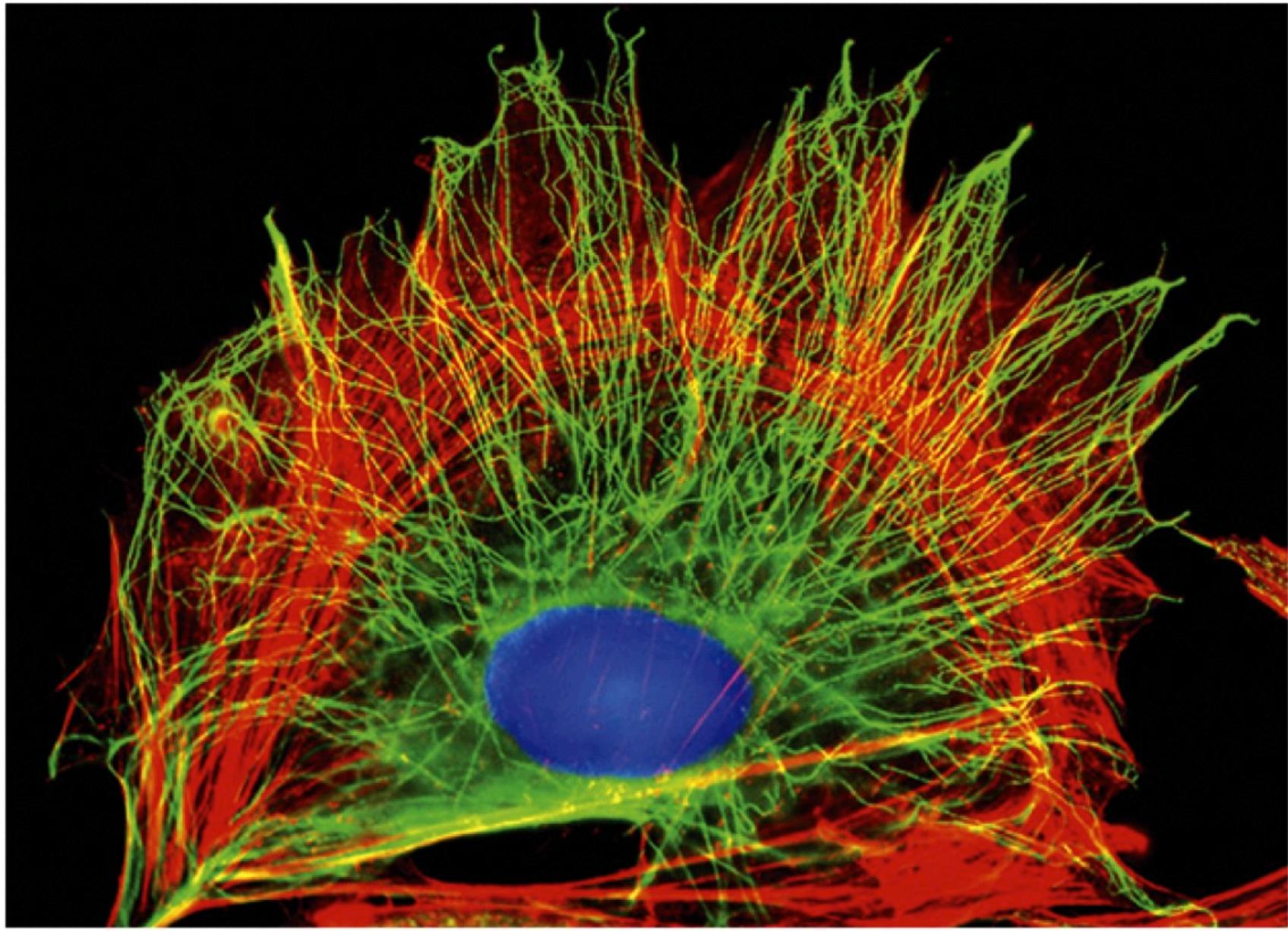


The Cytoskeleton

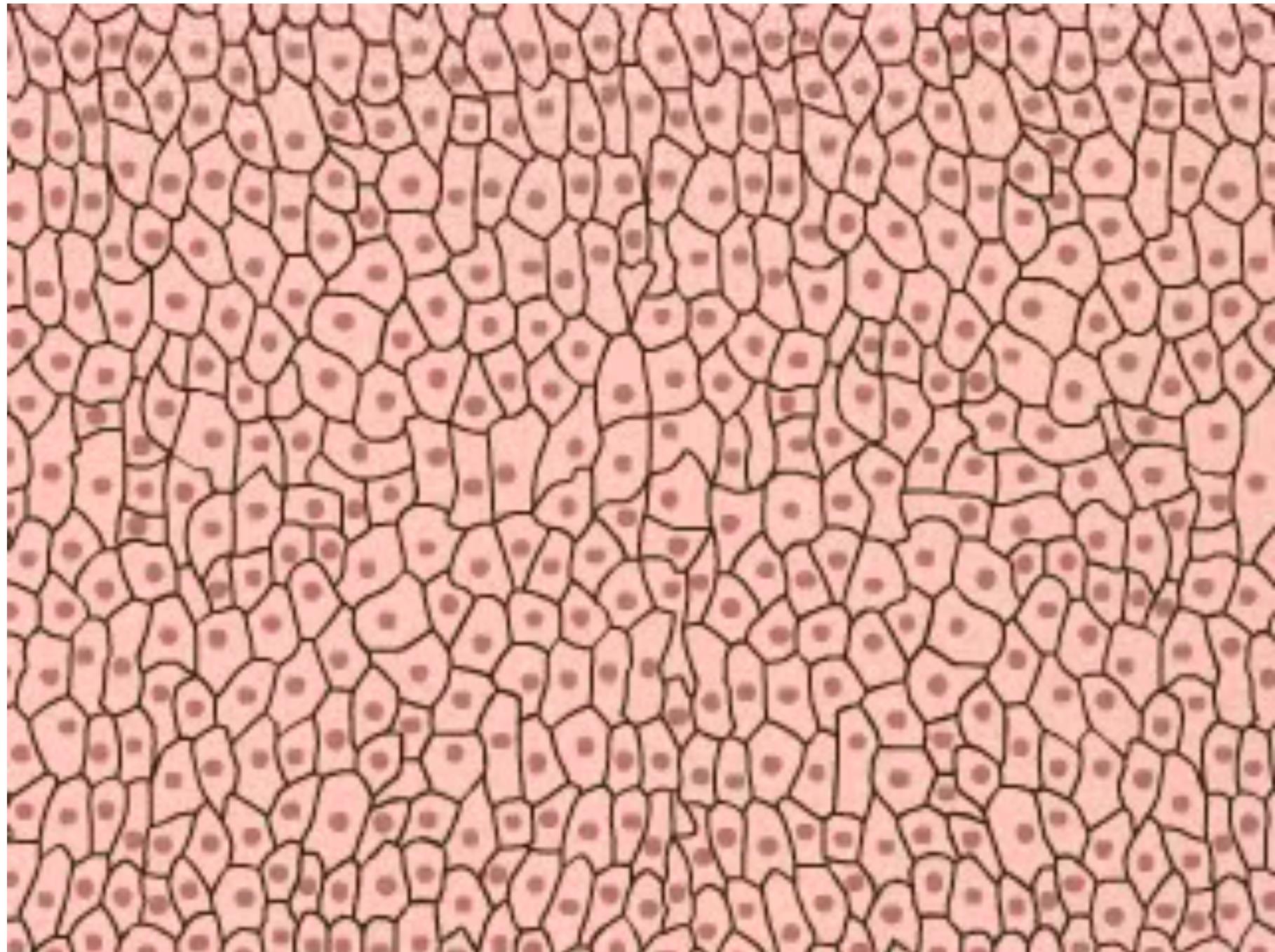


Biological Functions of Cytoskeletal Dynamics: Cell migration and homing as examples

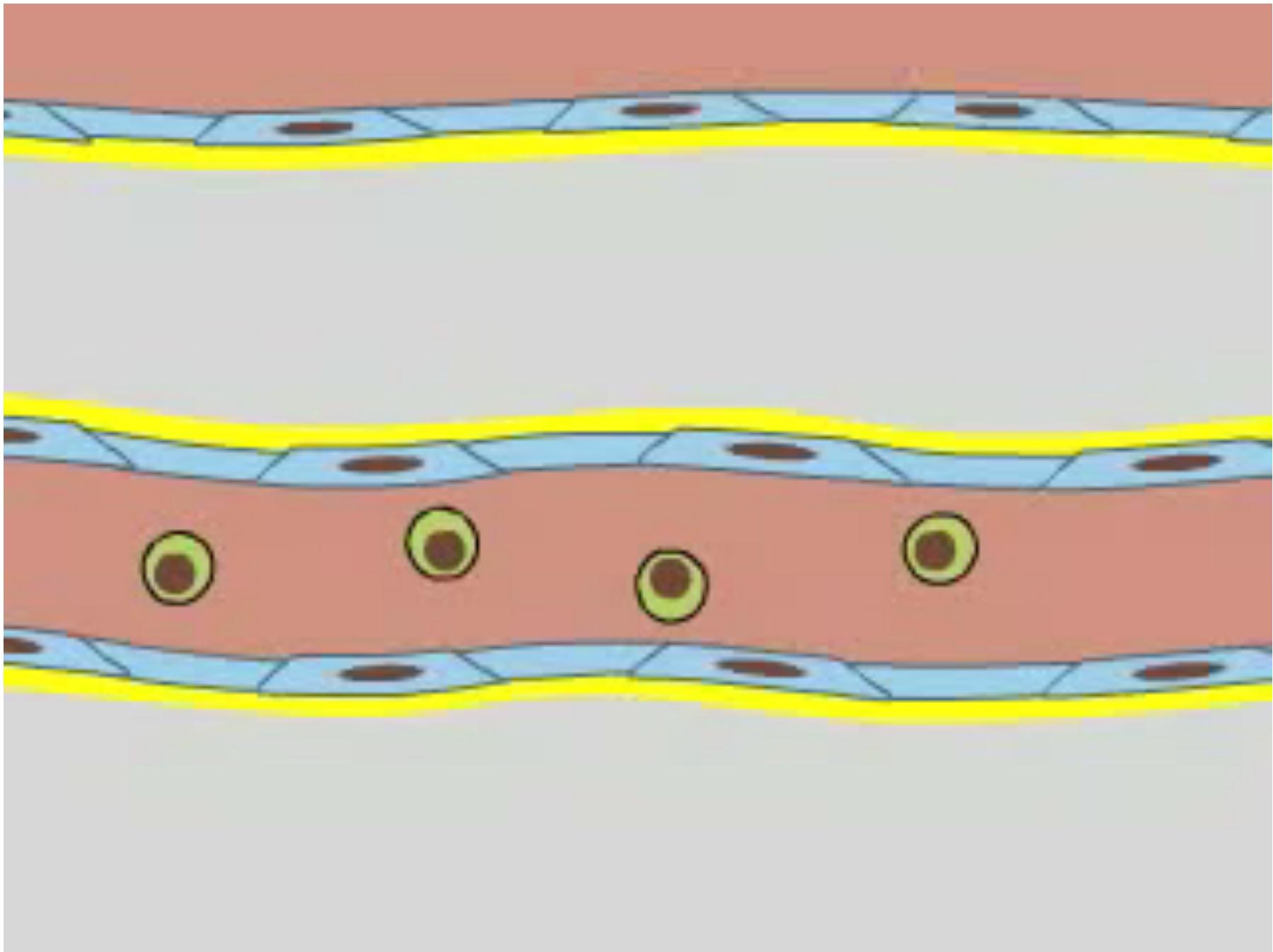
Cell migration and homing are important for normal physiological functions (e.g. development, chemotaxis, immunity, others) and also for pathological processes (e.g. infection, cancer metastasis, others).

