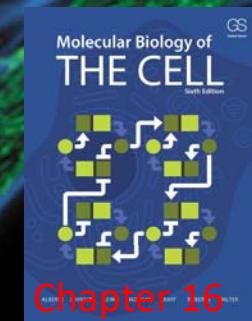


Cytoskeleton and Cell Movement I

-Lecture 13-

Outline

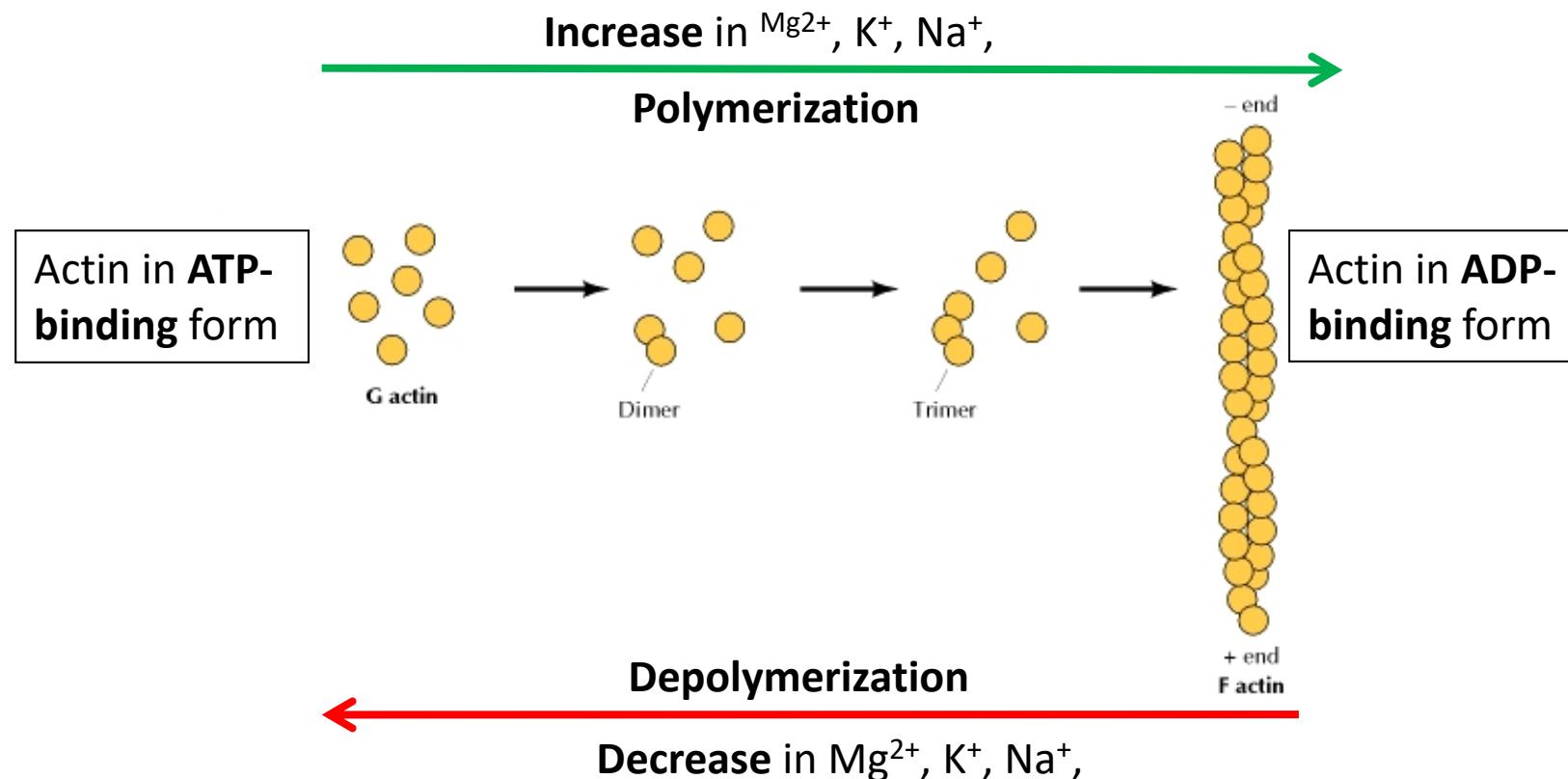
- I. Overview of cytoskeleton
- II. Microfilament and actin structures
- III. Dynamics of actin filaments
- IV. Mechanisms of actin filament assembly
- V. Organization of actin-based cellular structure
- VI. Myosins: actin-based motor proteins
- VII. Myosin-powered movements
- VIII. Cell migration: mechanisms,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



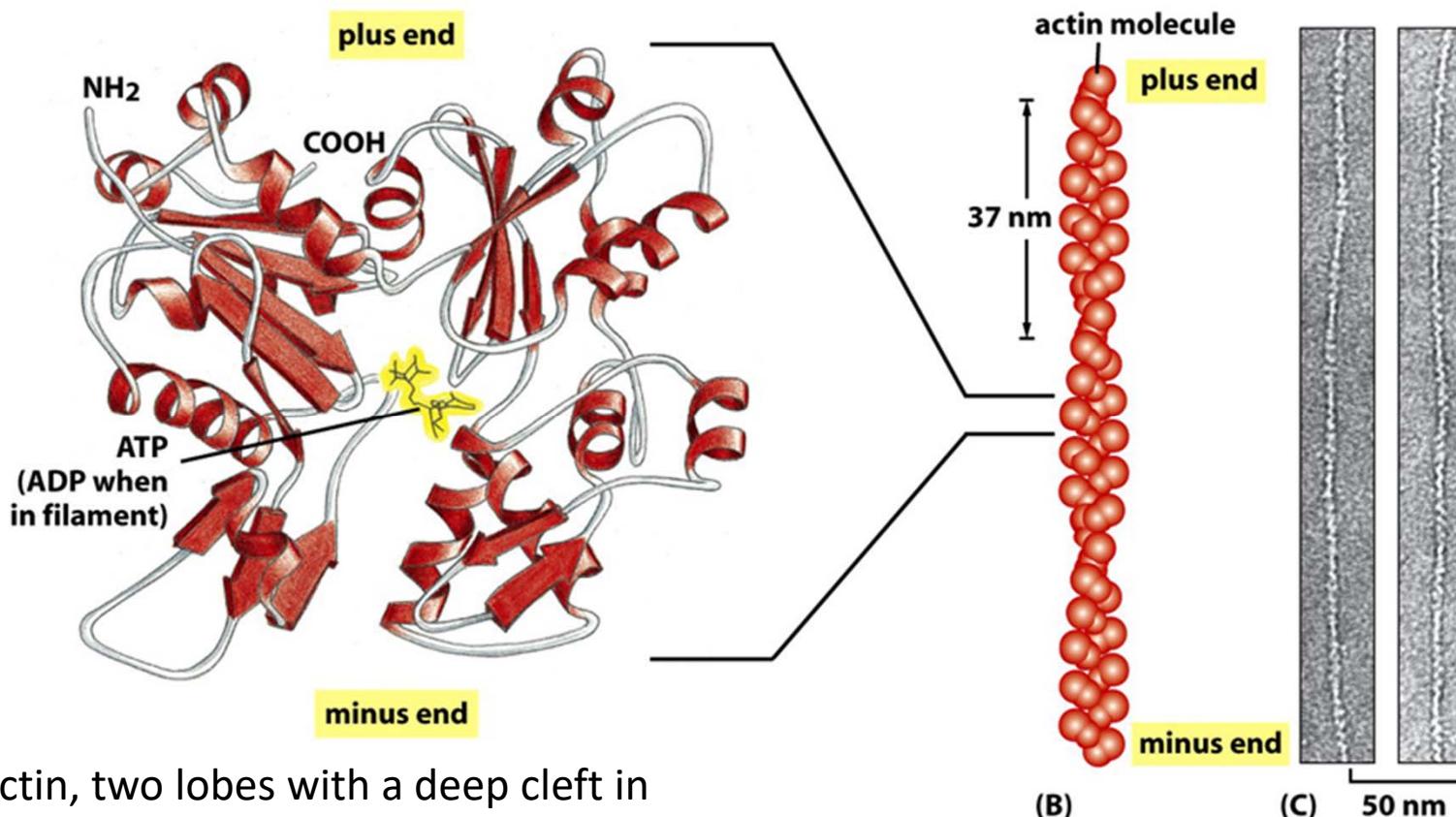
G-actin (globular) and F-actin (filamentous)

Actin exists in two forms:

- 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)

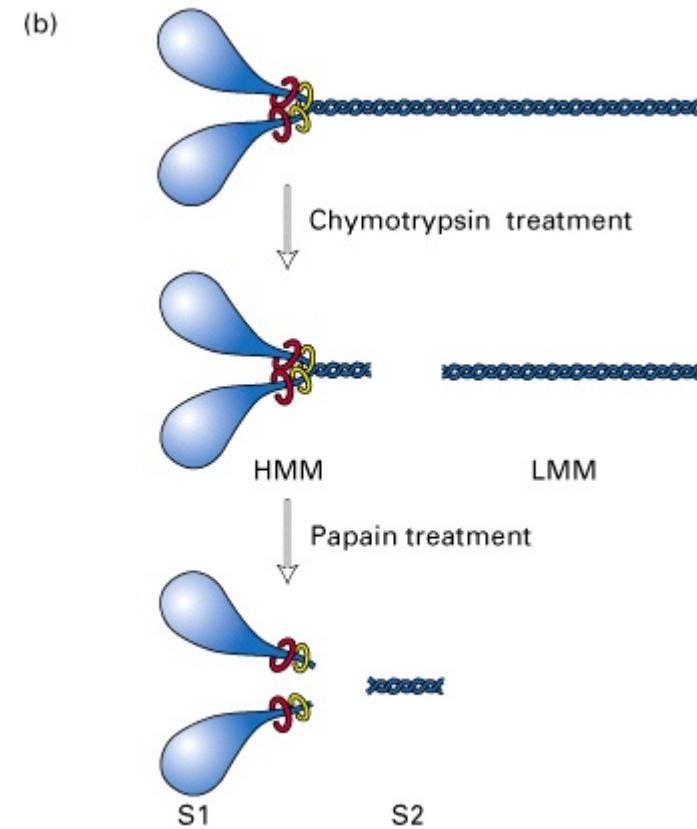
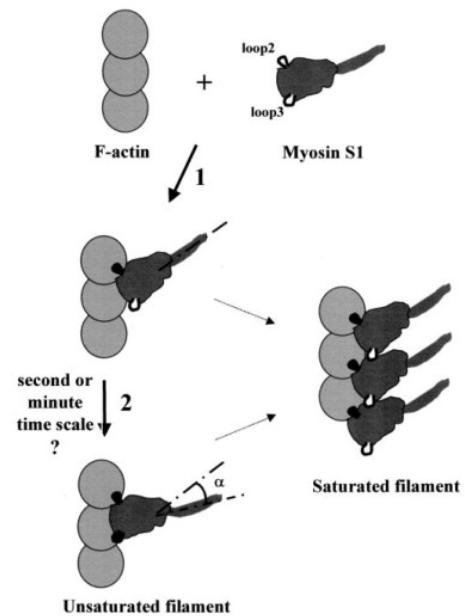
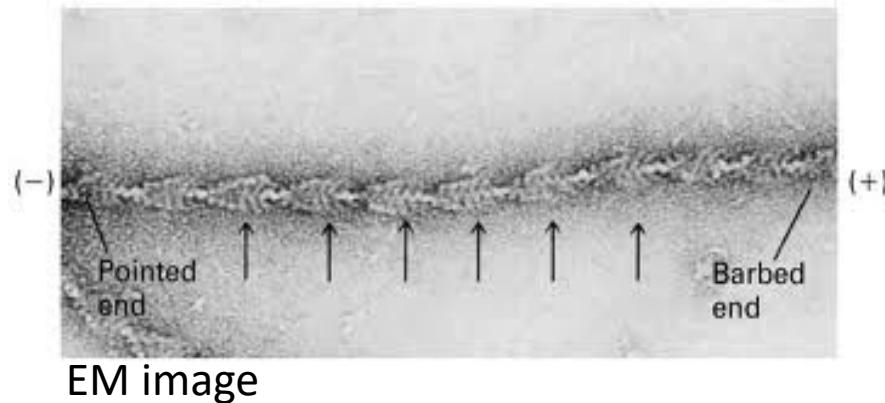
F-actin has structural and functional polarity

Actin 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**.

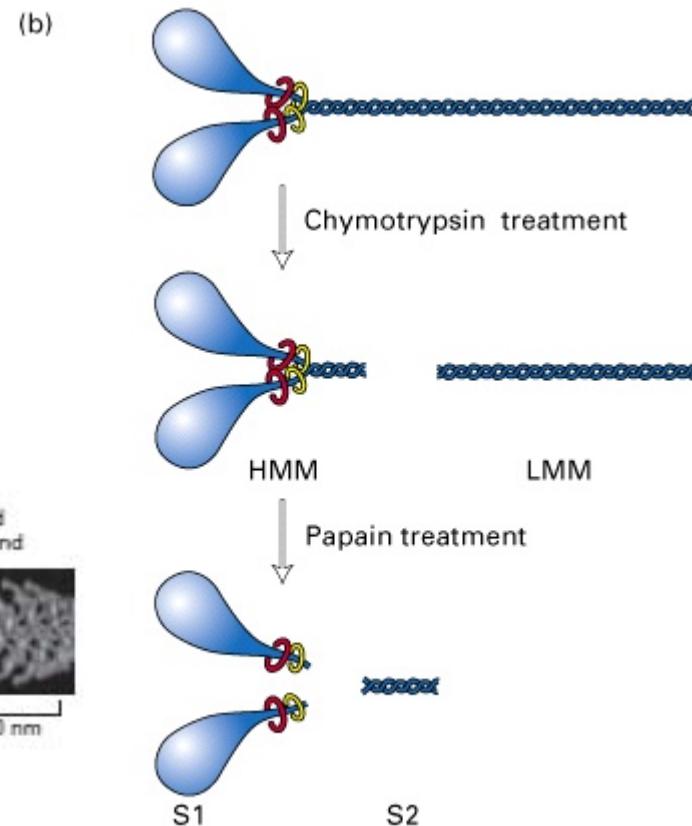
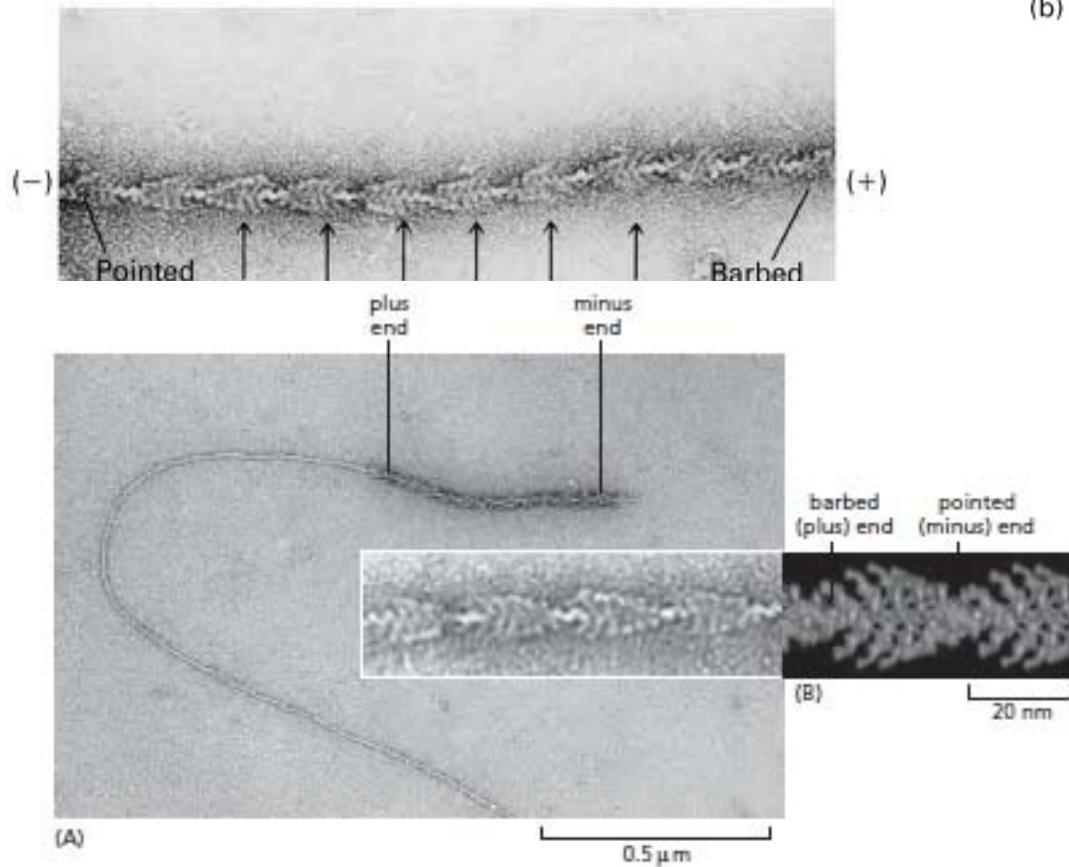
How to demonstrate the polarity of an actin filament?

Myosin S1 decoration experiment



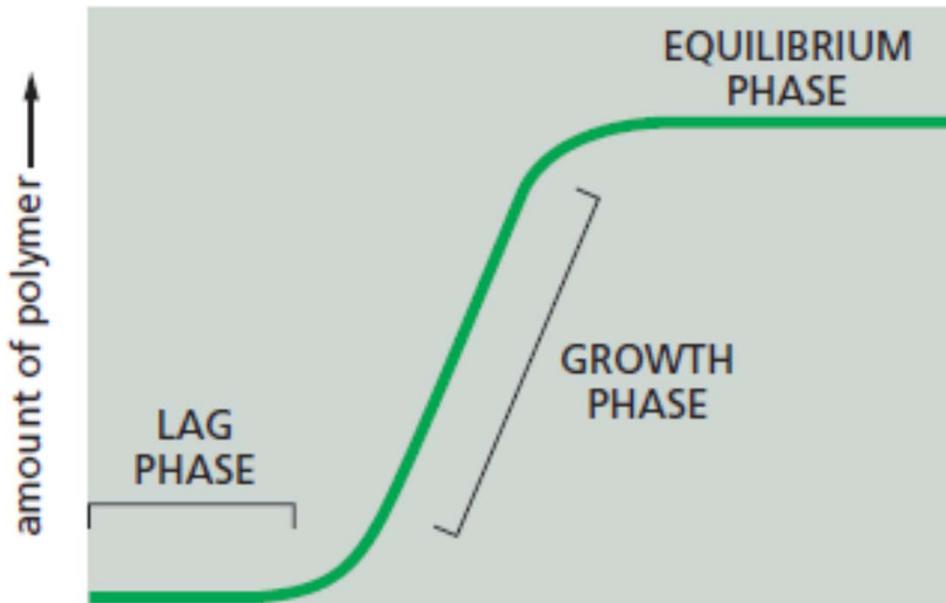
How to demonstrate the polarity of an actin filament?

