Lecture 13. Cytoskeleton and Cell Movement I

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. Mysoin-powered movements
VIII. Cell migration: mechanisms, signaling, and chemotaxis

Questions to be answered:

What is cytoskeleton?

What are the functions of cytoskeleton?

How is it assembled and regulated?

01.1-keratocyte_dance.mov

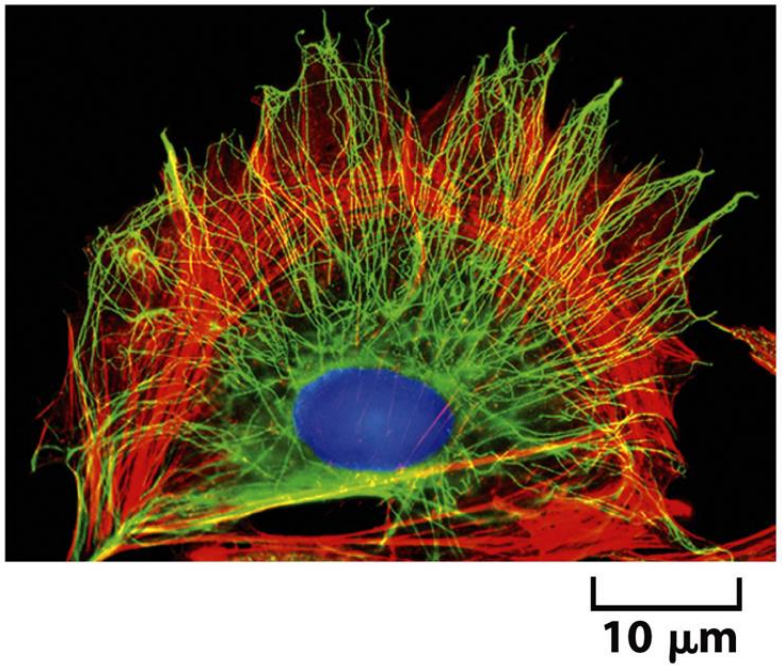
16.2-neutrophil_chase.mov

I. Overview of cytoskeleton

1). types of cytoskeleton
2). functions of cytoskeleton
3). Common regulation of cytoskeleton

Types of cytoskeleton system

- Microfilament--- basic unit: Actin
- Microtubule---basic unit: tubulin
- Intermediate filament-basic unit: keratin, vimentin, lamin, etc.



Cytoskeleton in an epithelial cell

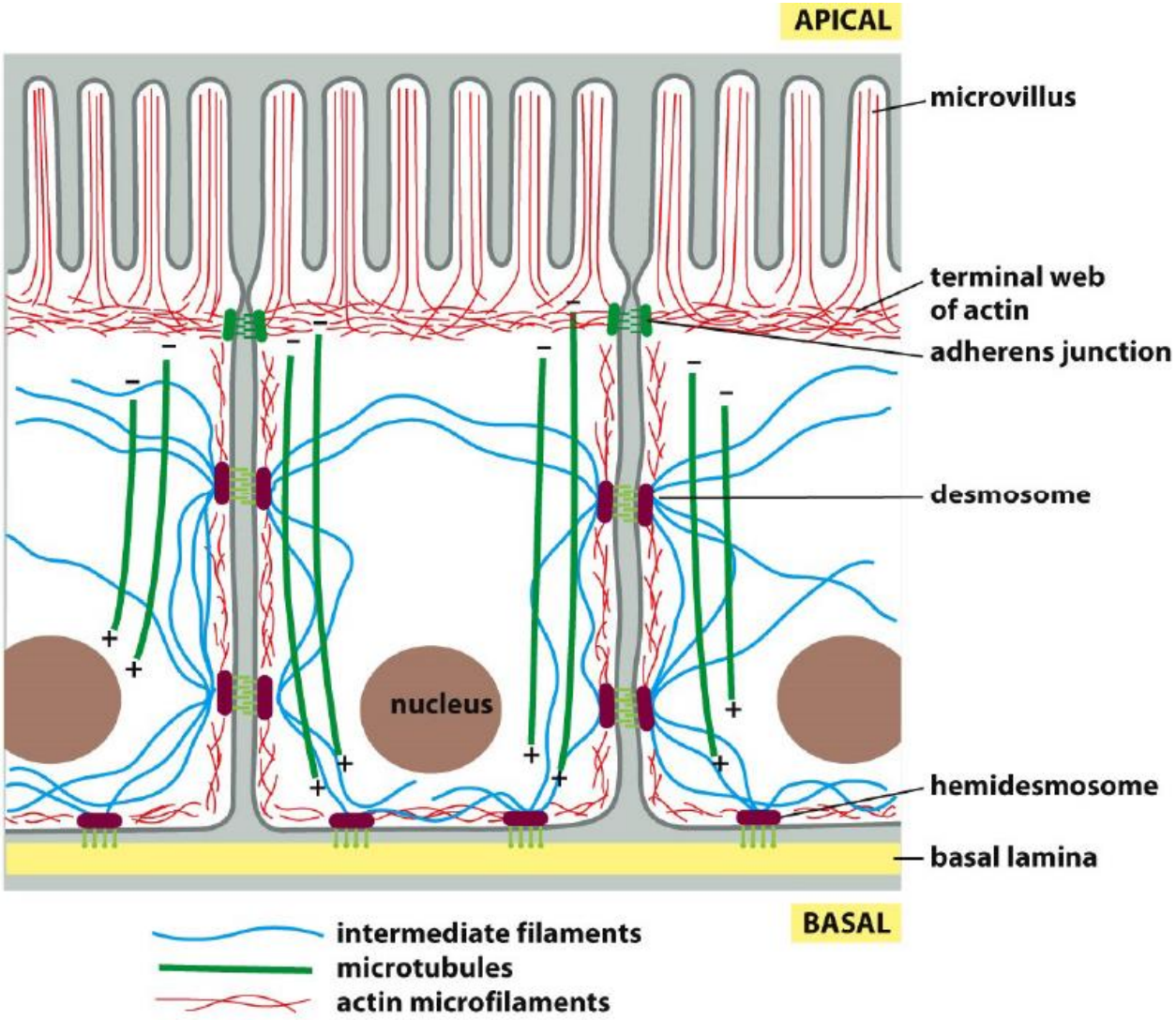
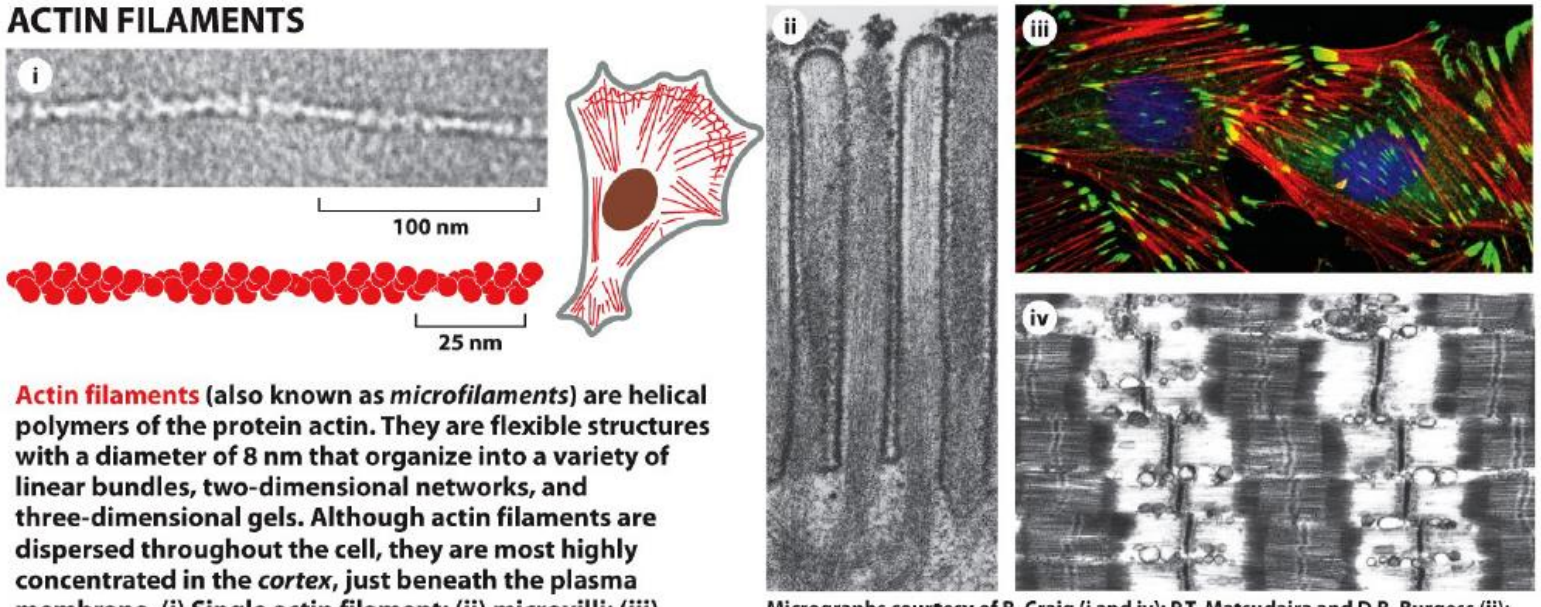


Figure 16-4 Molecular Biology of the Cell 6e (© Garland Science 2015)

Microfilaments

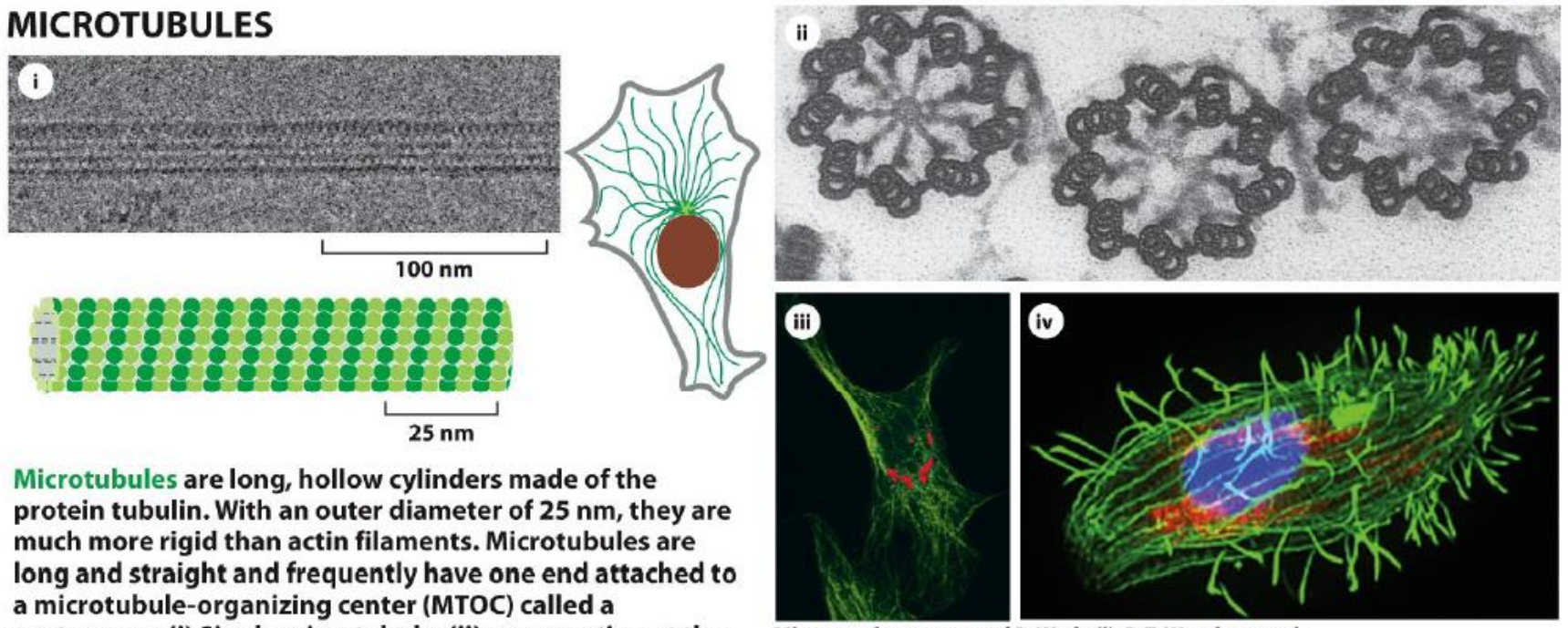


Actin filaments (also known as *microfilaments*) are helical polymers of the protein actin. They are flexible structures with a diameter of 8 nm that organize into a variety of linear bundles, two-dimensional networks, and three-dimensional gels. Although actin filaments are dispersed throughout the 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.

Panel 16-1 (part 1) Molecular Biology of the Cell 6e (© Garland Science 2015)

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

microtubules

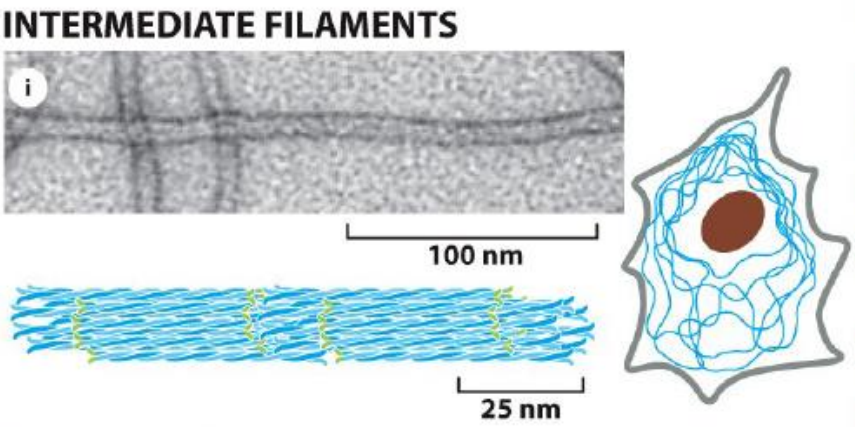


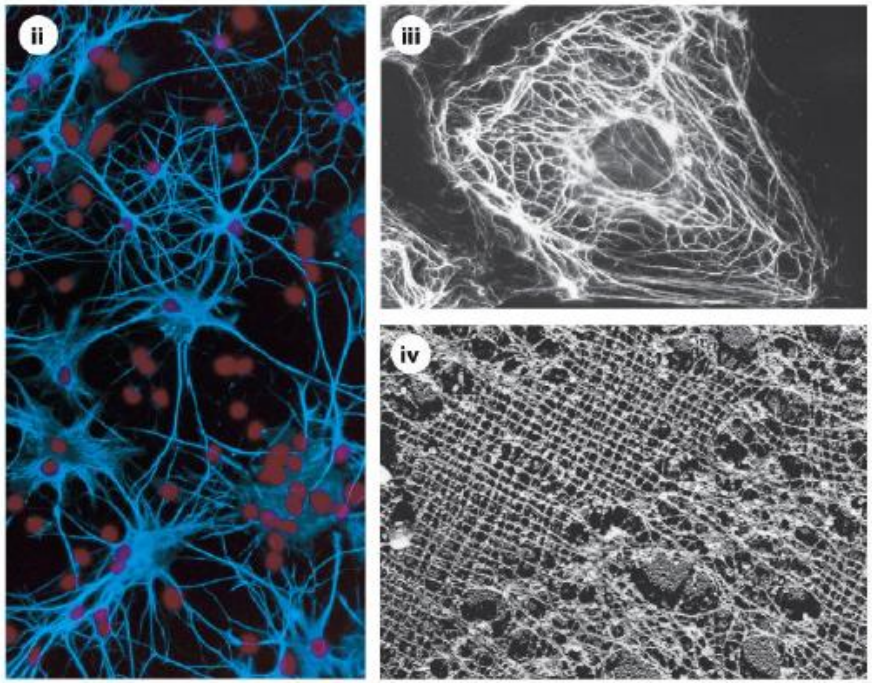
Microtubules are long, hollow cylinders made of the protein tubulin. With an outer diameter of 25 nm, they are much more rigid than actin filaments. Microtubules are long and straight and frequently have one end attached to a microtubule-organizing center (MTOC) called a *centrosome*. (i) Single microtubule;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

Panel 16-1 (part 2) Molecular Biology of the Cell 6e (© Garland Science 2015)

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

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).