**Both MT and F-actin dynamics are required
for most cell migration and homing events.**

Cell Migration-Mediated Wound Healing: movie

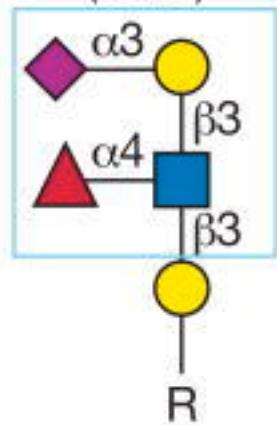


Cell Homing-Mediated Immunity: movie

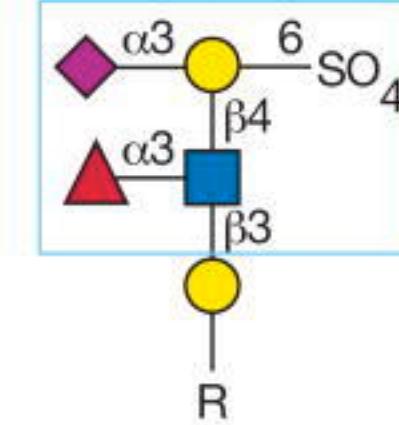


The Selectin Ligands Required for Leukocyte Homing and Inflammation

Sialyl Lewis^a
(SLe^a)



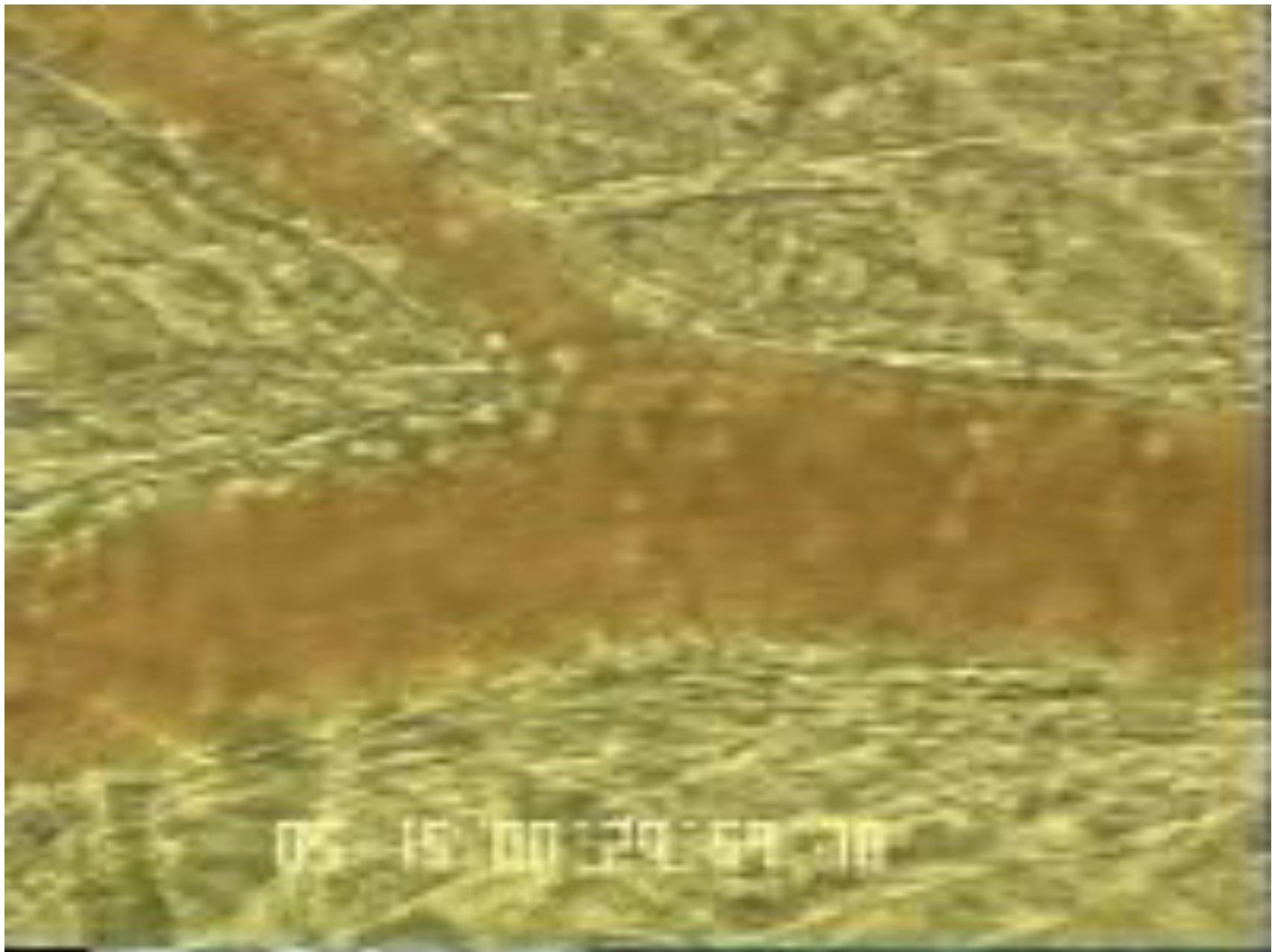
6'-Sulfo-Sialyl Lewis^x
(6'-Sulfo-SLe^x)



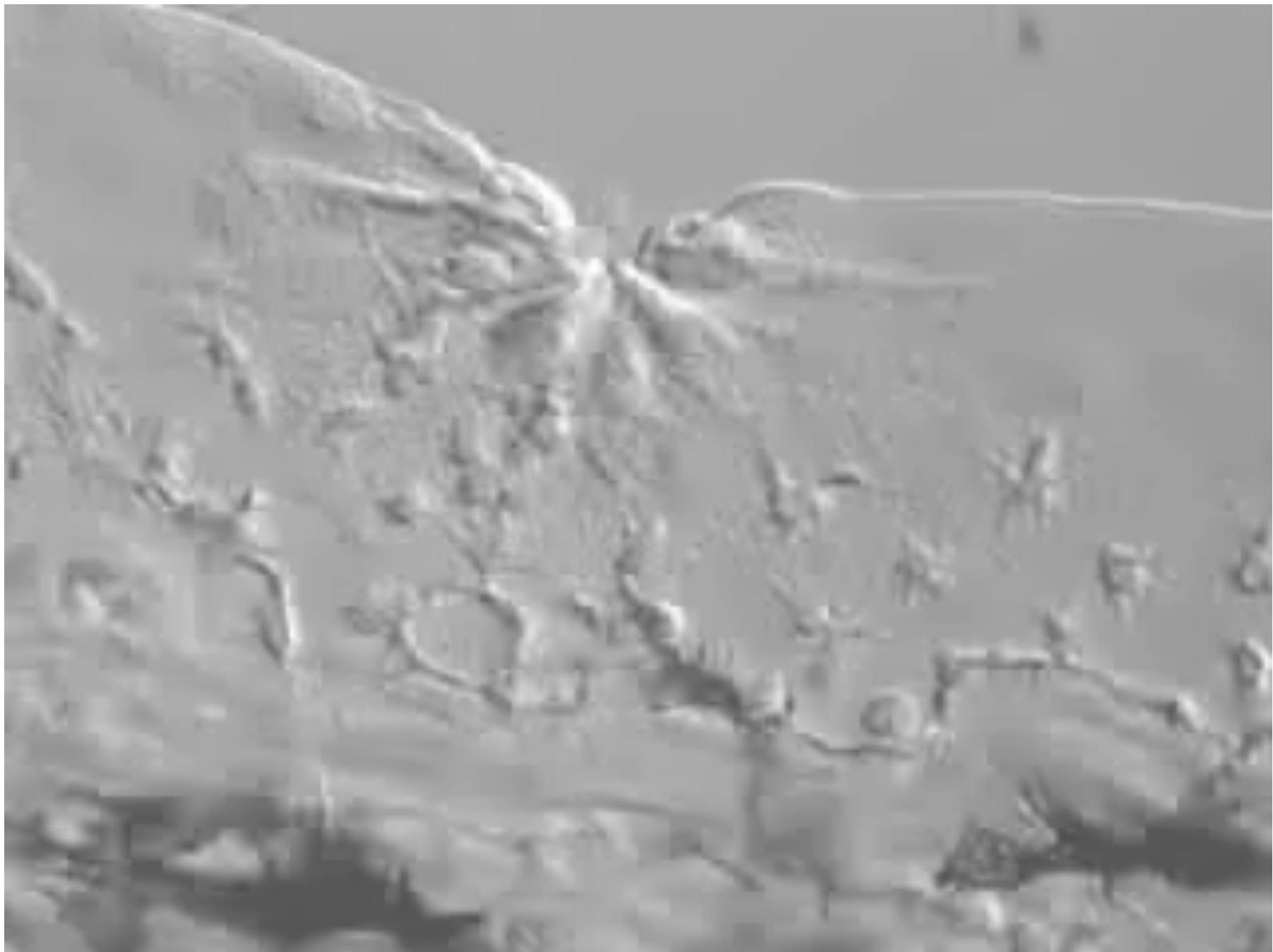
- Galactose (Gal)
- N-Acetylgalactosamine (GalNAc)
- ▲ Galactosamine (GalN)
- Glucose (Glc)
- N-Acetylglucosamine (GlcNAc)
- ▲ Glucosamine (GlcN)
- Mannose (Man)
- N-Acetylmannosamine (ManNAc)
- ▲ Mannosamine (ManN)

- ★ Xylose (Xyl)
- ◆ N-Acetyleneuraminic acid (Neu5Ac)
- △ N-Glycolyneuraminic acid (Neu5Gc)
- ◆ 2-Keto-3-deoxynononic acid (Kdn)
- ▲ Fucose (Fuc)
- ◆ Glucuronic acid (GlcA)
- ◆ Iduronic acid (IdoA)
- ◆ Galacturonic acid (GalA)
- ◆ Mannuronic acid (ManA)

Selectin-Mediated Leukocyte Rolling on Inflamed Vascular Endothelium (movie)

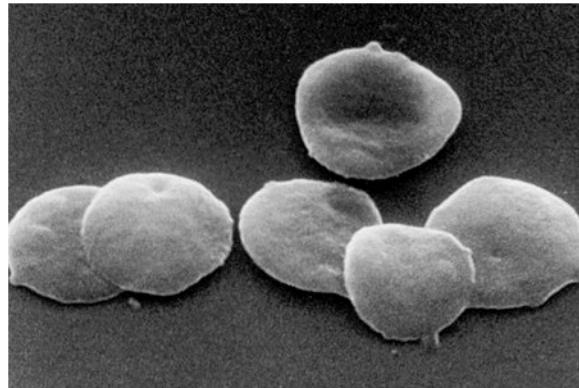


Leukocyte homing in wound detection/repair: movie

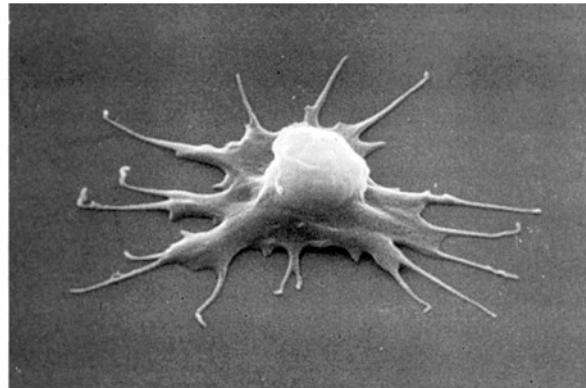


Besides cell migration and homing, another example showing the importance of cytoskeleton rearrangement:

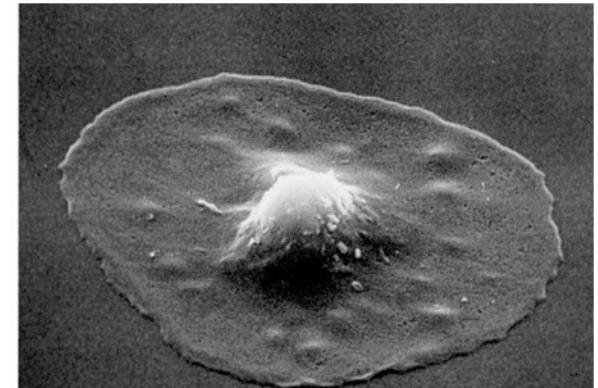
Platelets change shape during blood clotting (thrombosis) via complex rearrangements of the actin cytoskeleton connected to the plasma membrane.



Resting platelets have a discoid shape.



After exposure to extracellular agents, platelets attach to the substratum & start to extend themselves, a process driven by F-actin rearrangement.



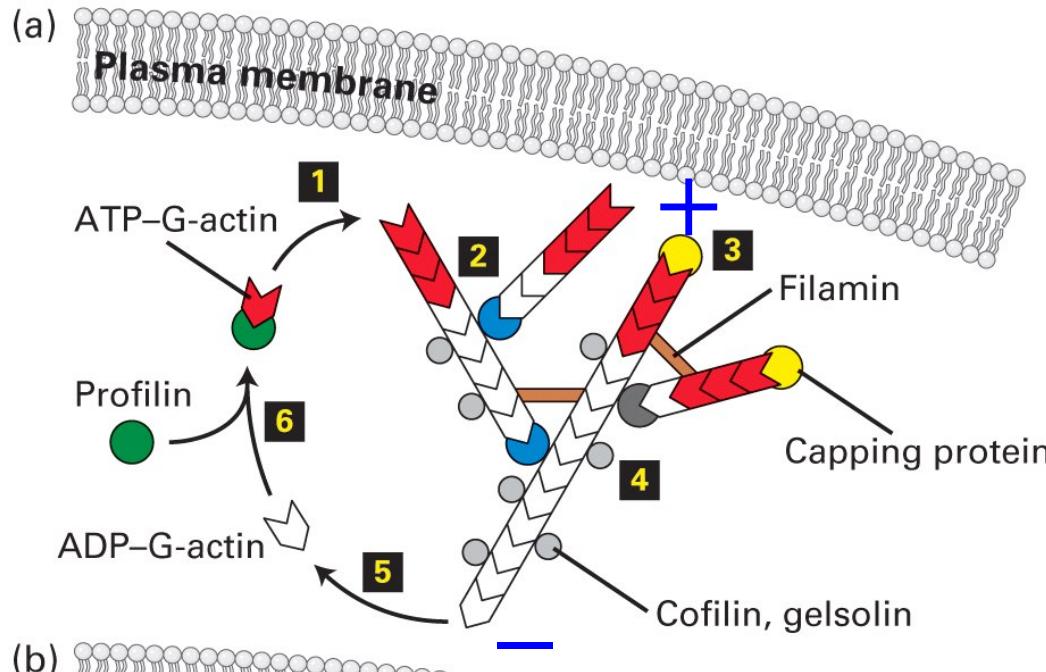
Finally, platelets spread out to promote thrombosis.

