlarged to show the arrangement of actin filaments:

Stress fibers are contractile and exert tension

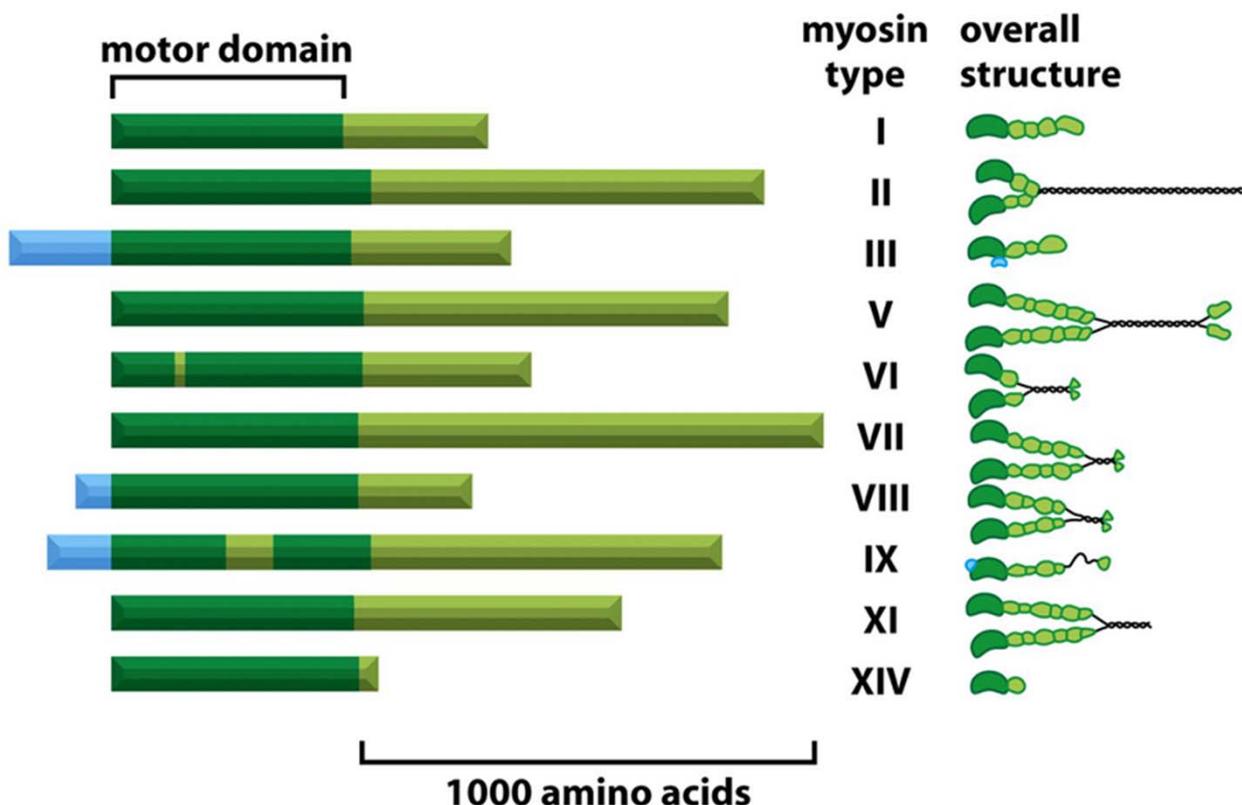
Actin cortex underlies the plasma membrane and consists of gel-like networks

Dendritic actin networks enable membrane protrusion at **lamellopodia**.

Filopodia are spike-like projections of the plasma membrane that allow a cell to explore its environment.

VI. Myosins: Actin-based motor proteins

A large family of motor proteins that can move along actin filaments, with ATP hydrolysis activity, >40 members



Is cytoskeleton network analogous to city traffic?



Actin and myosin perform a lot of functions in non-muscle cells,
usually towards the “+” end, only myosin VI moves to the “-” end

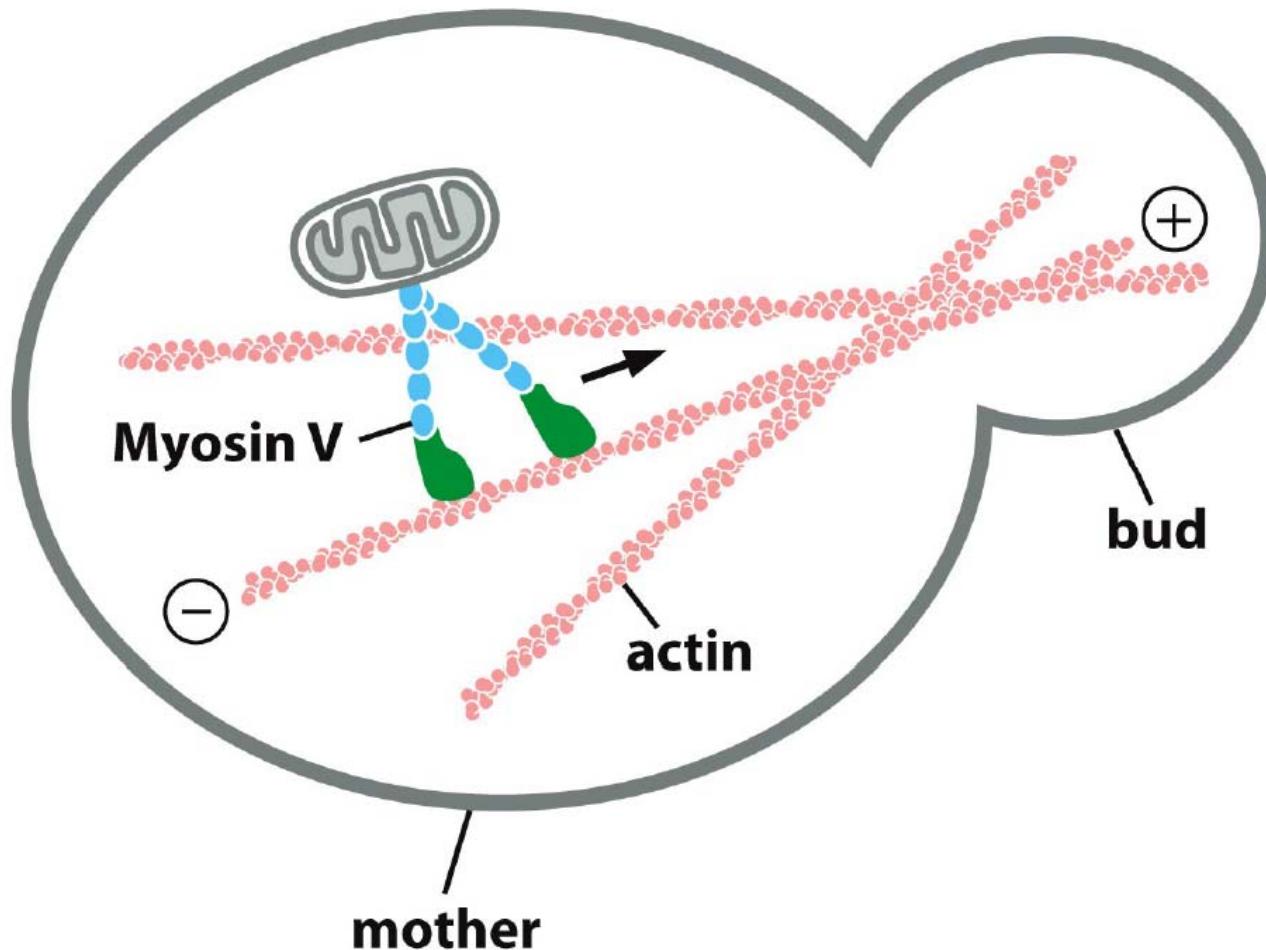
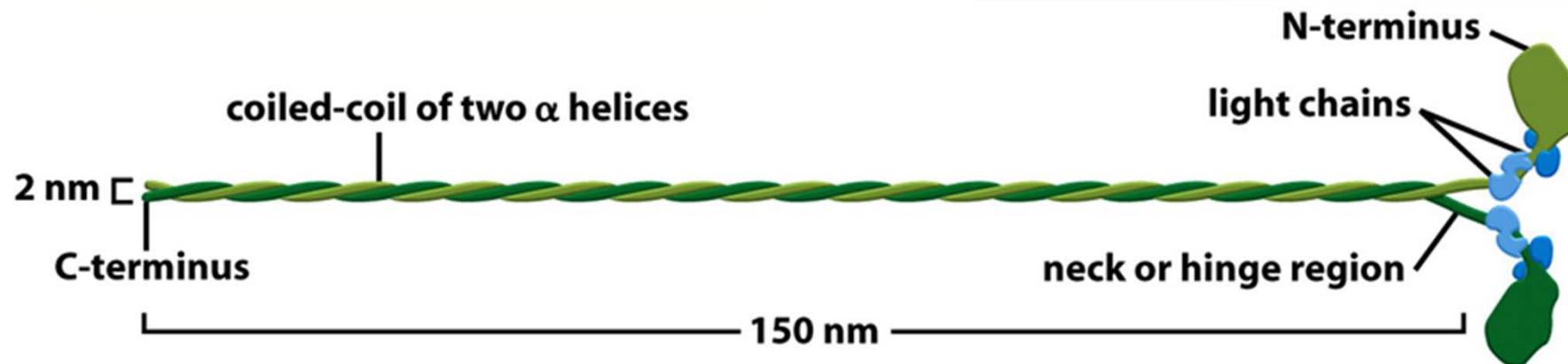