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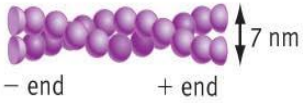
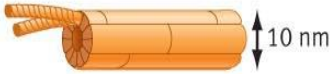
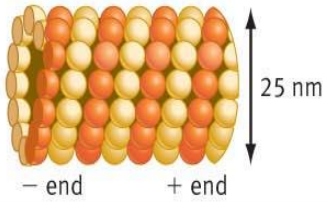
A brief summary for the components of the cytoskeleton

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 	• 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
Intermediate filaments	Fibers wound into thicker cables 	Keratin or vimentin or lamin or others 	• maintain cell shape by resisting tension (pull) • anchor nucleus and some other organelles
Microtubules	Hollow tube 	α- and β-tubulin dimers 	• 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

Semiflexible
Motors
polarized

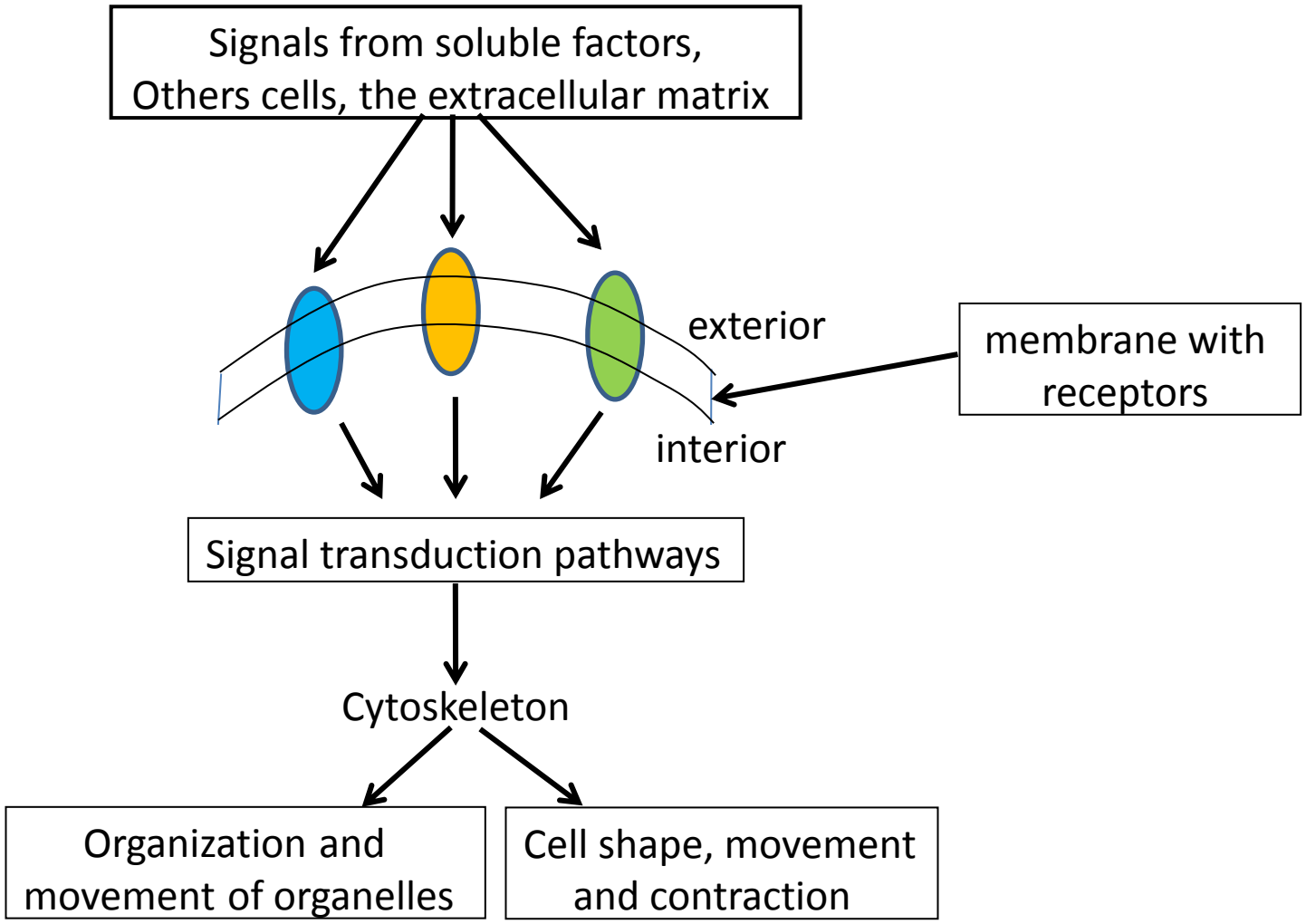
Flexible
No motor
unpolarized

Stiff rods
Motors
Polarized

functions of Cytoskeleton

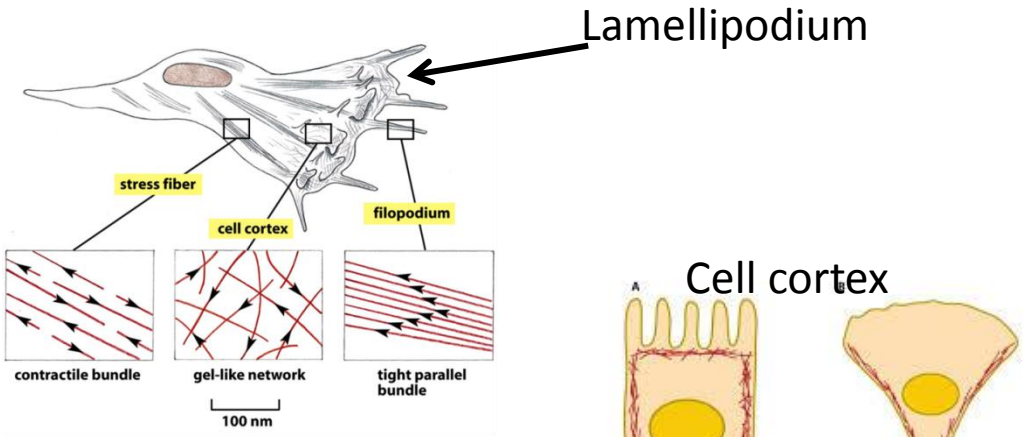
- Determine cell shapes and provide structure support
- Play roles in cell migration
- Anchor sites for organelle organization and enzymes in specific location in cells
- Phagocytosis
- Cell polarity
- Cell division/cytokinesis, etc

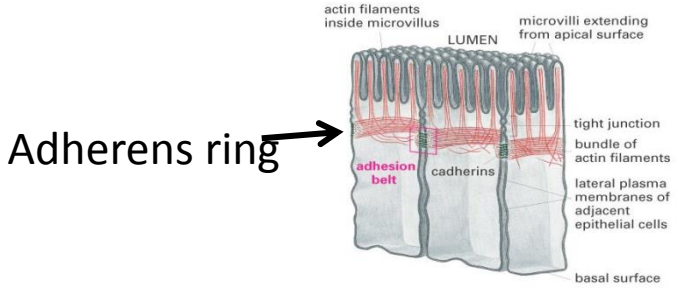
Regulation of cytoskeleton function by cell signaling in time and space

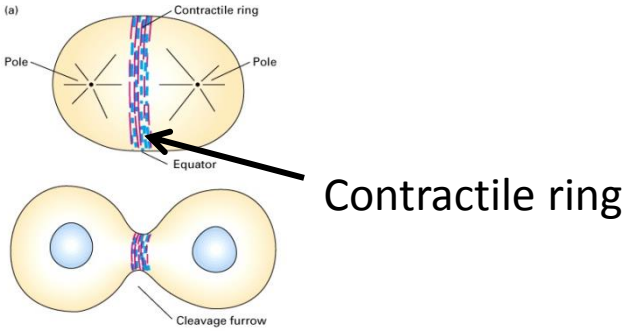


II. Microfilaments and actin structures

- Microvilli
- Cell cortex
- Adherens belt
- Filopodia
- Lamellipodium/leading edge
- Stress fibers
- Phagocytosis
- Moving endocytic vesicles
- Contractile ring







Actin

- Highly **conserved** across species, 80% homology between Amebas and animals
- Most **abundant** protein in cells (1-5 % cellular protein in non-muscle cells, 10% in muscle cells)
- **Three isoforms** (α-actin, β-actin, γ-actin)

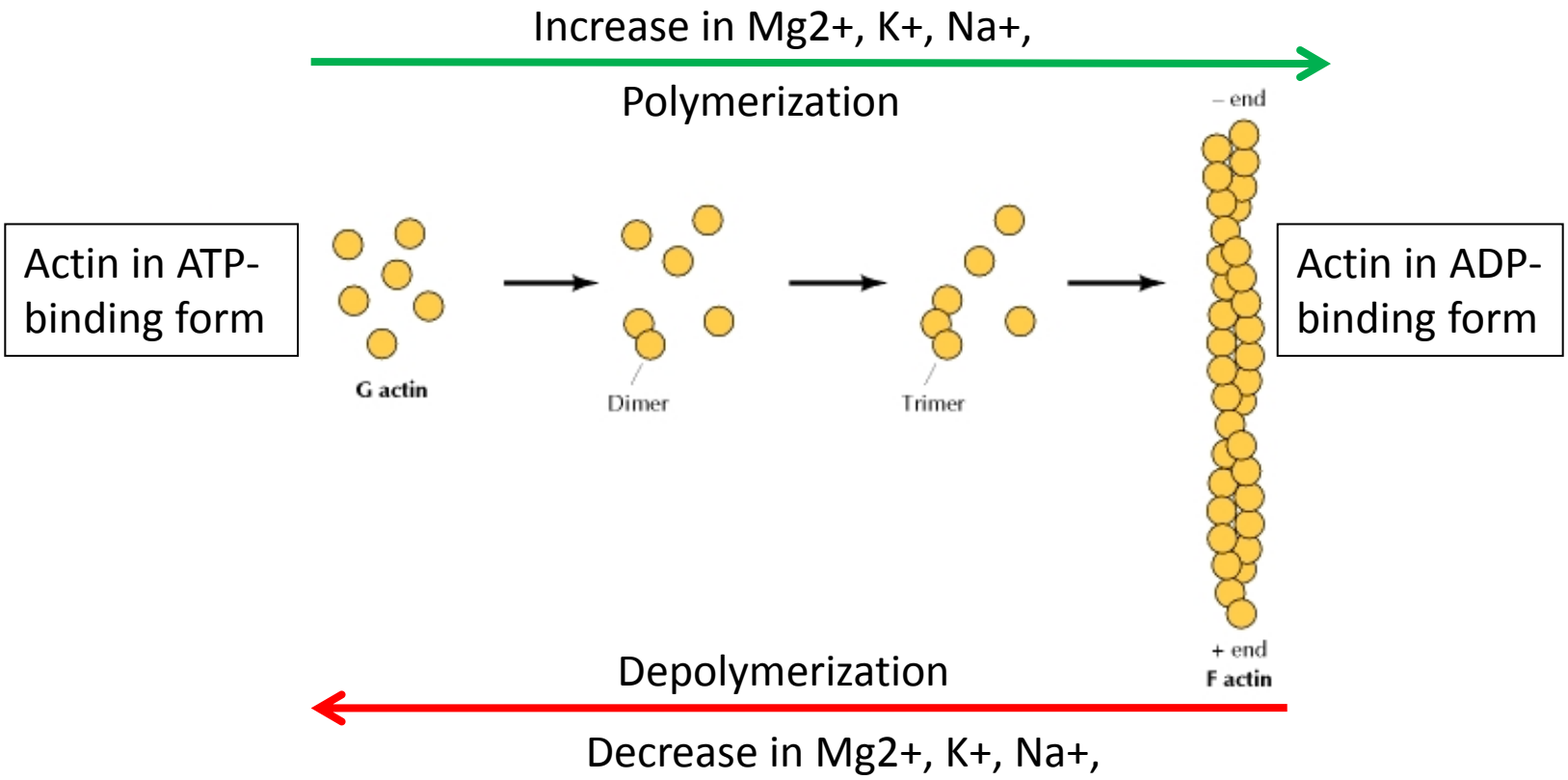
  α-actin--- contractile structure

  β-actin--- leading edge and cell cortex

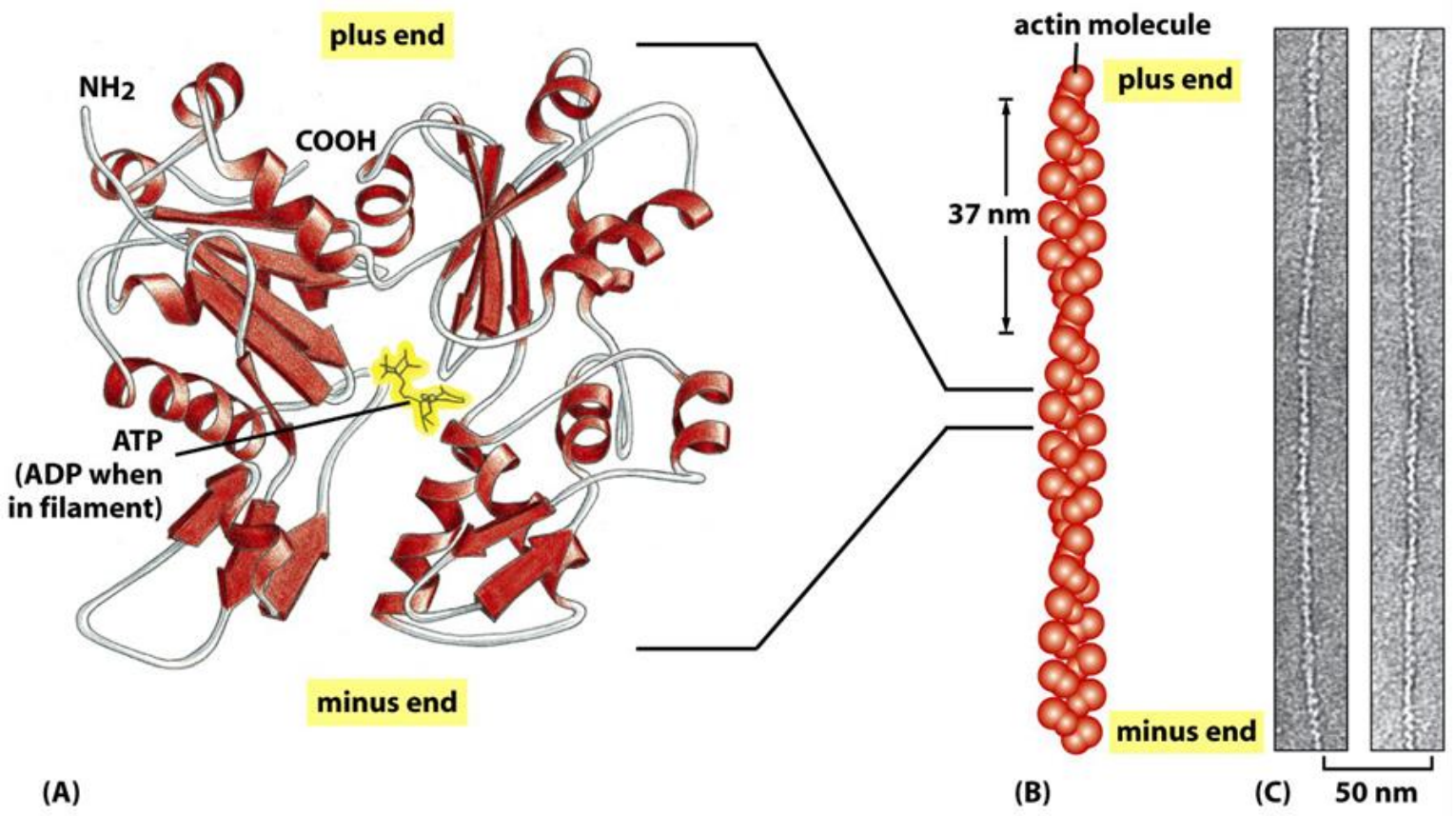
  γ-actin---stress fibers

G-actin and F-actin

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



Structures of monomeric G-actin and F-actin filaments



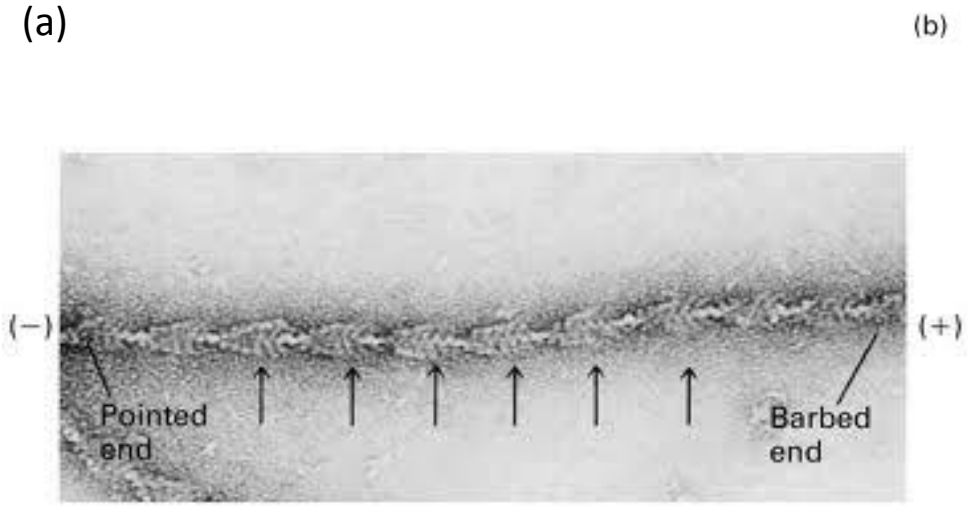
G-actin, two lobes with a deep cleft in between, binds to ADP/ATP and Mg2+

F-actin has structural and functional polarity

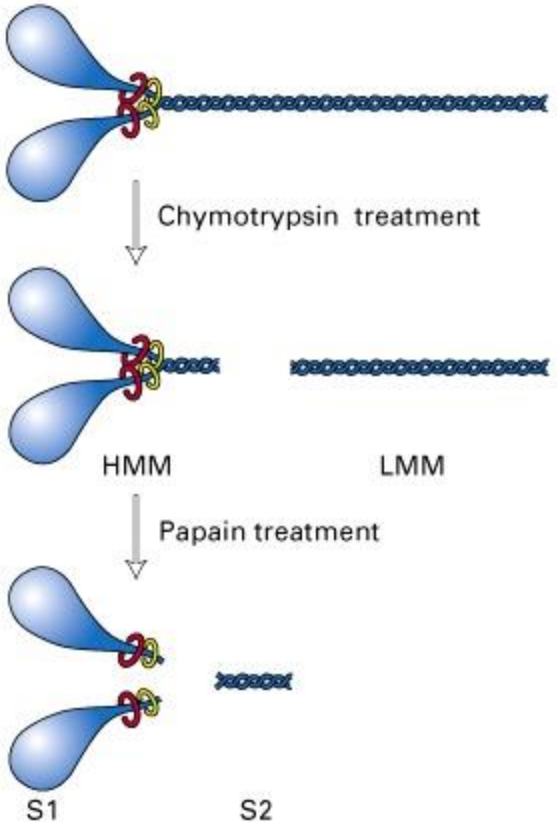
- All actin subunits are oriented the same way.
- “+” end: end that is favored for addition of actin subunits; ATP-binding cleft of the terminal actin subunits contacts the neighboring subunits
- “-” end: end that is favored for subunit dissociation; ATP-binding cleft of the terminal actin subunits is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

How to demonstrate the polarity of an actin filament?