Regulation of the Cytoskeletal Filaments

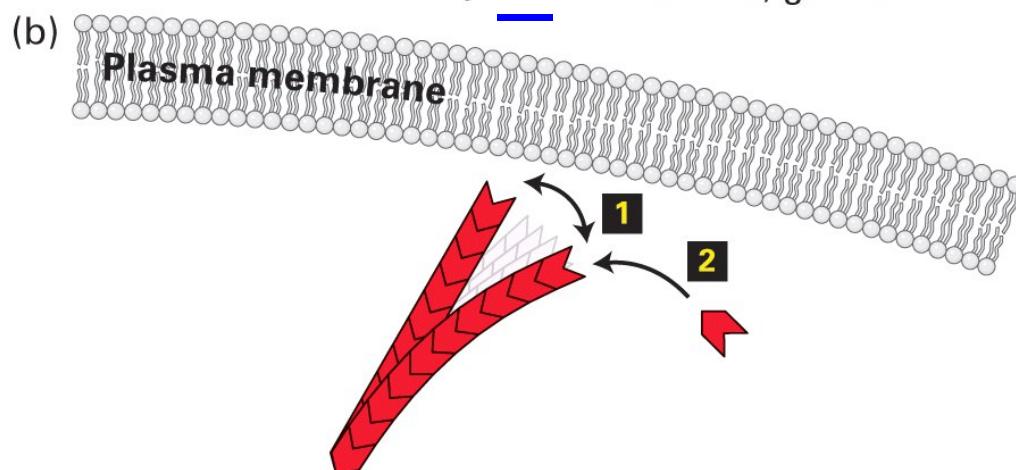
**We will mainly use F-actin assembly/disassembly as an example.
The same principles can be applied to MT.**

F-Actin Regulation

We will discuss some of the regulatory factors involved



The polymerization of F-actin from its plus end functions as an engine to push the plasma membrane forward.



The depolymerization of F-actin from its minus end generates a pool of free subunits to allow the filament growth at the plus end

↑
direction of cell movement

Key Points

There are many cytoskeleton regulators inside a cell which cannot be described in detail in this course.

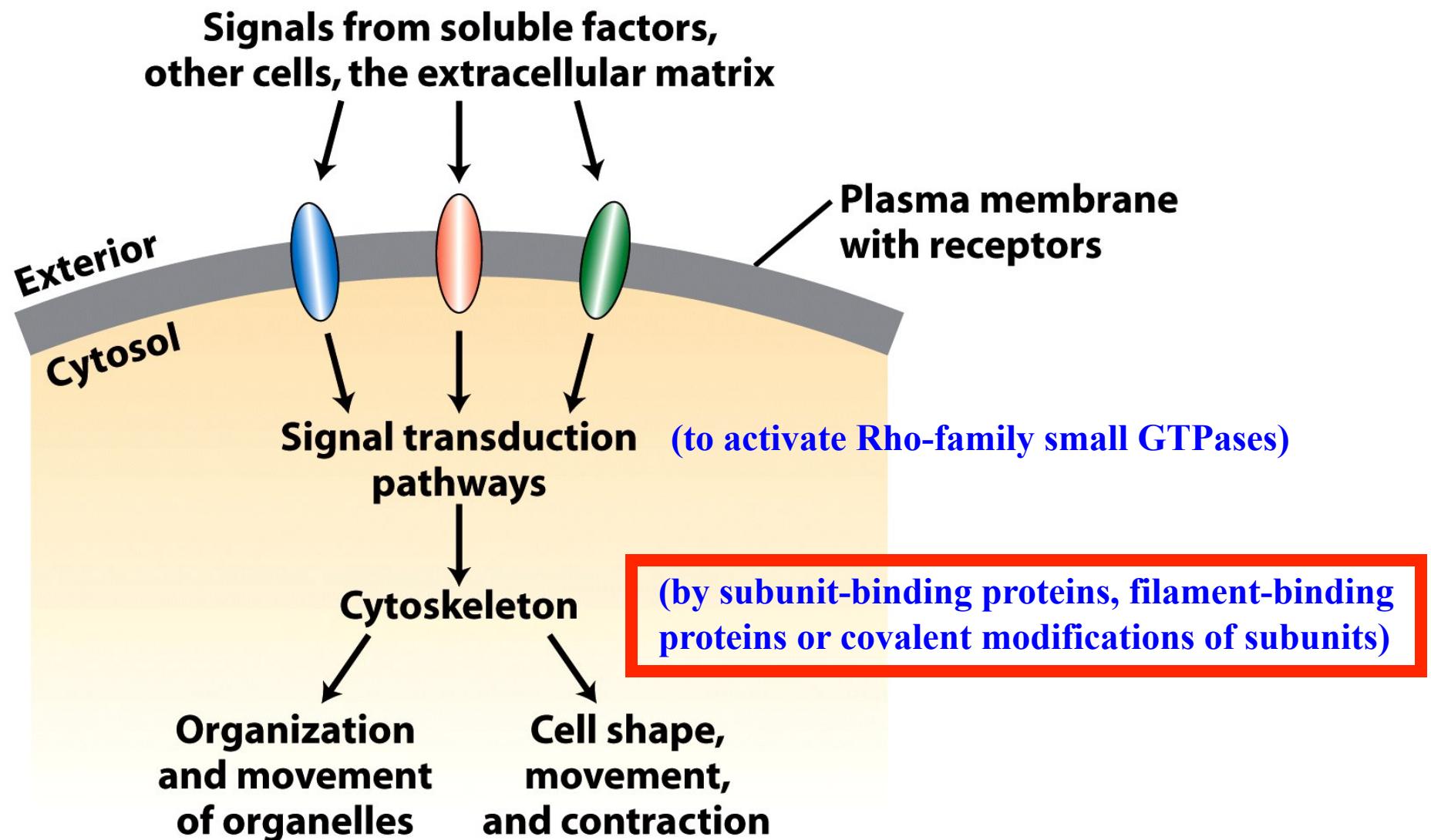
It is more important to understand the following basic principles:

How are the rearrangements of different cytoskeletons coupled to different signals?

&

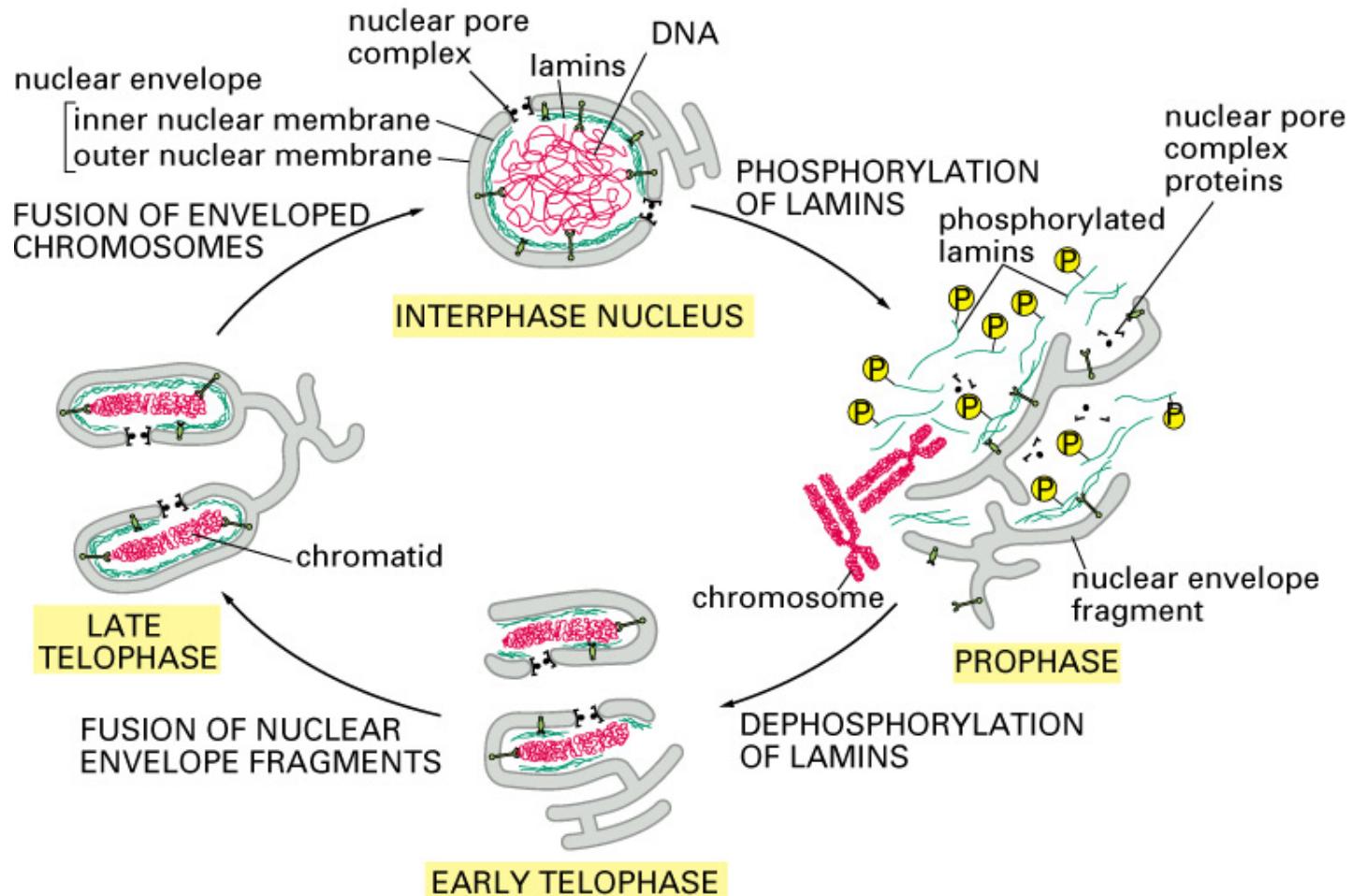
What are the general strategies a cell uses to regulate the assembly/disassembly of different cytoskeletons?

How does the extracellular environment communicate through the cytoskeleton?



Intermediate filaments are more stable compared to F-actin and MT, but they are still dynamic

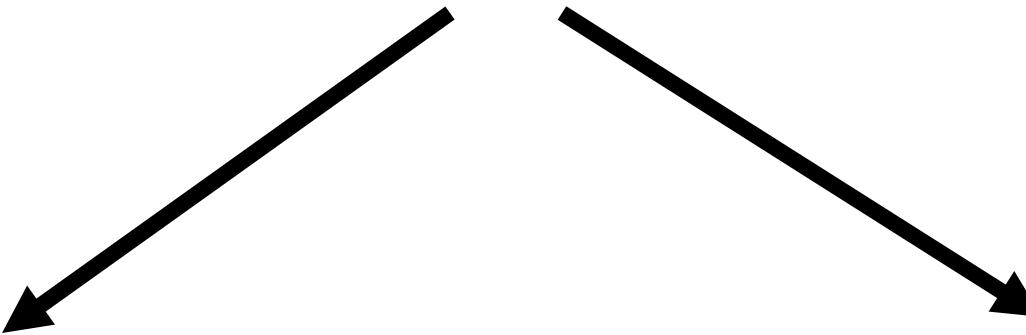
Phosphorylation of lamin (a nuclear IF) contributes to the breakdown of the nuclear envelope during mitosis, which is important for the segregation of replicated chromosomes.



How Cells Regulate Their Cytoskeletal Filaments

**covalent modifications of the building subunits
(such as phosphorylation of lamins)**

by filament- or subunit-associated accessory proteins



**Some accessory proteins
have a general role in
all cell types**

**Others have a
cell type-specific role**

How do accessory proteins regulate cytoskeletal dynamics?

Affecting filament nucleation

Binding to the free subunits

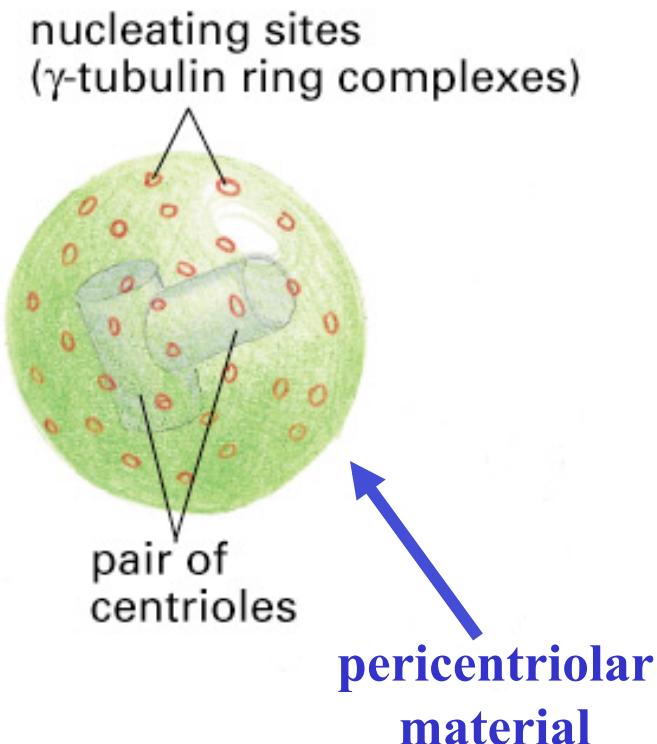
Binding along the filament side

Binding at the filament ends

The Essential Role of the MTOC in the Nucleation of MT in non-dividing (interphase) cells

MTOC is a region of the cytoplasm, usually lying near the nucleus, that initiates the majority of a cell's microtubules. They define the position of microtubule initiation and both the number and orientation of microtubules they initiate.