Figure 16-41b Molecular Biology of the Cell 6e (© Garland Science 2015)

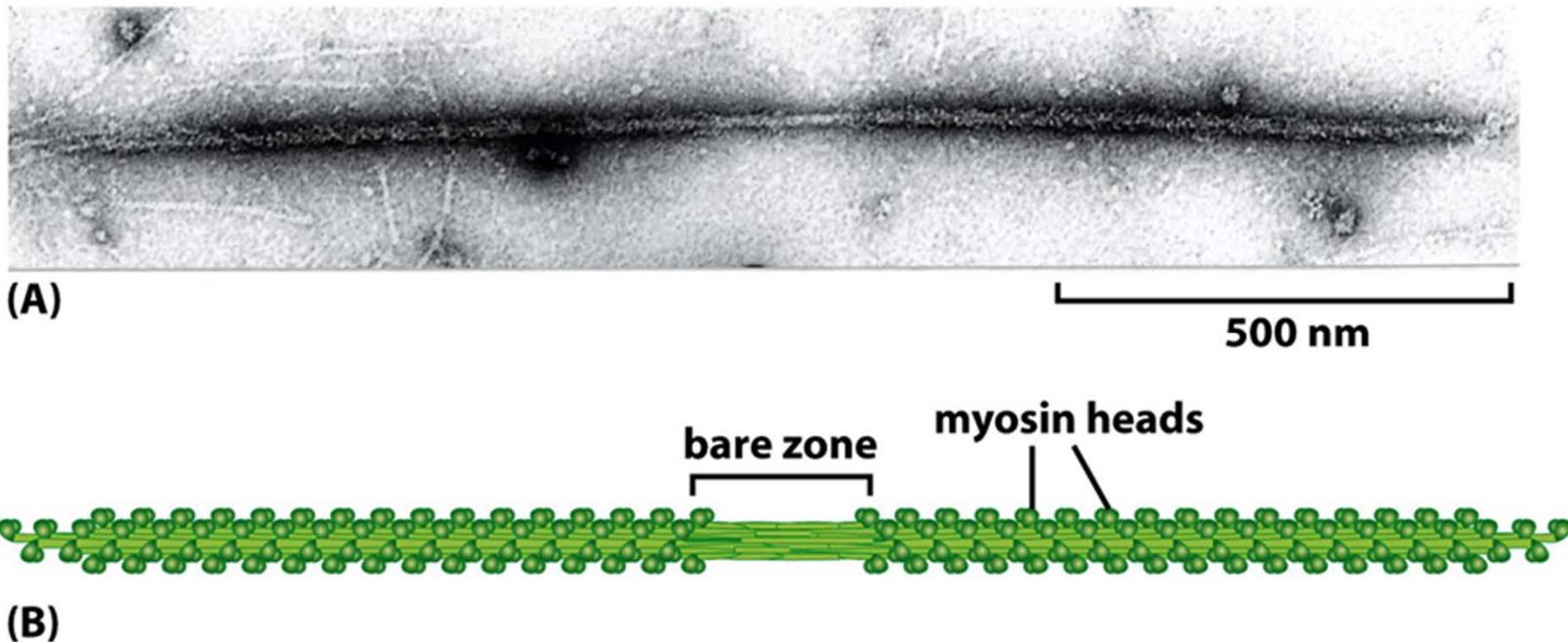
Structure of Myosin II



1. Head : S1 fragment , ATPase activity, actin binding sites
2. Neck : light chains binding
3. Tail: intertwining of two tail helices

2 heavy chains (**abt. 2000 amino acids long**)
2 essential light chains
2 regulatory light chains

Myosin II are arranged in a bipolar manner in skeletal muscle



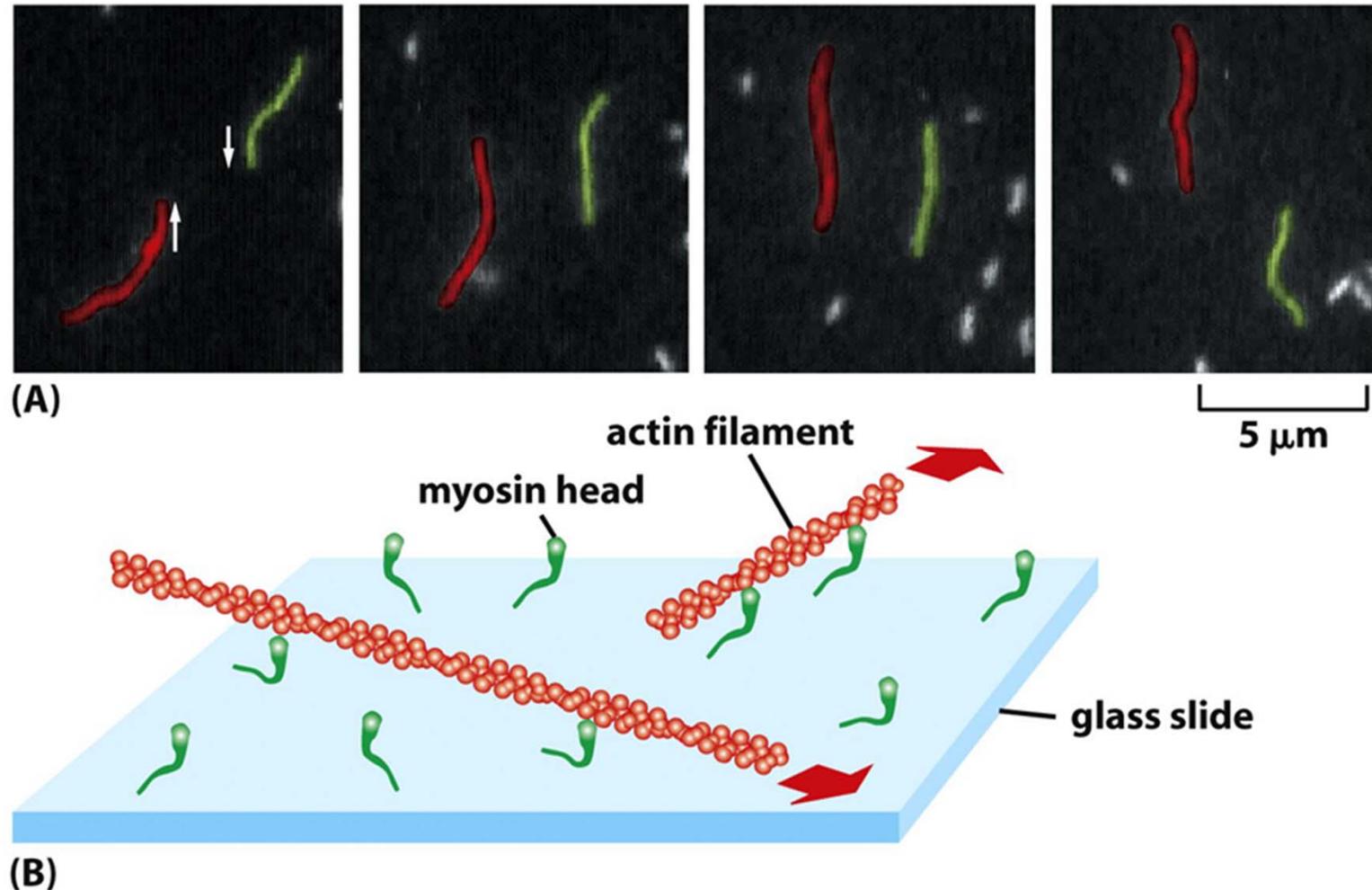
The myosin II bipolar thick filament in muscle.