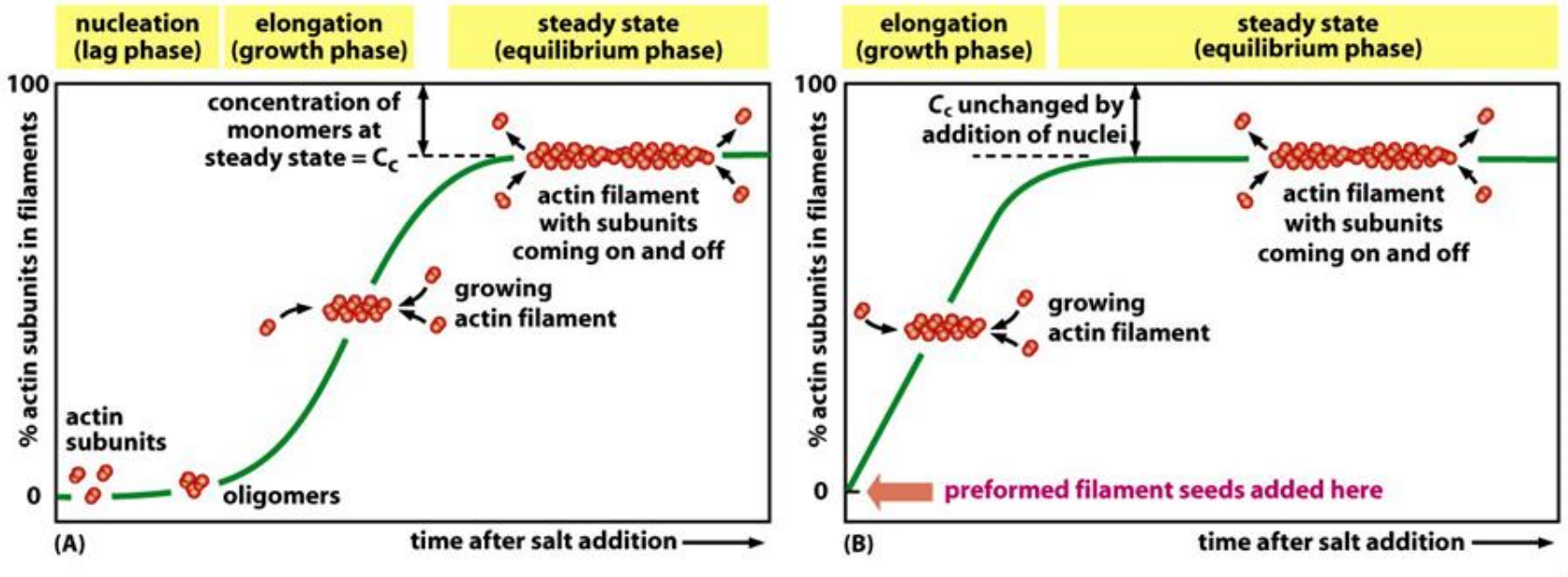
Myosin S1 decoration experiment



EM image



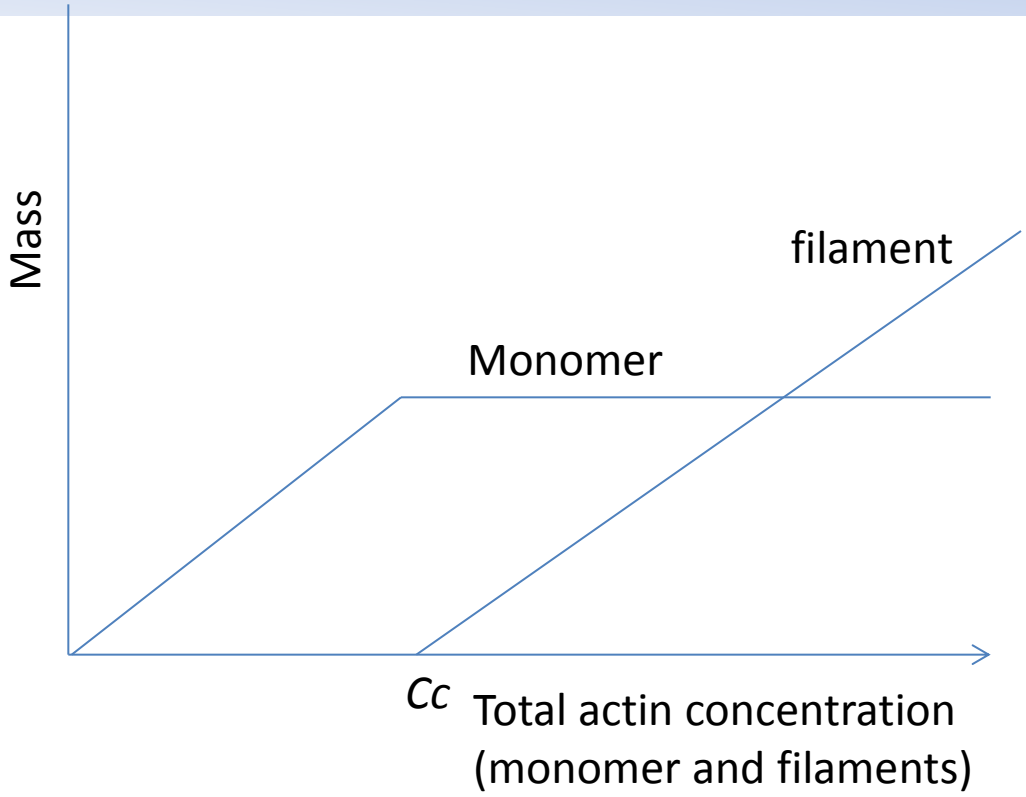
II. Dynamics of actin filaments



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 concentrations (*Cc*)

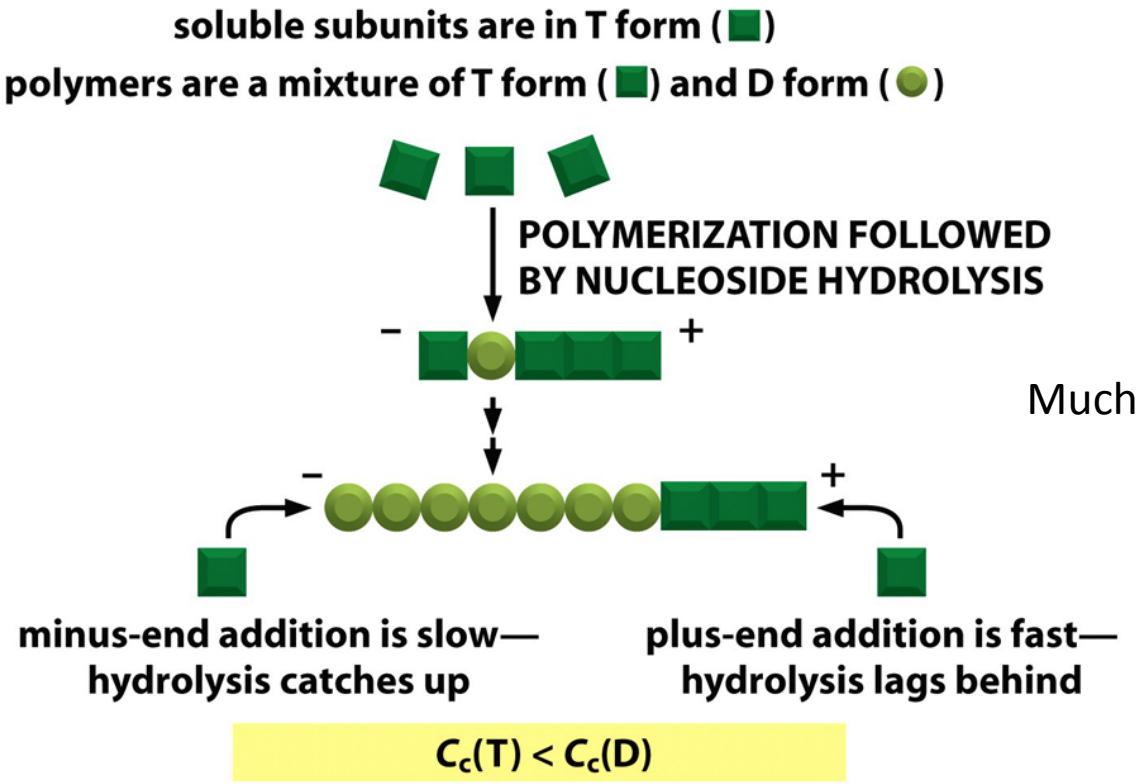


In cells, G-actin levels can be 0.1-0.4mM, *Cc* is ~0.2µM

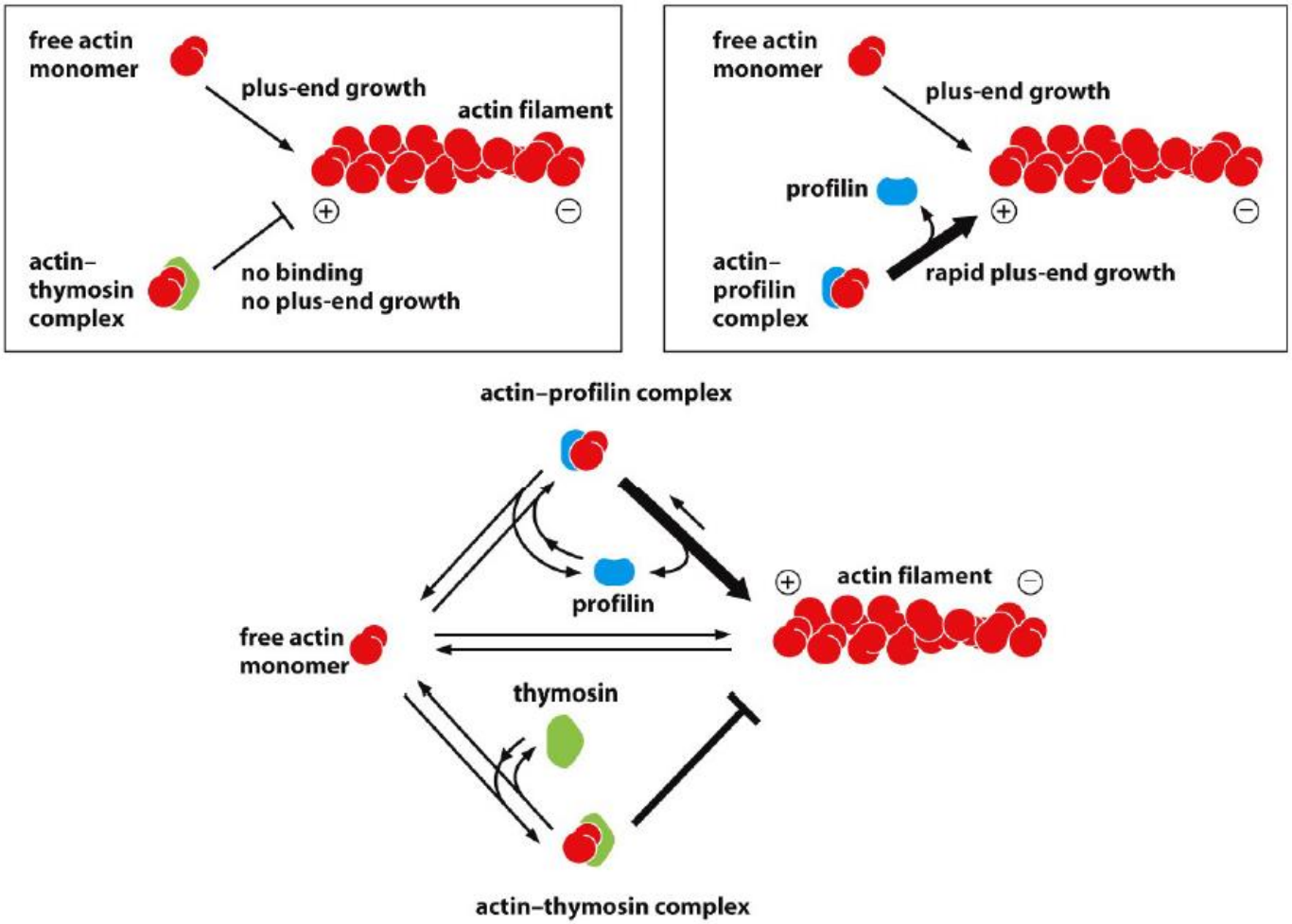
Definition of *Cc*:
Concentration of free G-actin at which the assembly onto a filament end is balanced by loss from that end.

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 THYMOSIN FOR BINDING TO ACTIN MONOMERS AND PROMOTES ASSEMBLY

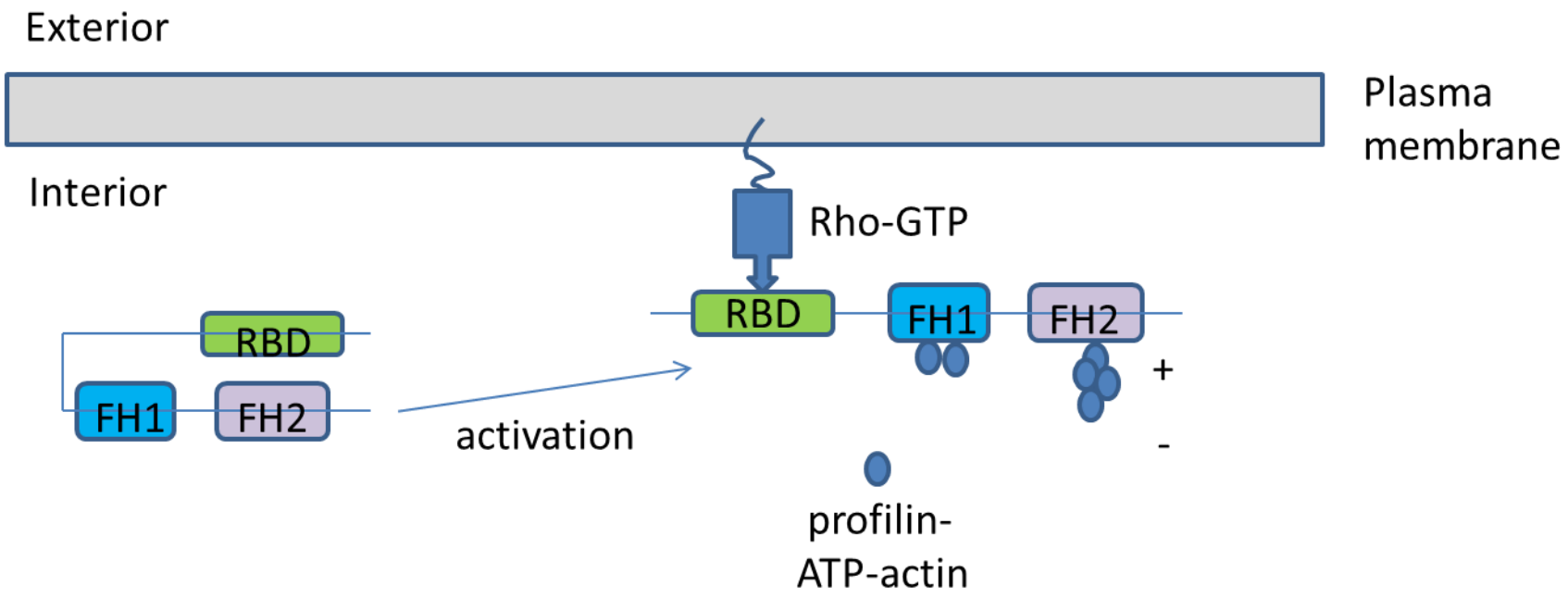
Figure 16-15 Molecular Biology of the Cell 6e (© Garland Science 2015)

IV. Mechanisms of actin filament assembly

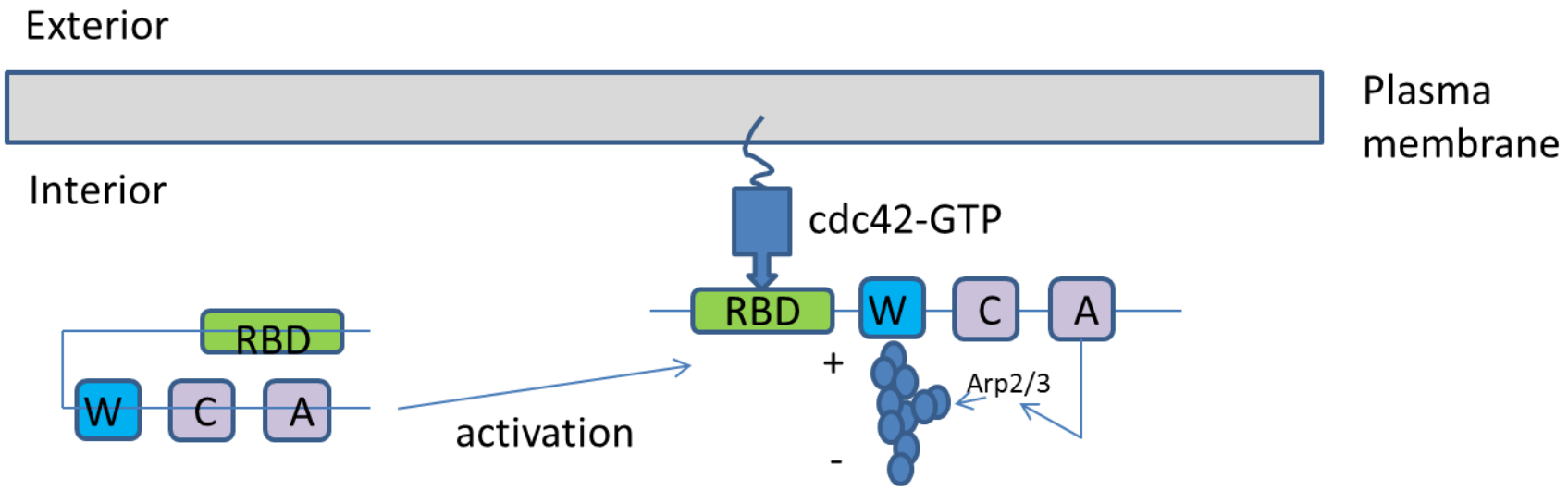
" nucleation" is the rating limiting step,
what is controlling this critical step?