The centrosome functions as the MTOC in most animal cells.



a pair of centrioles:
a barrel shaped organelle composed of MT. It organizes the pericentriolar material

pericentriolar material:
organizing the microtubules in both interphase and mitotic cells via the γ -TuRC
(γ tubulin ring complex)

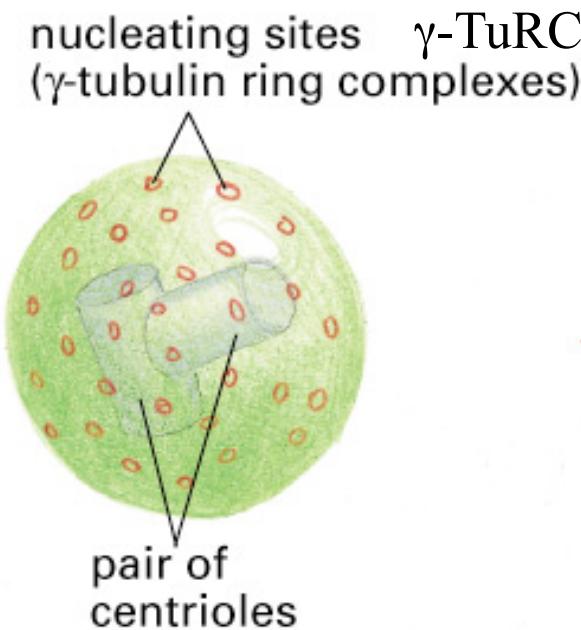
γ TuRC Nucleates Microtubules at the MTOC

Number of MTs:

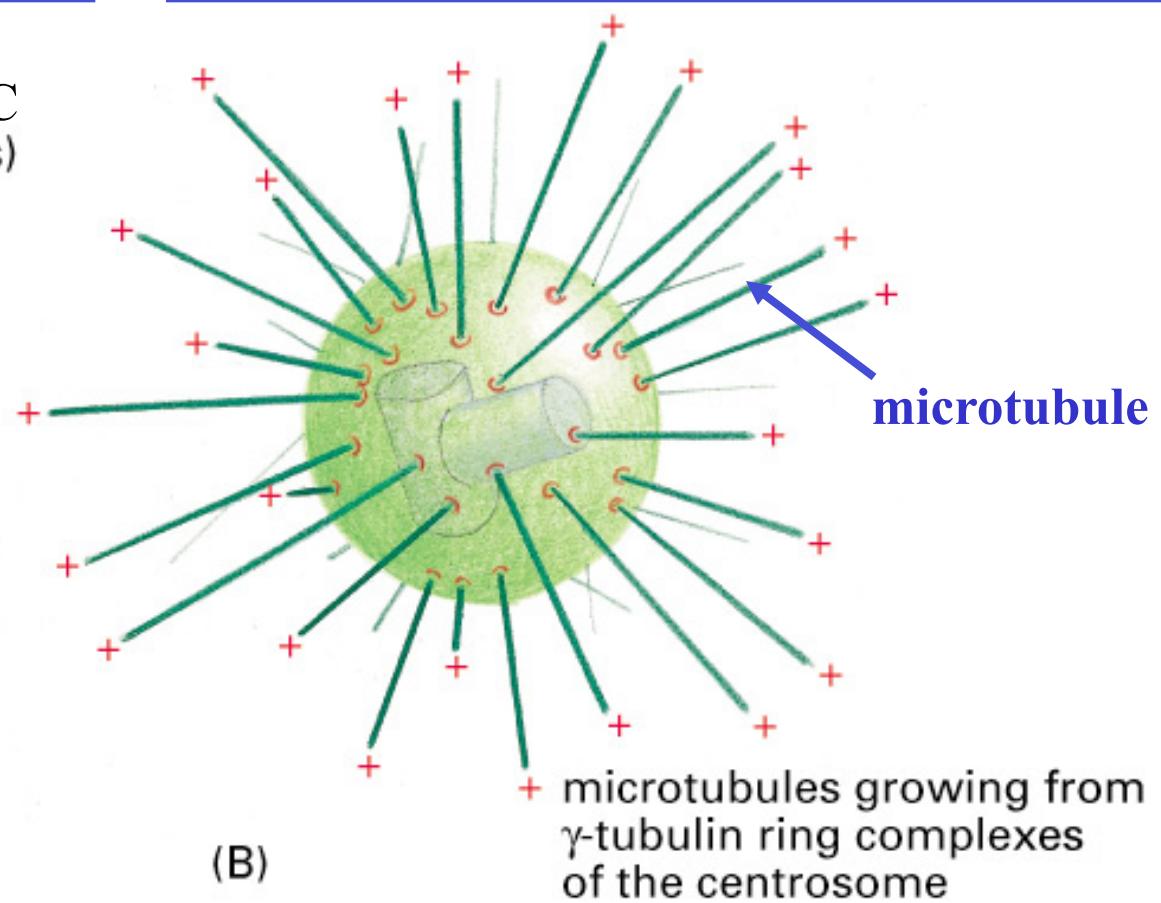
There are a limited number
of γ -TuRC complexes

Orientation of MTs:

Minus ends of microtubules are anchored
at the γ -TuRC of the centrosome



(A)

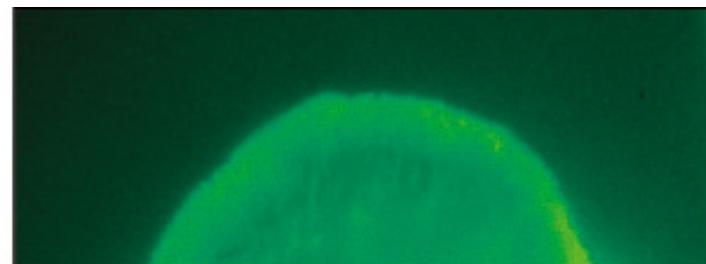


(B)

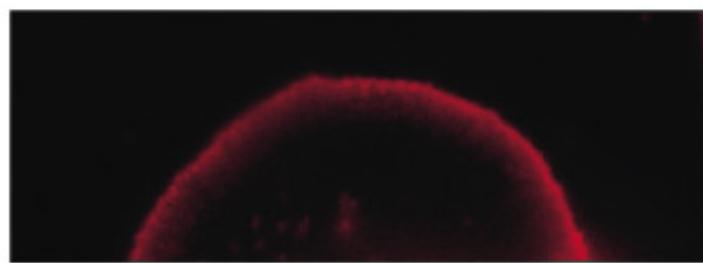
The minus ends of most cellular microtubules are anchored to the MTOC

Unlike Microtubules, F-actin Often Nucleates Near the PM (i.e. Cortex) in vivo

Observation of the newly assembled F-actin by adding the FITC-labeled phalloidin (labeling F-actin) and rhodamin-labeled G-actin into a permeabilize cell. The former is used to visualize the total F-actin (including the new & old ones, whereas the latter is used to detect the newly assembled F-actin)



(A)



(B)

(Phalloidin: a fungus toxin which binds to F-actin)

total actin
(by FITC-phalloidin)
a green fluorescent molecule

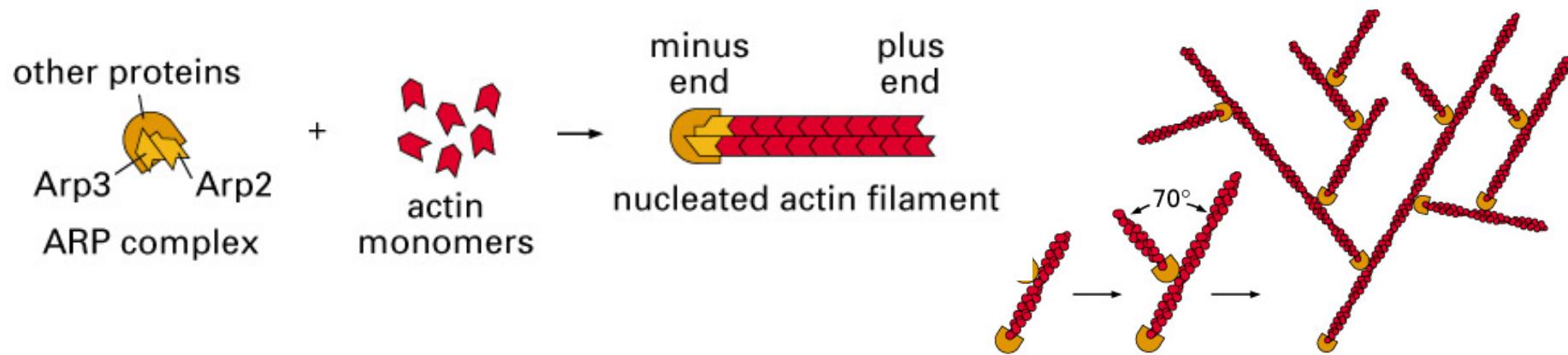
newly polymerized actin
(by rhodamin-actin)
a red fluorescent molecule

What nucleates F-actin at the cortex?

Two protein complexes can nucleate F-actin

ARP (actin related protein) complex:
Nucleates branched F-actin

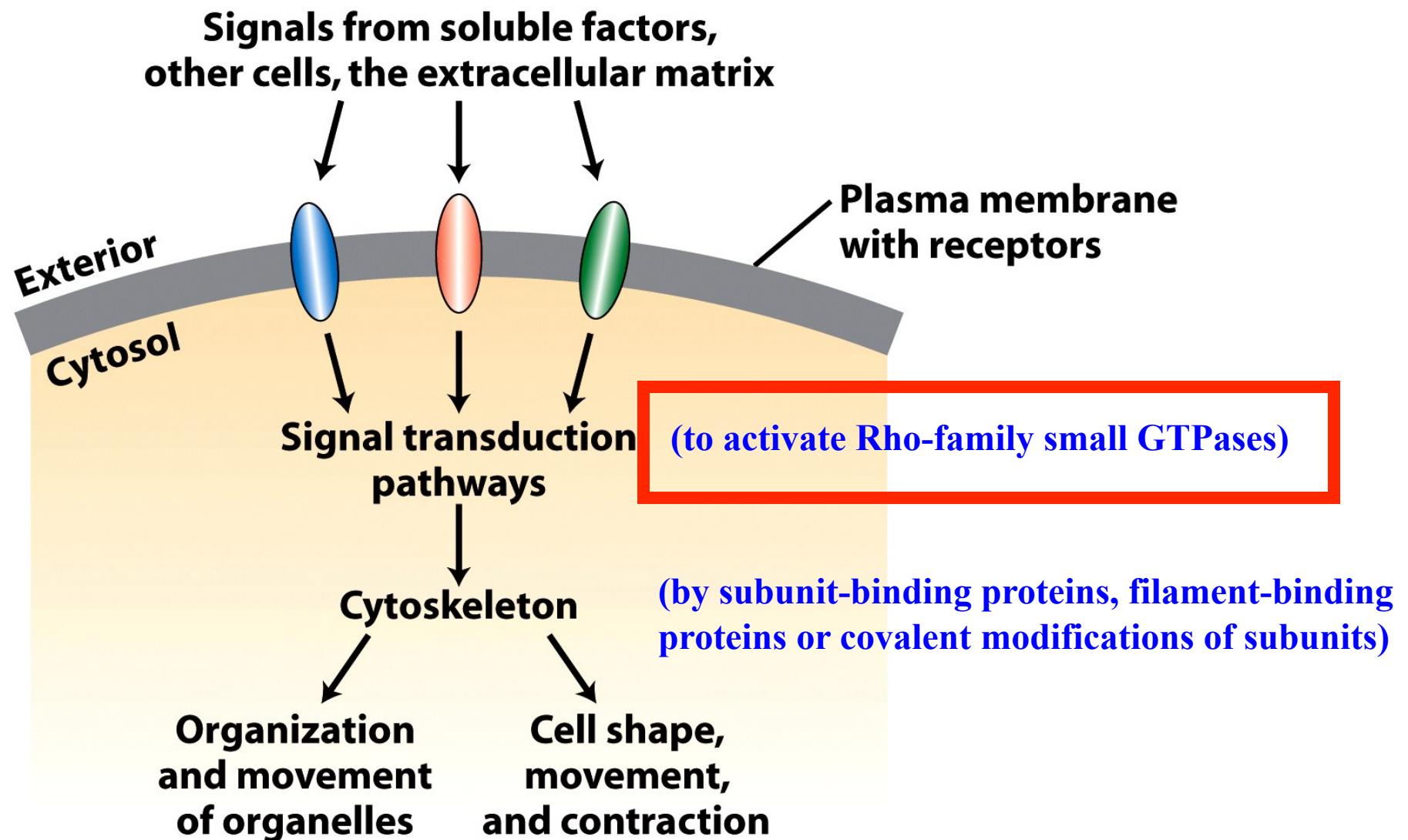
ARP complexes serve as the nuclei allowing the filament growth from the sides of pre-existing filaments. Upon binding to the pre-existing filament, ARP2 & 3 change their conformation to resemble the plus end, providing a template for the assembly of a new filament (ARP protects the – end of branched F-actin)



Formin protein (not shown here): nucleates unbranched F-actin.
Formin does not cap the minus end of F-actin. Instead, it associates with the growing plus end of F-actin.