(A) Electron micrograph of a myosin II thick filament isolated from frog muscle.

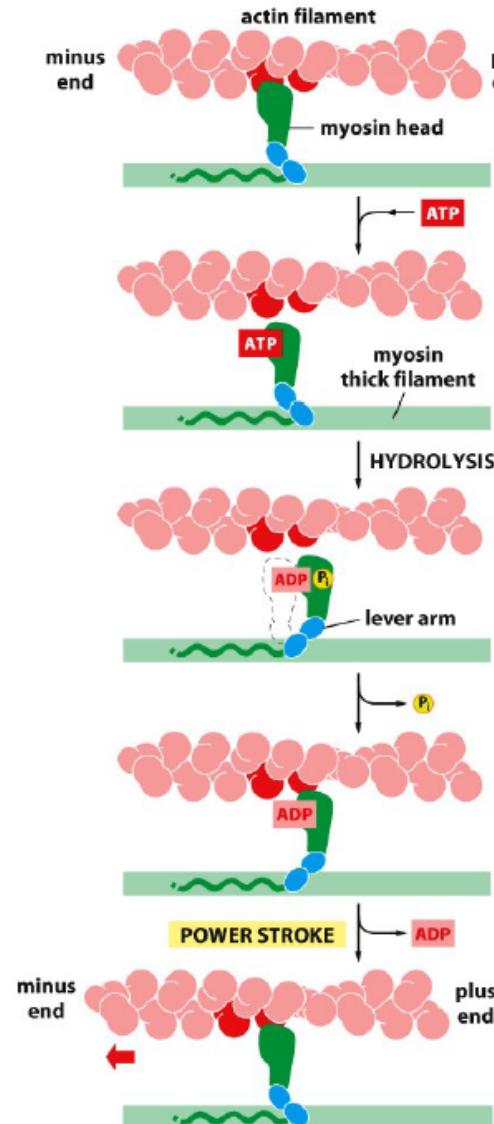
Note: the central bare zone, which is free of head domains.

(B) Schematic diagram, not drawn to scale. The myosin II molecules aggregate by means of their tail regions, with their heads projecting to the outside of the filament. The bare zone in the center of the filament consists entirely of myosin II tails.

Myosin head drives actin movement



How ATP hydrolysis couples myosin conformation change in causing movement along actin



ATTACHED At the start of the cycle shown in this figure, a myosin head lacking a bound nucleotide is locked tightly onto an actin filament in a *rigor* configuration (so named because it is responsible for *rigor mortis*, the rigidity of death). In an actively contracting muscle, this state is very short-lived, being rapidly terminated by the binding of a molecule of ATP.

RELEASED A molecule of ATP binds to the large cleft on the “back” of the head (that is, on the side furthest from the actin filament) and immediately causes a slight change in the conformation of the actin-binding site, reducing the affinity of the head for actin and allowing it to move along the filament. (The space drawn here between the head and actin emphasizes this change, although in reality the head probably remains very close to the actin.)

COCKED The cleft closes like a clam shell around the ATP molecule, triggering a movement in the lever arm that causes the head to be displaced along the filament by a distance of about 5 nm. Hydrolysis of ATP occurs, but the ADP and inorganic phosphate (Pi) remain tightly bound to the protein.

FORCE-GENERATING Weak binding of the myosin head to a new site on the actin filament causes release of the inorganic phosphate produced by ATP hydrolysis, concomitantly with the tight binding of the head to actin. This release triggers the power stroke—the force-generating change in shape during which the head regains its original conformation. In the course of the power stroke, the head loses its bound ADP, thereby returning to the start of a new cycle.

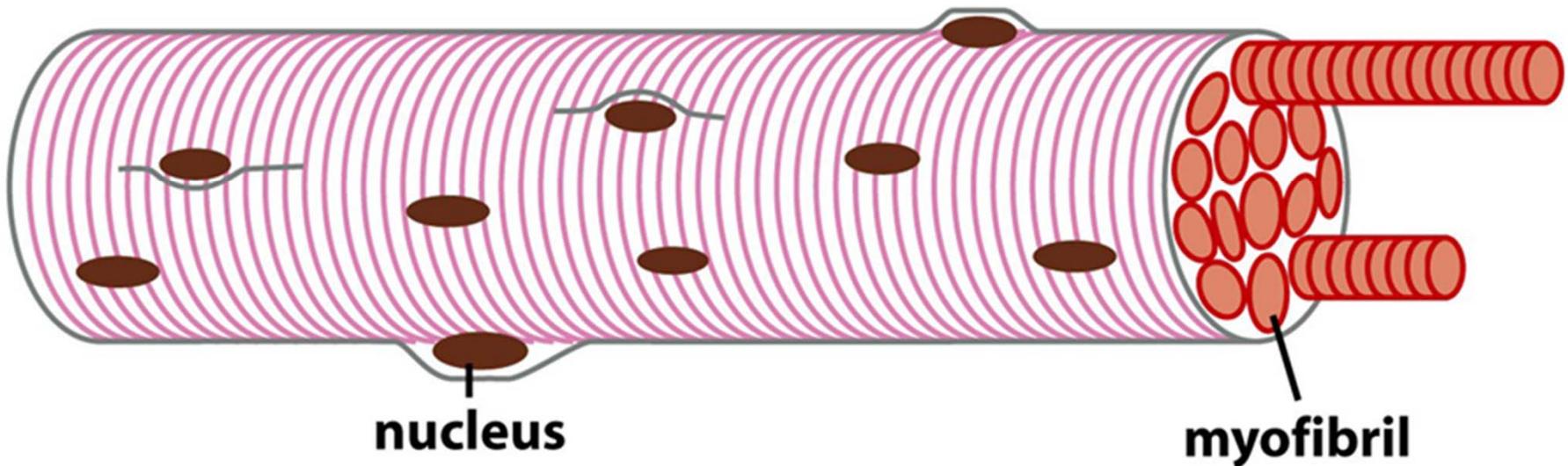
ATTACHED At the end of the cycle, the myosin head is again locked tightly to the actin filament in a rigor configuration. Note that the head has moved to a new position on the actin filament.

VII. Myosin-powered movements

1. Mechanism of muscle contraction: Myosin II
 - 1). Structure of skeletal muscle
 - 2). Mechanism of contraction
 - 3). Regulation of muscle contraction by Ca^{2+} and cAMP
2. Mechanism of vesicle/organelle transport: Myosin V

1). Detailed structure of muscle

The structure of muscle cell:

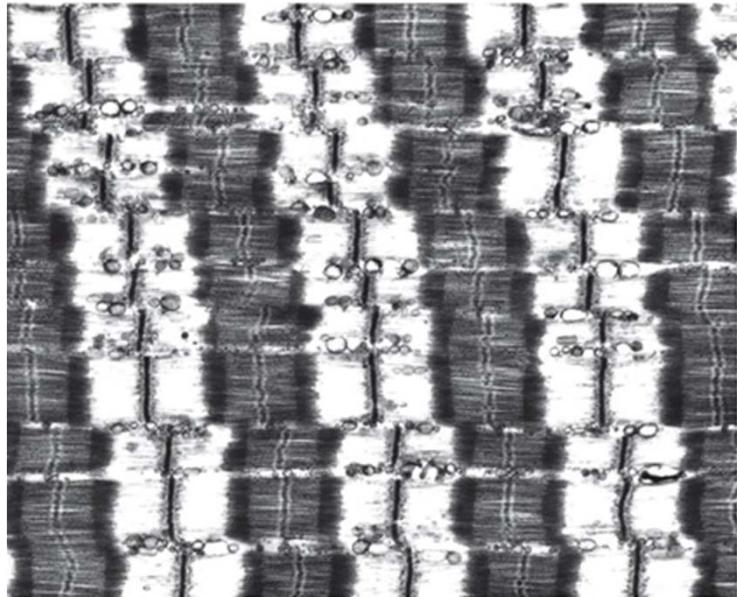


These huge multinucleated cells form by the fusion of many muscle cell precursors, called myoblasts.