- Two major classes of actin nucleating proteins:
  1. Formin protein family: long filament assembly
  2. Arp2/3 complex: branched filament assembly

Regulation of formins by Rho-GTPs



How is Arp2/3 complex regulated by WASp



Formin mediates straight filament assembly

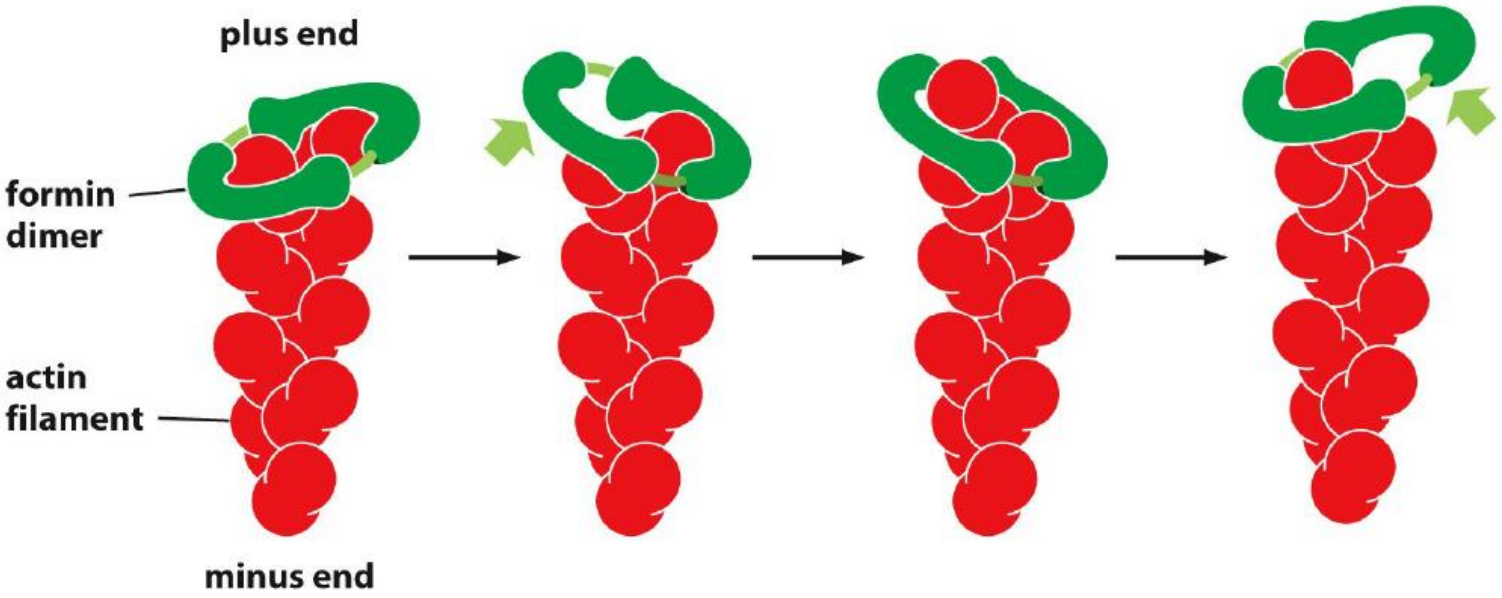


Figure 16-17 Molecular Biology of the Cell 6e (© Garland Science 2015)

Arp2/3 mediates branched filament assembly

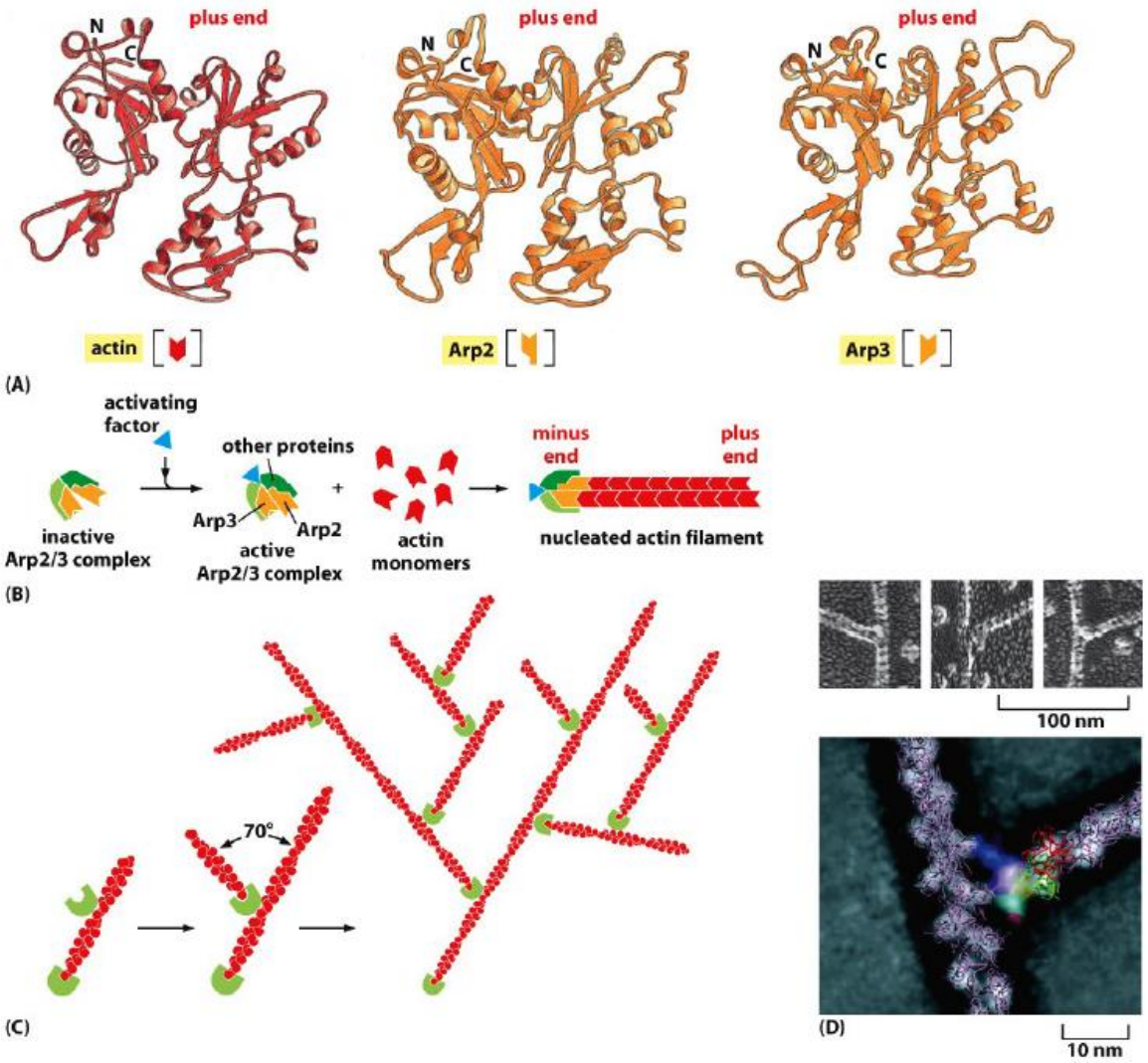
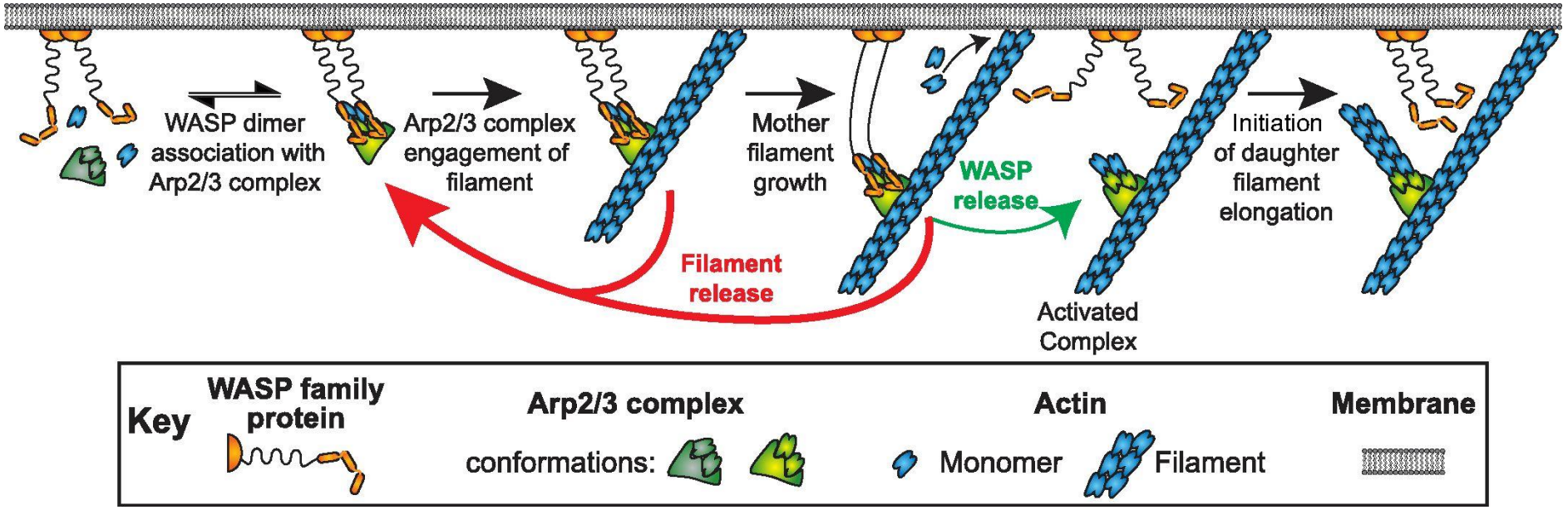


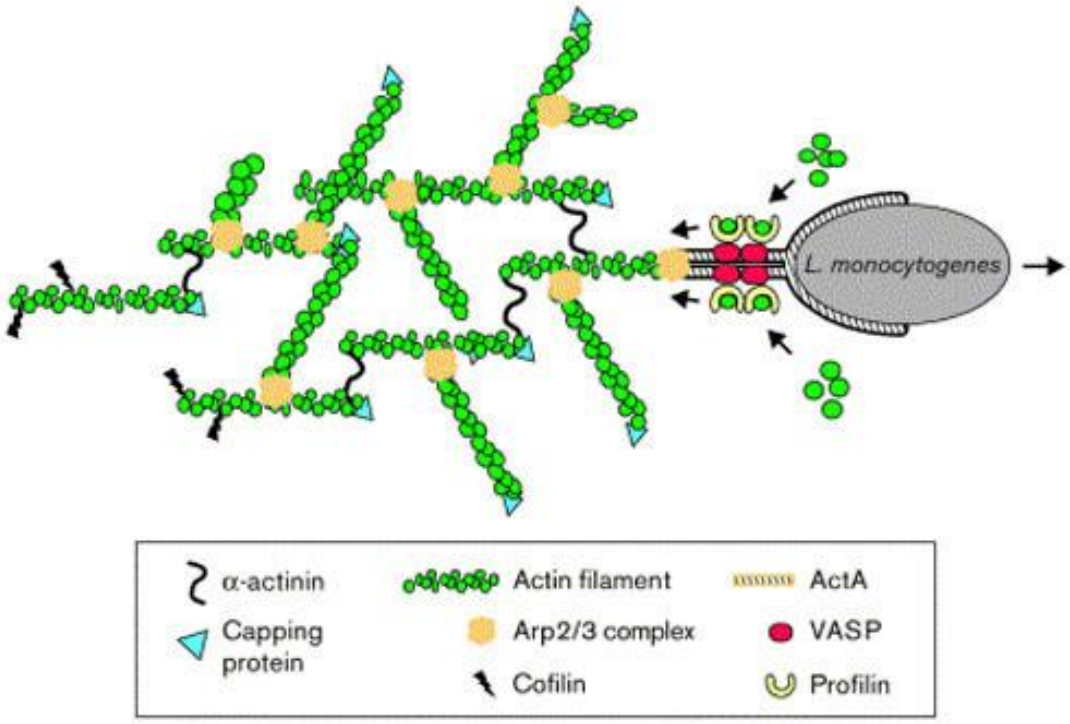
Figure 16-16 Molecular Biology of the Cell 6e (© Garland Science 2015)

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 cell surface protein ActA functions as a NPF, which interacts with VASP
Help to recruit Arp2/3 and enhance ATP-actin assembly.

Toxins that perturb actin dynamics

- Microfilament depolymerization drugs:

1. Cytochalasin D: a fungal alkaloid binds to "+" end of F-actin, blocks addition of subunits.

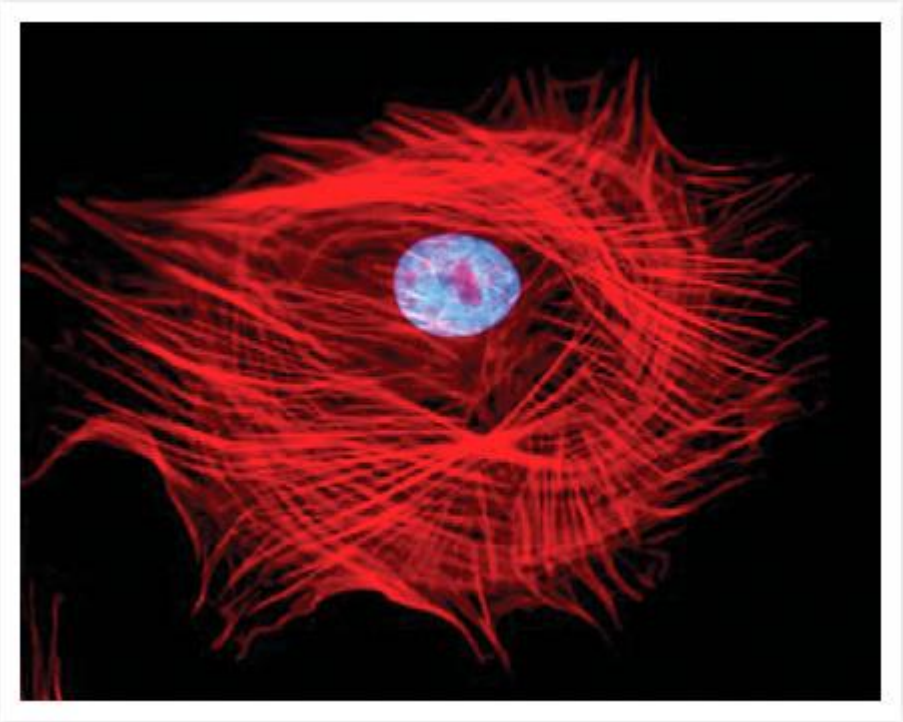
2. Latrunculin: binds to and sequesters G-actin, inhibiting its addition into a filament end.

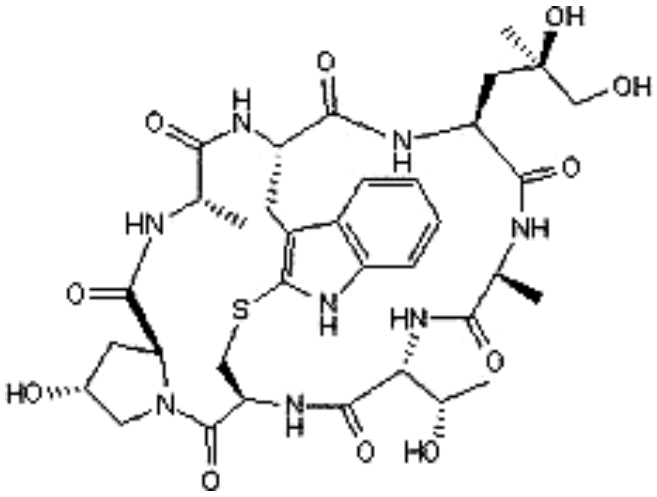
- microfilament polymerization drugs:

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.