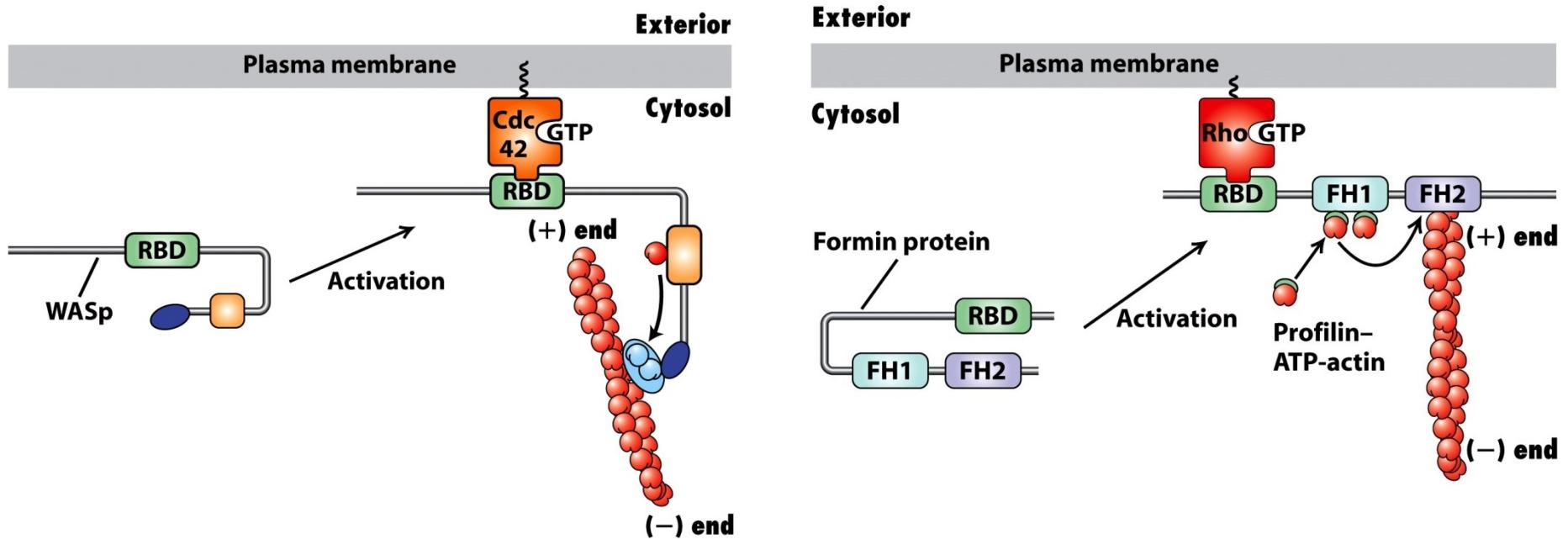
We will see later why different forms of F-actin are needed
How are the activities of ARP and Formin regulated?

How does the extracellular environment communicate with the cytoskeleton?

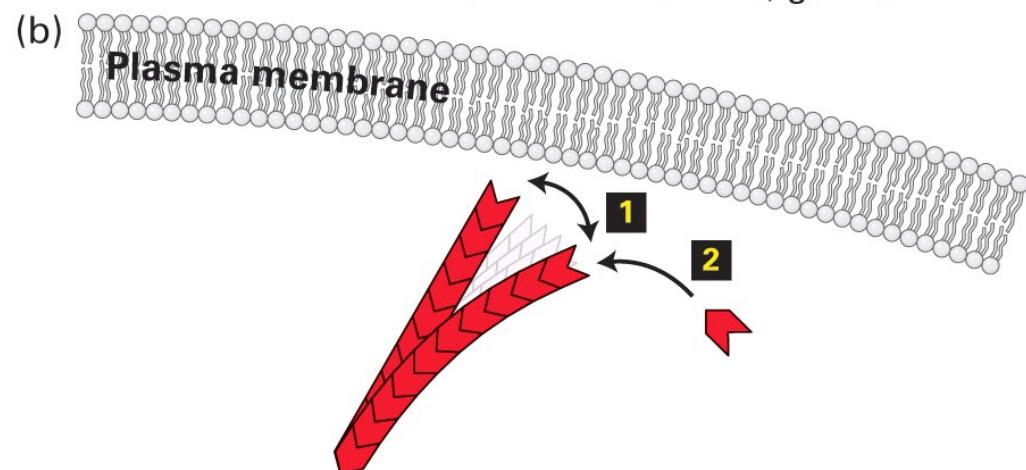
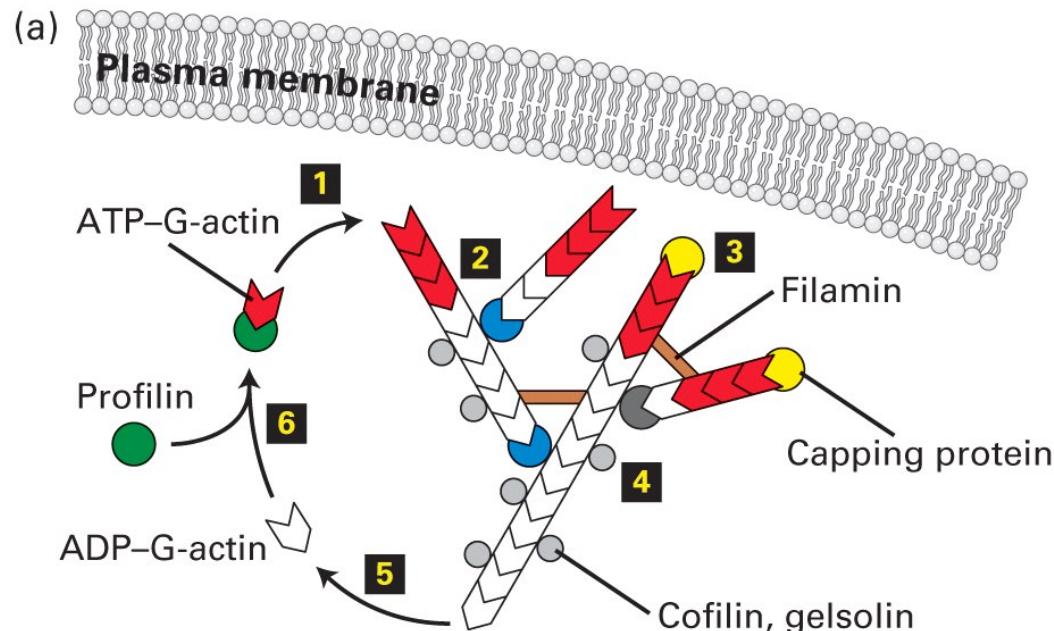


Regulation of ARP and Formin

A specific activated GTPase binds to WASp/ARP complex or Formin, leading to their conformation change and subsequent activation



F-actin polymerization “pushes” membrane extensions to cause cell movement



movement direction

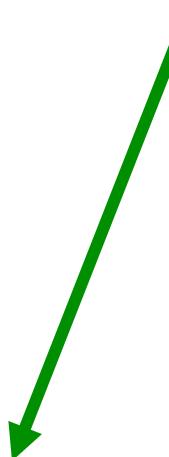
ARP and/or Formin (not shown) promote the synthesis of new F-actin at the plus ends to form membrane protrusions to cause cell movement.

Accessory proteins: Affecting filament nucleation

Binding to the free subunits

Binding along the filament side

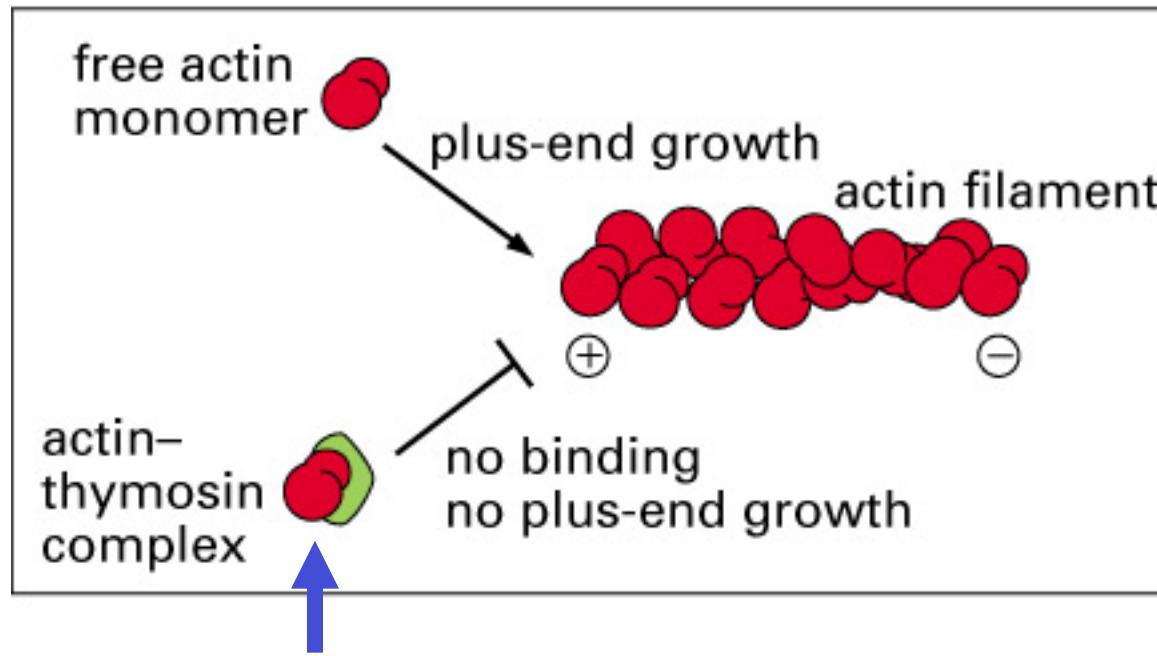
Binding at the filament ends



As a few examples, we will only discuss two G-actin binding proteins with opposite effects, and a tubulin-binding protein whose activity can be modulated by phosphorylation. The same principles can be applied to other cases.

Some subunit-binding proteins inhibit the formation of filaments

Thymosin is one major actin monomer-binding protein in cells.
It inhibits the elongation of F-actin.

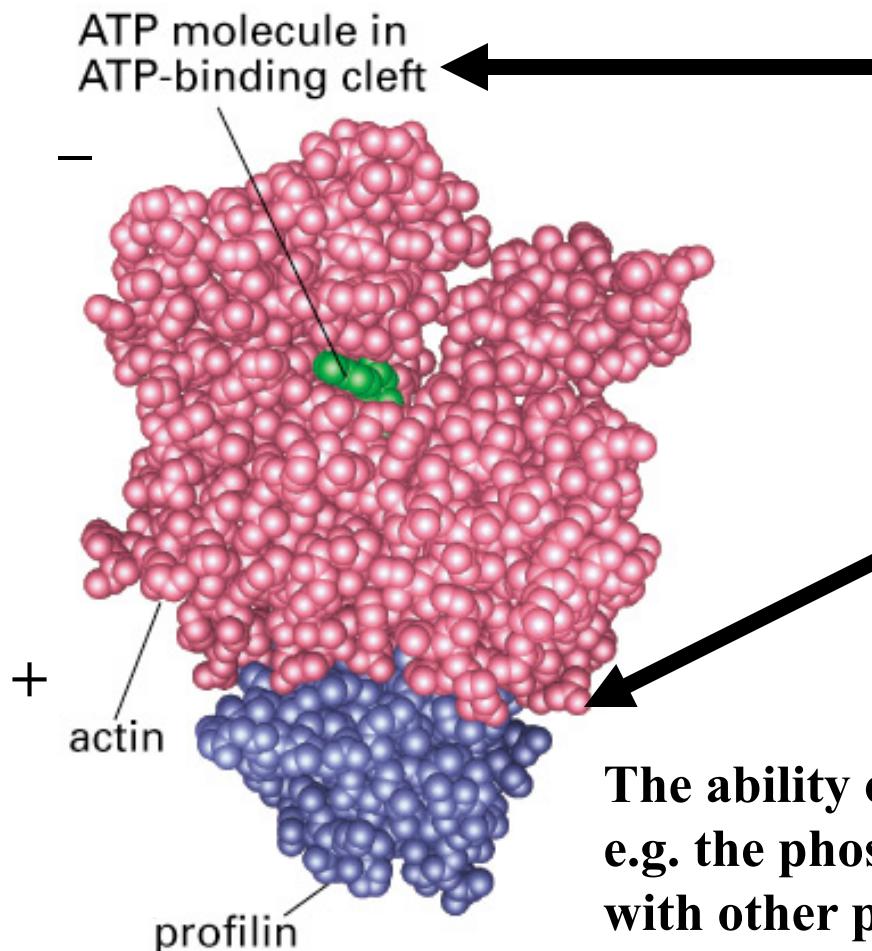


cannot hydrolyze or exchange the bound nucleotide & cannot be added to F-actin

Thymosin sequesters free G-actin and makes them unavailable for adding to the filaments. However, this sequestration also provides a large pool of reserve G-actin when it is needed.