Myosin S1 decoration experiment



II. Dynamics of actin filaments

The time course of actin polymerization:



Three stages:

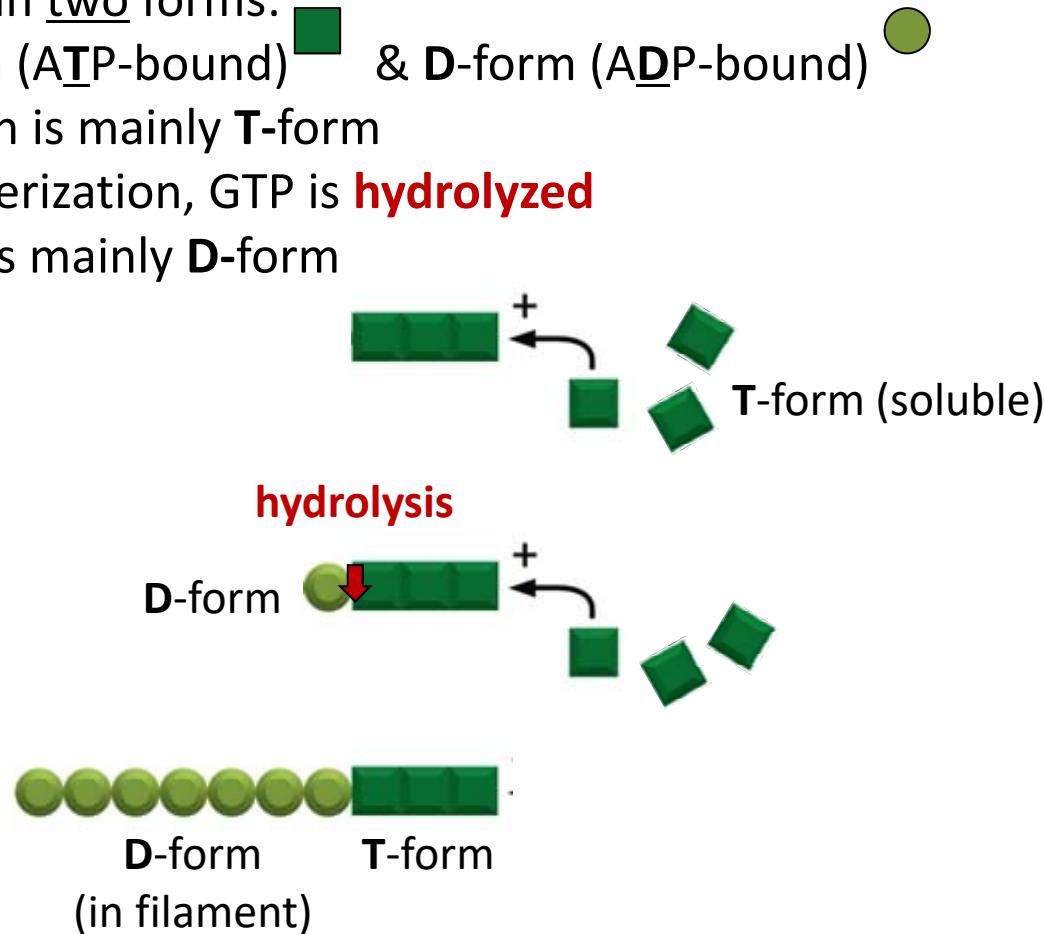
1. **Nucleation:** formation of 3 subunits as seeds for polymerization
(This is the rating-limiting step)
2. **Elongation:** rapid polymerization from the nucleated seeds
3. **Steady-state:** addition and removal are balanced, no net increase.

Molecular mechanism of filament polymerization

Prerequisites for understanding the dynamics:

1) Difference between soluble and filamentous actin:

- Actin exists in two forms:
 T-form (ATP-bound)  & D-form (ADP-bound) 
- **Soluble** actin is mainly T-form
- After polymerization, GTP is **hydrolyzed**
- **Filament** has mainly D-form

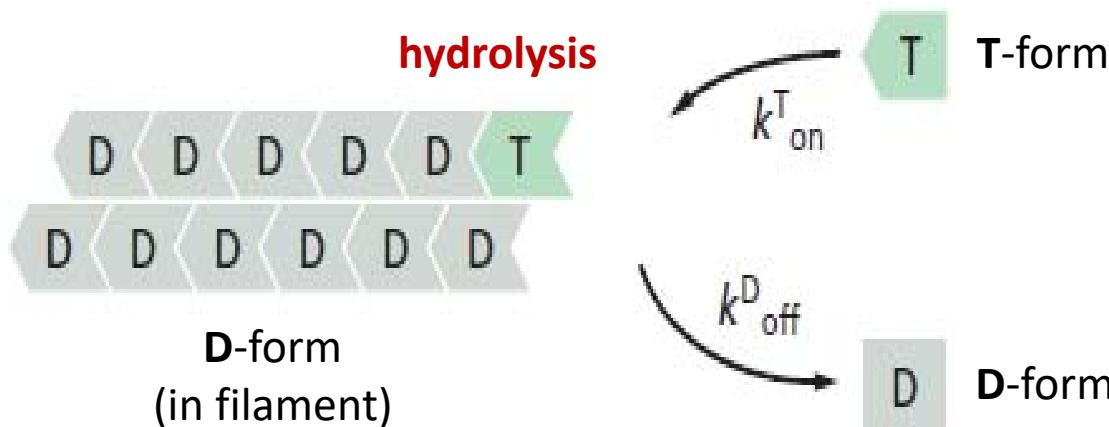


Molecular mechanism of filament polymerization

Prerequisites for understanding the dynamics:

2) Balance between assembly and dissociation at an end of the filament:

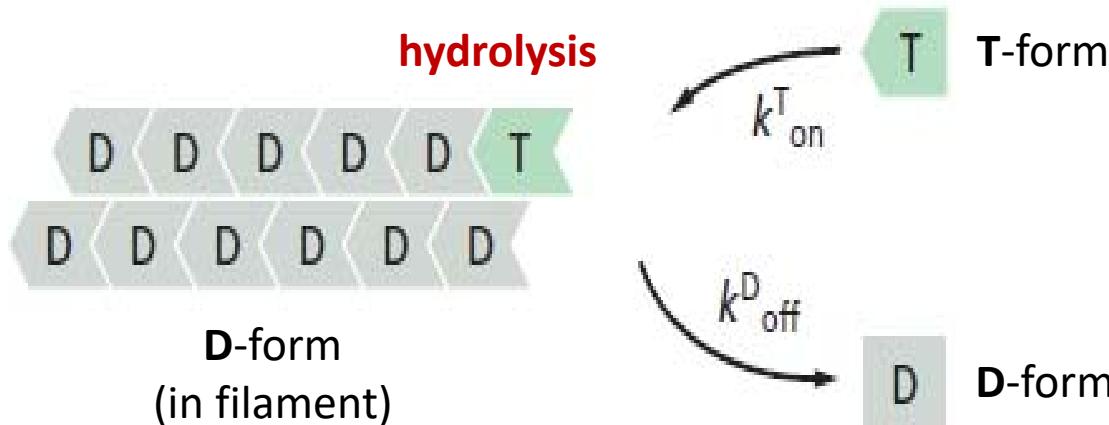
- T-form has **high binding affinity** but D-form has **low binding affinity**
 - T-form will get “on” the **end of the filament**
 - T-form will get “off” the **end of the filament**



Molecular mechanism of filament polymerization

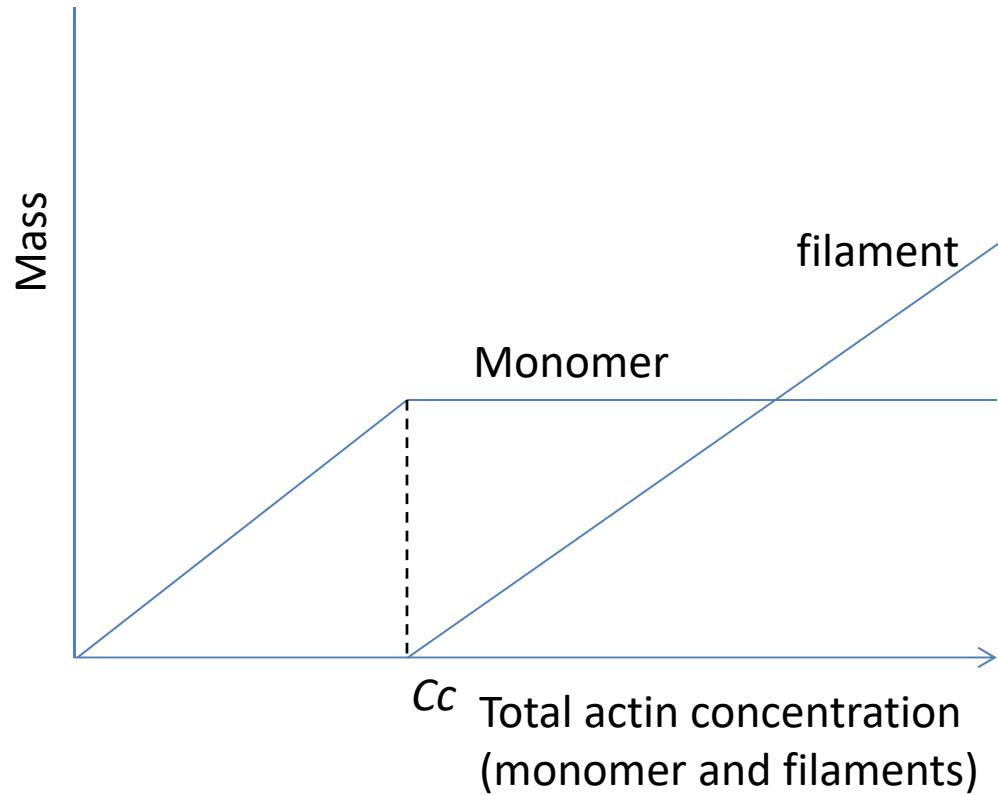
Prerequisites for understanding the dynamics:

2) Balance between assembly and dissociation at an end of the filament:



- Association (“on”) of the T-form is proportional to the concentration of the soluble actin:
- Dissociation (“off”) of D-form does not depend on the concentration
- If $k^T_{on} c > k^D_{off}$ then it will growth, if $k^T_{on} c < k^D_{off}$ then it will shrink
but if $k^T_{on} c = k^D_{off}$ then is equilibrium (critical concentration C_c)

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 critical concentration C_c :

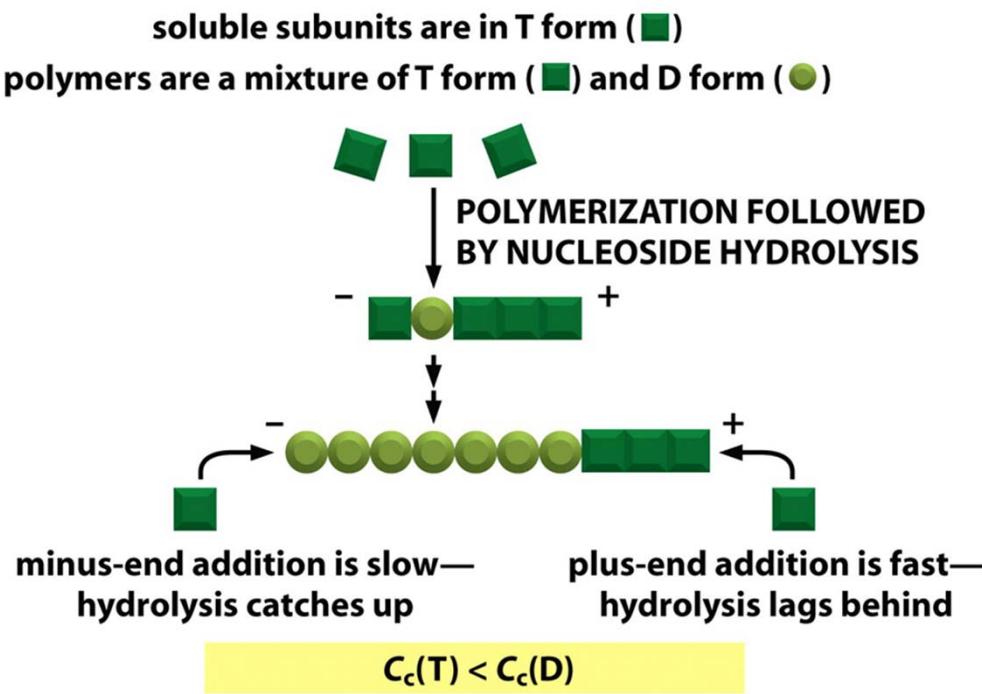
Concentration of free G-actin at which the assembly onto a filament end is balanced by loss from that end.

Molecular mechanism of filament polymerization

Prerequisites for understanding the dynamics:

2) Actin filaments have two different ends and we have to consider both!

- (+) and (-) ends have different critical concentrations (C_c): because of different binding affinities for binding to T-forms at (+) end vs. binding to D-forms at the (-) end

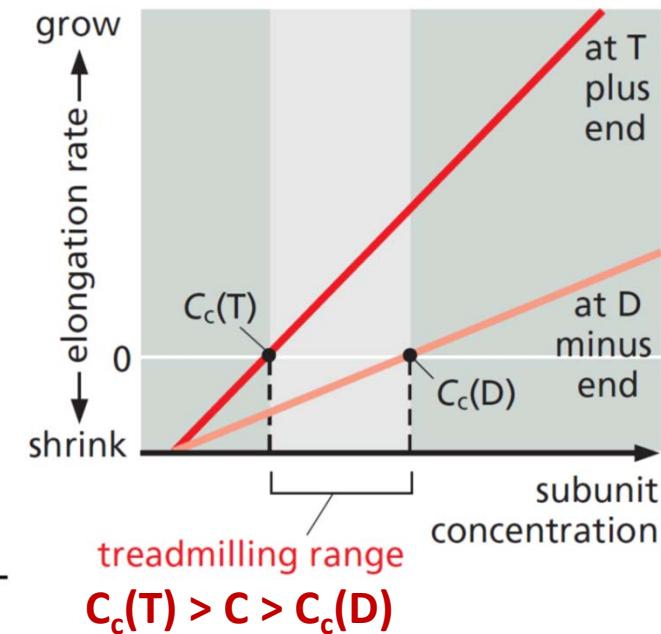
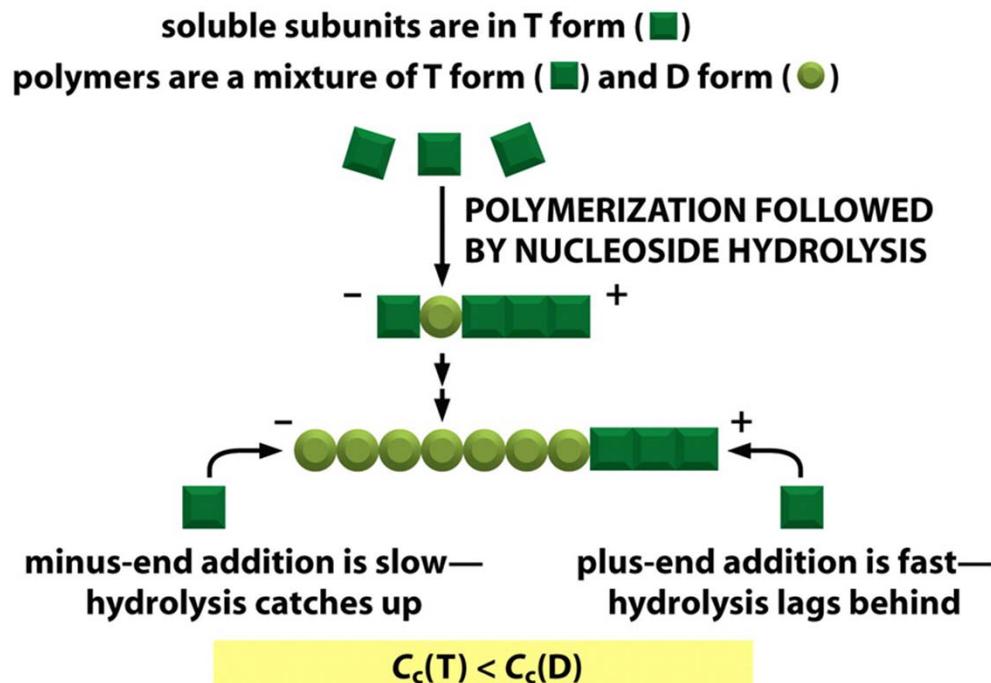


Molecular mechanism of filament polymerization

Prerequisites for understanding the dynamics:

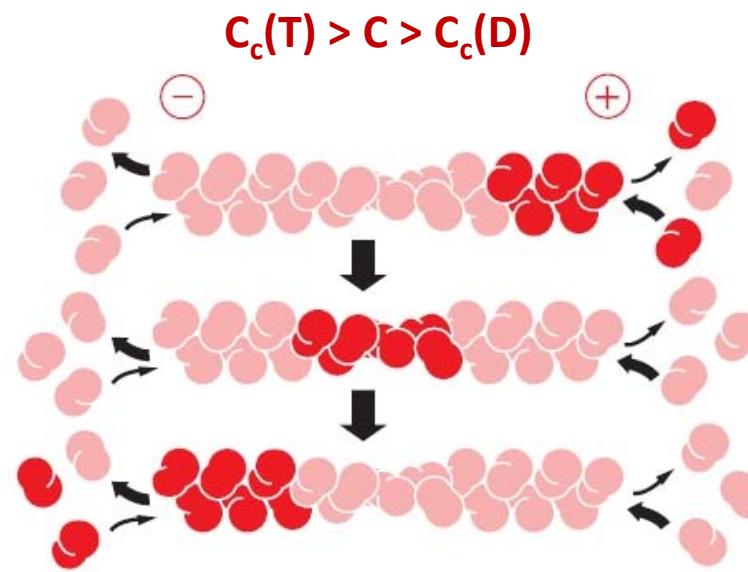
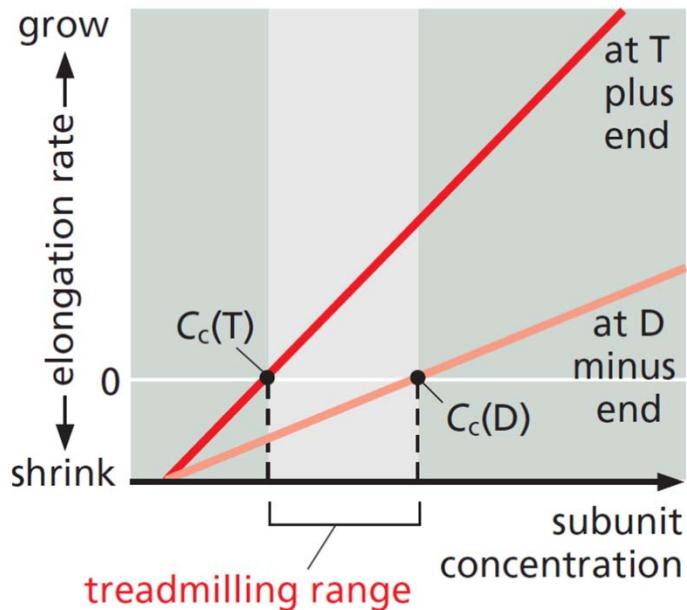
2) Actin filaments have two different ends and we have to consider both!