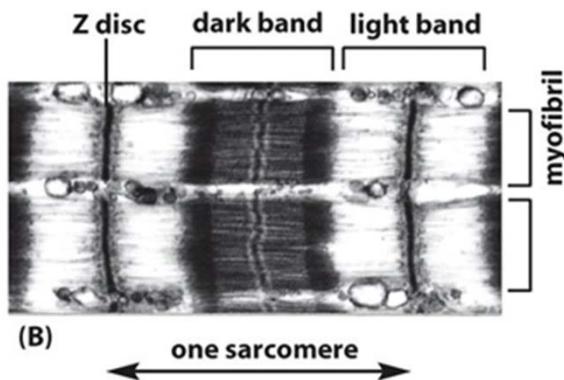
Here, a single muscle cell is depicted.

In an adult human, a muscle cell is typically $50 \mu\text{m}$ in diameter and can be up to several centimeters long.

Skeletal muscle myofibrils

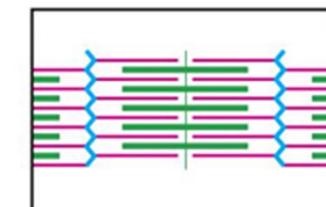
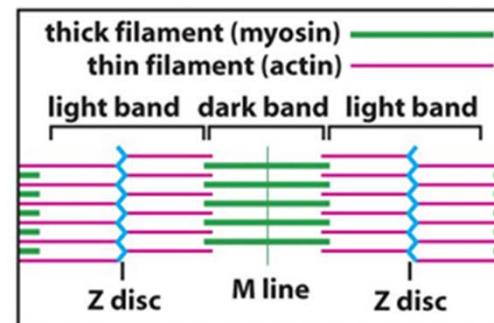


longitudinal section through a skeletal muscle cell of a rabbit, showing the regular pattern of cross-striations
2 μ m



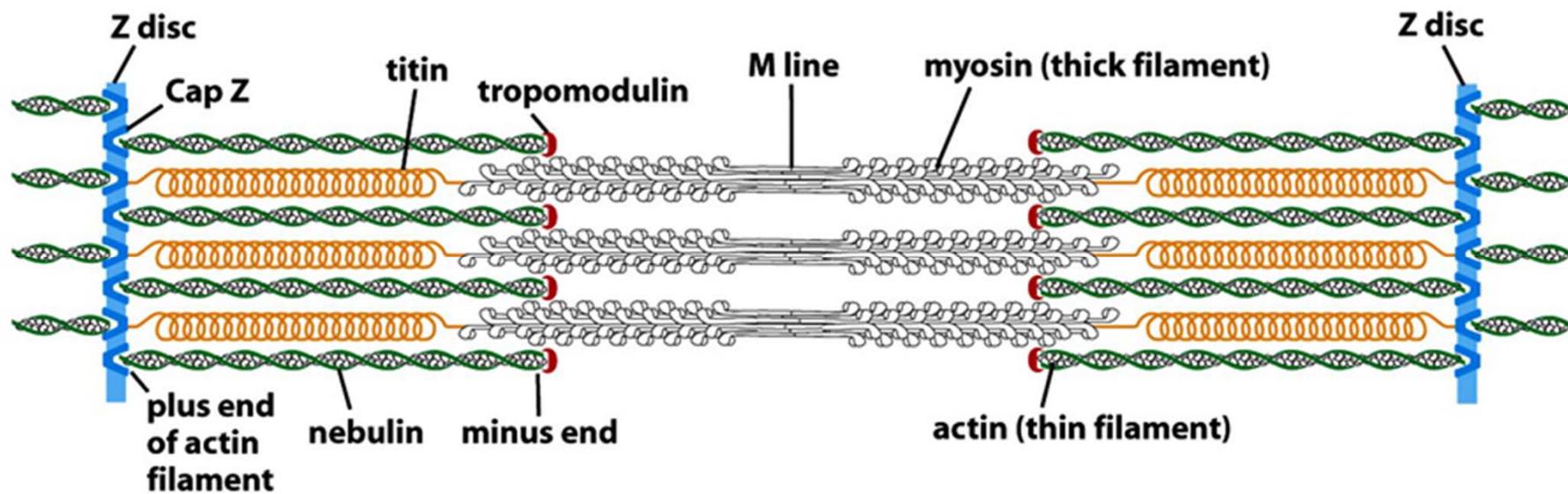
The Z discs, at each end of the sarcomere, are attachment sites for the plus ends of actin filaments (thin filaments)

The M line, or midline, is the **location of proteins that link adjacent myosin II filaments** (thick filaments) to one another.



When the sarcomere contracts, the **actin and myosin filaments slide past one another without shortening!**

Organization of accessory proteins in a sarcomere



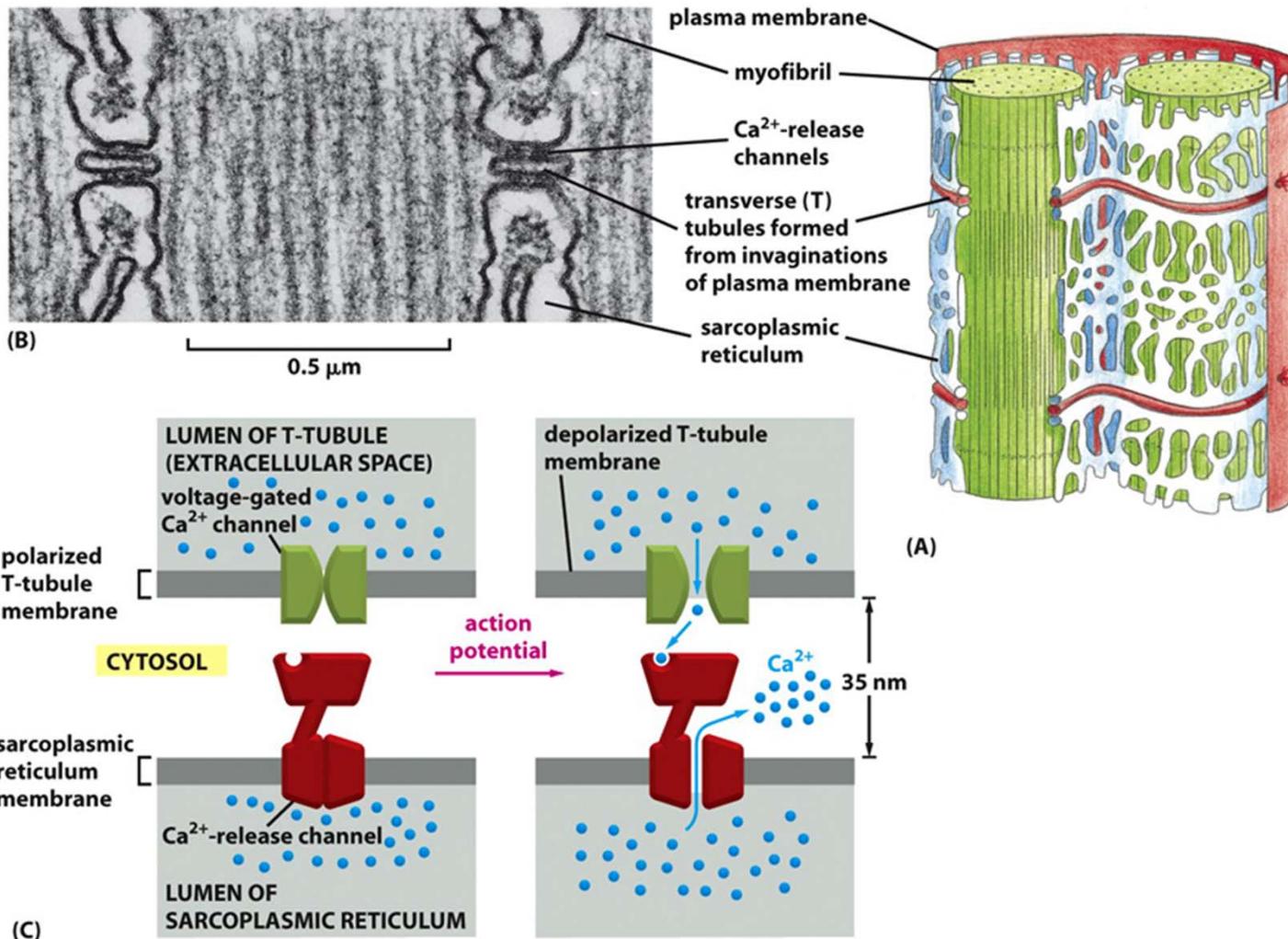
Nebulin provide scaffold and structural support, molecular ruler.