Some subunit-binding proteins promote the formation of filaments

Profilin promotes filament elongation by facilitating the replacement of ADP by ATP in G-actin and allows the G-actin to be added at the plus end.

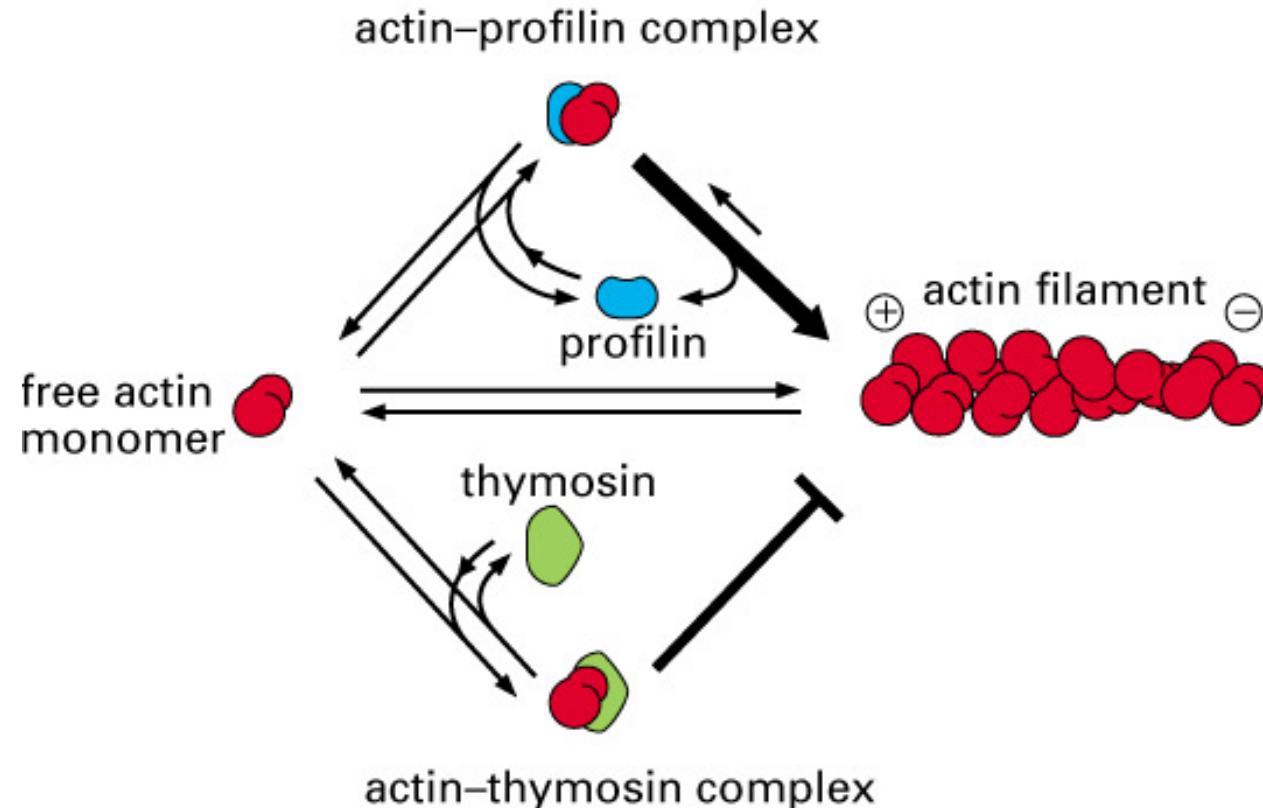


The minus end of G-actin is available for binding to the plus end of F-actin. Once added to the F-actin, the profilin will dissociate from the G-actin

The plus end of G-actin is masked by profilin and thus unavailable for binding to the minus end of F-actin

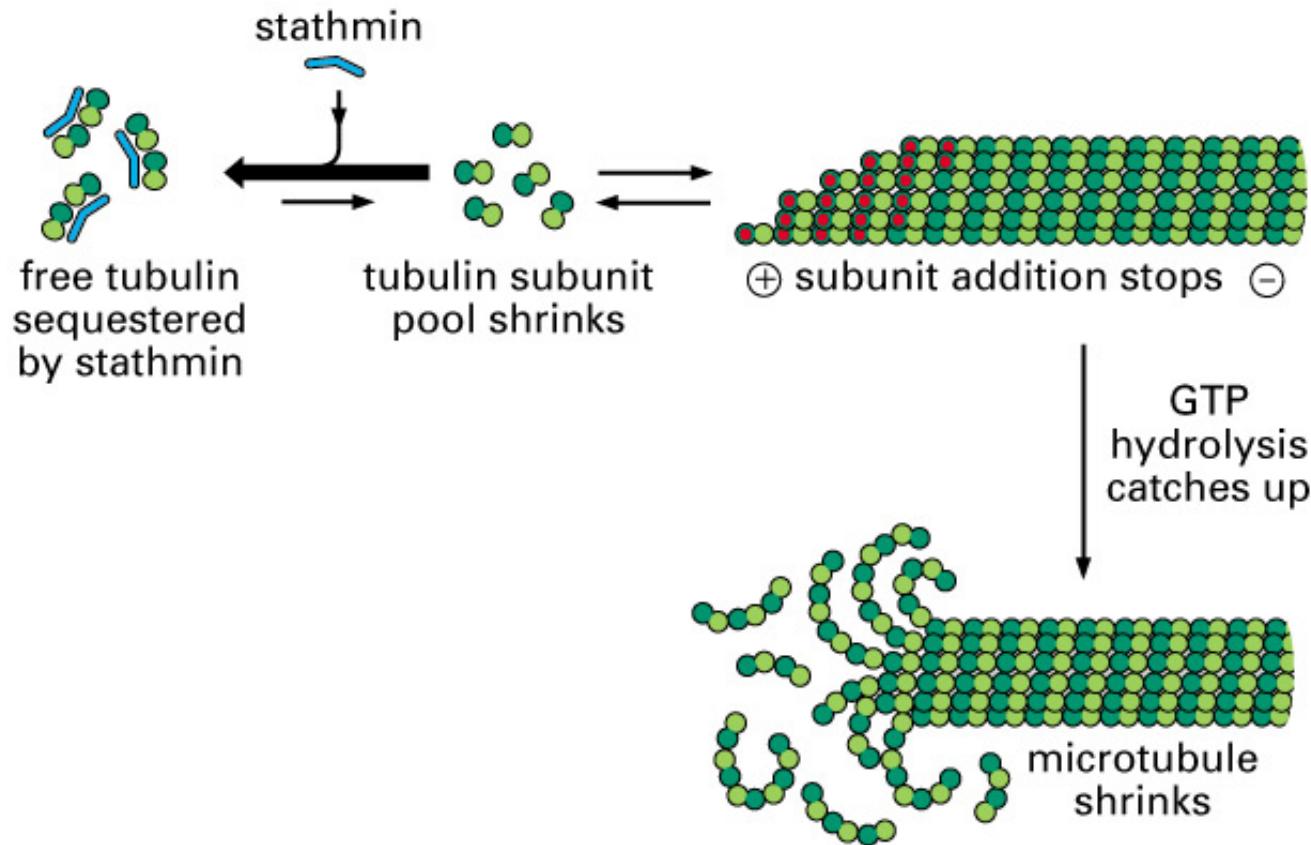
The ability of profilin to bind G-actin can be regulated, e.g. the phosphorylation of profilin or its association with other proteins or phosphoinositides, can regulate the function of profilin

A G-actin can bind to thymosin or profilin, but not to both



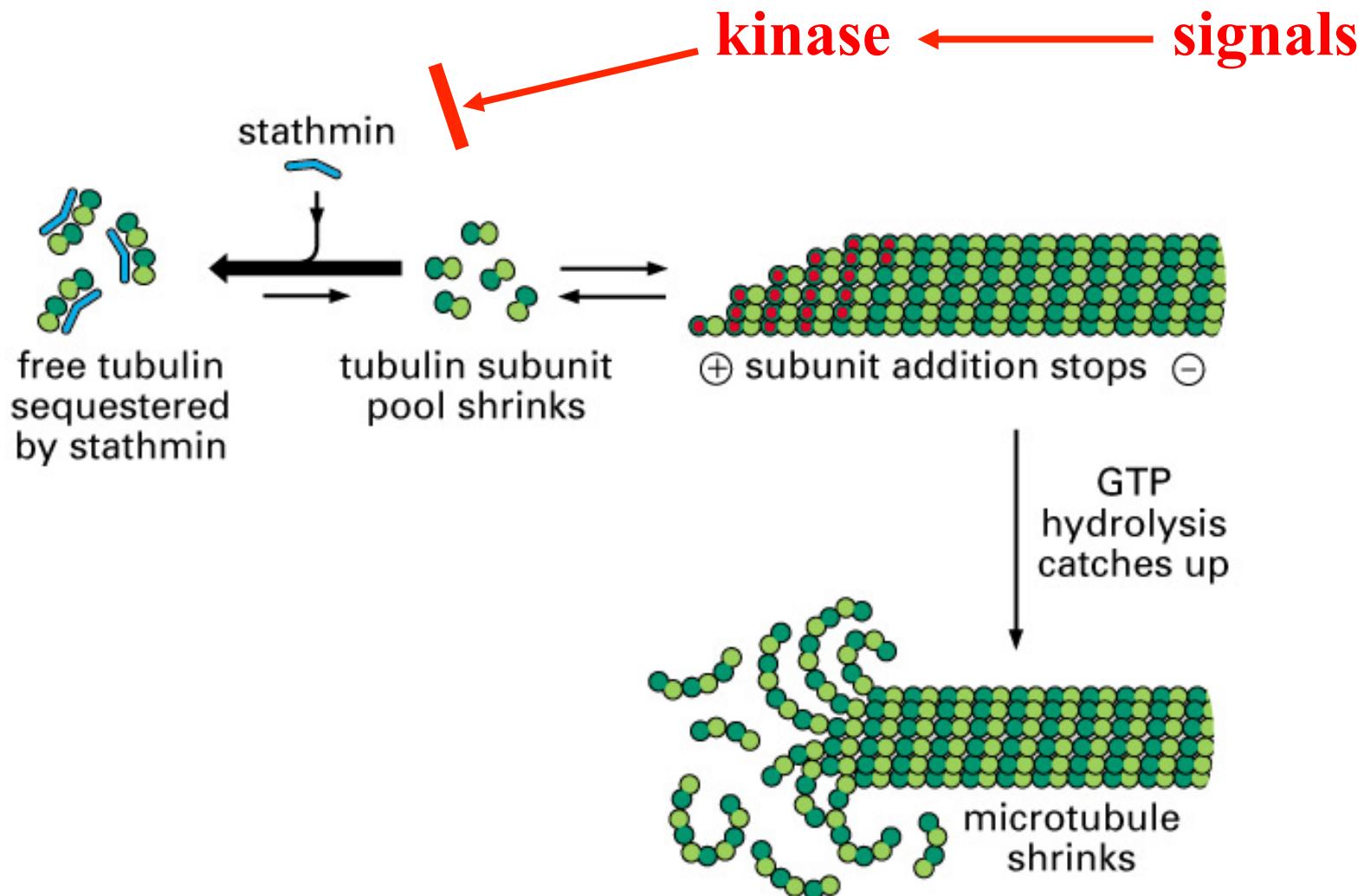
PROFILIN COMPETES WITH THYMOSIN
FOR BINDING TO ACTIN MONOMERS
AND PROMOTES ASSEMBLY

Another Protein Stathmin Sequesters Free Tubulin

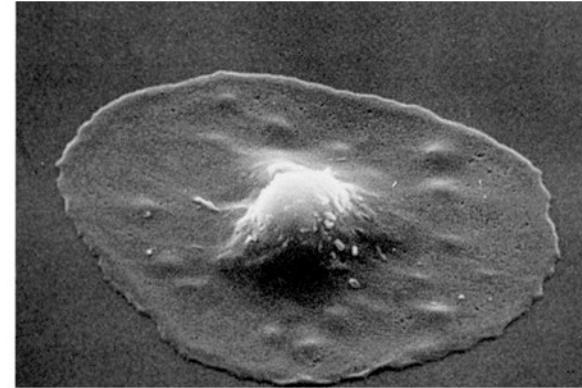
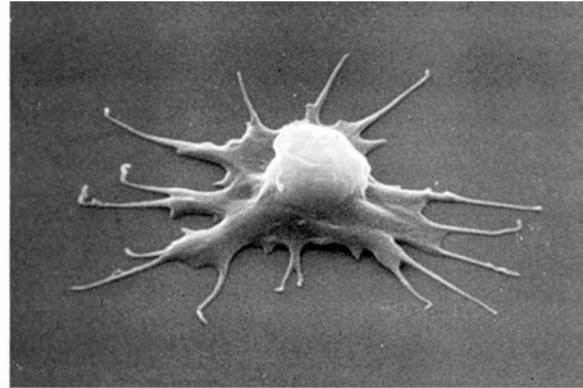
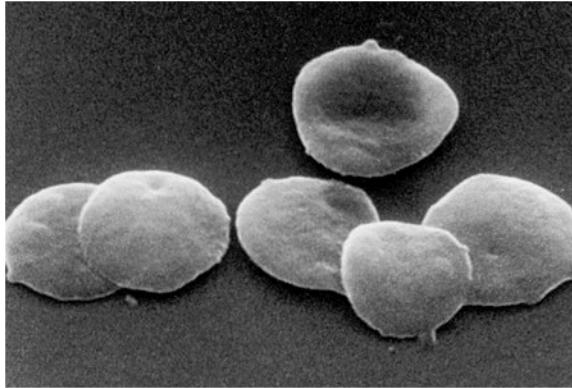


Similar to the effect of Thymosin in the actin filaments

Stathmin Activity Is Regulated by Phosphorylation



Platelets change shape during blood clotting via complex rearrangements of actin cytoskeleton connected to the plasma membrane



Resting platelets have a discoid shape.