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

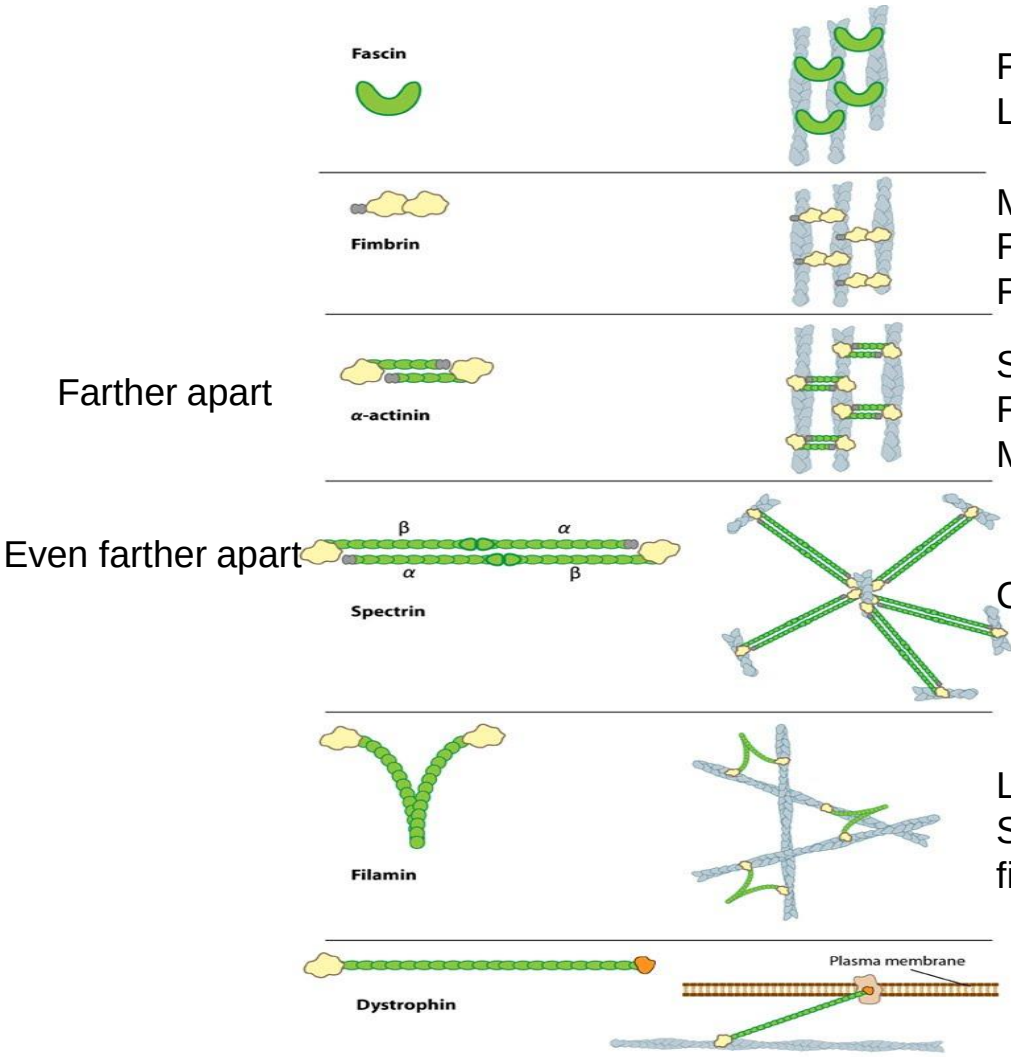




V. Organization of actin-based cellular structures

Various actin filament crosslinking proteins:

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



Actin network in cells

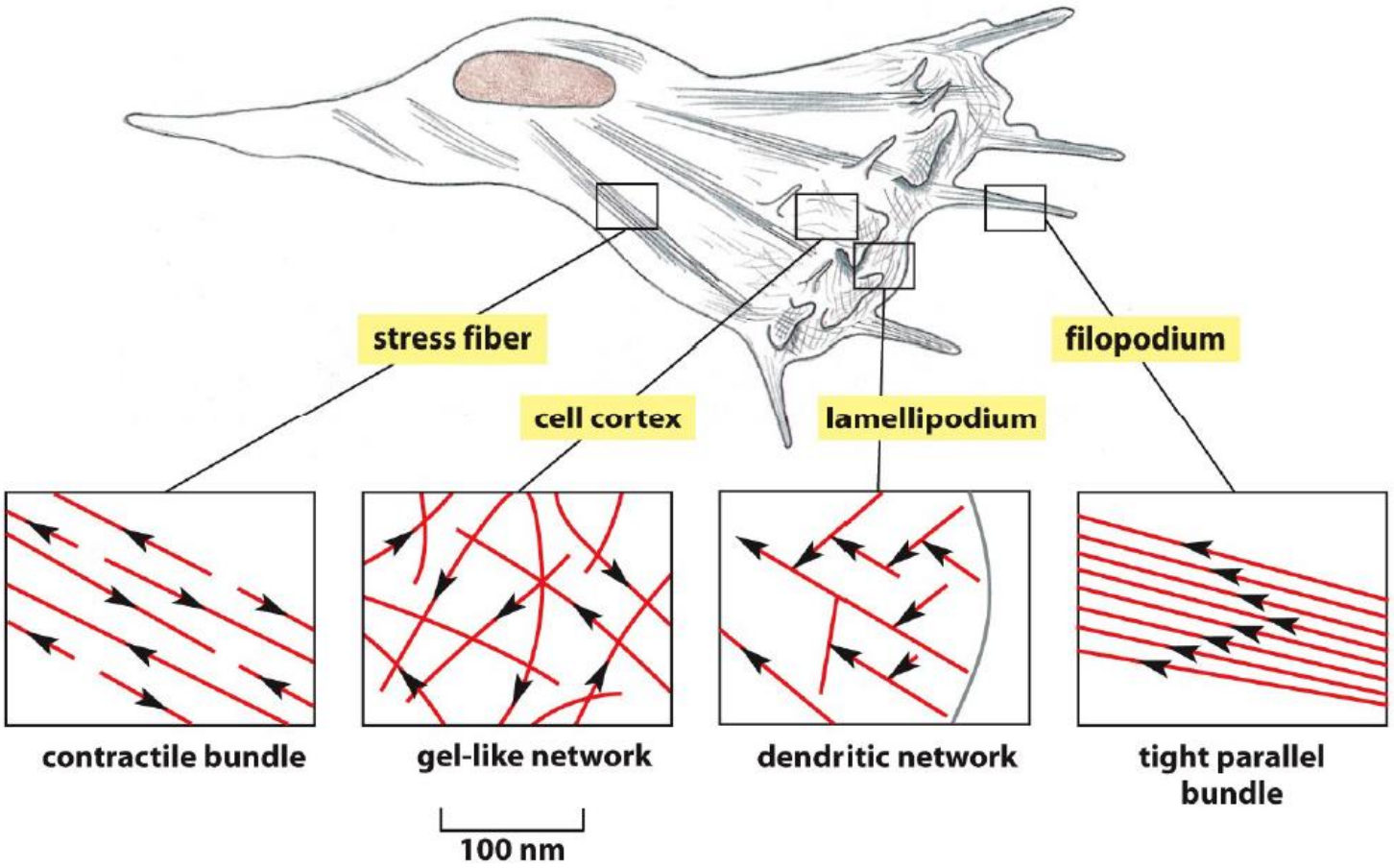
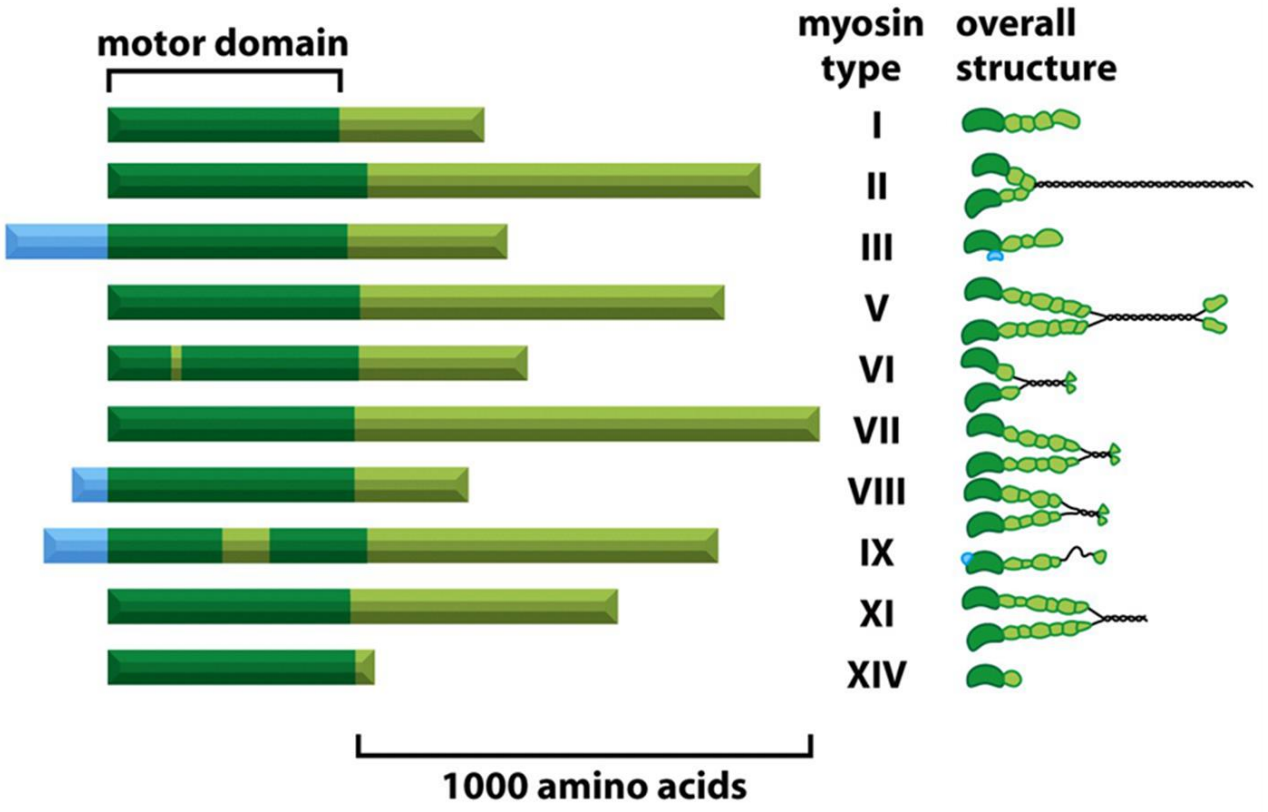


Figure 16-21 Molecular Biology of the Cell 6e (© Garland Science 2015)

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

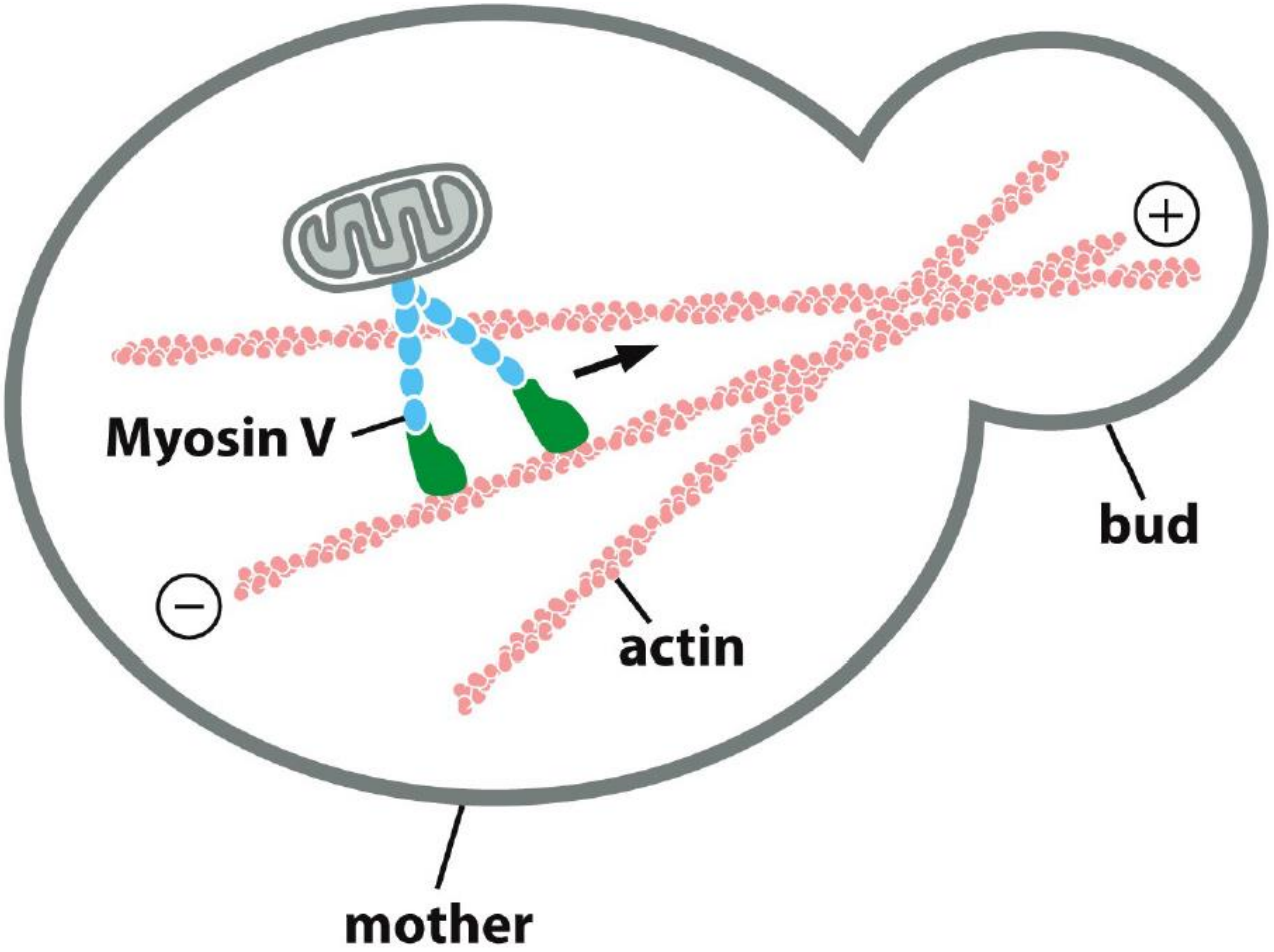
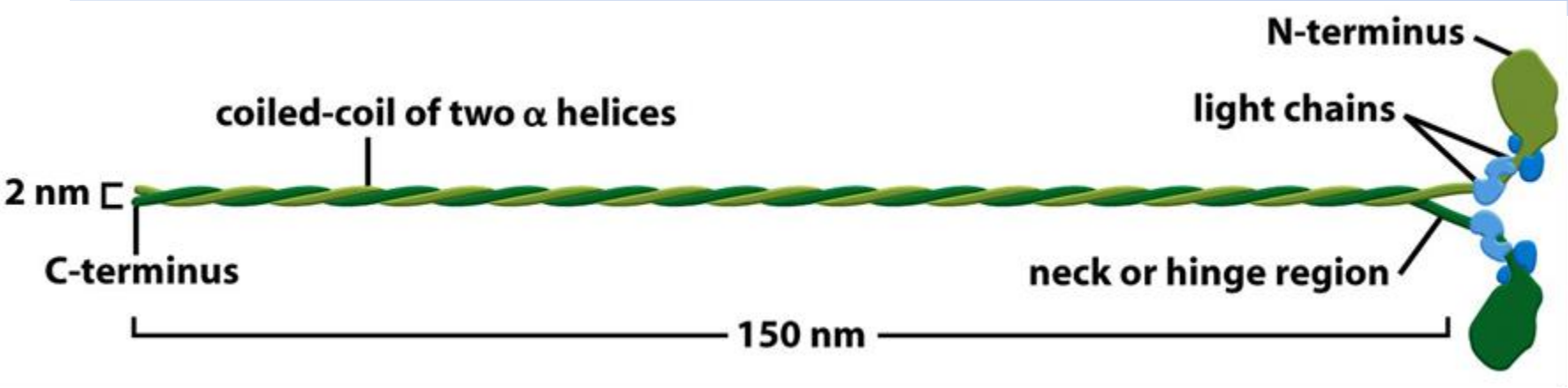


Figure 16-41b Molecular Biology of the Cell 6e (© Garland Science 2015)