- (+) and (-) ends have different critical concentrations (C_c):
because of different binding affinities for binding to T-forms at (+) end
vs. binding to D-forms at the (-) end



Actin treadmilling $C_c(T) > C > C_c(D)$: net flux of molecules

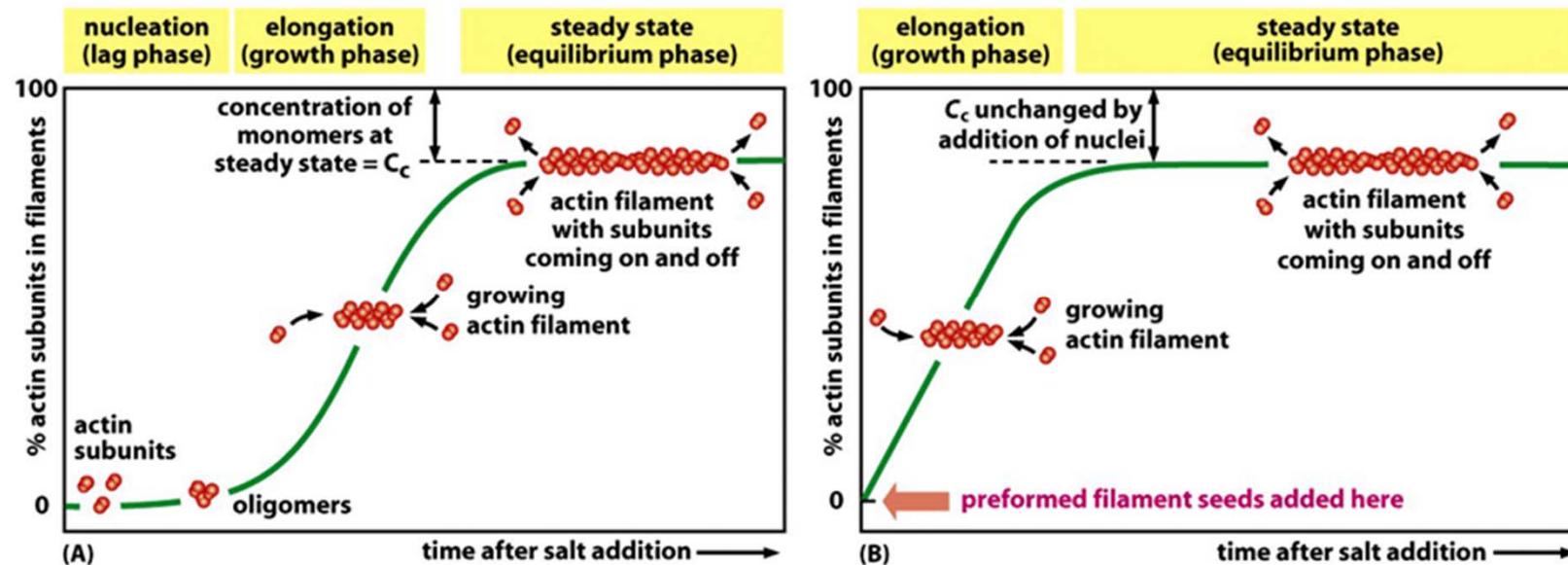
- At this steady state, subunits undergo a net assembly at the plus end and a net disassembly at the minus end at an identical rate.



The polymer maintains a **constant length**, even though there is a **net flux** of subunits through the polymer, termed treadmilling.

Molecular mechanism of filament polymerization

The time course of actin polymerization:



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.

Actin monomer availability controls actin filament assembly

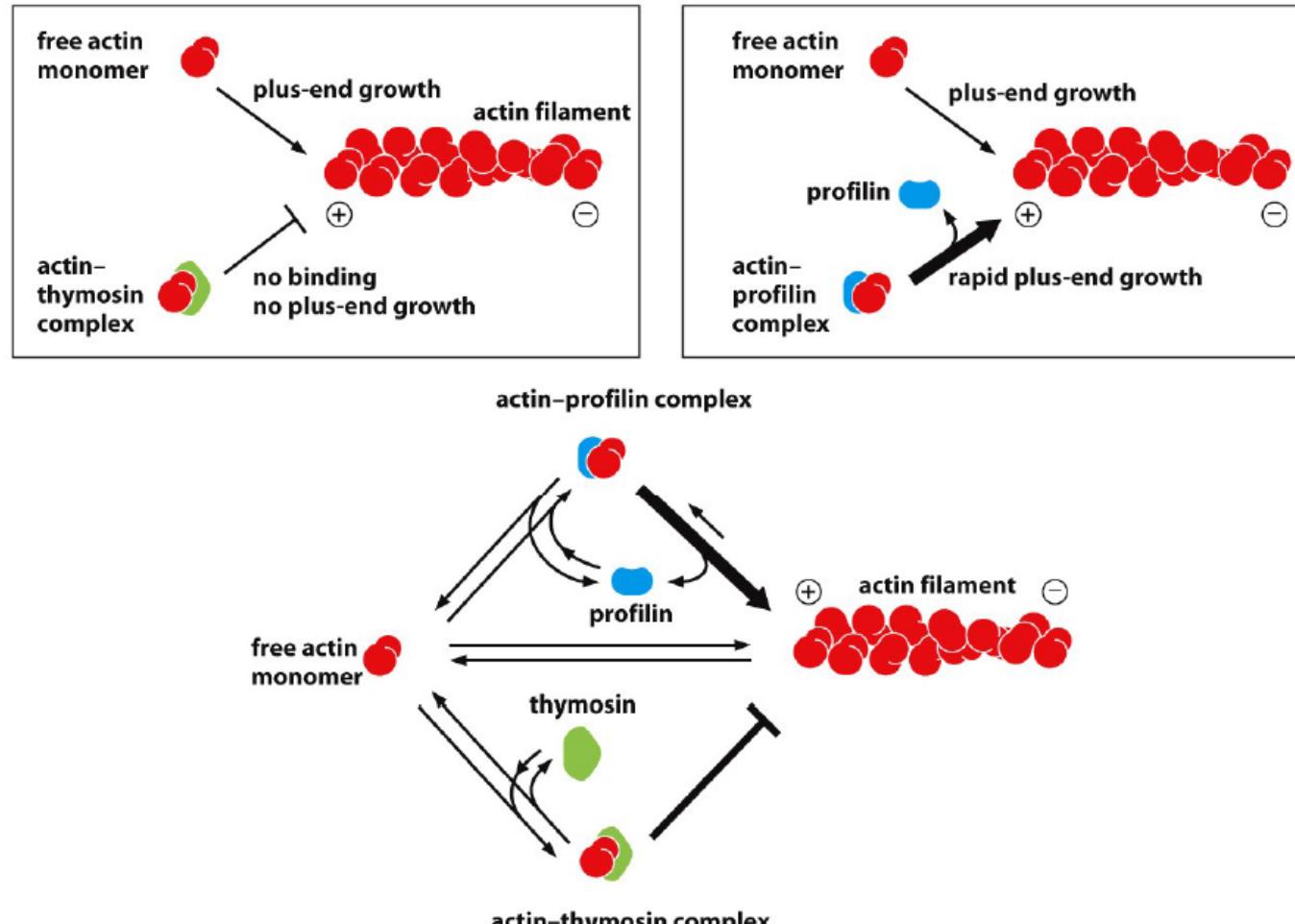


Figure 16-15 Molecular Biology of the Cell 6e (© Garland Science 2015)

IV. Mechanisms of actin filament assembly

The “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**: assembly of **long** filaments
- **Arp (actin-related protein) 2/3 complex**: **branched** filament assembly

Formin mediates straight filament assembly

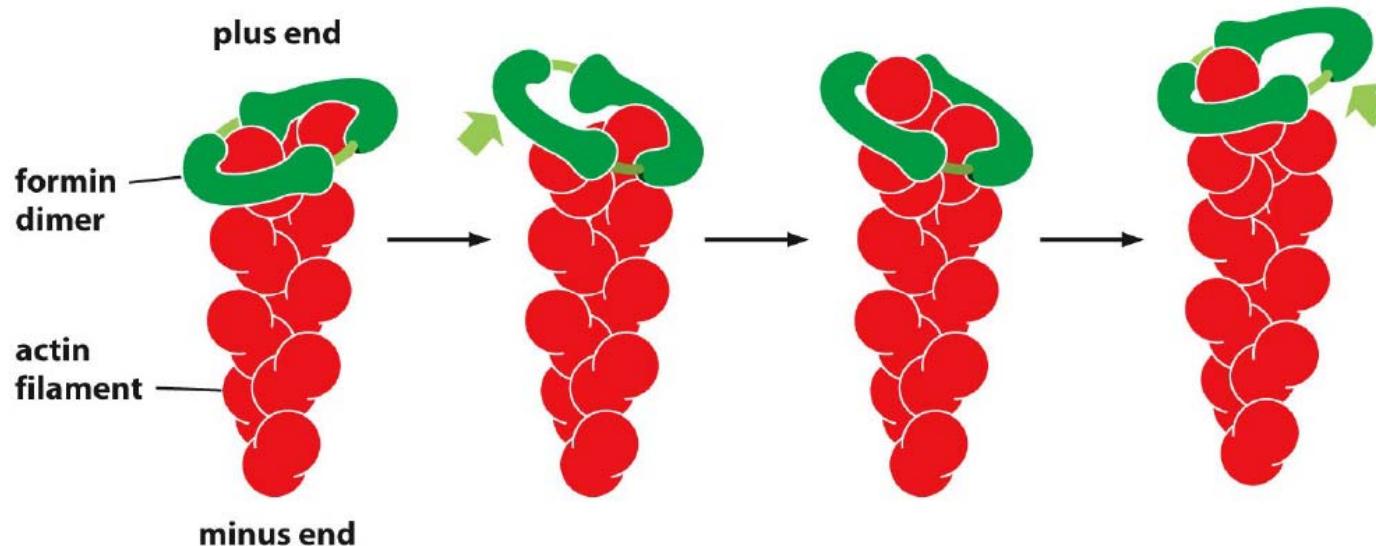
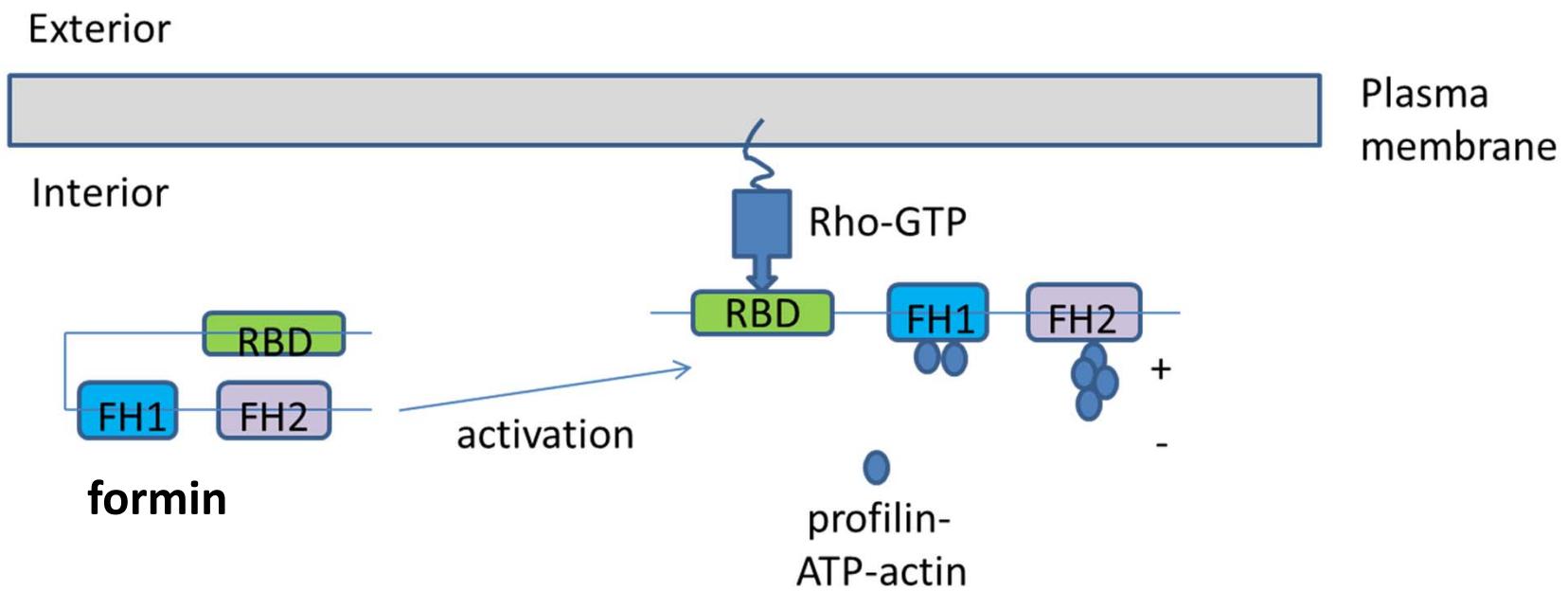


Figure 16-17 Molecular Biology of the Cell 6e (© Garland Science 2015)

- 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**

Regulation of formins by Rho-GTPases

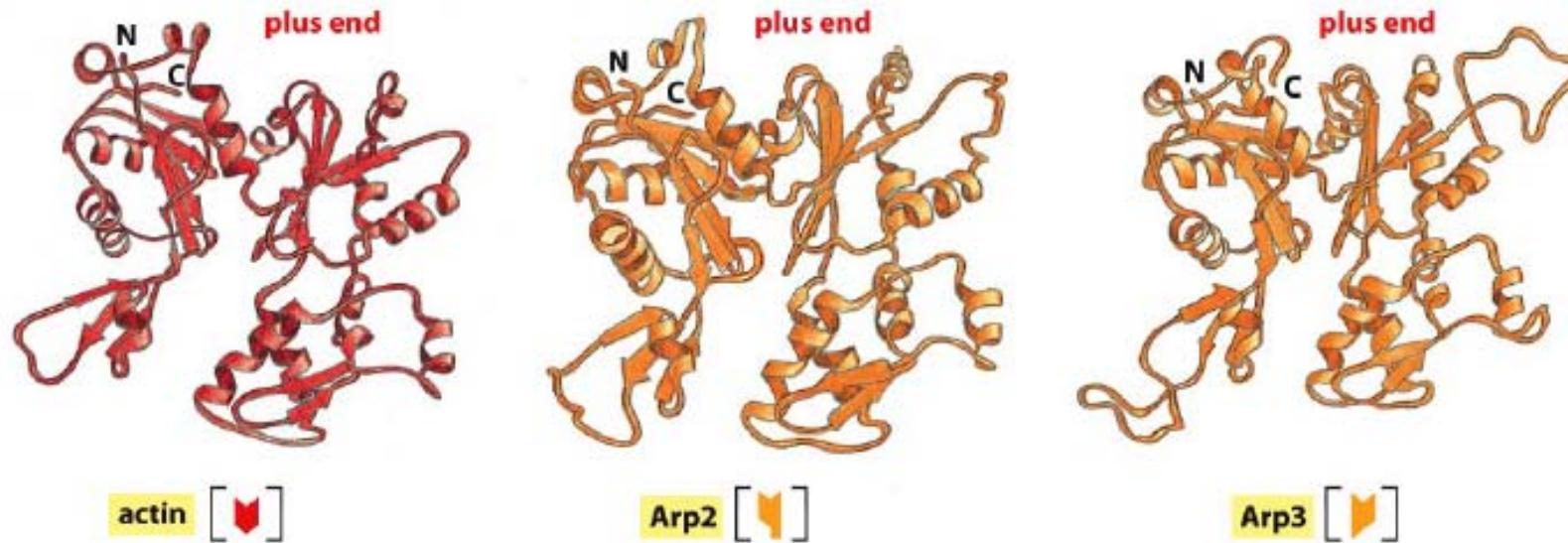


RBD: Rho-binding domain

FH: Formin homology domain

Arp2/3 mediates branched filament assembly

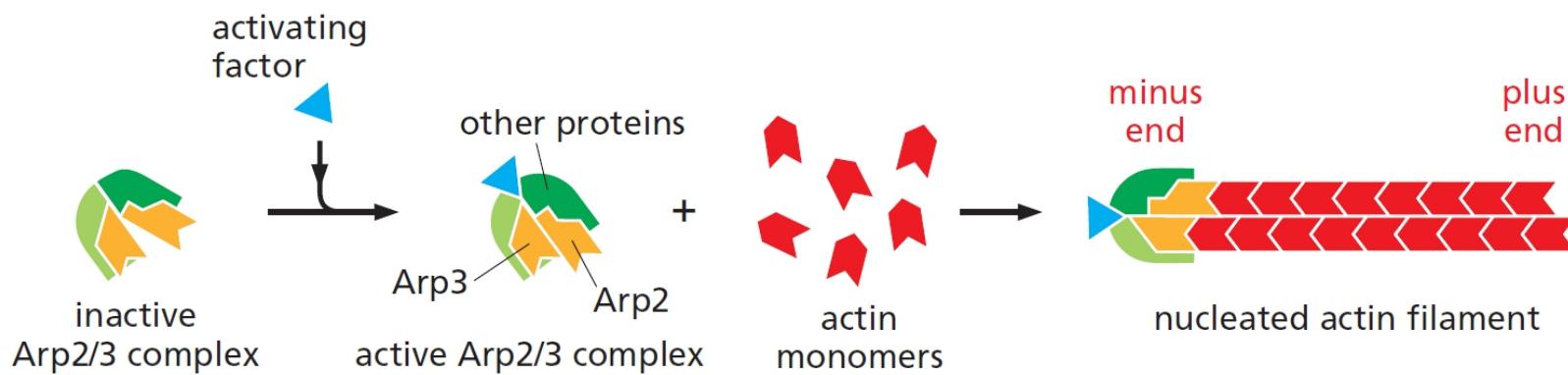
Comparison of structures: actin versus actin-related protein (Arp) 2 and 3