Titin is a molecular spring

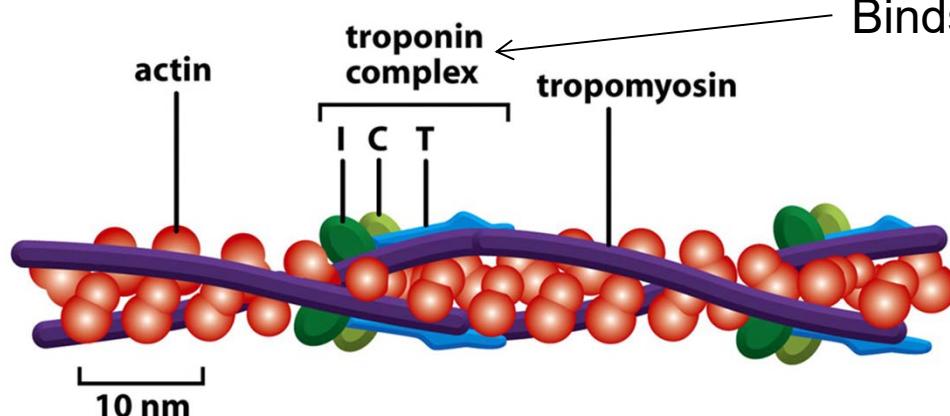
Cap Z and α – actinin on the Z-line

Tropomodulin on the minus end.

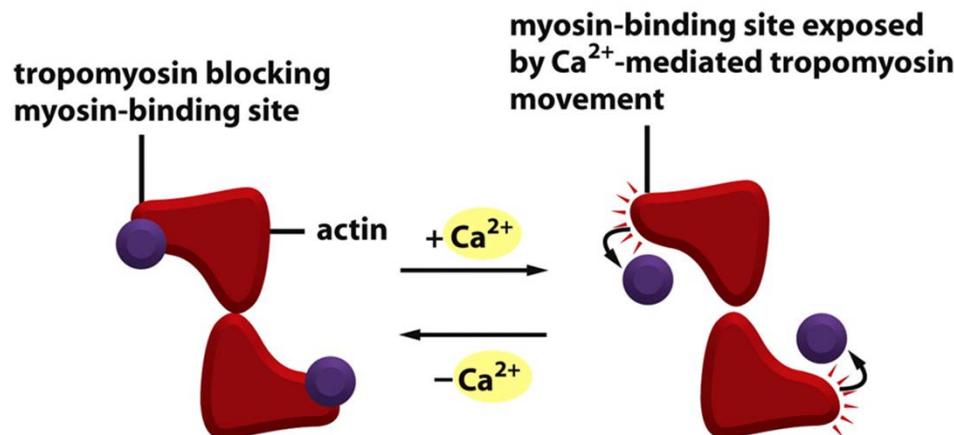
T tubules(invagination from PM) relay the action potential (signal to contract) from the PM to the sarcoplasmic reticulum, to all myofibrils of the cell



2) The control of skeletal muscle contraction by the actin binding proteins troponin and tropomyosin



troponins T, I, and C
(Tropomyosin-binding, Inhibitory, and Ca^{2+} -binding activities)



Binds to 4 Ca^{2+}

resting muscle:

the troponin I-T complex **pulls the tropomyosin out of its normal binding groove to block binding of myosin heads (no force generating action)**

High Ca_{2+} : troponin C causes troponin I to **release its hold on actin**. This allows the tropomyosin molecules to slip back into their normal position so that the myosin heads can walk along the actin filaments