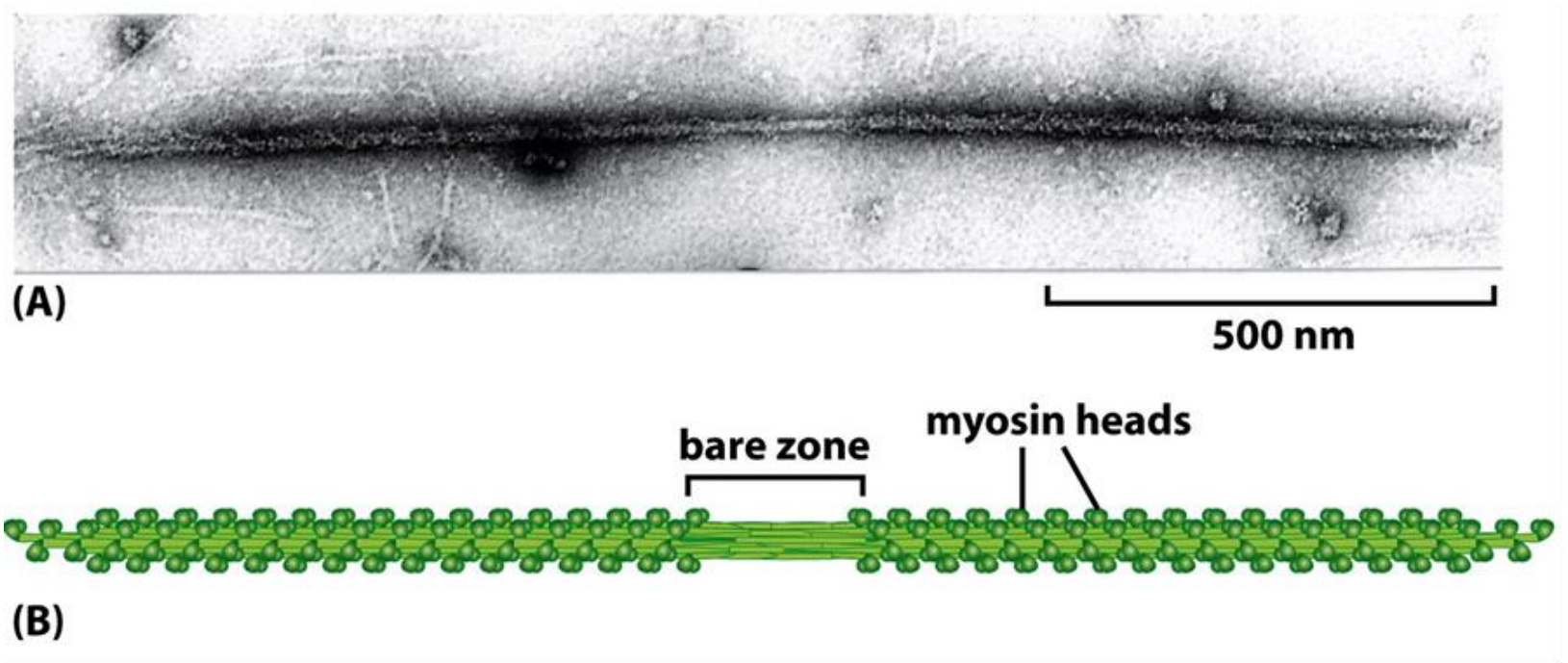
Structure of Myosin II



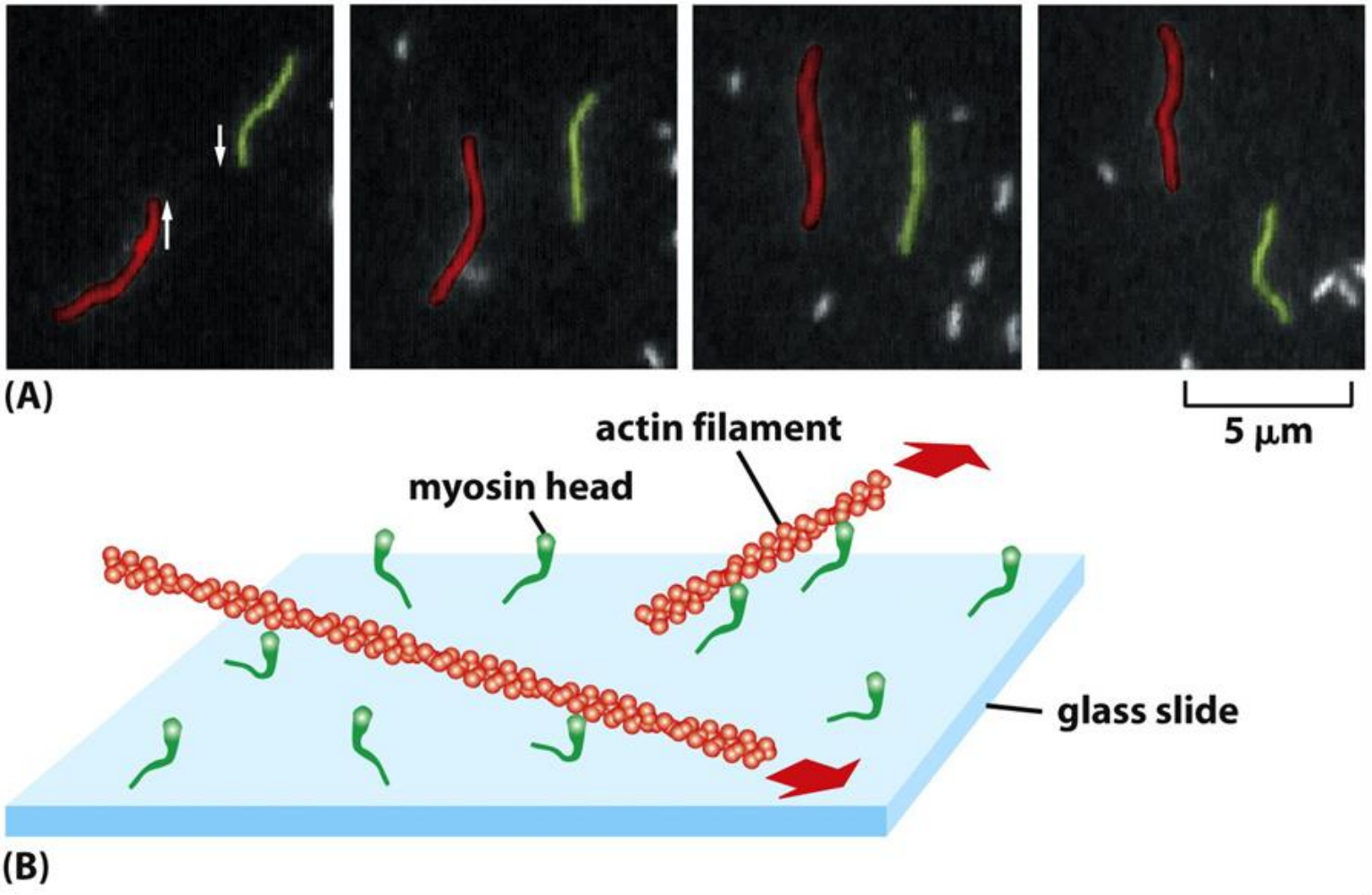
1. Head : S1 fragment , ATPase activity, actin binding sites
2. Neck : light chains binding
3. Tail: intertwining of two tail helices

2 heavy chains
2 essential light chains
2 regulatory light chains

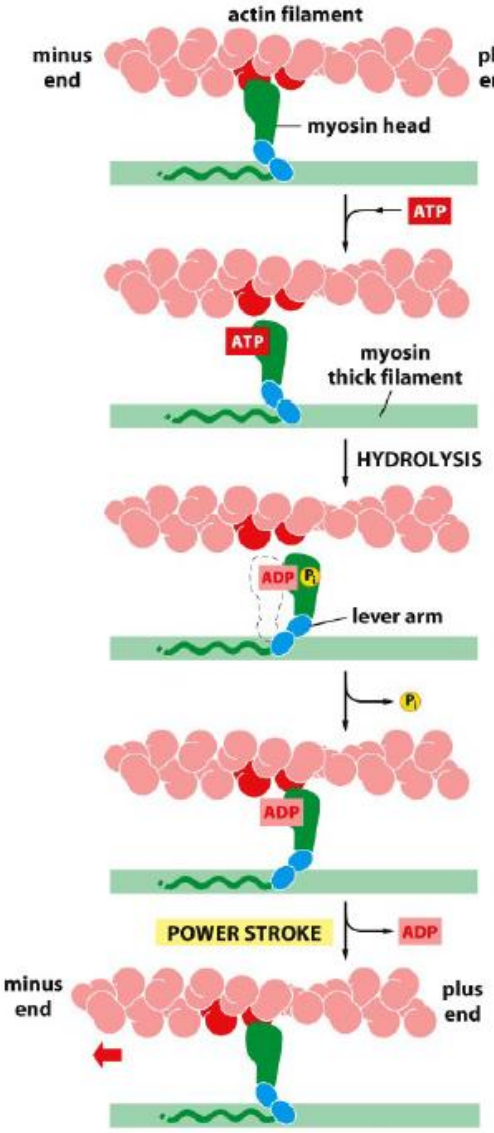
Myosin II are arranged in a bipolar manner in skeletal muscle



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 (P_i) 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.

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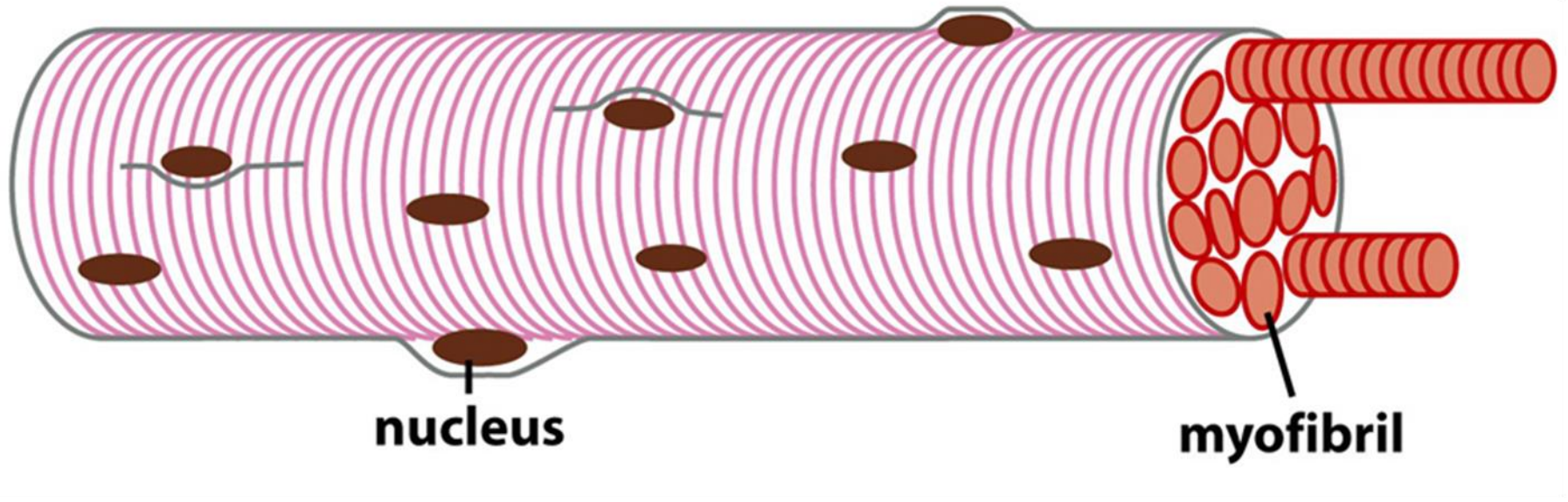
VII. Myosin-powered movements

1. Mechanism of muscle contraction: Myosin II
    1). Structure of skeletal muscle
    2). Mechanism of contraction
    3). Regulation of muscle contraction by Ca2+ and cAMP

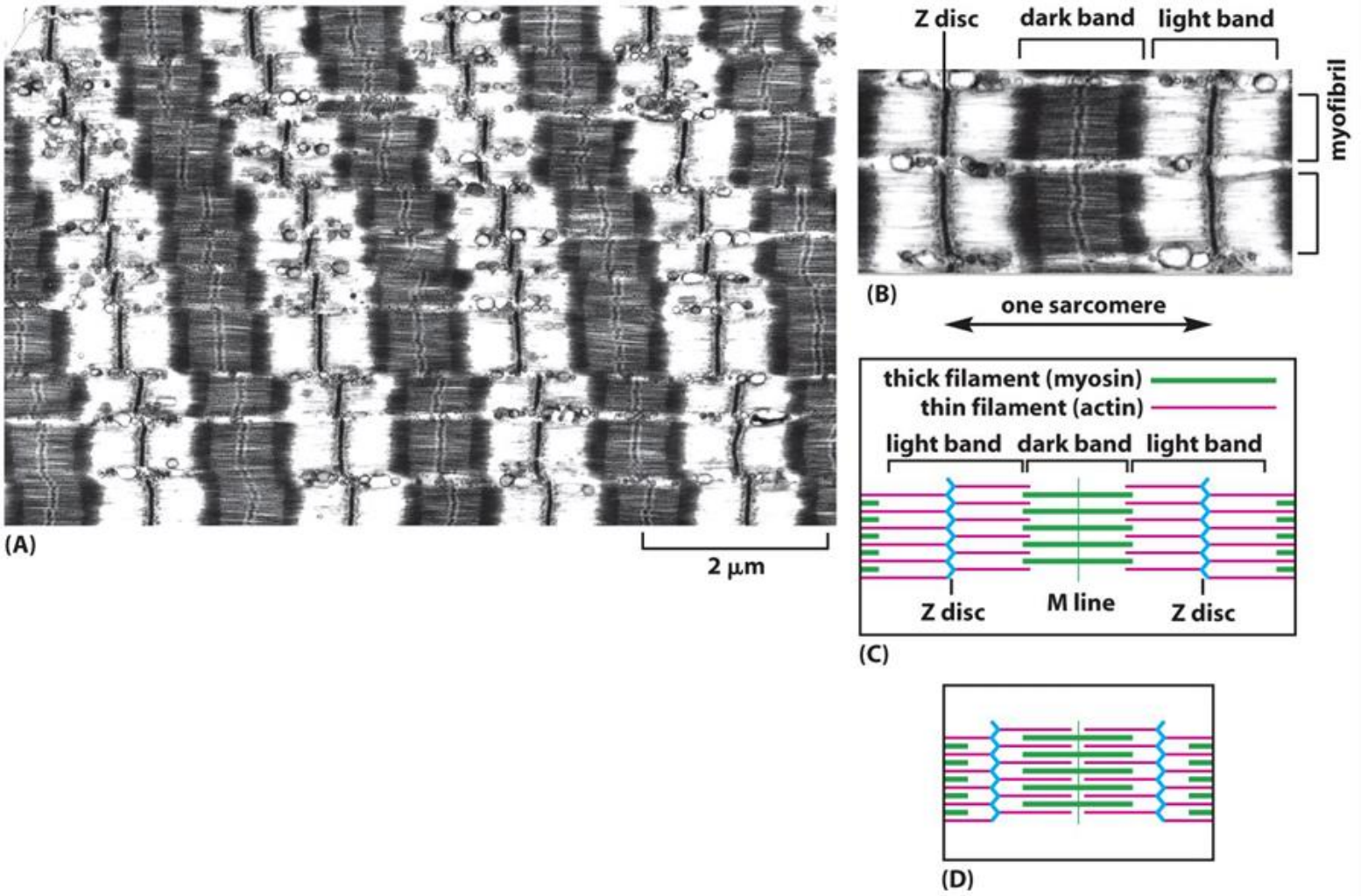
2. Mechanism of vesicle/organelle transport: Myosin V

1). Detailed structure of muscle

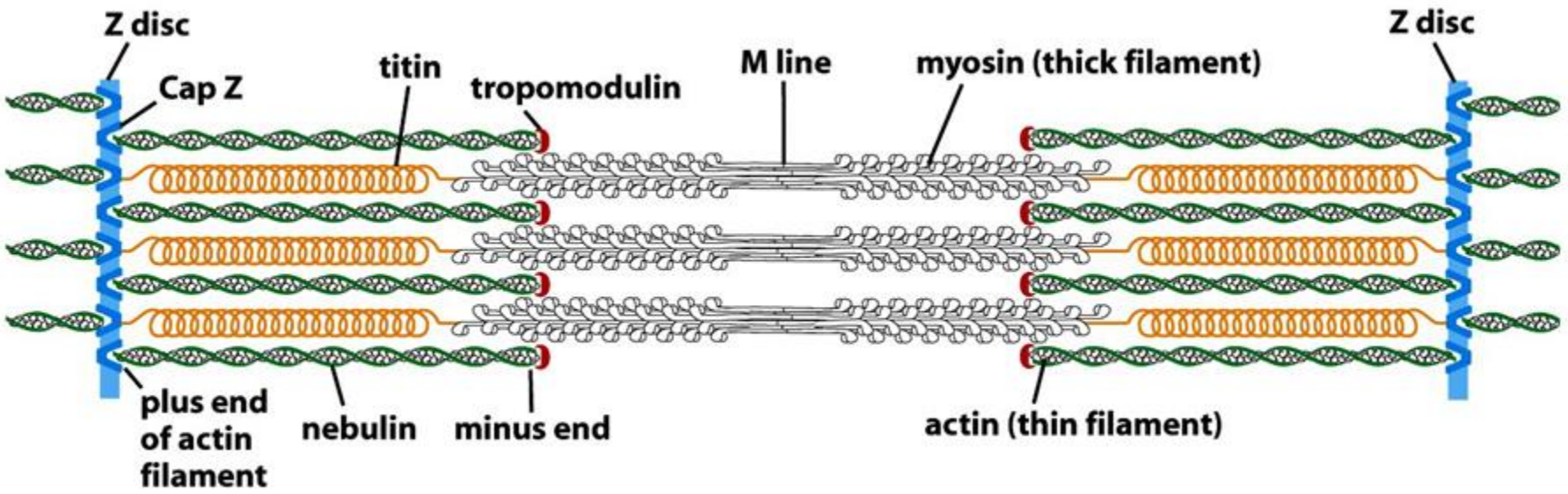
The structure of muscle cell:



Skeletal muscle myofibrils

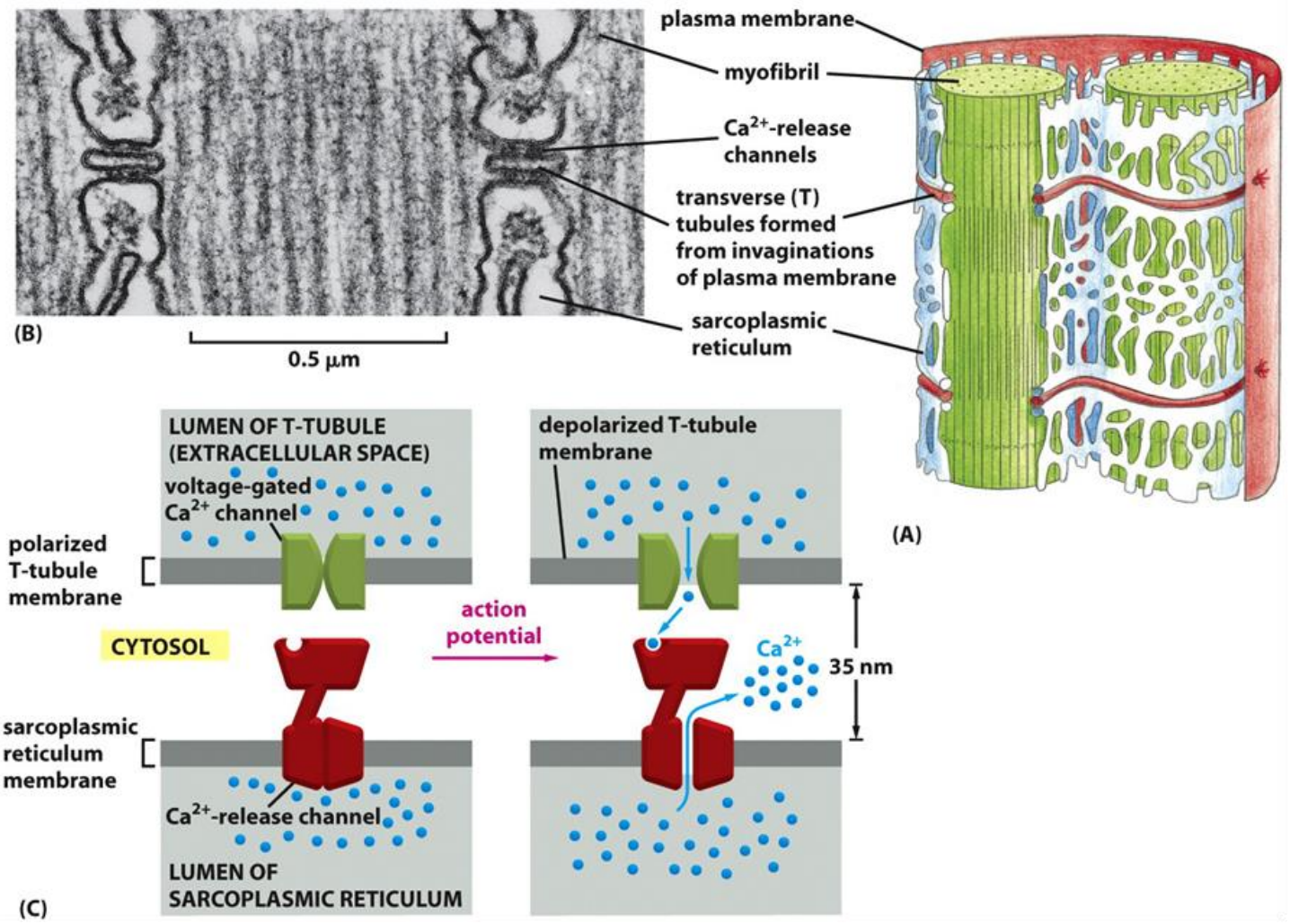


Organization of accessory protiens in a sarcomere

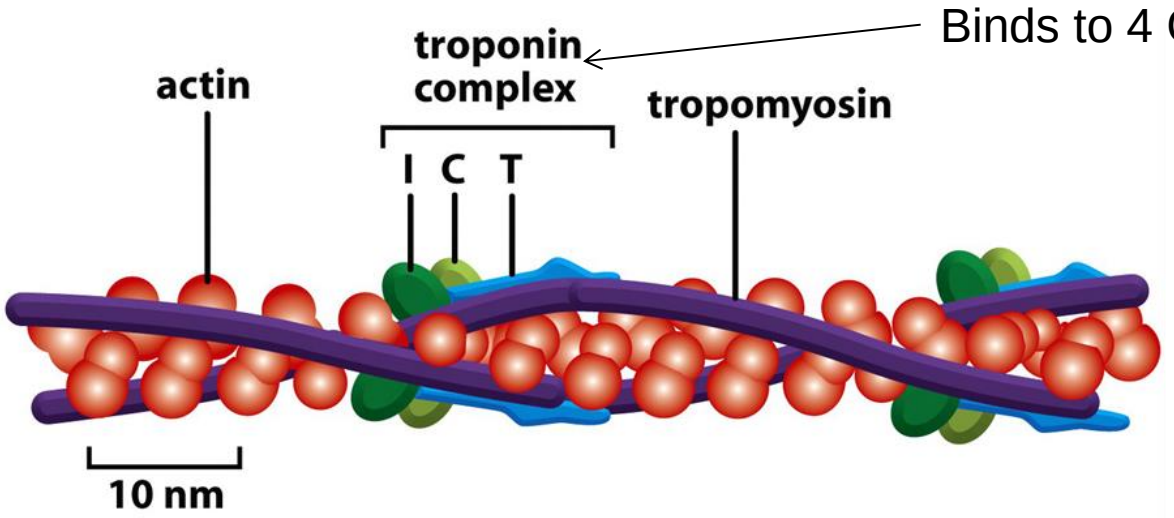


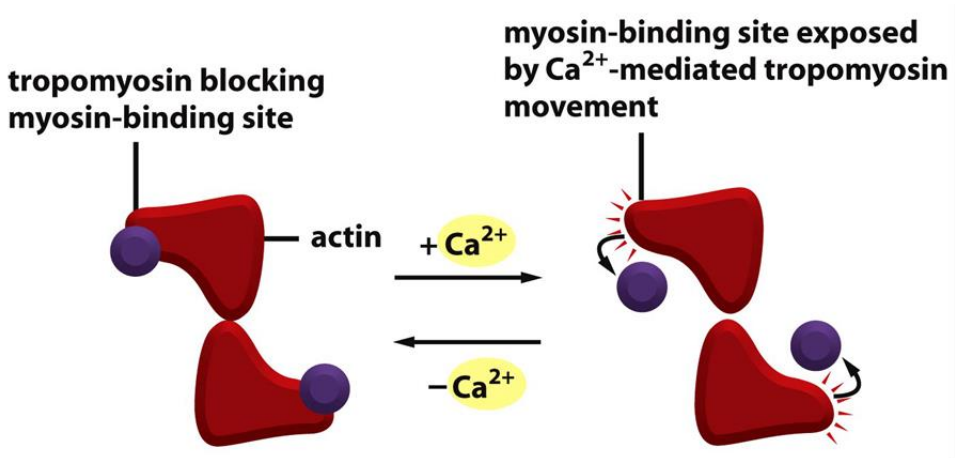
Nebulin provide scaffold and structural support, molecular ruler.
Titin is a molecular spring
Cap Z and $\alpha - actinin$ on the Z-line
Tropomodulin on the minus end.

T tubules(invagination from plasma membrane) and the Sarcoplasmic Reticulum



2) The control of skeletal muscle contraction by troponin and tropomyosin





3) Muscle contraction is additionally regulated by myosin II phosphorylation

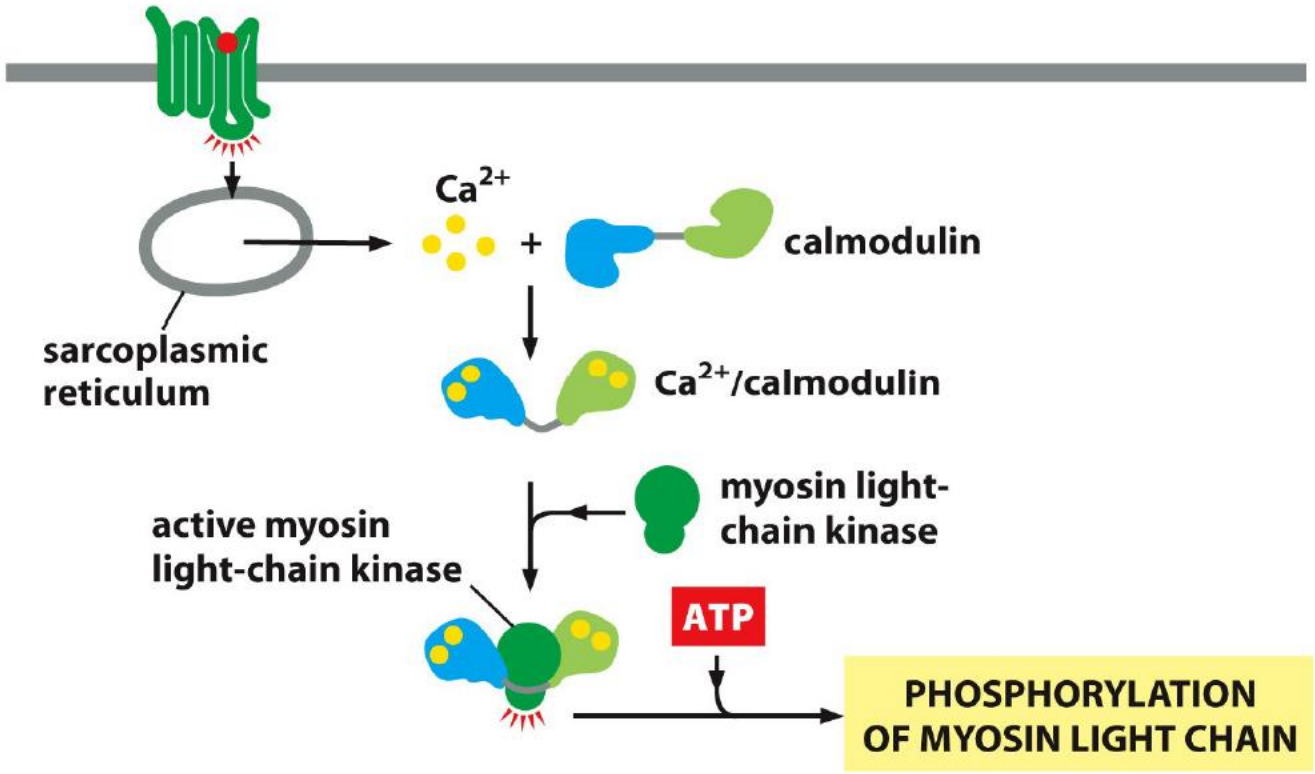
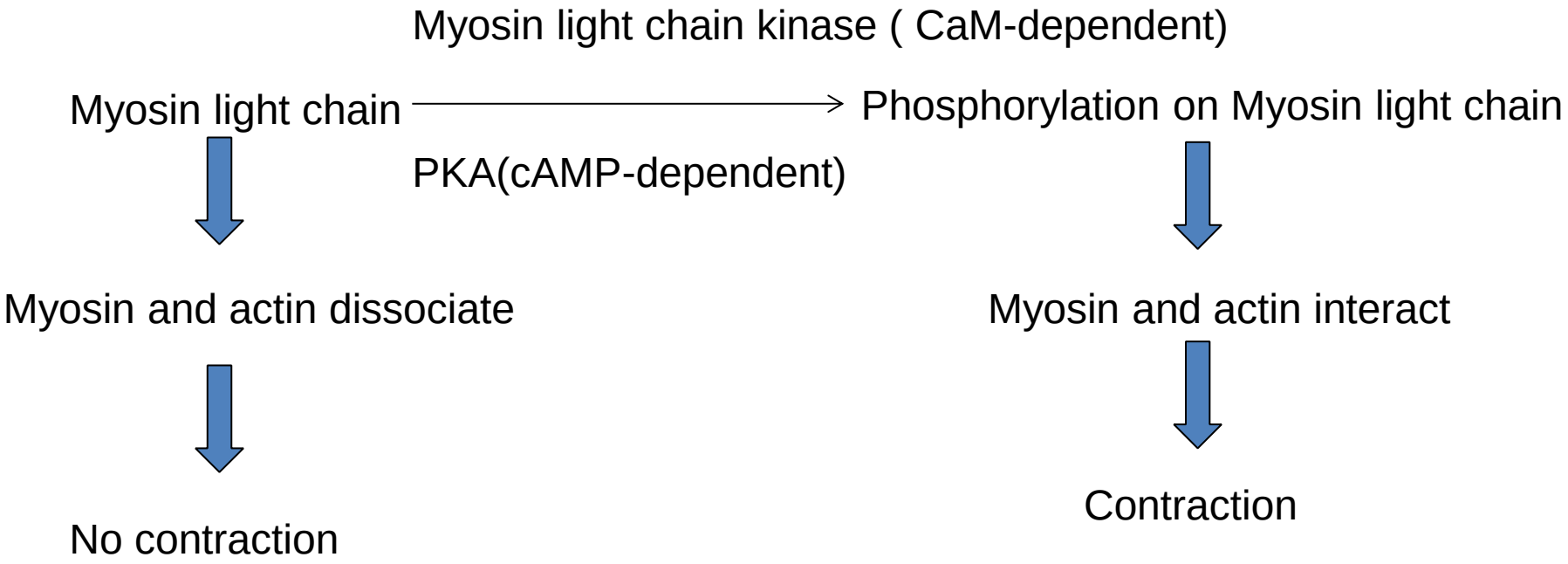
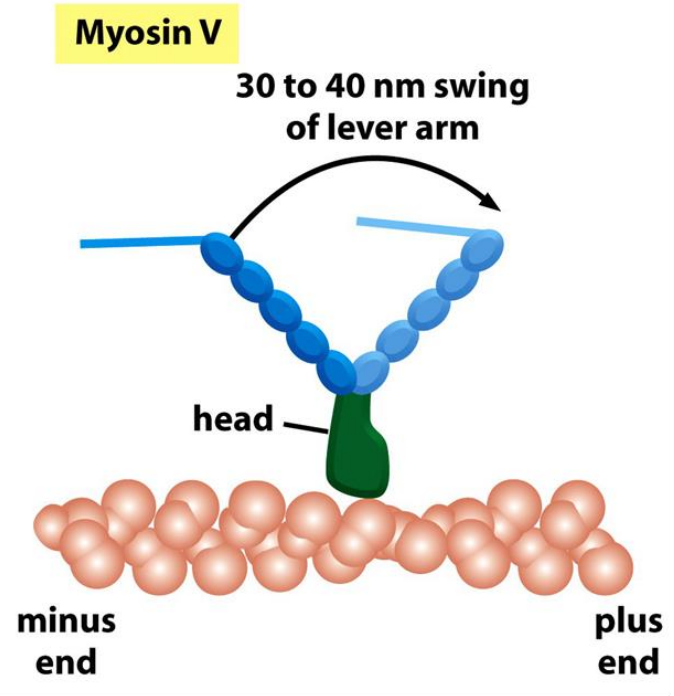
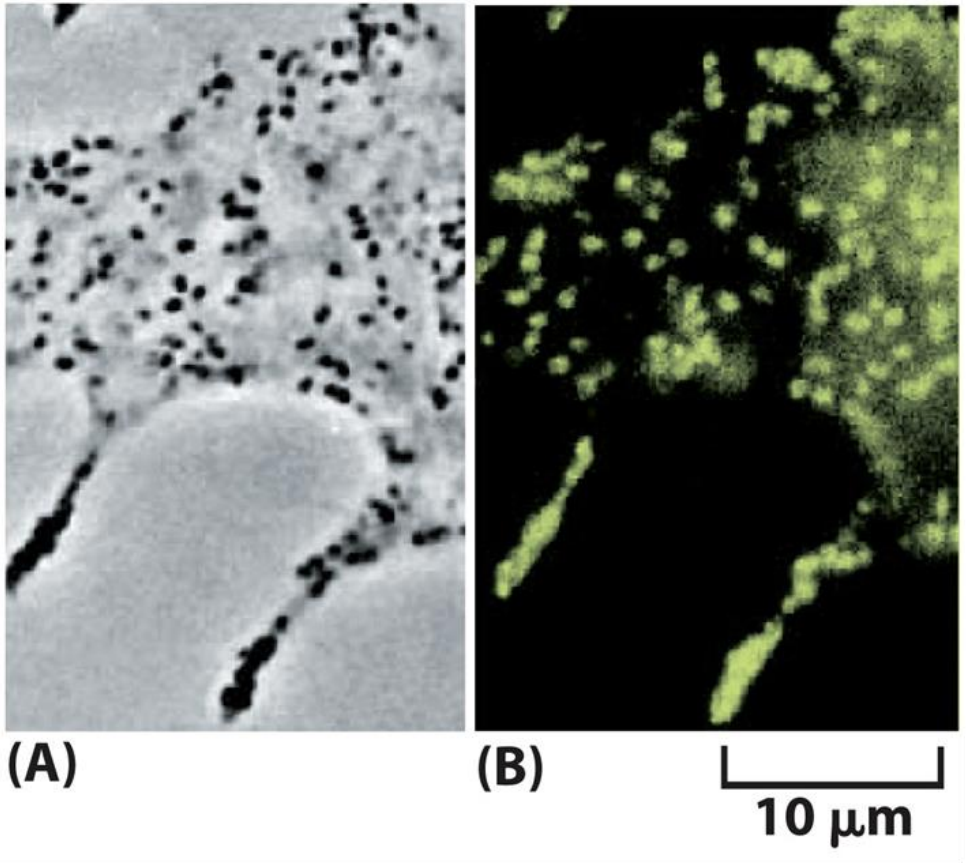


Figure 16-37a Molecular Biology of the Cell 6e (© Garland Science 2015)