Arp2/3 similar to actin at (+) end but **differences** at sides and (-) end
prevent spontaneous filament formation

Arp2/3 mediates branched filament assembly

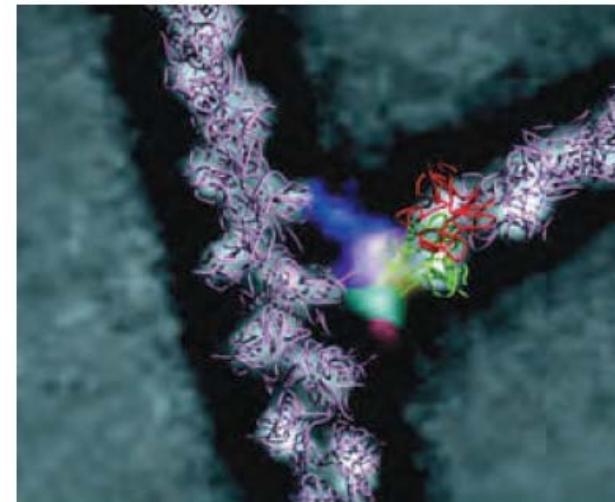
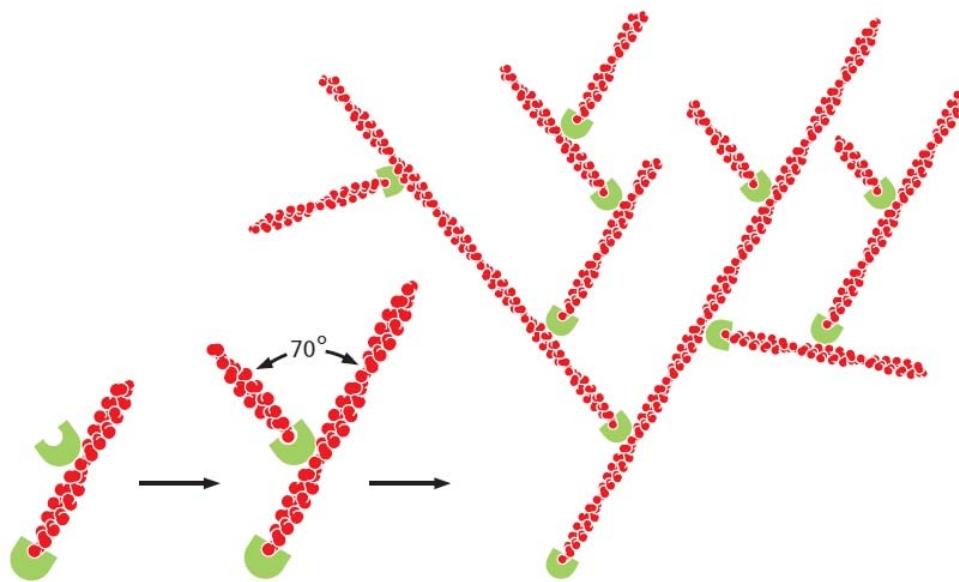
An **activating factor** is required to bring Arp2 and Arp3 **together**



- The activated Arp2/3 complex resembles a (+) end of a filament and is used for the actin filament assembly

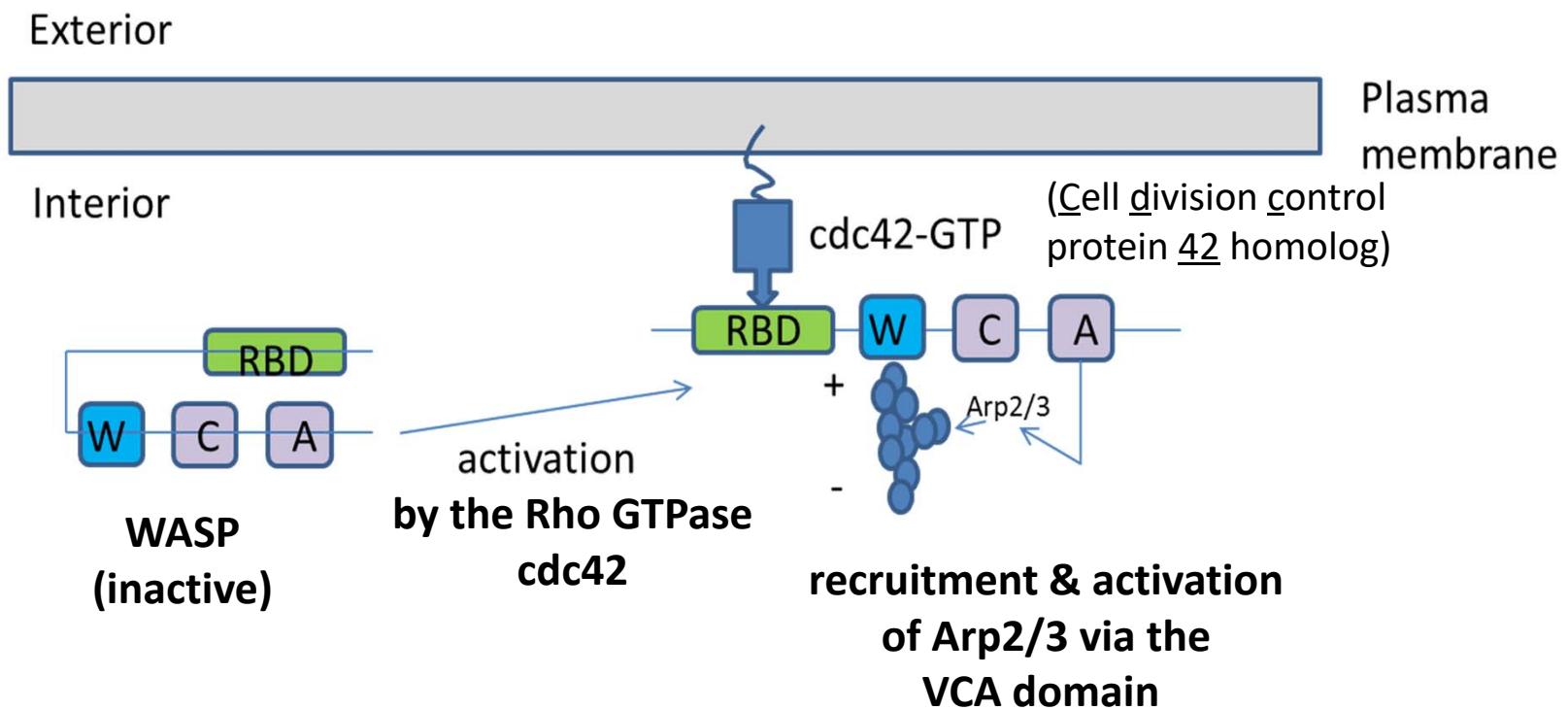
Arp2/3 mediates branched filament assembly

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

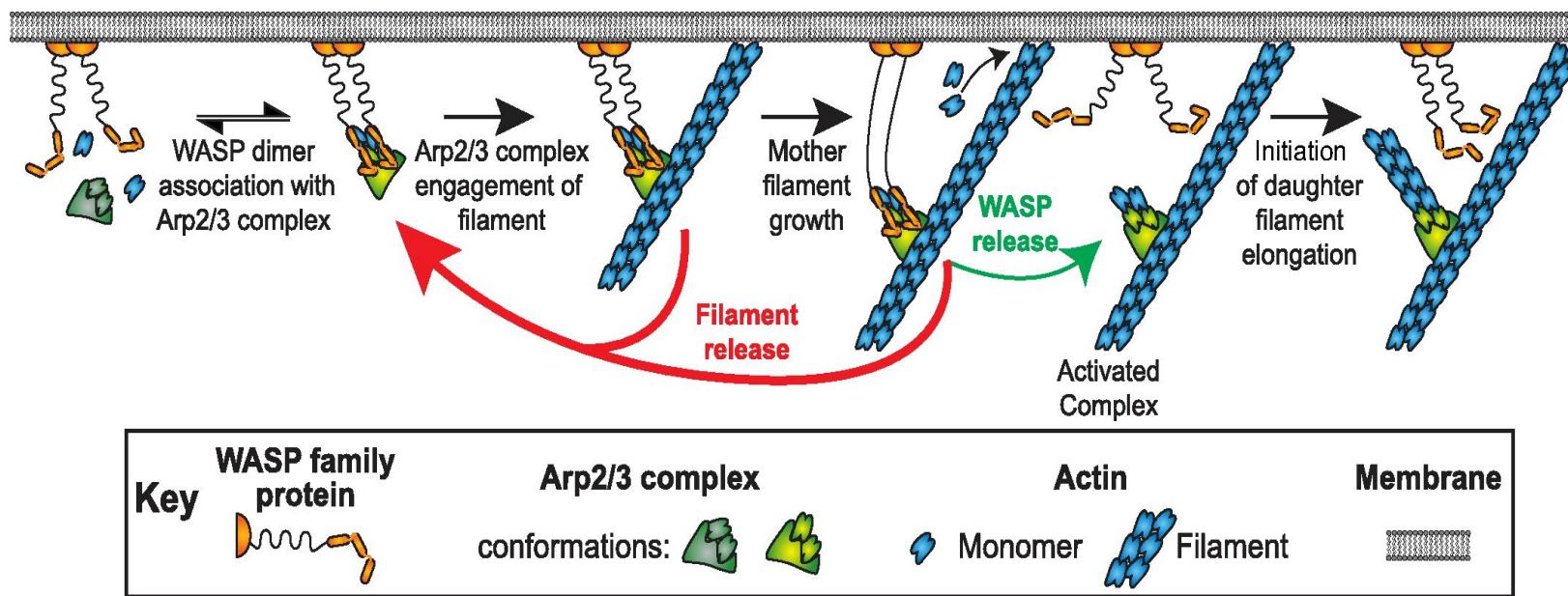


(D)
Branching: actin (pink) with
Arp2/3 complex fitted according
to electron density.
Mother filament runs from top to
bottom, daughter filament
branches to the right

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

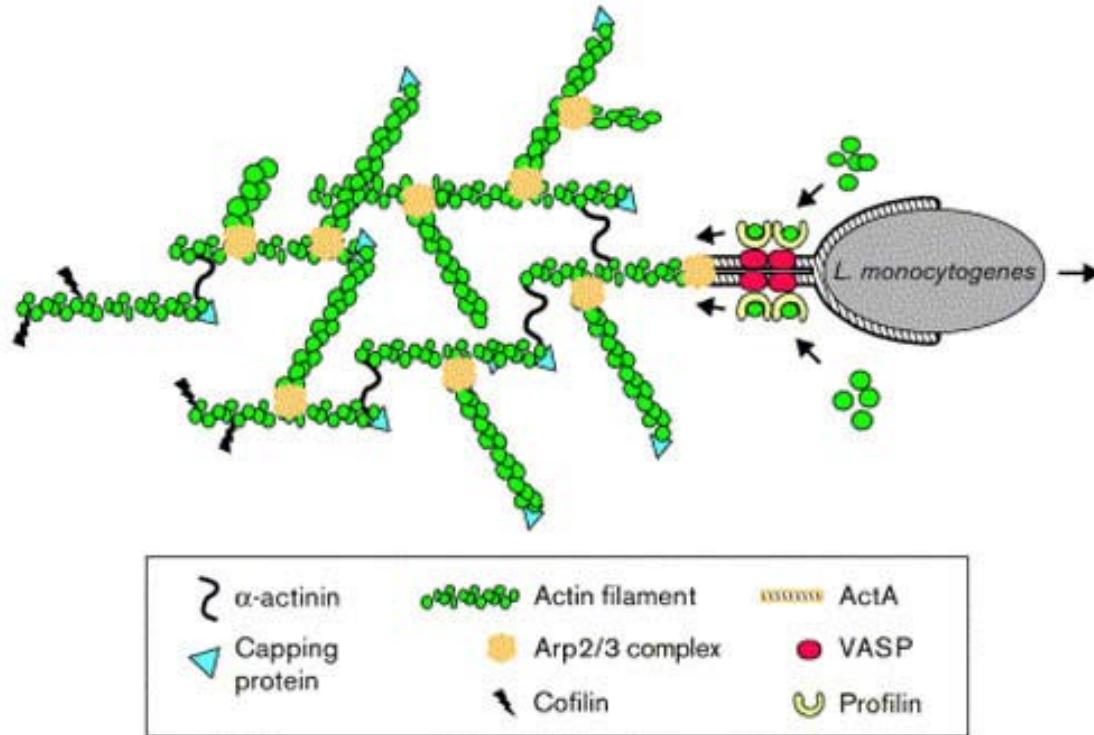


Actin nucleation by the Arp2/3 complex



new filament and old filament have an angle of 70 degrees

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** to recruit **Arp2/3** to enhance ATP-actin assembly.

The recruited Arp2/3 complex nucleates the assembly of actin filaments.

This generates force and pushes 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"

Many toxins target actin dynamics

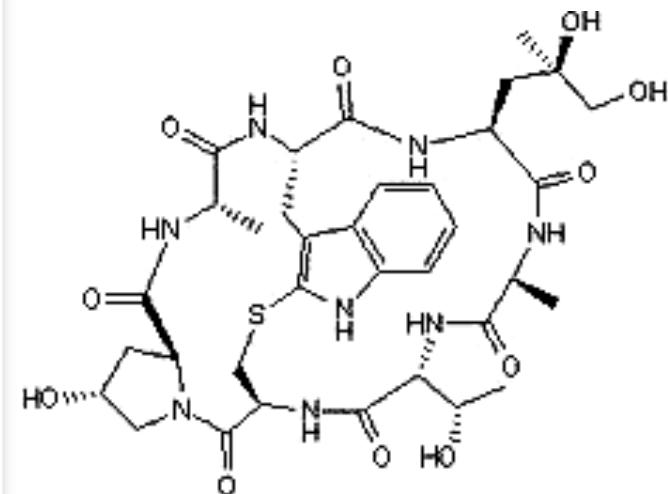
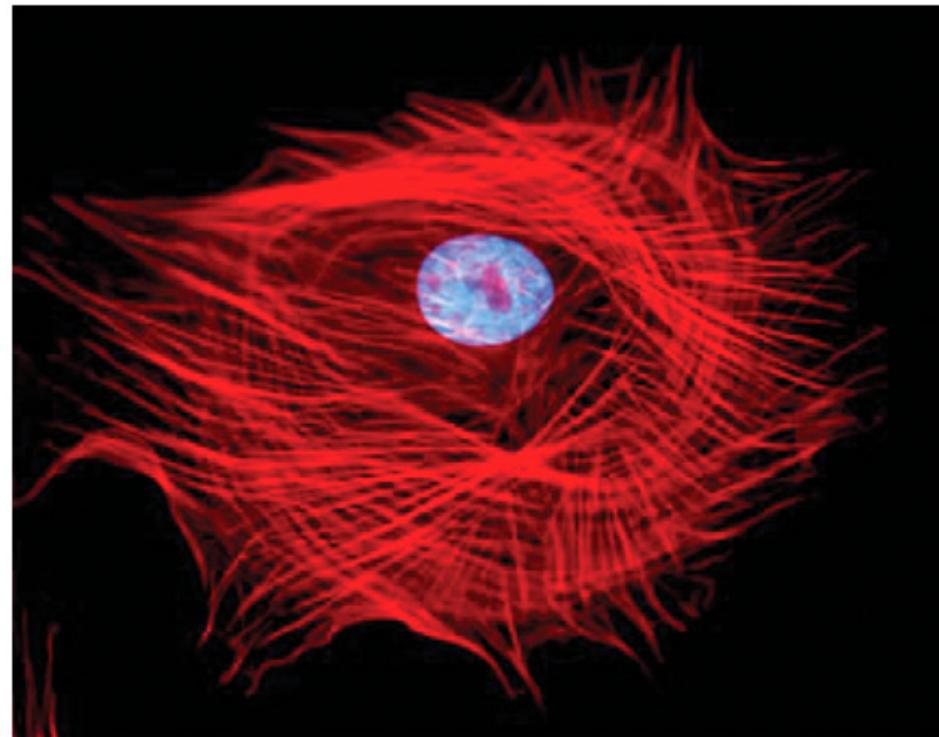
Microfilament depolymerization drugs:

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 depolymerization**.