3) Muscle contraction is additionally regulated by myosin II phosphorylation

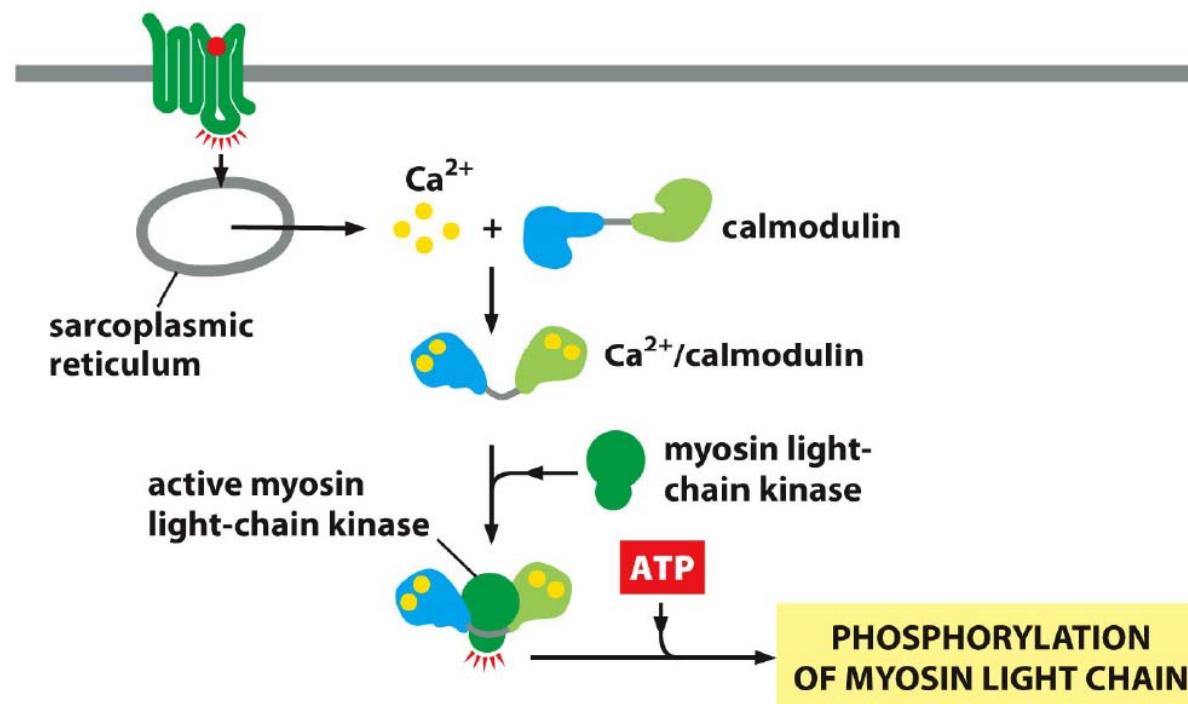
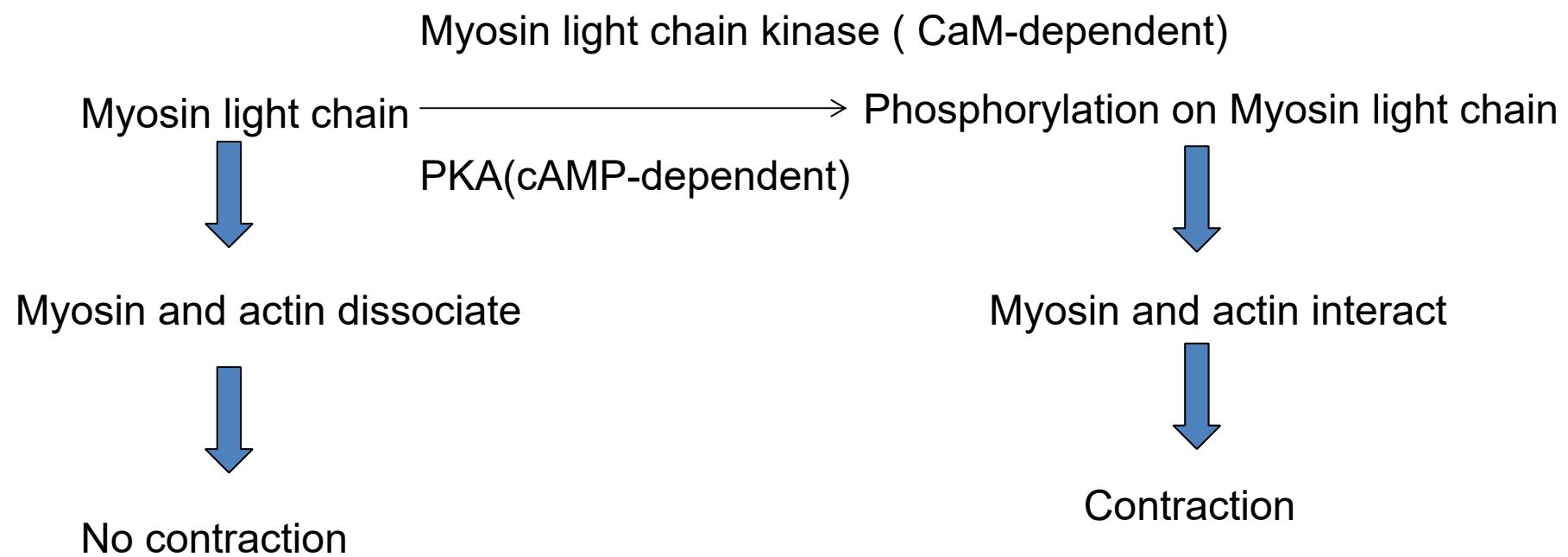
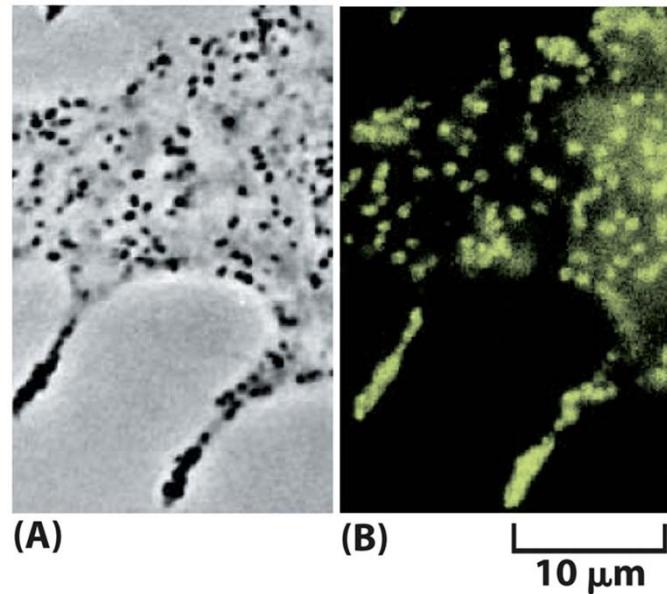
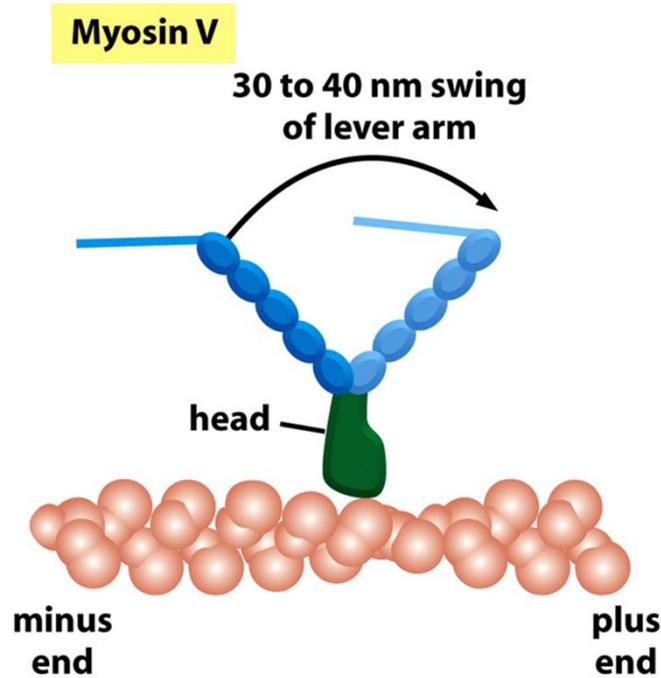


Figure 16-37a Molecular Biology of the Cell 6e (© Garland Science 2015)

Upon muscle stimulation by activation of cell-surface receptors, Ca^{2+} released into the cytoplasm from the sarcoplasmic reticulum (SR) **binds to calmodulin**. **Ca^{2+} -bound calmodulin** then **binds myosin light-chain kinase (MLCK)**, which phosphorylates myosin light chain, stimulating myosin activity.



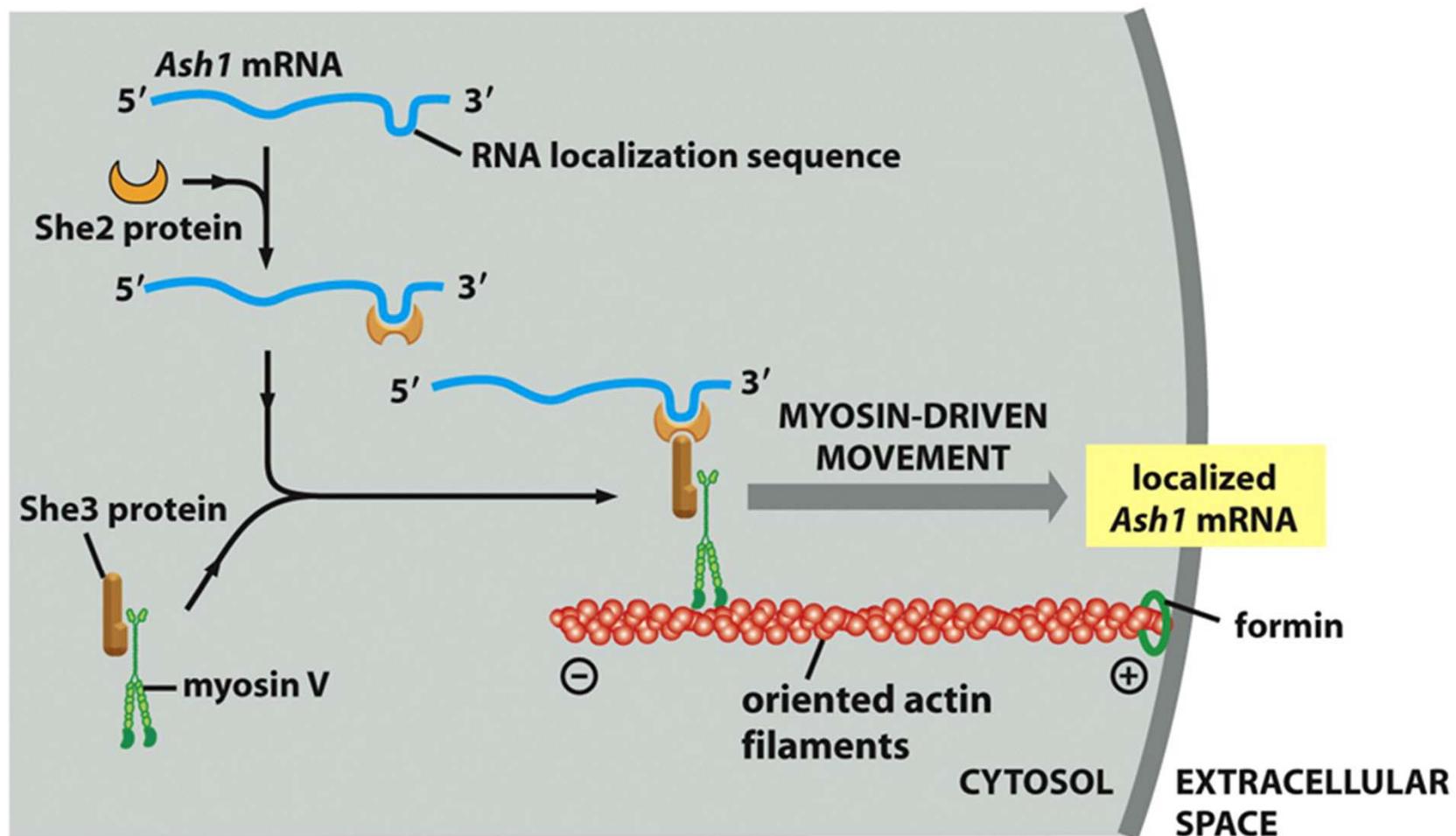
2. Myosin V for organelle/mRNA transport



Myosin V motors carry a wide range of cargoes including mRNA, ER, and secretory vesicles along the actin cables.