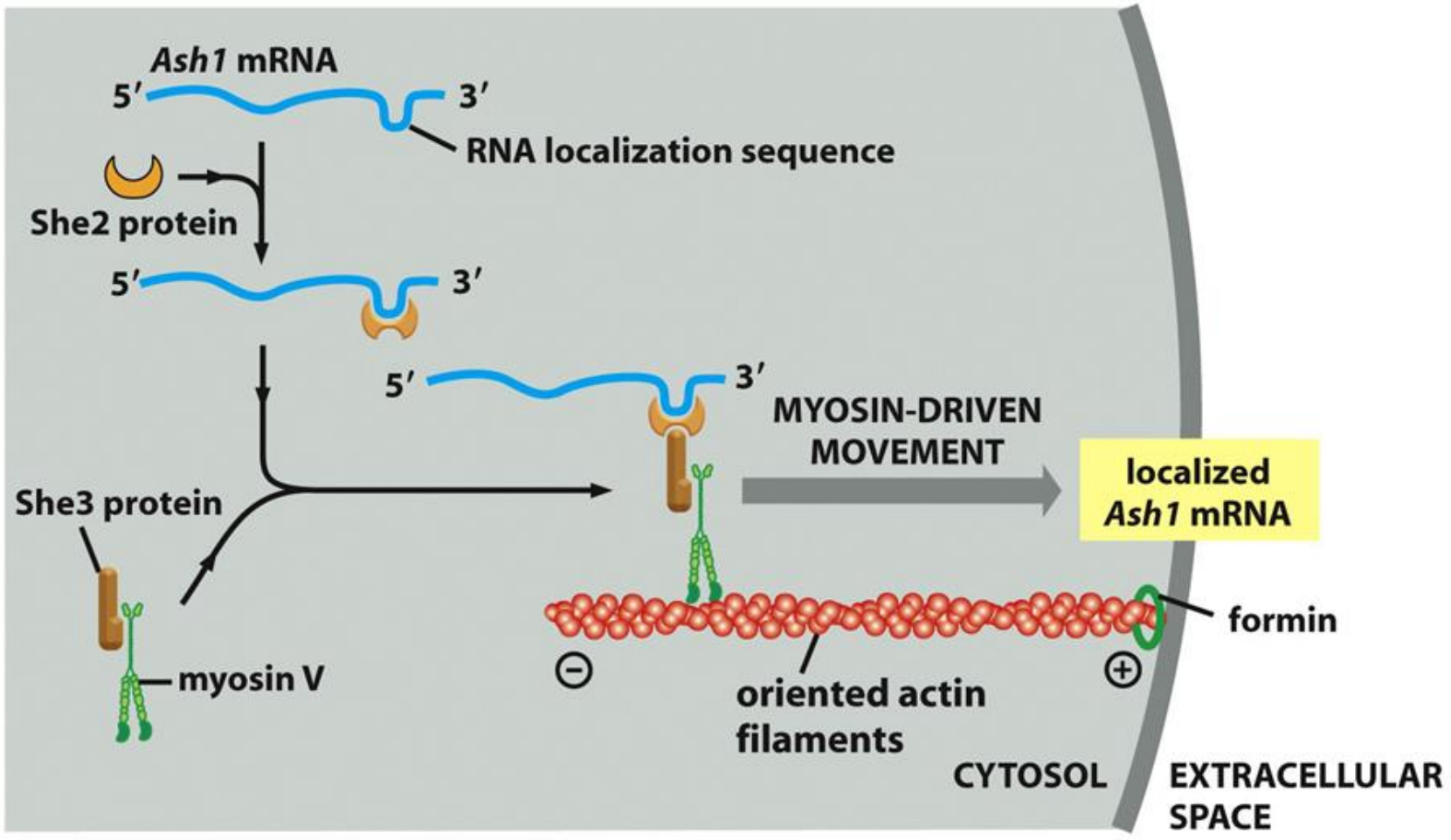

Myosin light chain kinase (CaM-dependent)
Myosin light chain
Phosphorylation on Myosin light chain
PKA(cAMP-dependent)
Myosin and actin dissociate
Myosin and actin interact
No contraction
Contraction

2. Myosin V for organelle/mRNA transport





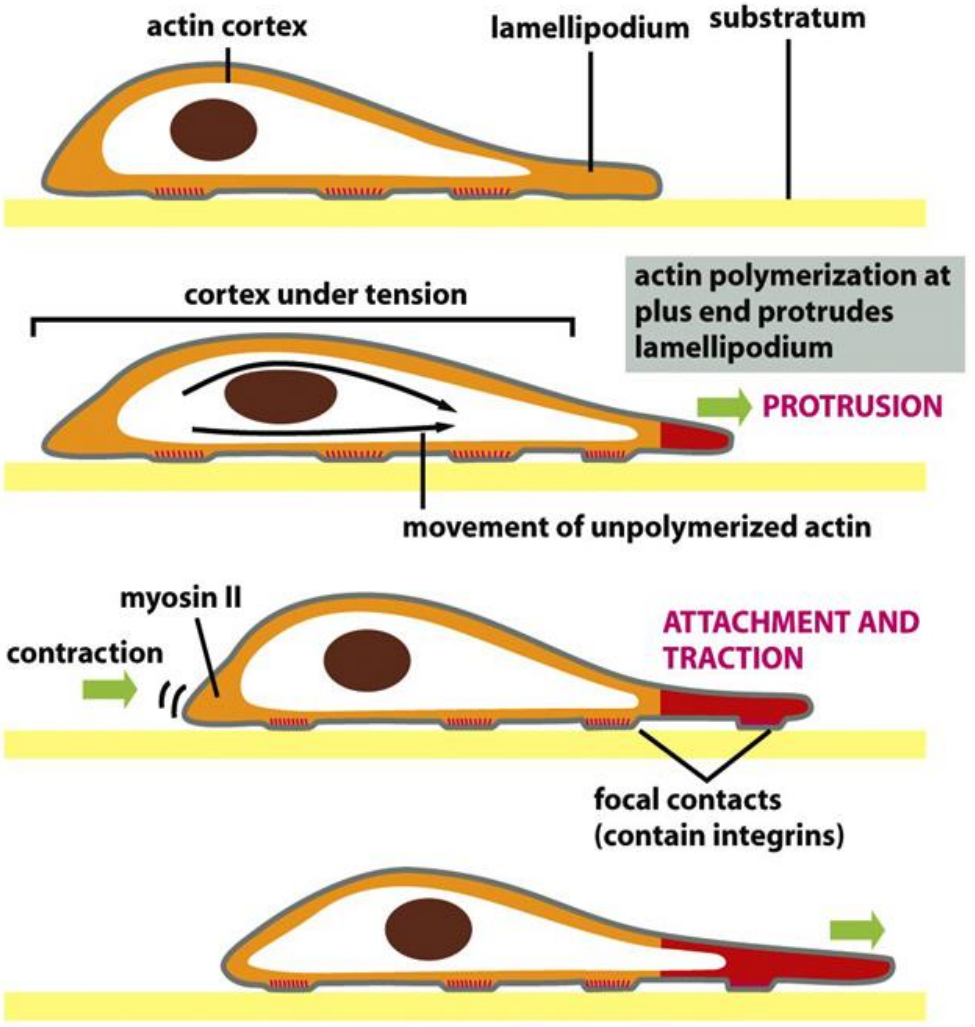
Localized mRNA by Myosin V



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



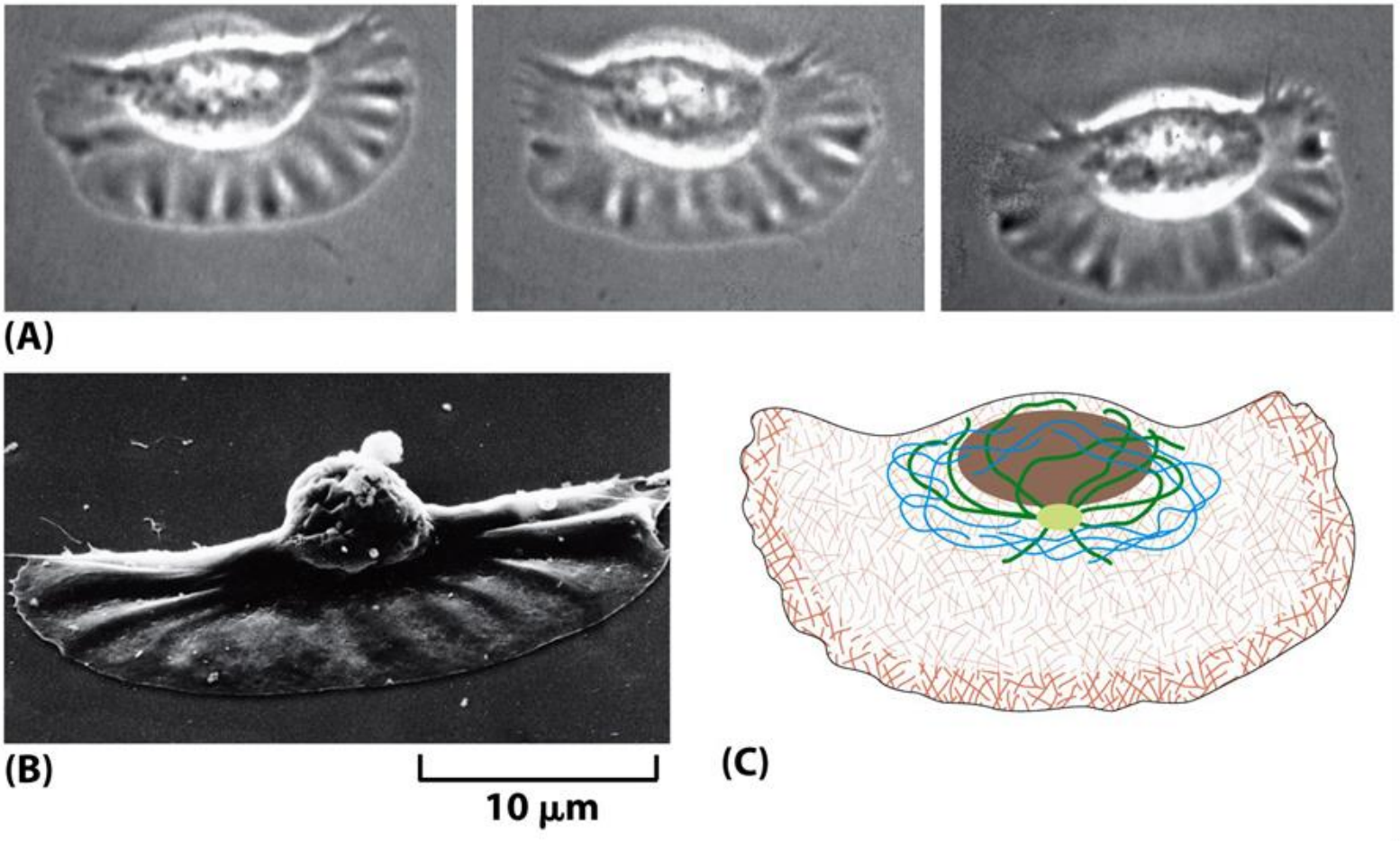
Filopodia, lamellipodia, pseudopodia

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

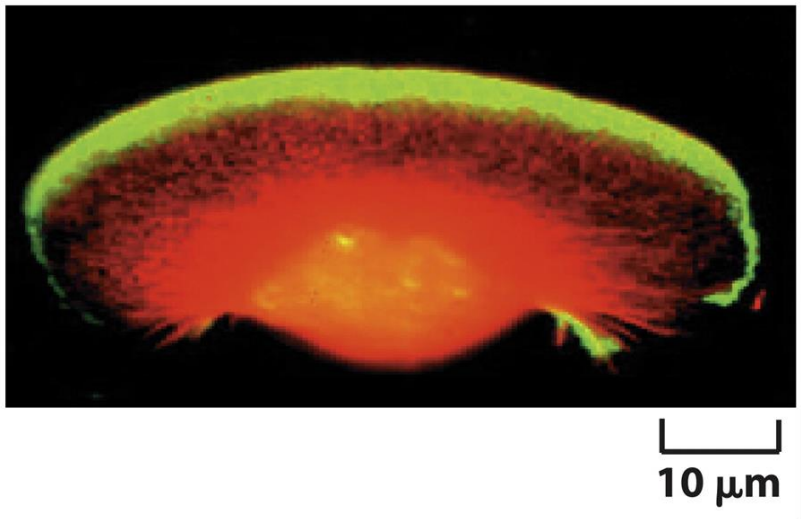
Lamellipodia: two dimensional, sheet like structures, cross-linked mesh of actin filaments lie parallel to the solid substratum, epithelia, fibroblast, and some neurons.

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

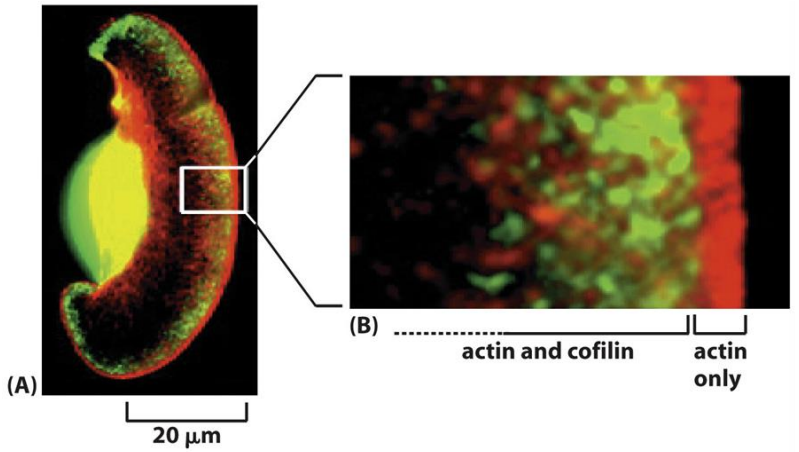
Cell leading edge in migration



Localization of different actin regulation proteins in the leading edge

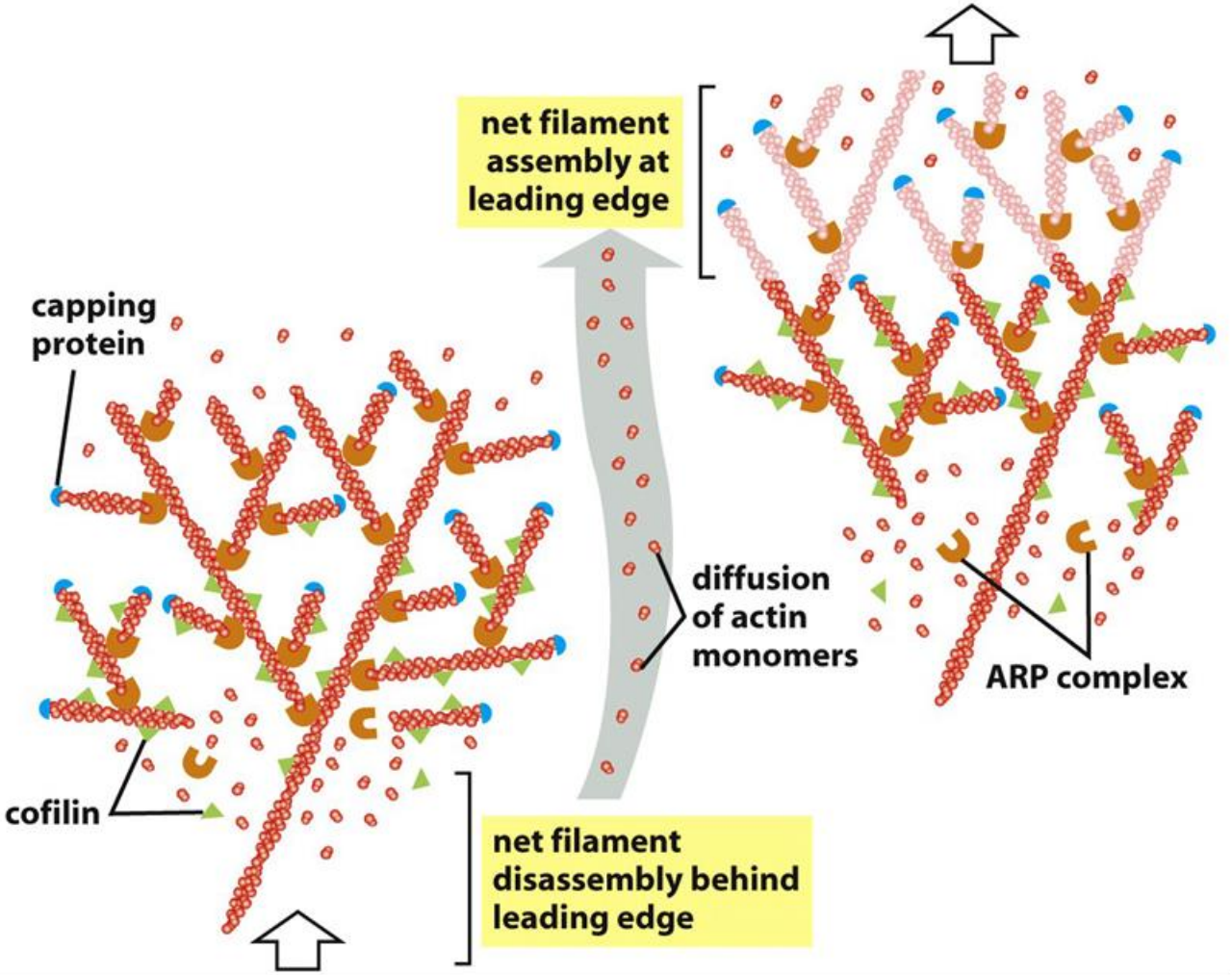


Green: Arp2/3
Red: phalloidin-F-actin



Green: cofilin
Red: F-actin –phalloidin

How actin cause protrusions in leading edge?



Neutrophil in chemotaxis