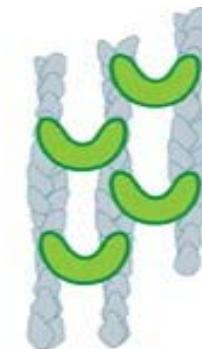
Phalloidin has been used extensively in research for fluorescence-labelling of actin filaments (F-actin)



V. Organization of actin-based cellular structures

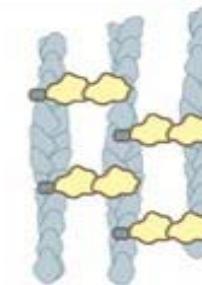
Actin filaments are cross-linked by various proteins:

Fascin



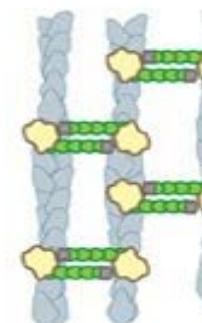
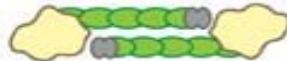
Filopodia
leading edge

Fimbrin



Microvilli
Filopodia
Focal adhesion

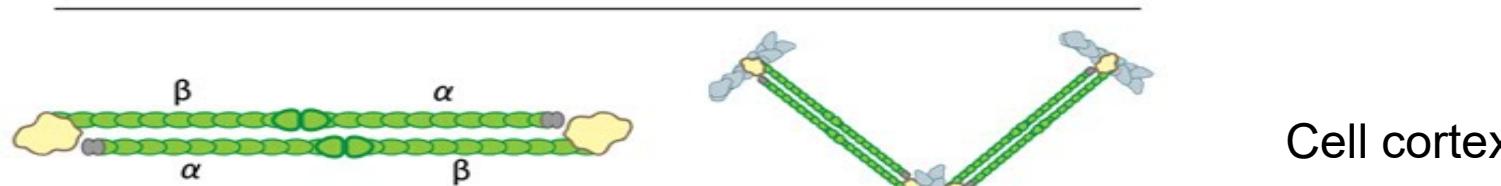
α -actinin



Stress fiber
Filopodia
Muscle Z line

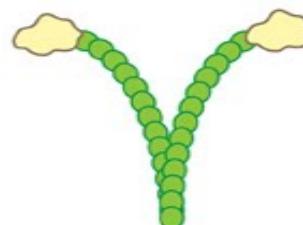
V. Organization of actin-based cellular structures

Actin filaments are cross-linked by various proteins:



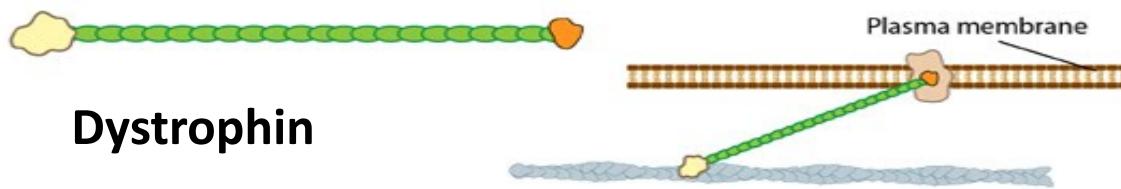
Spectrin

Cell cortex



Filaminin

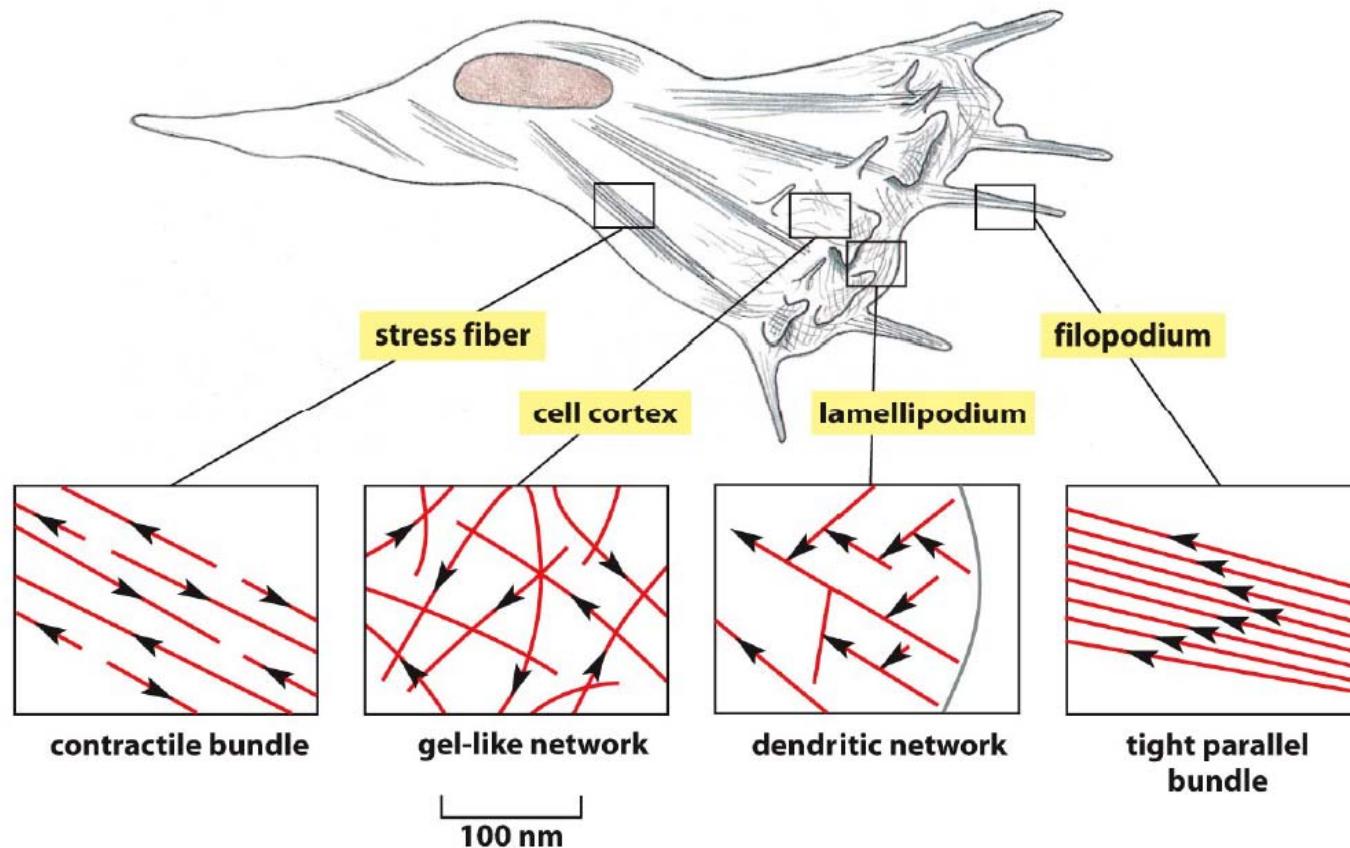
Leading edge
Stress fiber
filopodia



Dystrophin

Linking membrane
Proteins to
Actin cortex
In muscle

Single cells possess different actin networks in cells



Fibroblasts crawl in tissue-culture with four areas enlarged to show the arrangement of filaments:

Stress fibers are contractile and exert tension

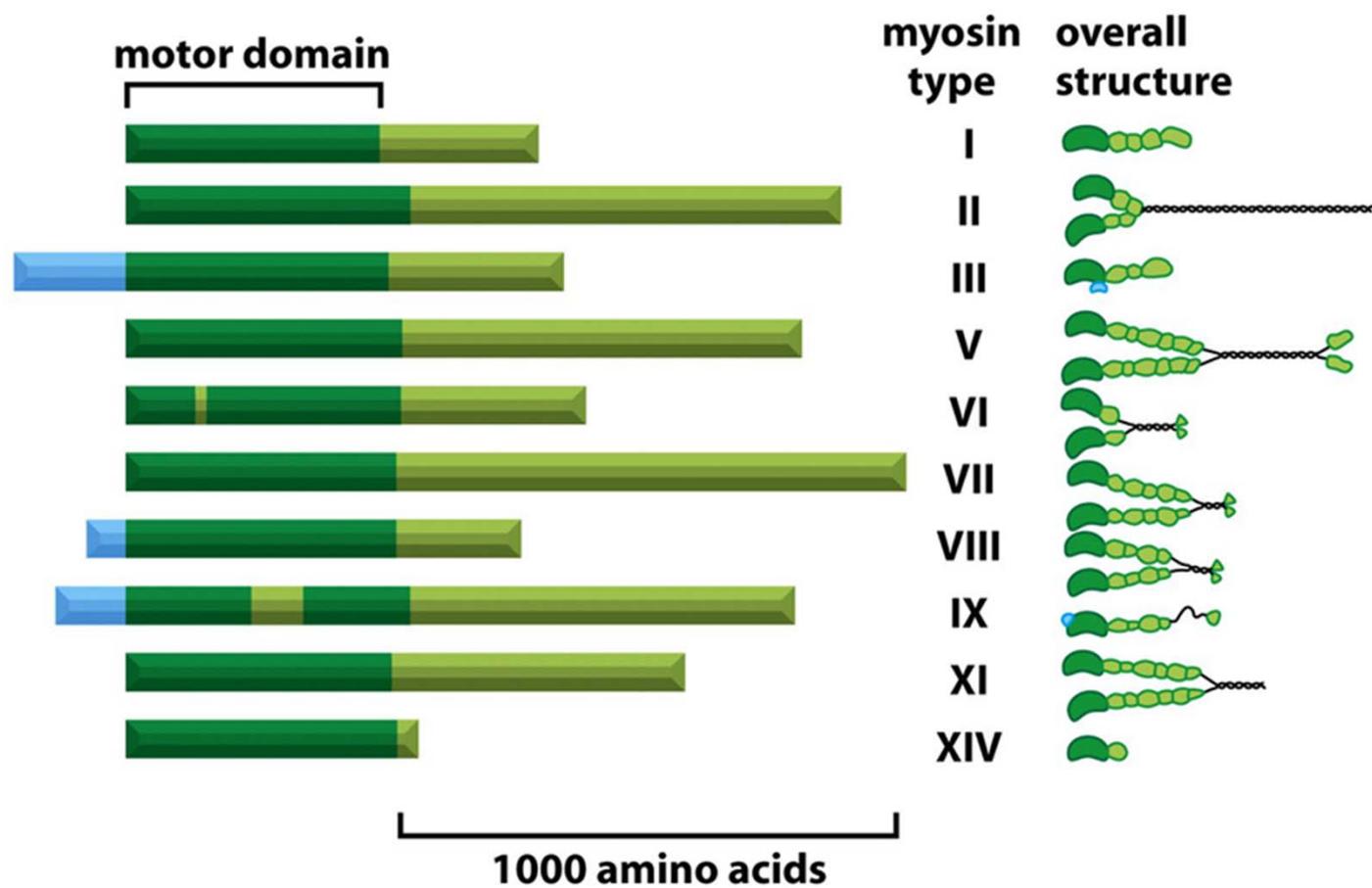
Actin cortex underlies the plasma membrane and consists of gel-like networks

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

Filopodia are spike-like projections of the plasma membrane to explore the 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

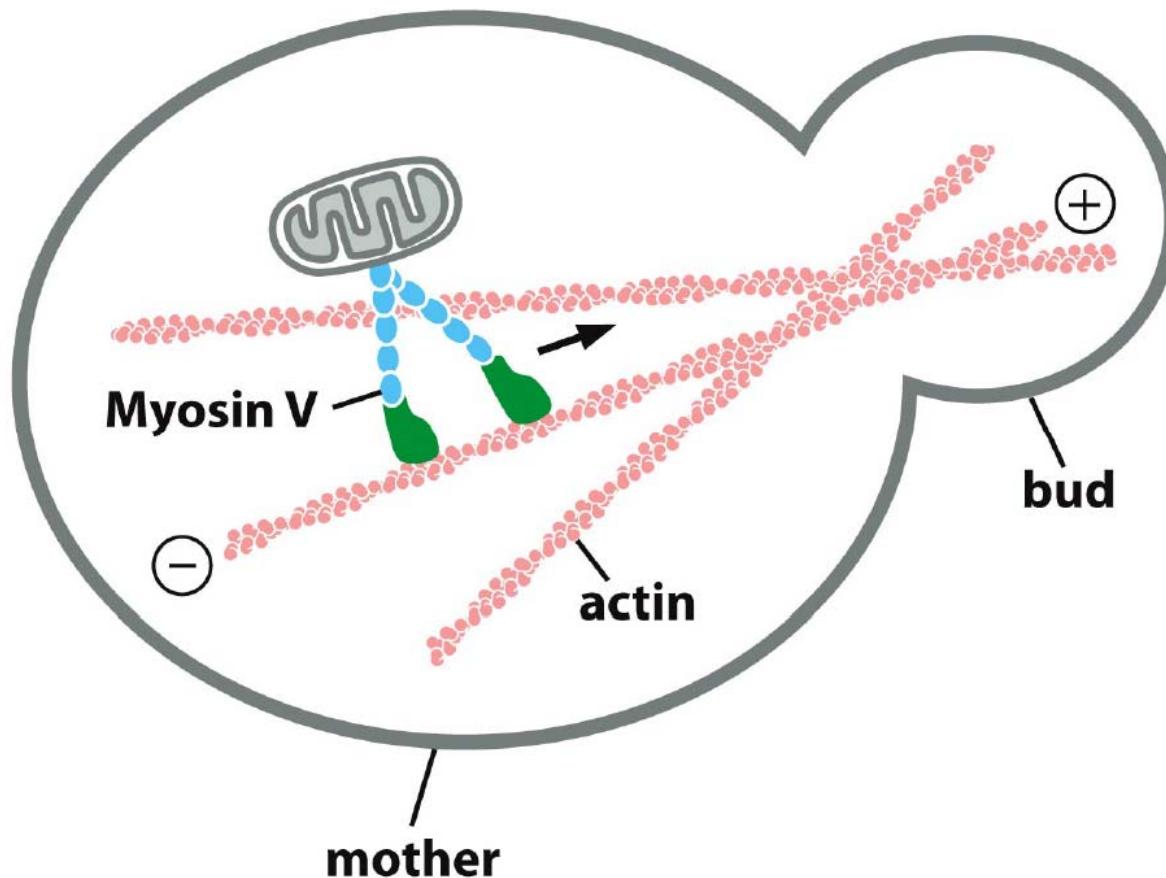
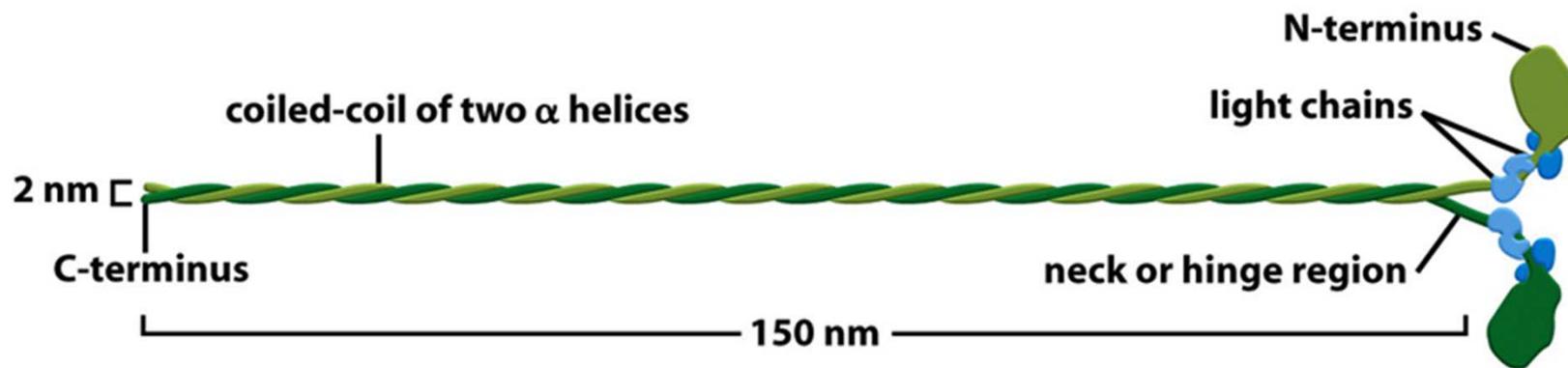


Figure 16-41b Molecular Biology of the Cell 6e (© Garland Science 2015)

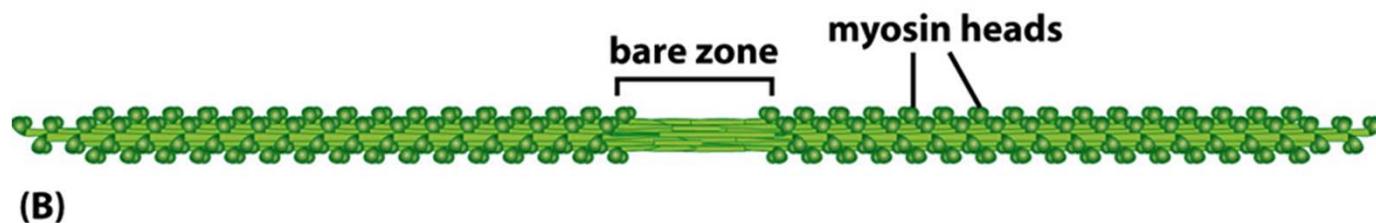
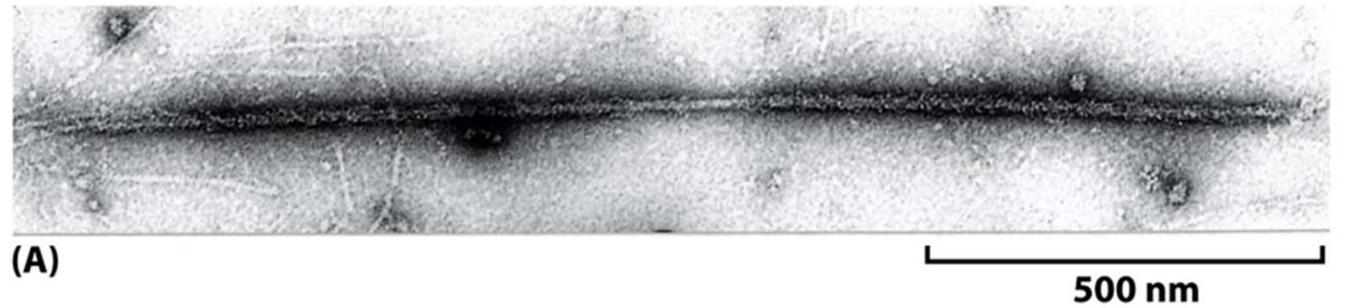
Structure of Myosin II



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



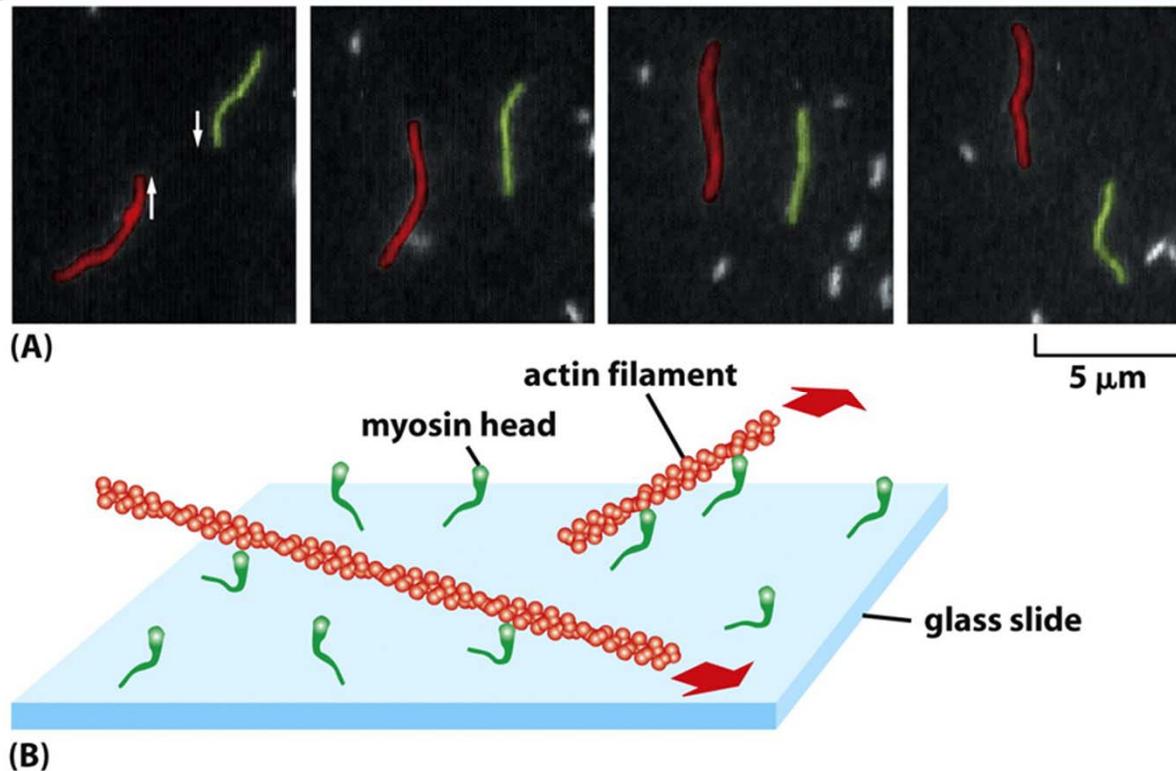
The myosin II bipolar thick filament in muscles.