Myosin V mediates the partitioning of organelles such as peroxisomes and mitochondria between mother and daughter cells

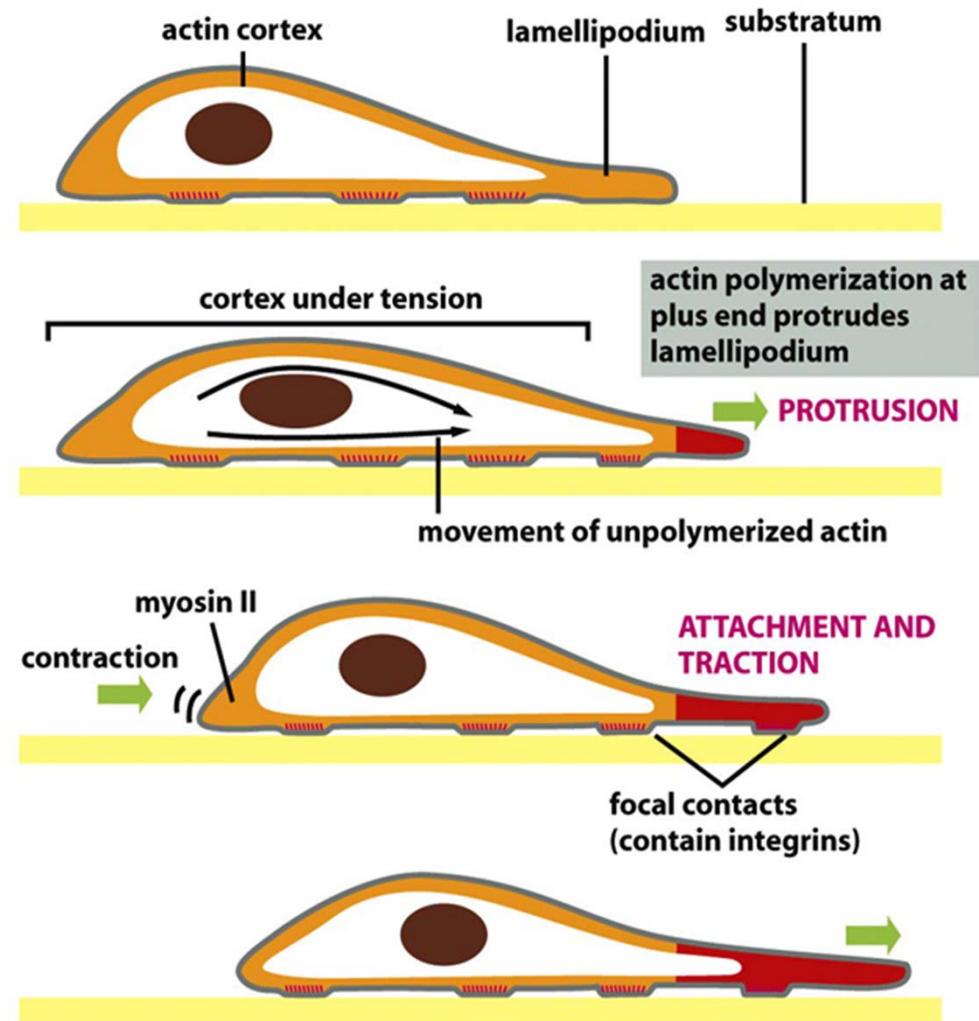
Localized mRNA by Myosin V and the mRNA-binding adaptor proteins She2 and She3



VIII. Cell migration

Steps:

1. Focal adhesions, attachment
2. Extension
(Lamellipodium, Filopodia)
3. New attachment
(new focal adhesions)
4. Cell **contraction**
5. De-adhesion and
endocytic recycling



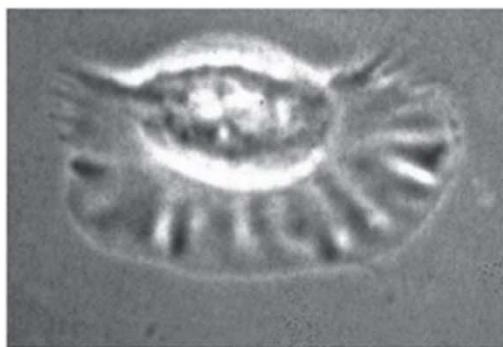
Filopodia, lamellipodia, pseudopodia

Filopodia: **one dimensional**. A core of **long, bundled actin filaments** and is dynamic
Formed by migrating growth cones and some fibroblasts.

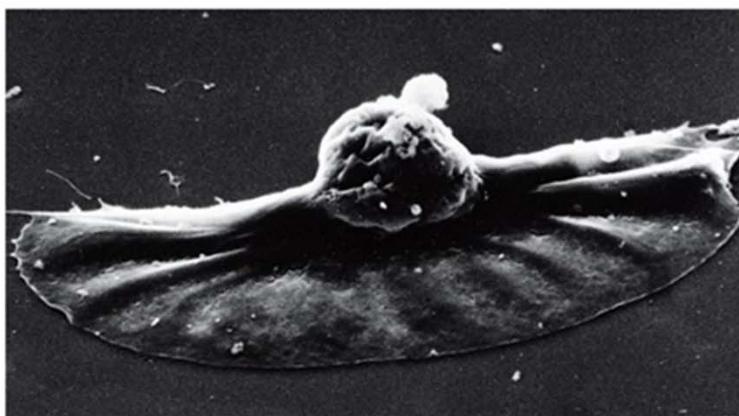
Lamellipodia: **two dimensional**, sheet like structures, **cross-linked mesh of actin filaments** lie parallel to the solid substratum, epithelia, fibroblast, and some neurons.

Pseudopodia: **three dimensional projections** filled with an **actin filament gel**, in **Amoebae and neutrophils**

Cell leading edge in migration

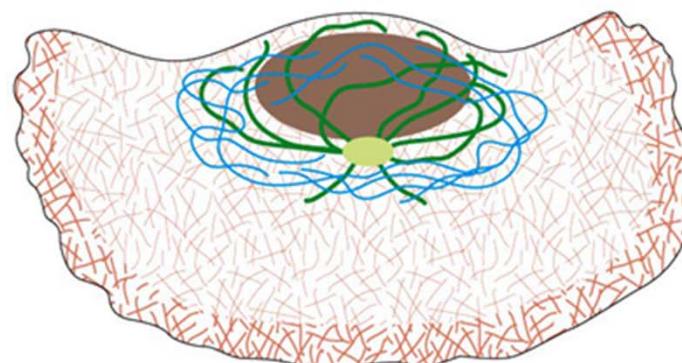


(A)



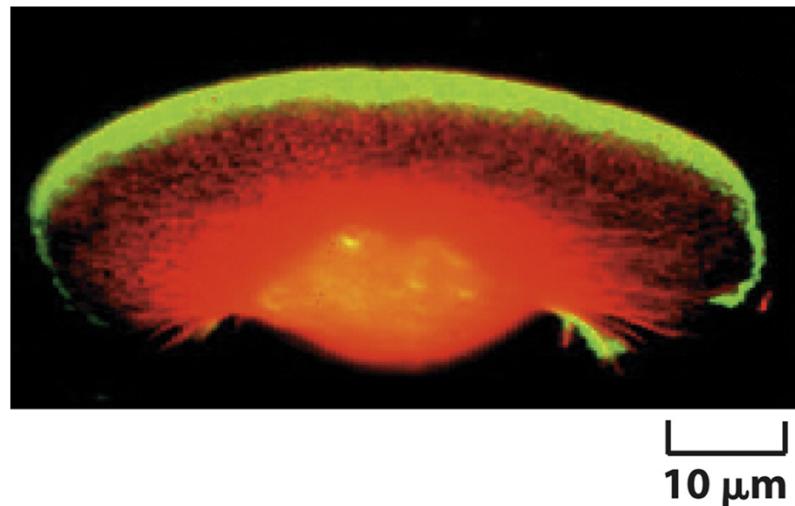
(B)