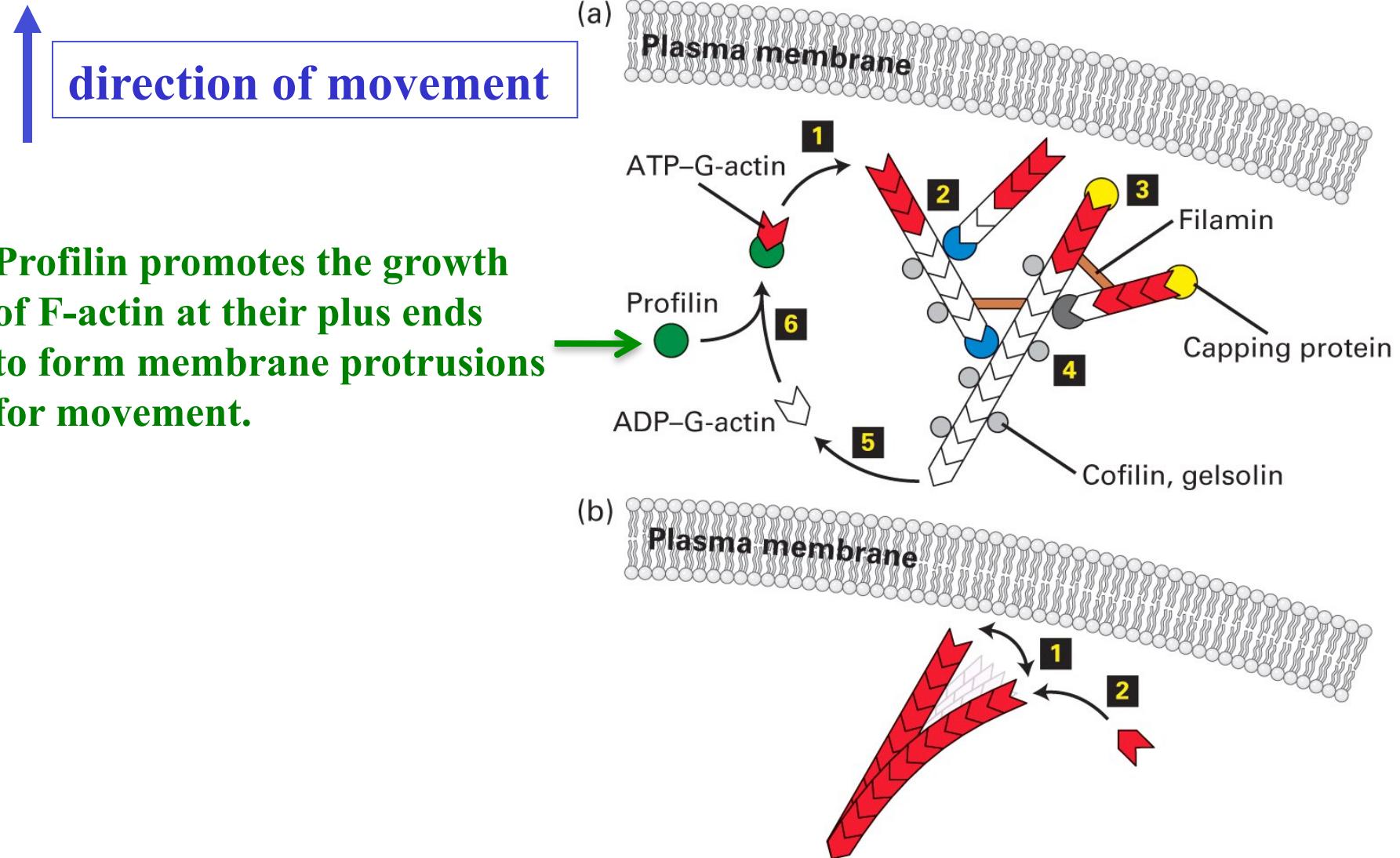
A large pool of G-actin in platelets (about 50%) is sequestered by the thymosin protein.

After exposed to clotting agents, platelets attach to the substratum & extend many filopodia.
(filopodia: a linear form of F-actin bundle)

Finally platelets spread out lamellipodia for clotting/thrombosis.
(lamellipodia is a sheet form of F-actin)

A burst of F-actin assembly occurs upon the activation of platelets and these F-actin are required for the blood clotting (the association between G-actin and thymosin is regulated by signals).

F-actin polymerization “pushes” membrane extension to cause cell movement



Accessory proteins: Affecting filament nucleation

Binding to the free subunits

Binding along the filament side

Binding at the filament ends

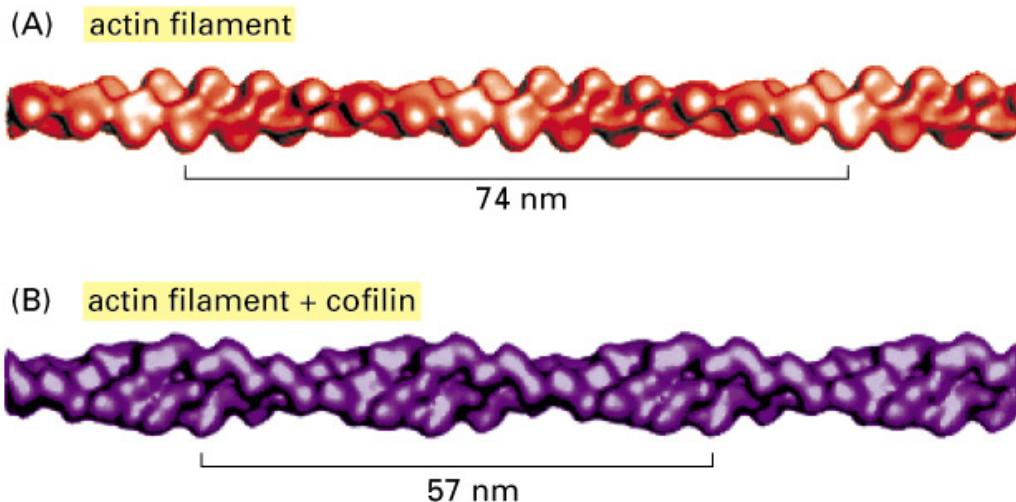
- A. affecting stability
- B. cross-linking
- C. severing



We will focus on examples for actin-filaments, the same principles can be applied to microtubules.

(A) Regulation of F-actin stability by proteins bound to the side of filaments

1. Cofilin (also called actin depolymerizing factor) destabilizing F-actin



Cofilin binding induces a conformational change of F-actin:
more twisted and weaker subunit-subunit interaction



more depolymerization
& easier to be severed

Cofilin selectively binds to “D” form of actin. Thus, it selectively destabilizes older actin filaments and increases their turnover rate.

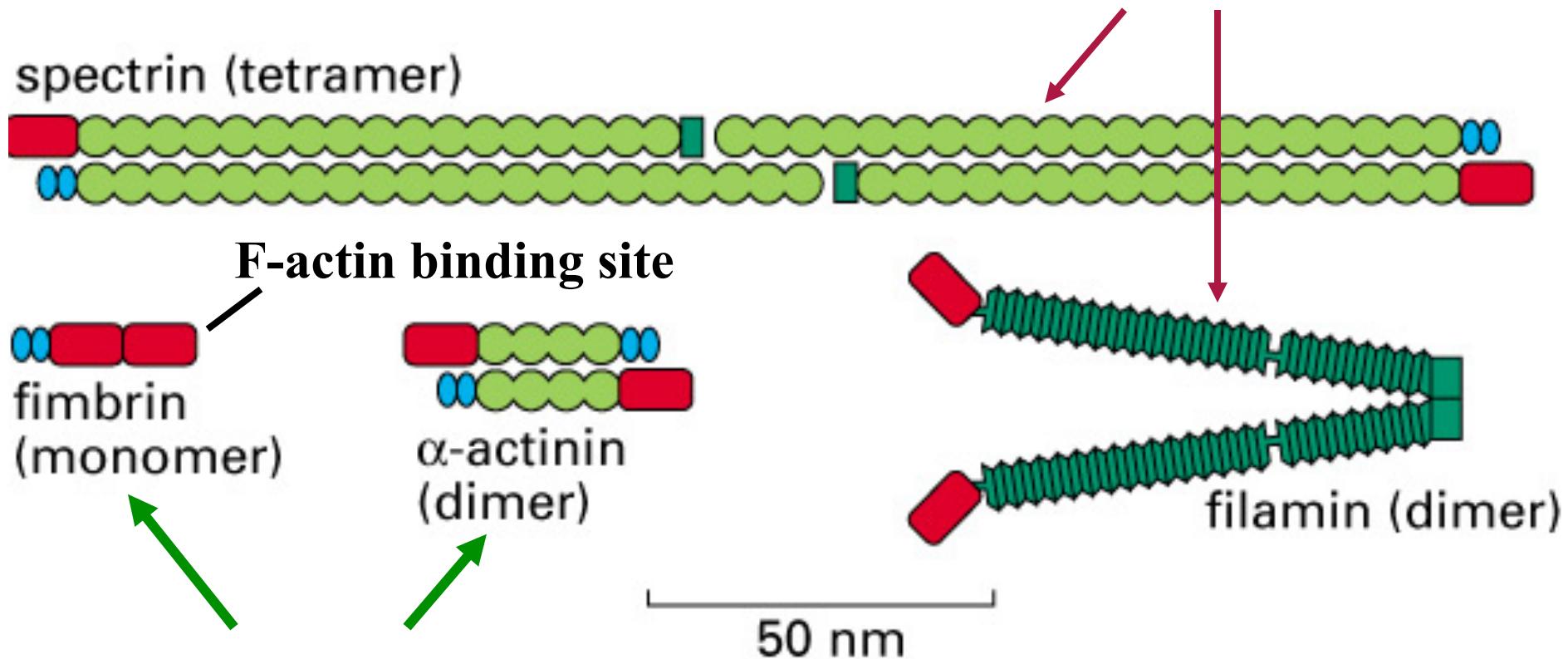
2. Tropomyosin: stabilizing F-actin by preventing the binding of other proteins to F-actin

(B) Cross-linking Proteins Controls the Forms of F-actin

At least two actin-binding sites (represented by the red boxes) are required to cross-link filaments.