(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.

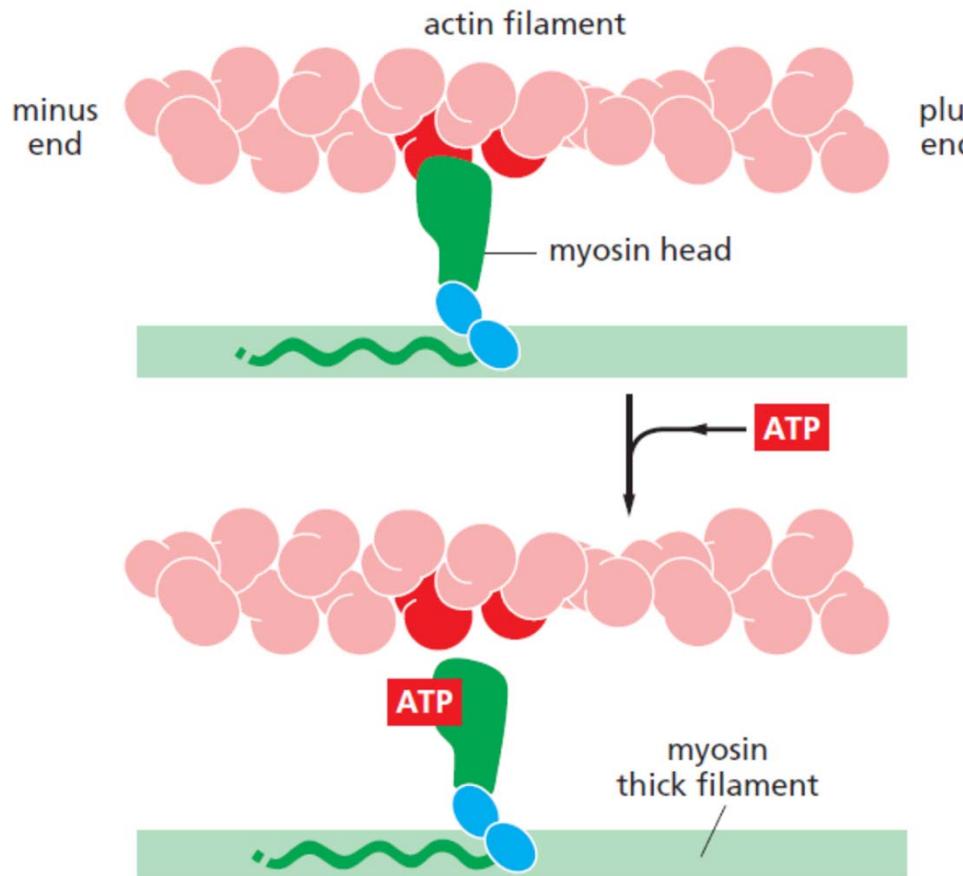
The bare zone in the center of the filament consists entirely of myosin II tails.

Myosin head drives actin movement



purified myosin heads were attached to a glass slide.
Phalloidin-labeled actin filaments were added and allowed to bind to the myosin heads.
When ATP was added, the actin filaments began to glide along the surface, owing to the many individual steps taken by each of the dozens of myosin heads bound to each filament.

ATP hydrolysis couples myosin conformation change to cause movement along actin filaments



Start:

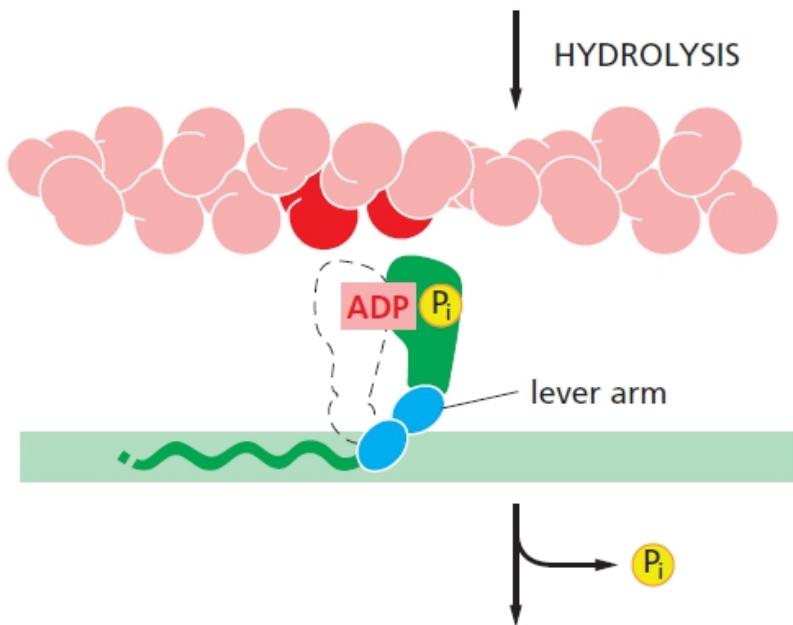
“Attached”:

- Myosin head is tightly locked to actin filament.
(Short-lived situation, terminated by ATP binding)

“Released”

- ATP-binding allows movement of the head along the actin filament

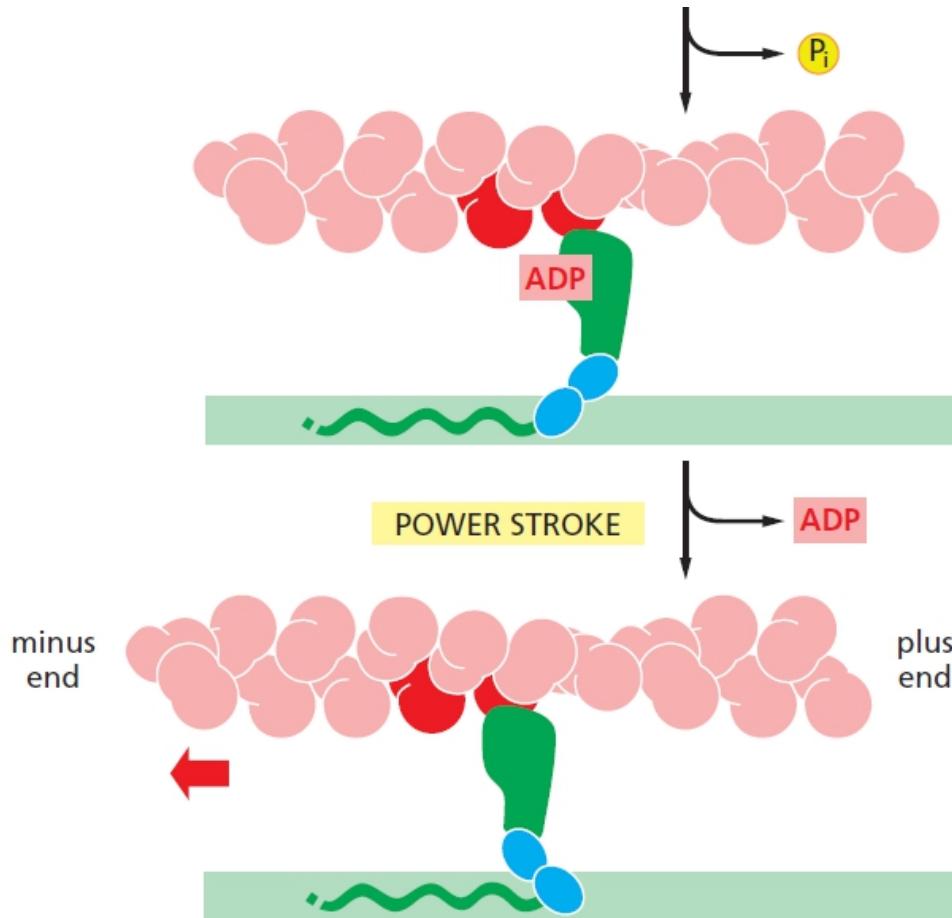
ATP hydrolysis couples myosin conformation change to cause movement along actin filaments



"Cocked"

- Upon ATP binding, the cleft encloses the ATP, triggers movement of the lever arm, causing the displacement of the head along the filament (5nm)
- After ATP hydrolysis, the inorganic phosphate (Pi) remains bound to the protein

ATP hydrolysis couples myosin conformation change to cause movement along actin filaments



"Force-generating"

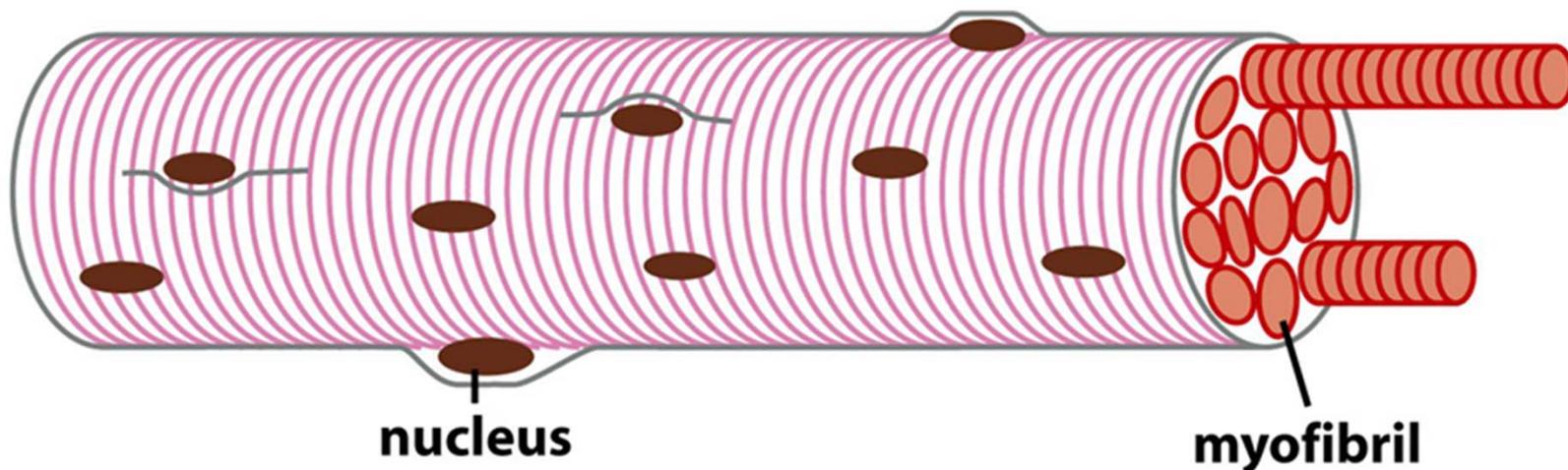
- Weak binding of the head to a new actin site causes release of P_i , which triggers tight actin binding, causing the "power stroke"
- During the power stroke, the head loses the bound ADP, thus returning to the "attached" start position

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:

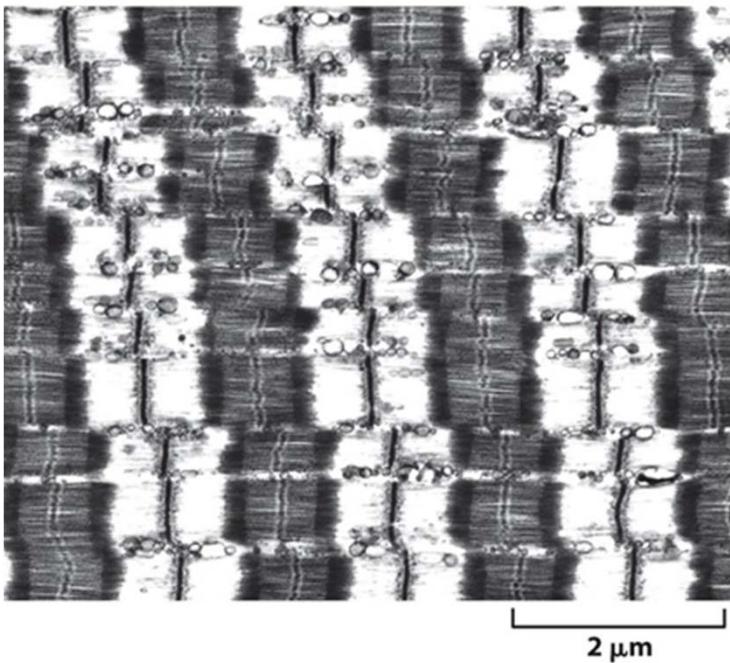


These huge **multinucleated** cells form by the **fusion** of many muscle cell precursors, called **myoblasts**.

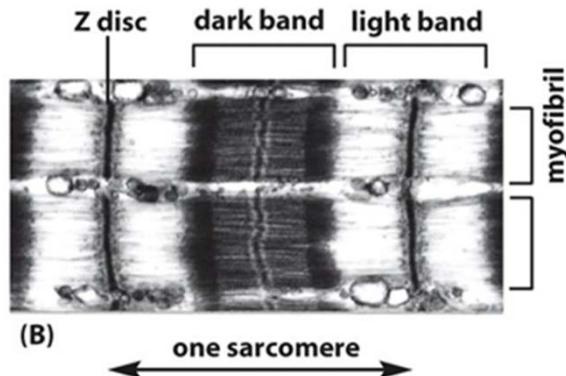
Here, a **single muscle cell** is depicted.

In an adult human, a muscle cell is typically **50 µm in diameter** and can be up to **several centimeters long**.

Skeletal muscle myofibrils

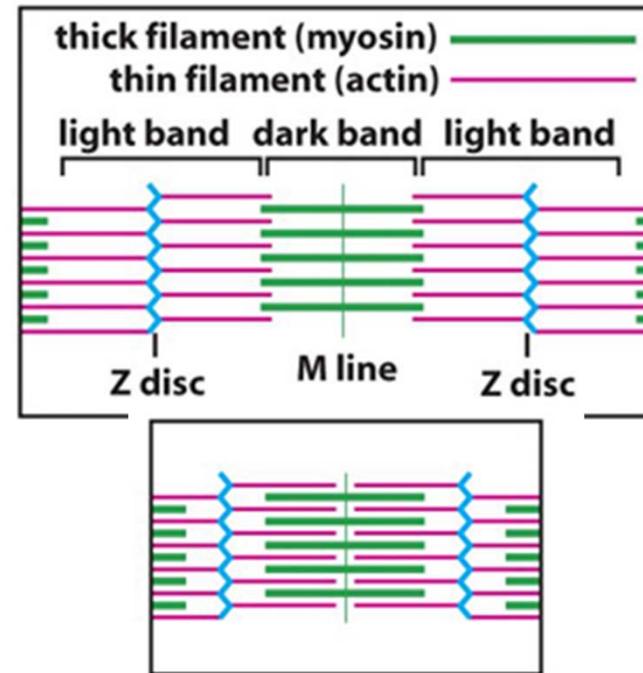


longitudinal section through a skeletal muscle cell of a rabbit, showing the regular pattern of cross-striations



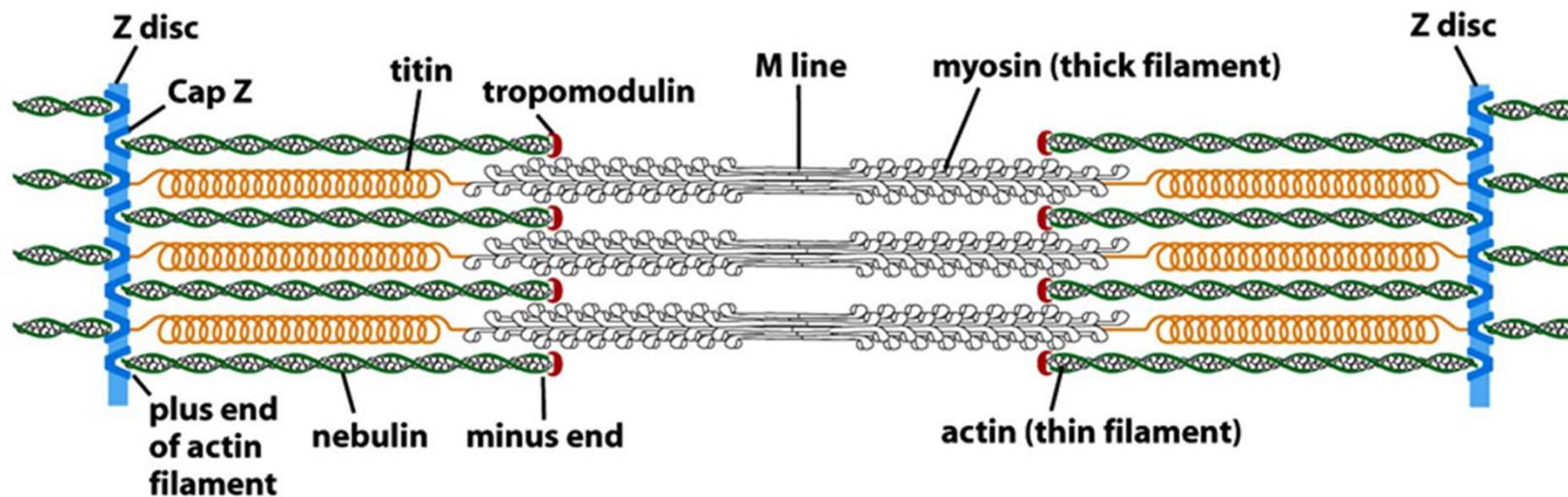
The **Z discs**, at each end of the sarcomere, are **attachment sites for the plus ends** of actin filaments (thin filaments)

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



Contraction: **actin and myosin filaments slide past one another without shortening!**