10 μm

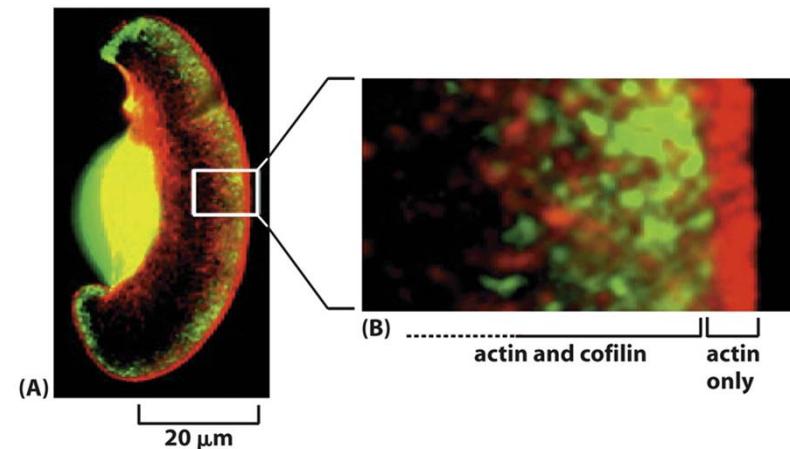


(C)

Localization of different actin regulation proteins in the leading edge

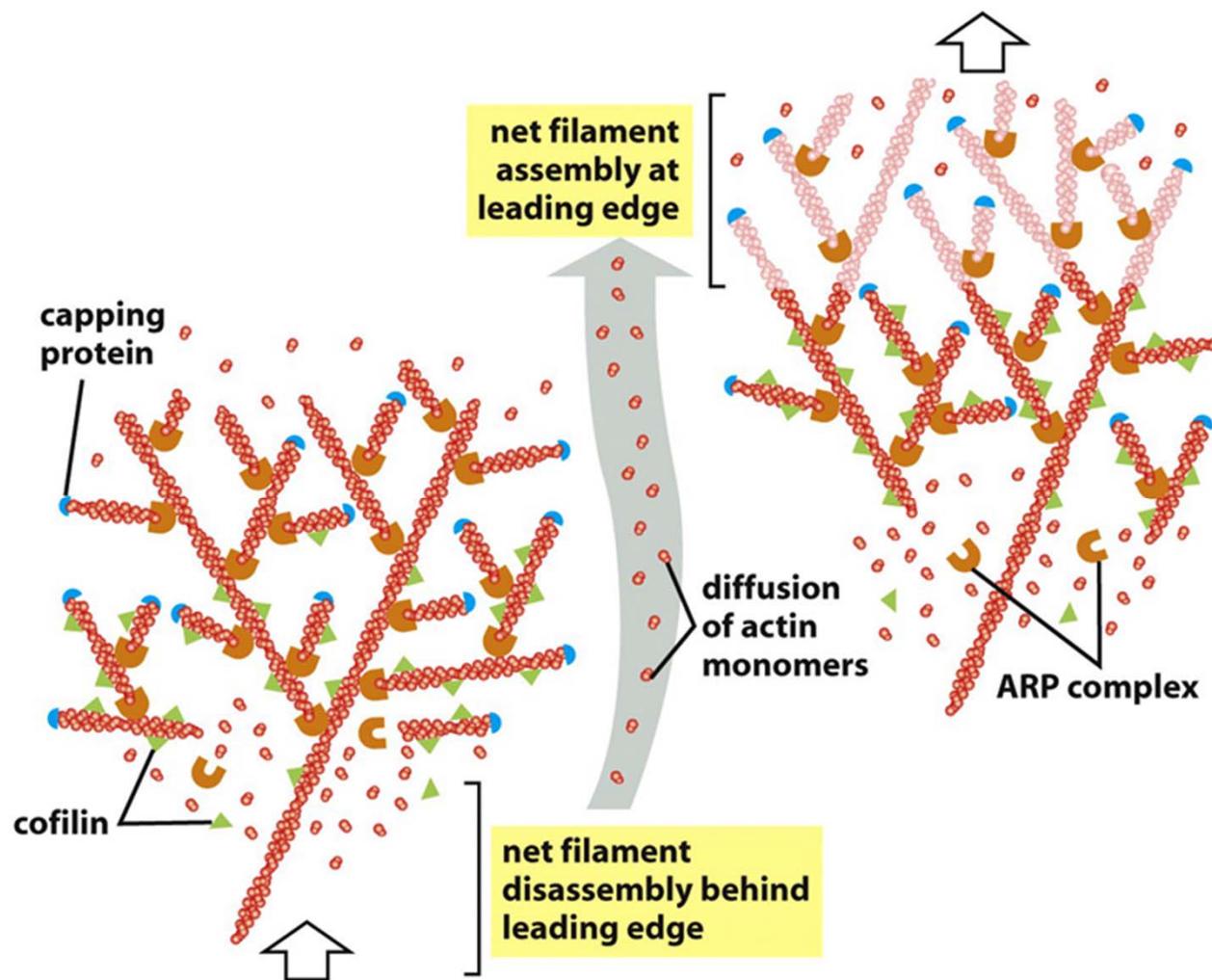


Green: Arp2/3
Red: phalloidin-F-actin



Green: cofilin
Red: F-actin –phalloidin

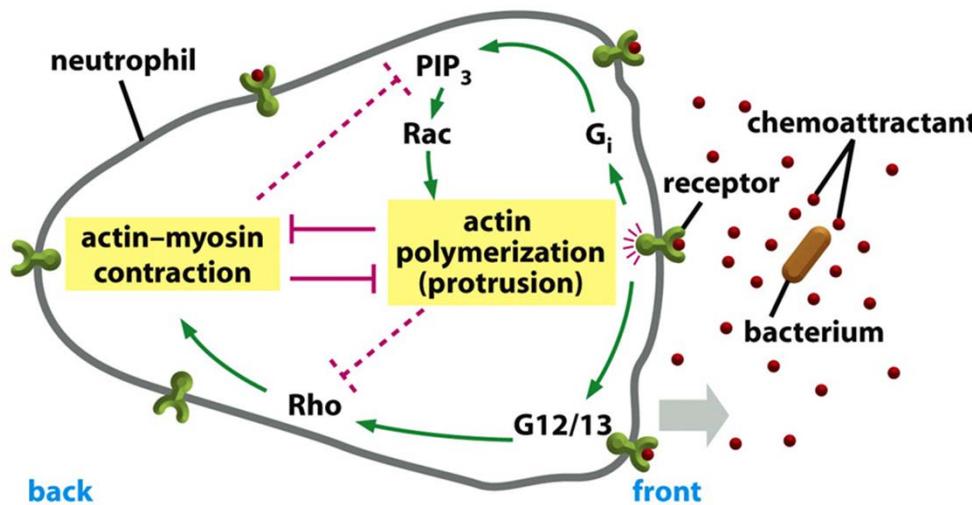
How actin cause protrusions in leading edge?



Involves the actin binding protein cofilin

Neutrophil in chemotaxis: one receptor signals in two pathways

Binding of bacterial molecules to G-protein-coupled receptors on the neutrophil stimulates directed motility:
TWO distinct signaling pathways contribute to the cell's polarization.



The same receptor also stimulates **a second signaling pathway**, via the trimeric G proteins **G12 and G13**, that triggers the **activation of Rho**.

At the front of the cell: stimulation of the **Rac pathway** leads, via the trimeric G protein **G_i**, to growth of protrusive actin networks. **Second messengers** within this pathway are shortlived, so protrusion is limited to the region of the cell closest to the stimulant.

The two pathways are mutually antagonistic. Since Rac-based protrusion is active at the front of the cell, Rho is activated only at the rear of the cell, stimulating contraction of the cell rear and assisting directed movement.