Organization of accessory proteins in a sarcomere



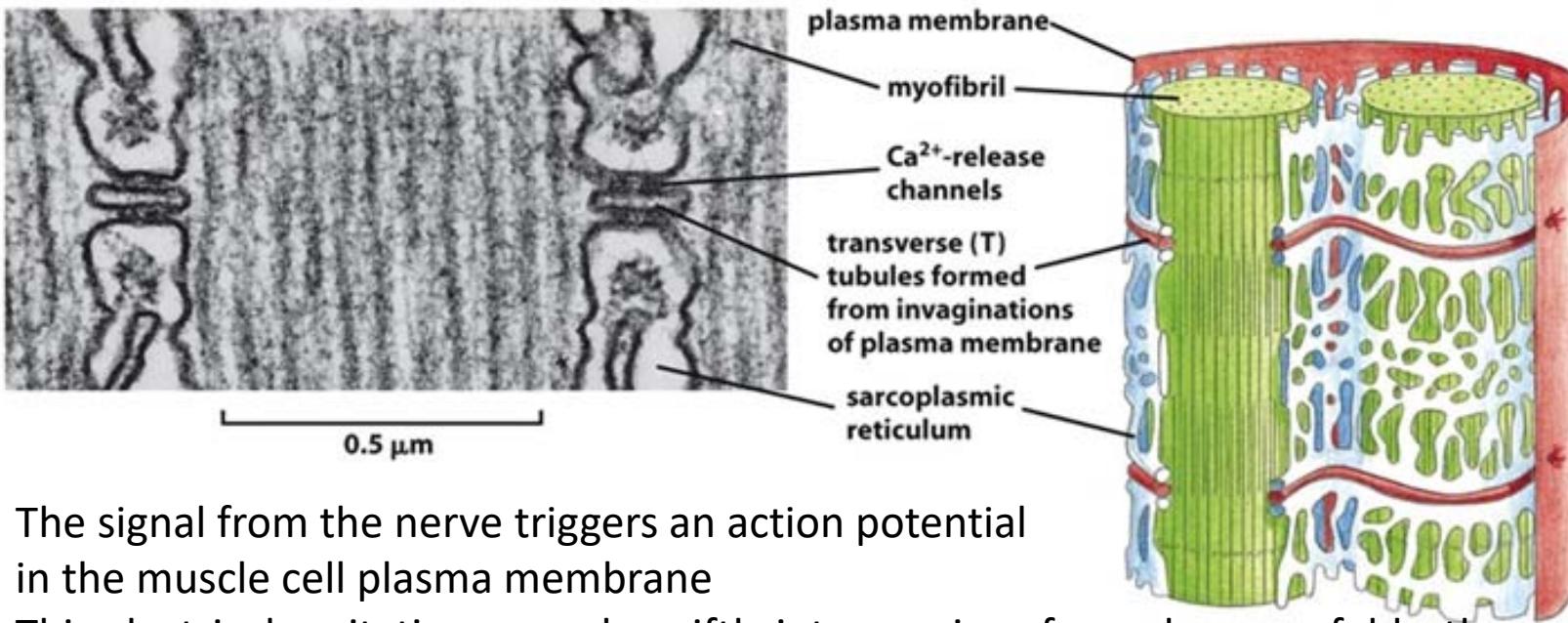
Nebulin provide scaffold and structural support, molecular ruler.

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



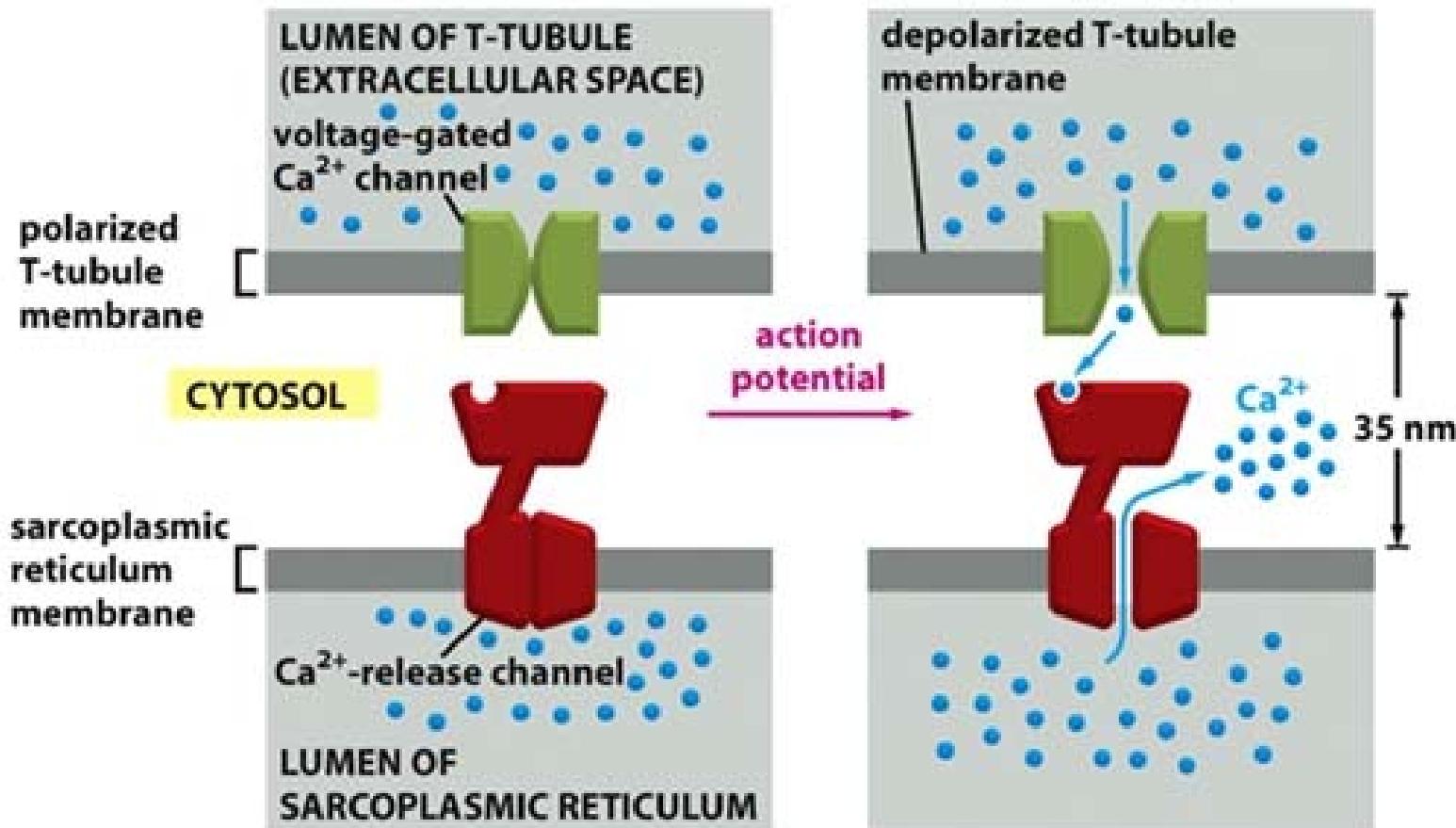
The signal from the nerve triggers an action potential in the muscle cell plasma membrane

This electrical excitation spreads swiftly into a series of membranous folds, the transverse tubules, or **T tubules**

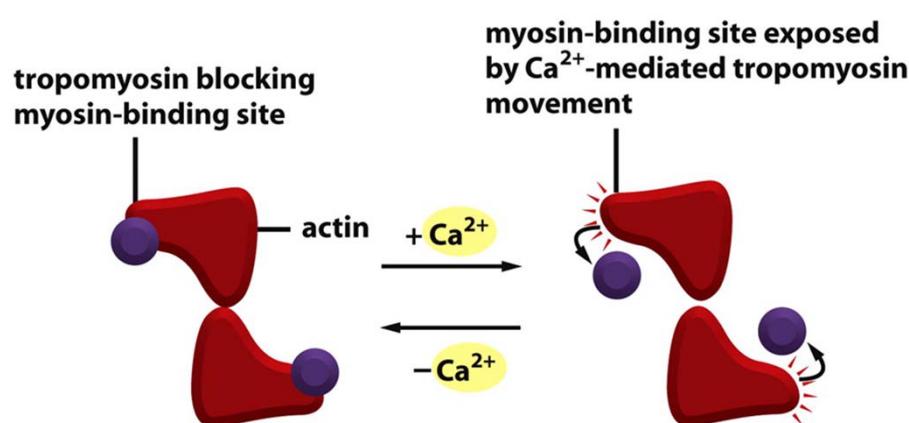
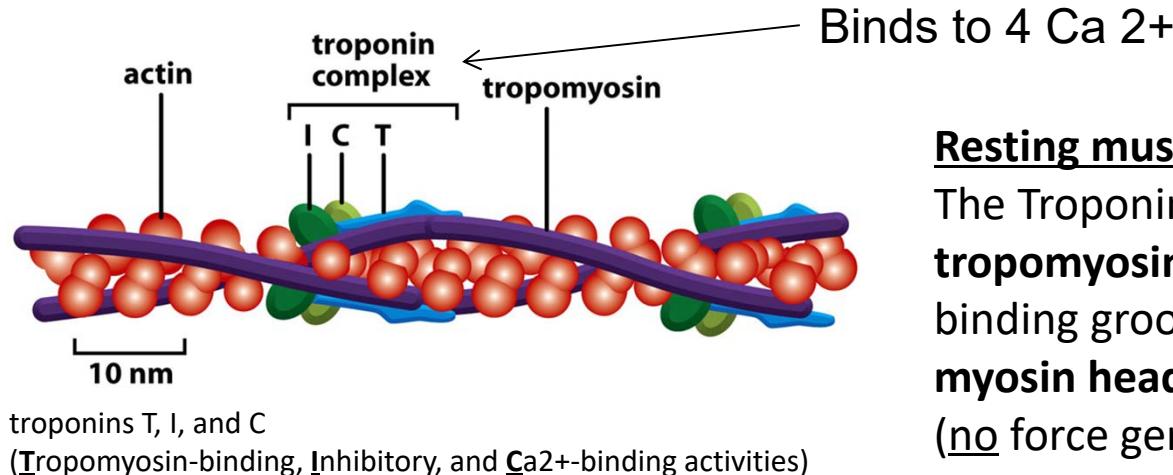
T tubules extend inward from the plasma membrane around **each** myofibril.

The signal is then relayed across a small gap to the **sarcoplasmic reticulum** that **surrounds each myofibril** like a net stocking

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



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 Ca²⁺:

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

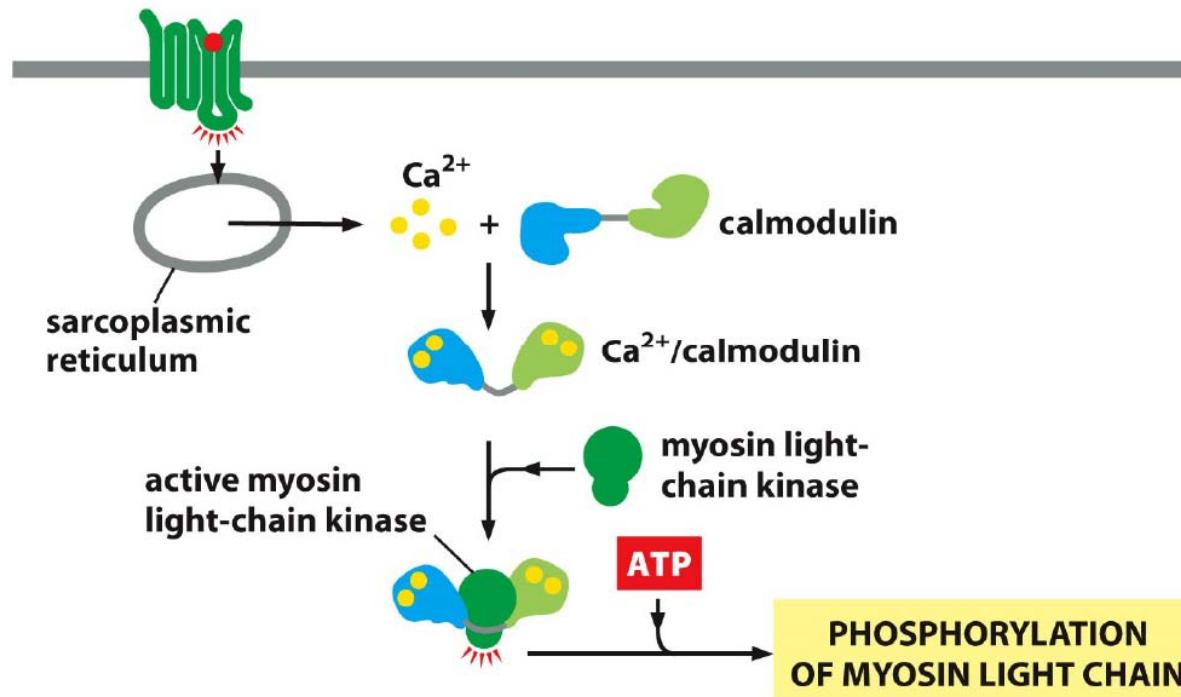
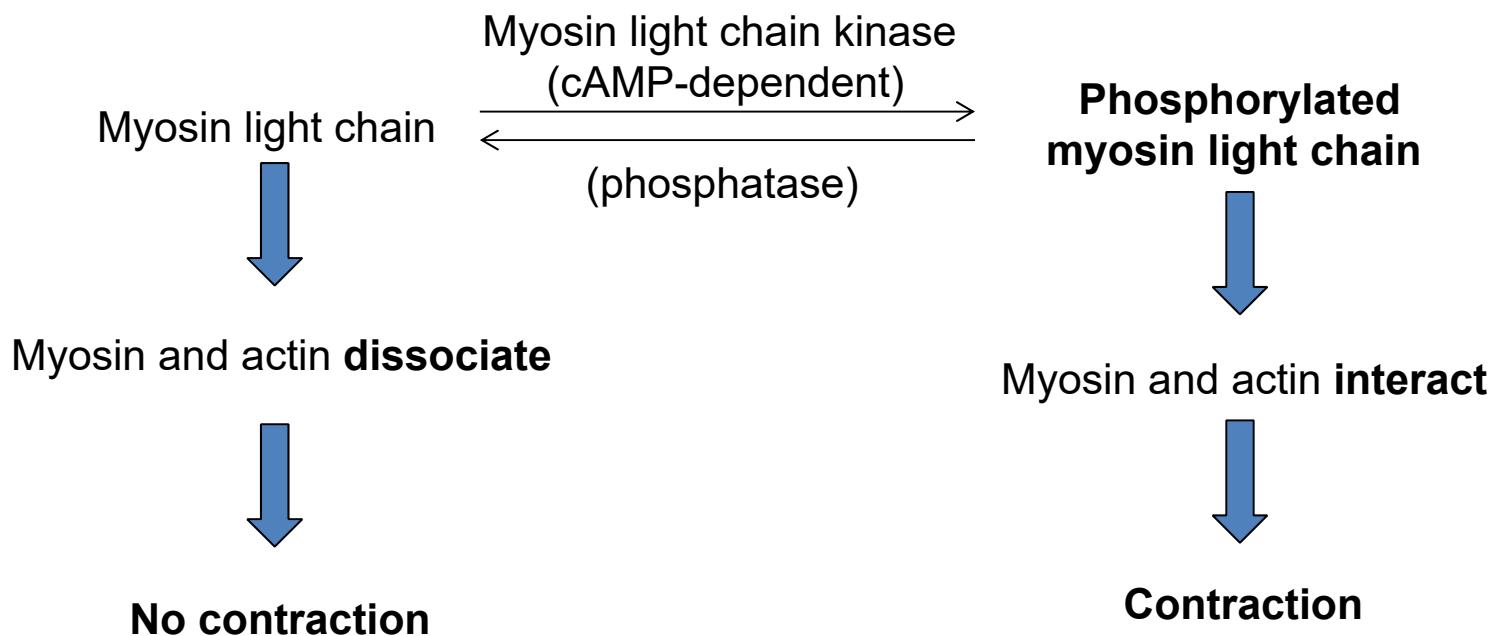


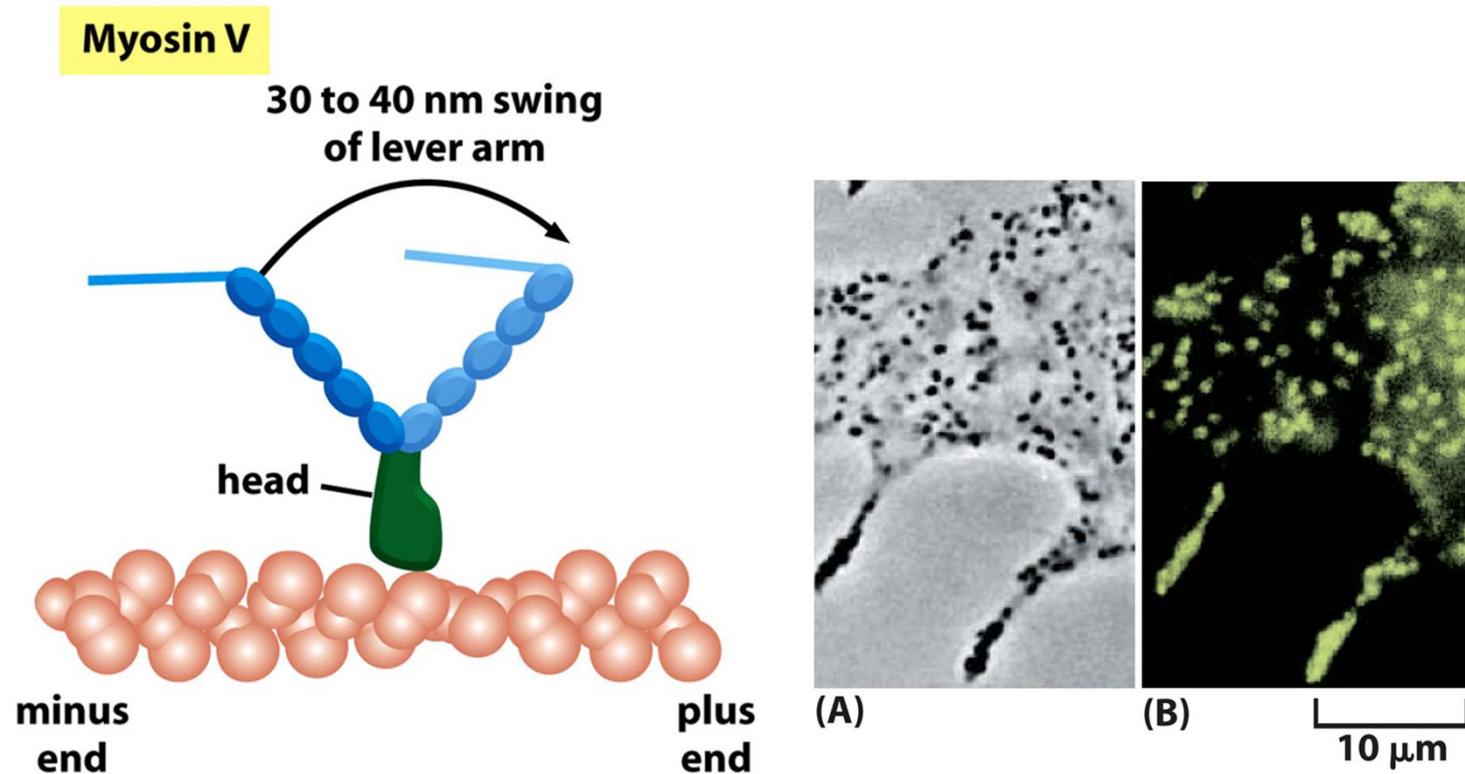
Figure 16-37a Molecular Biology of the Cell 6e (© Garland Science 2015)

Upon muscle stimulation by activation of cell-surface receptors, Ca^{2+} released into the cytoplasm from the sarcoplasmic reticulum (SR) binds to calmodulin. **Ca₂₊-bound calmodulin** then binds myosin light-chain kinase (MLCK), which **phosphorylates myosin light chain**, stimulating myosin activity.

3) Muscle contraction is additionally regulated by myosin II phosphorylation

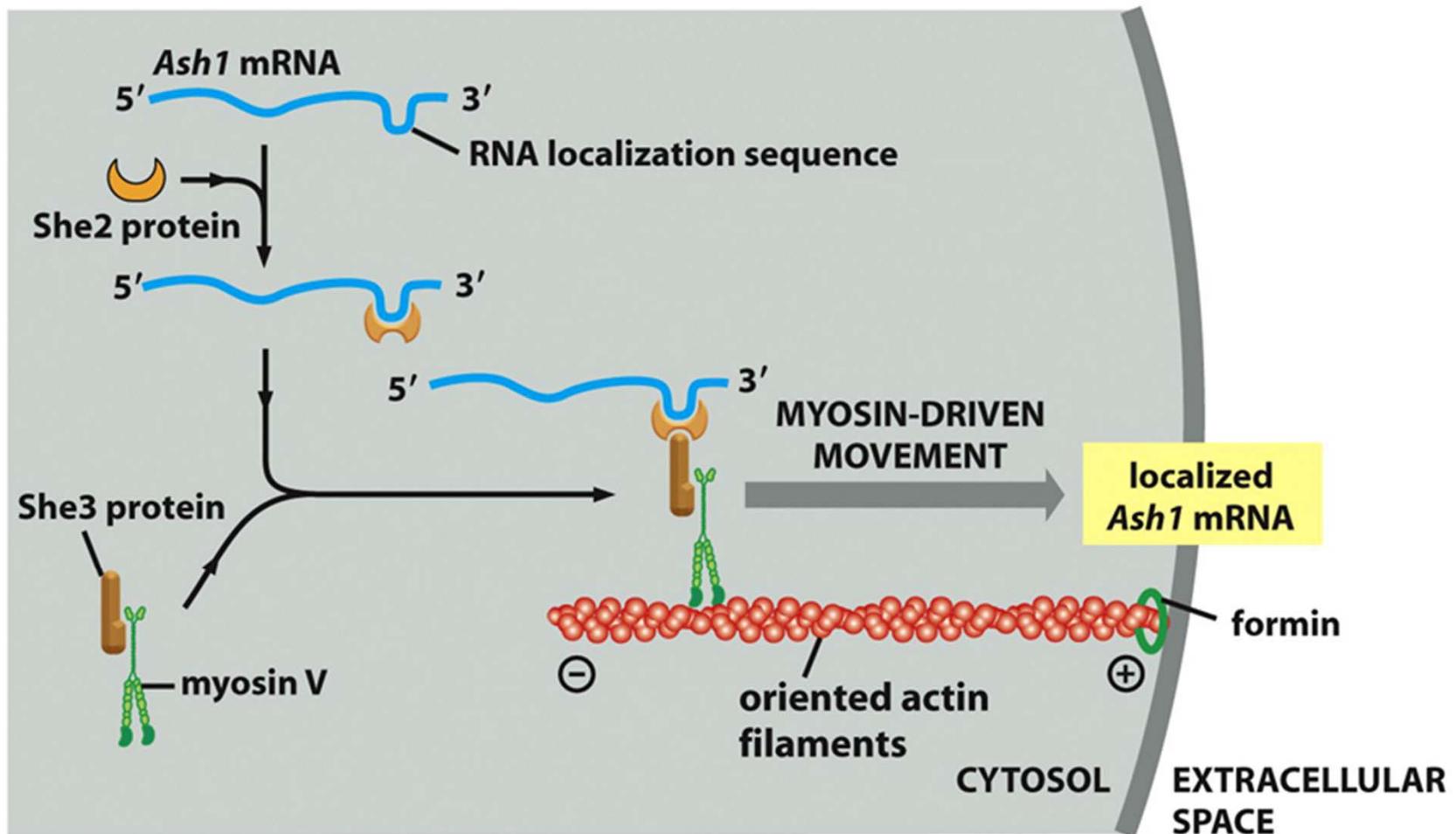


2. Myosin V for organelle/mRNA transport



- 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

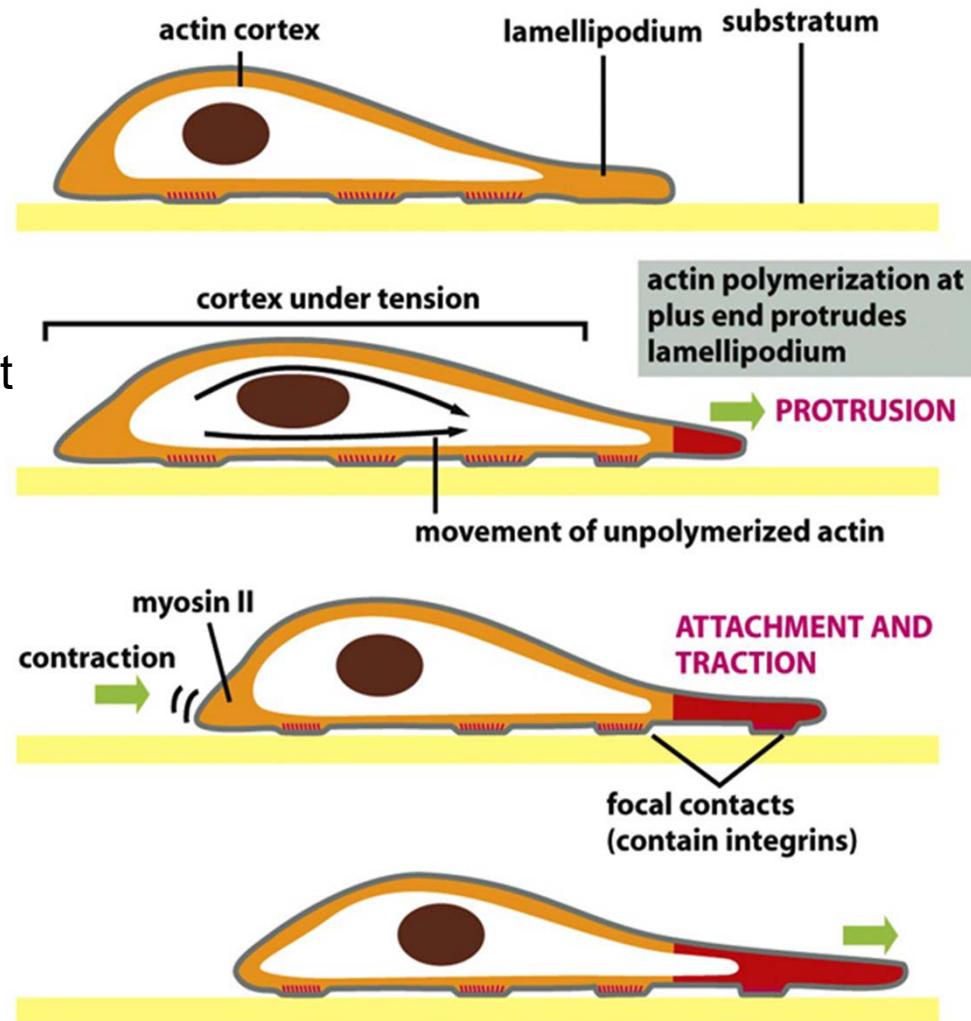
Localized mRNA by Myosin V and the mRNA-binding adaptor proteins She2 and She3



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
Occurrence: formed by migrating **growth cones** and by some **fibroblasts**.

Lamellipodia:

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

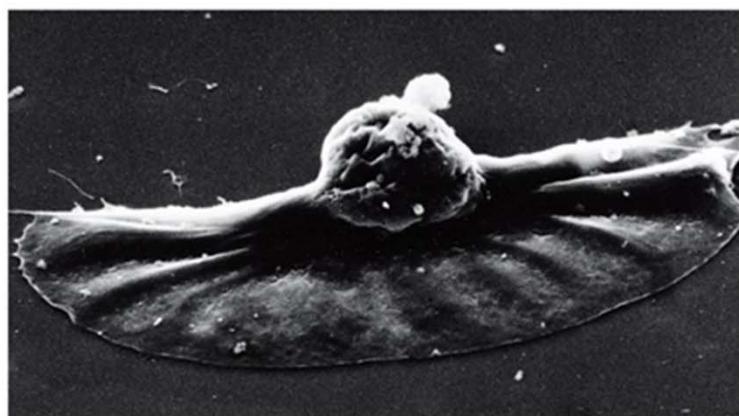
Pseudopodia:

- **three dimensional projections** filled with an **actin filament gel.**
Occurrence: **Amoebae and neutrophils**

Cell leading edge in migration

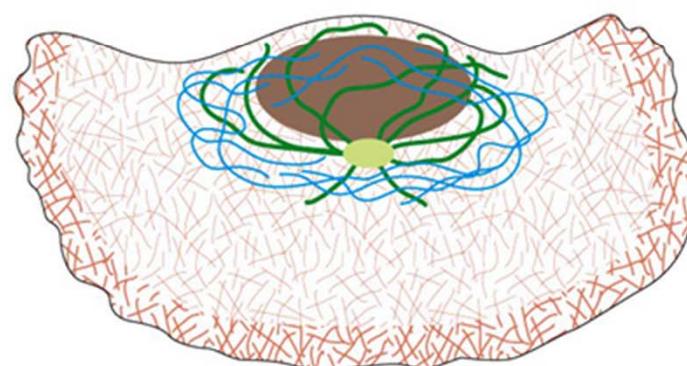


(A)



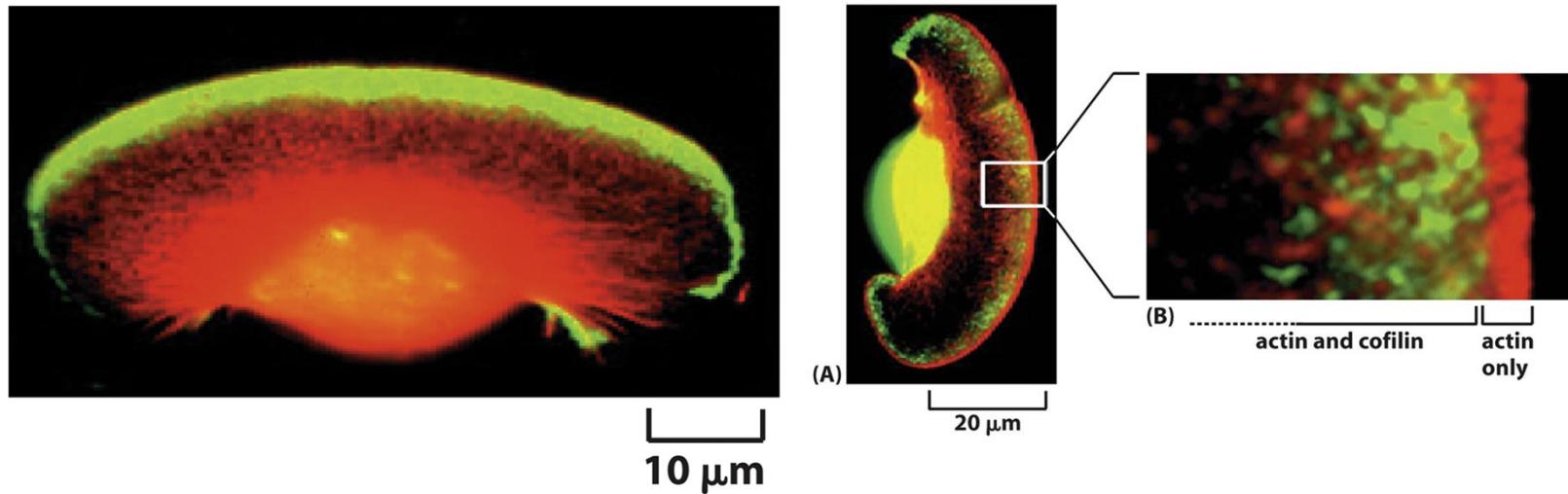
(B)

10 μm

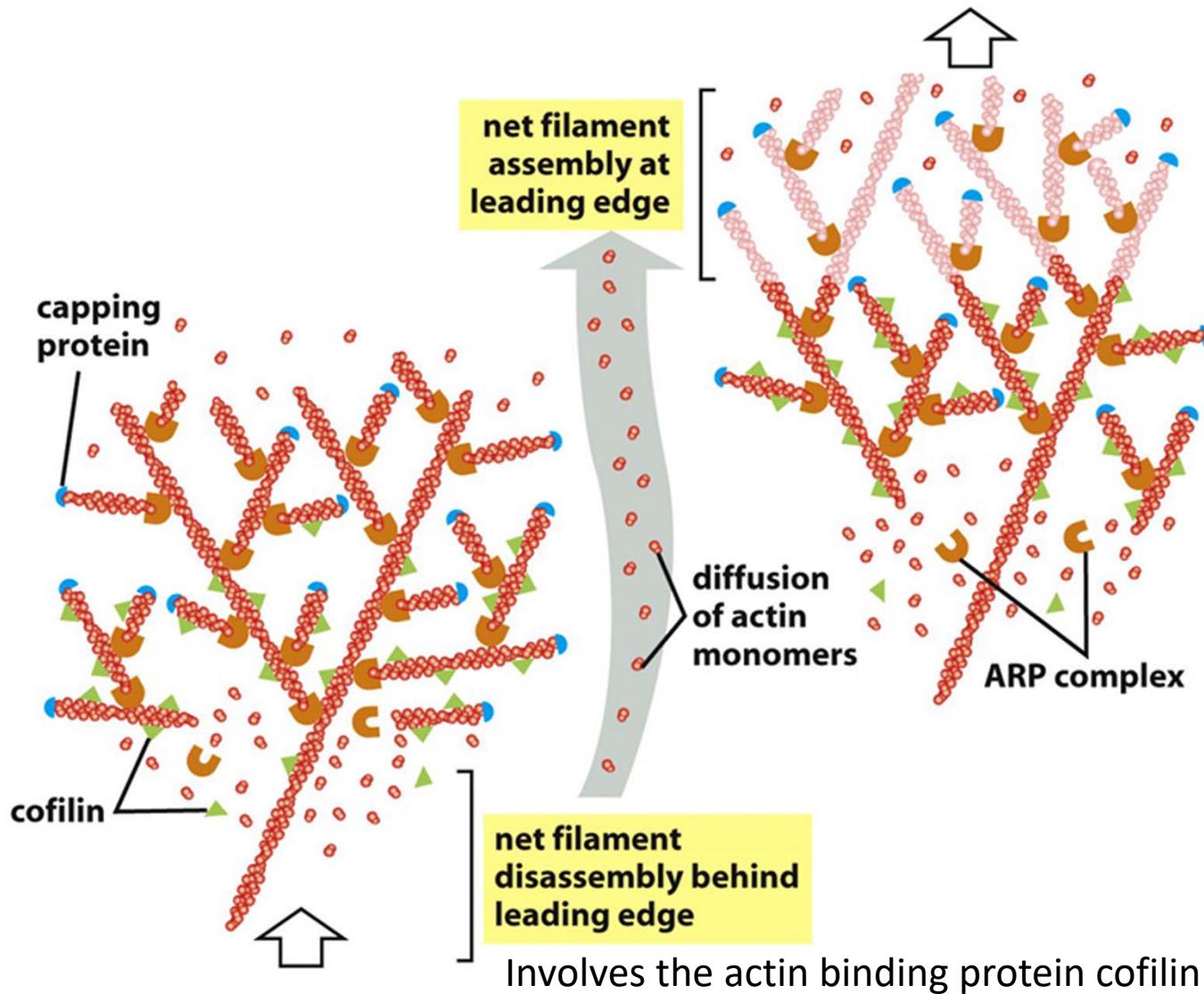


(C)

Localization of different actin regulation proteins in the leading edge

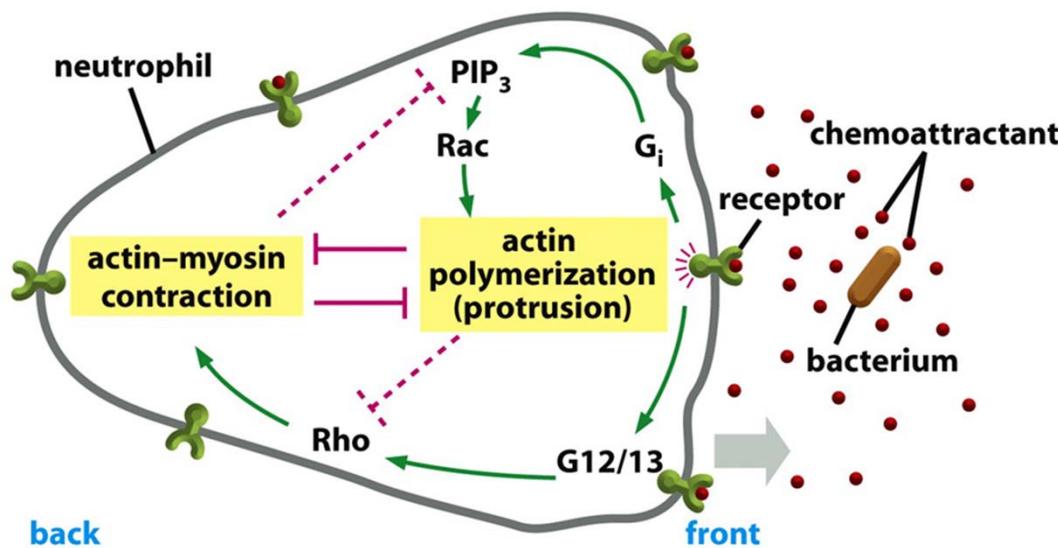


How actin cause protrusions in leading edge?



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.



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

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