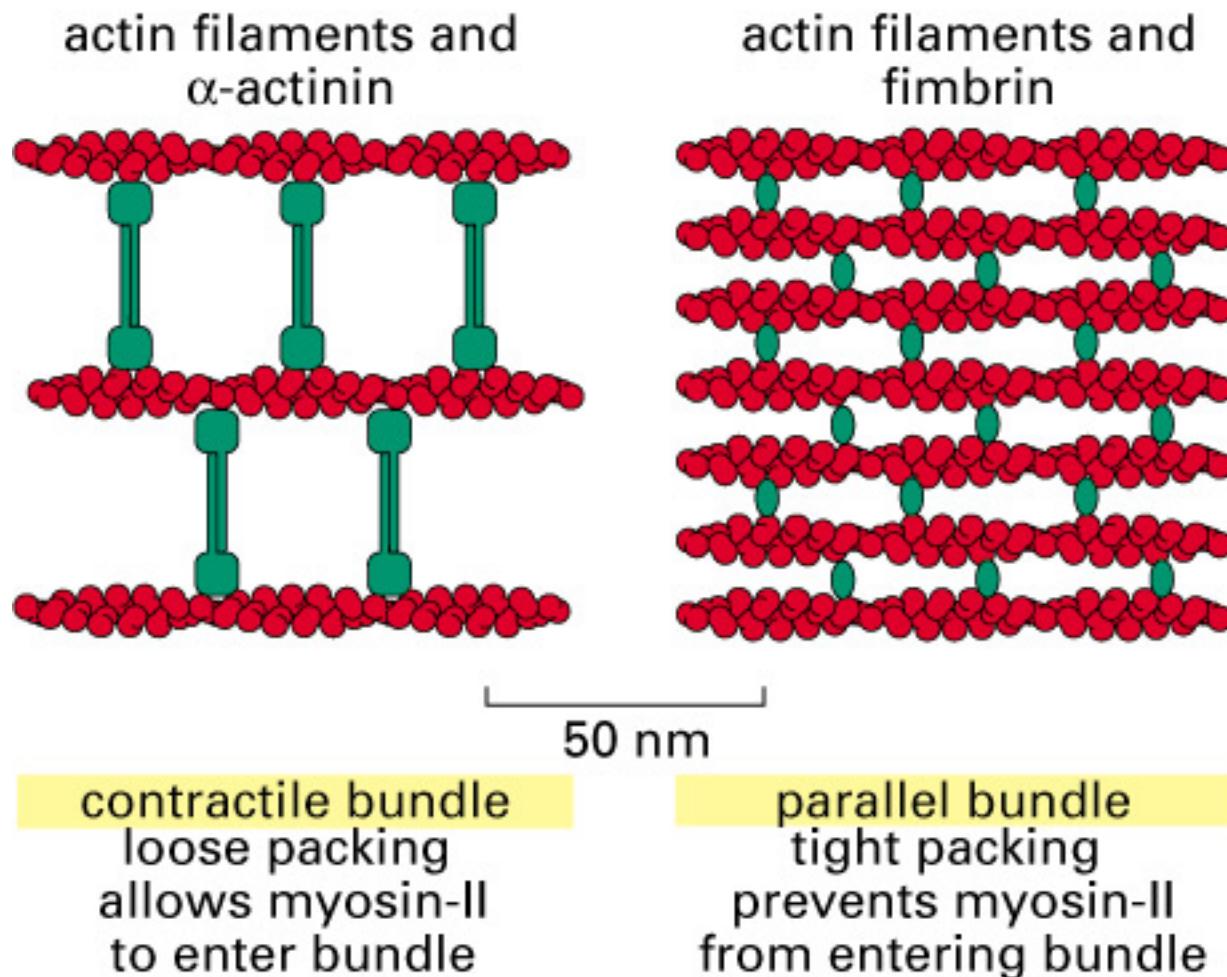
The spacing and arrangement of two actin binding sites determines the actin cross-linking patterns

flexible & bendable cross-linking proteins promote the formation of web or gel

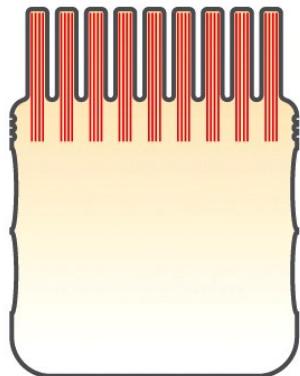


straight and stiff cross-linking proteins promote the formation of bundles

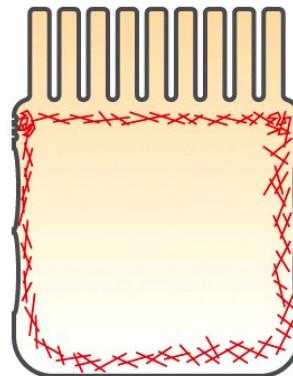
α -Actinin & Fimbrin Generate Different F-actin Bundles



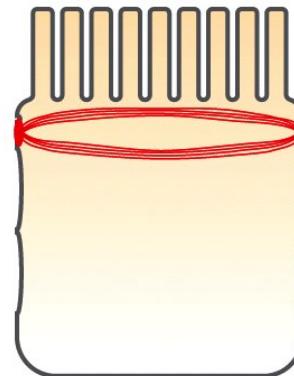
Different Forms of Cellular F-Actin



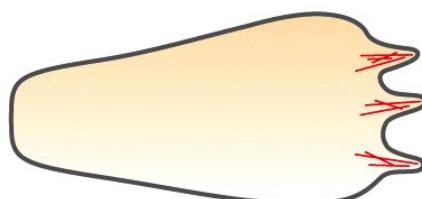
Microvilli



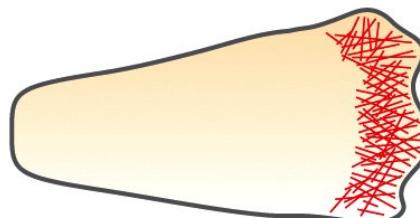
Cell cortex



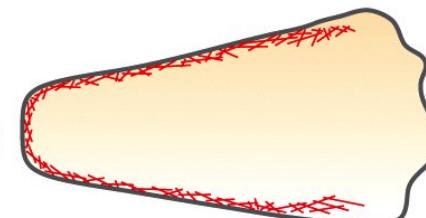
Adherens belt



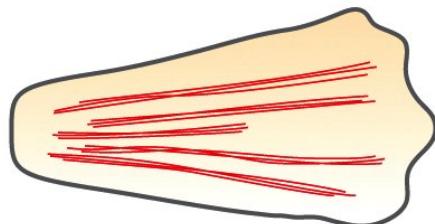
Filopodia



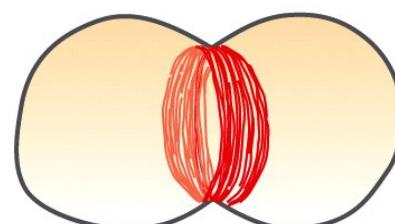
**Lamellipodium/
leading edge**



Cell cortex

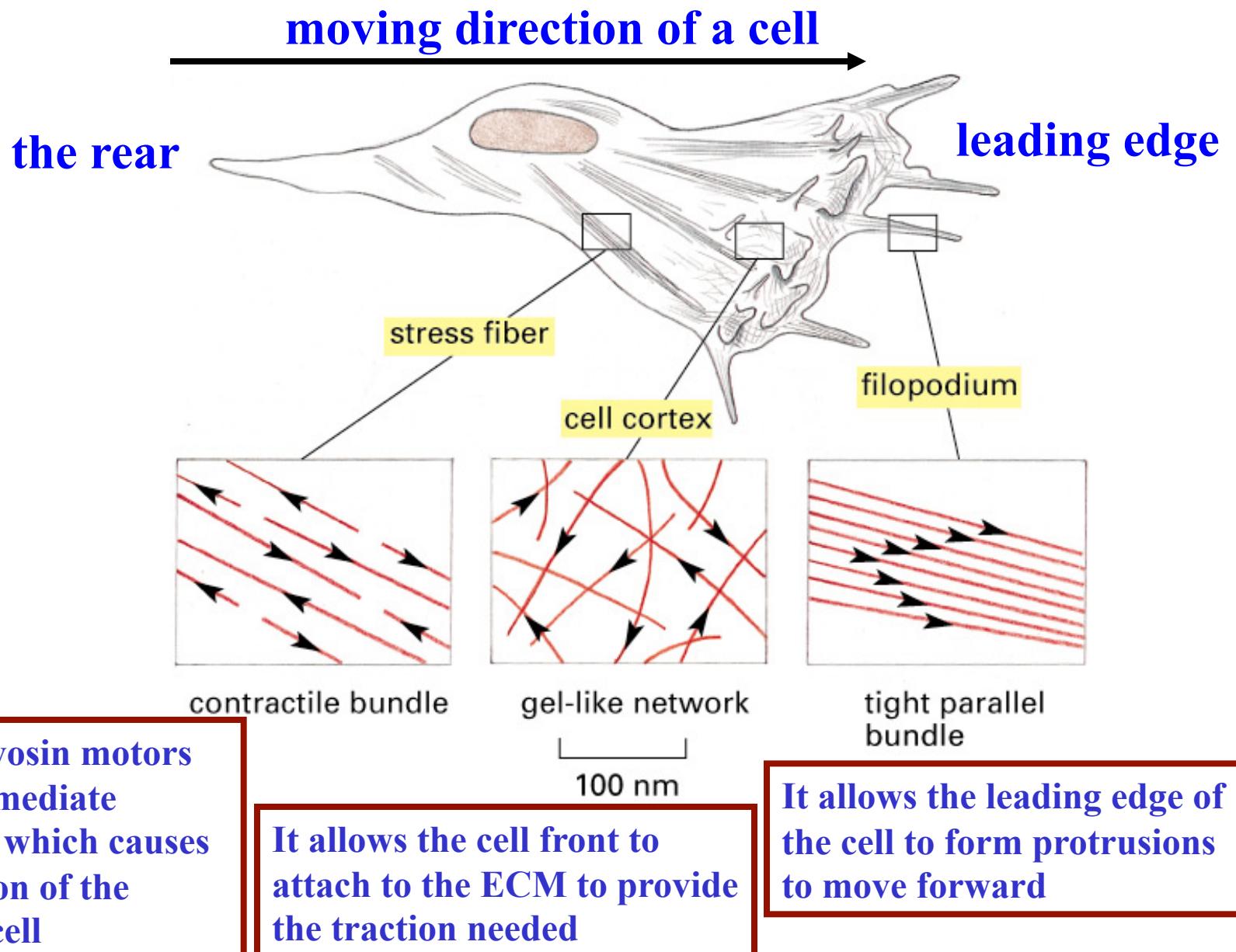


Stress fibers

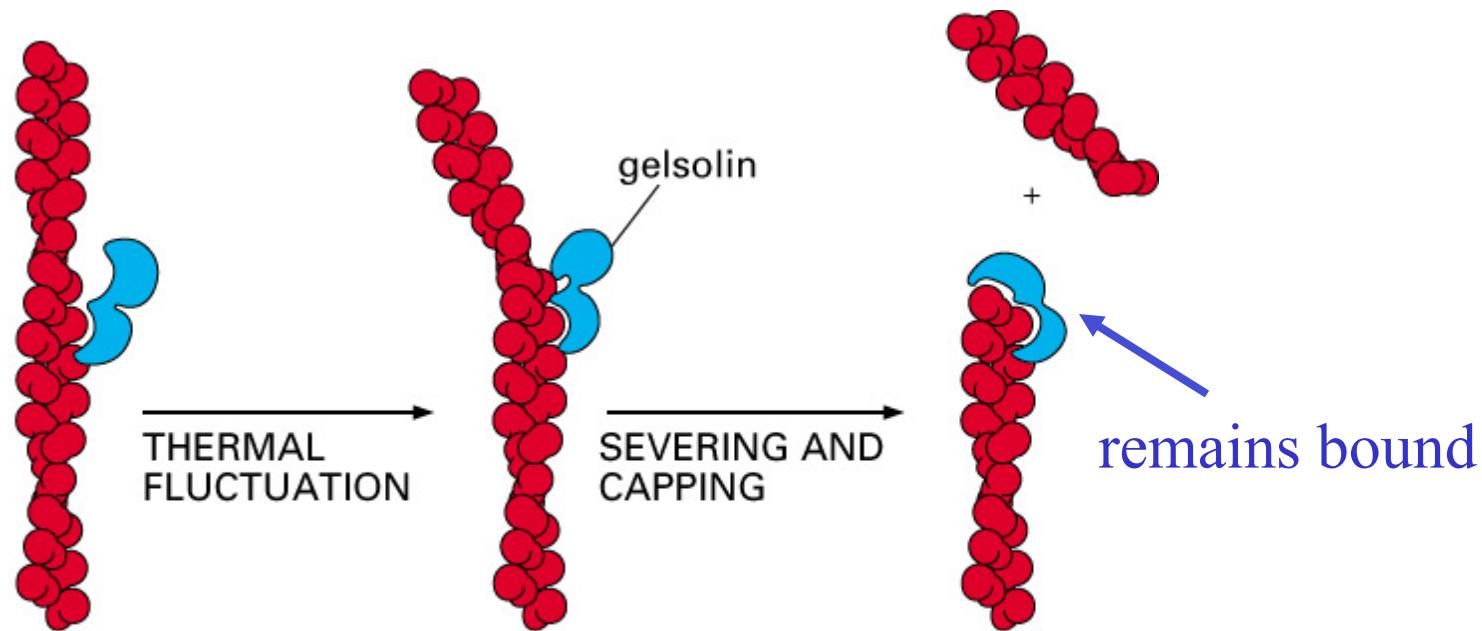


Contractile ring

Why Cross-Linking of F-Actin to Create Different Forms?



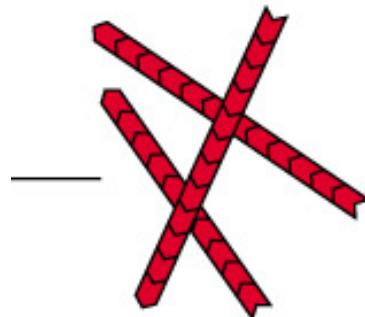
(C) Severing of F-Actin Filaments by Gelsolin



By remaining bound to the plus end of a broken actin filament, gelsolin also functions as a plus-end capping protein. Since the minus end of the broken filament is exposed, it can rapidly disassemble.

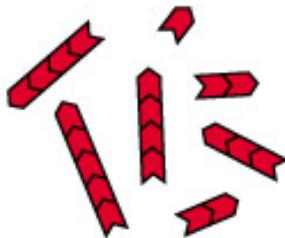
F-Actin Severing Proteins Increase the Rate of Filament Turnover

Gelsolin-mediated F-actin severing increases the turnover rate



in unsevered population,
actin filaments grow
and shrink relatively
slowly

a total of 6 turnover sites

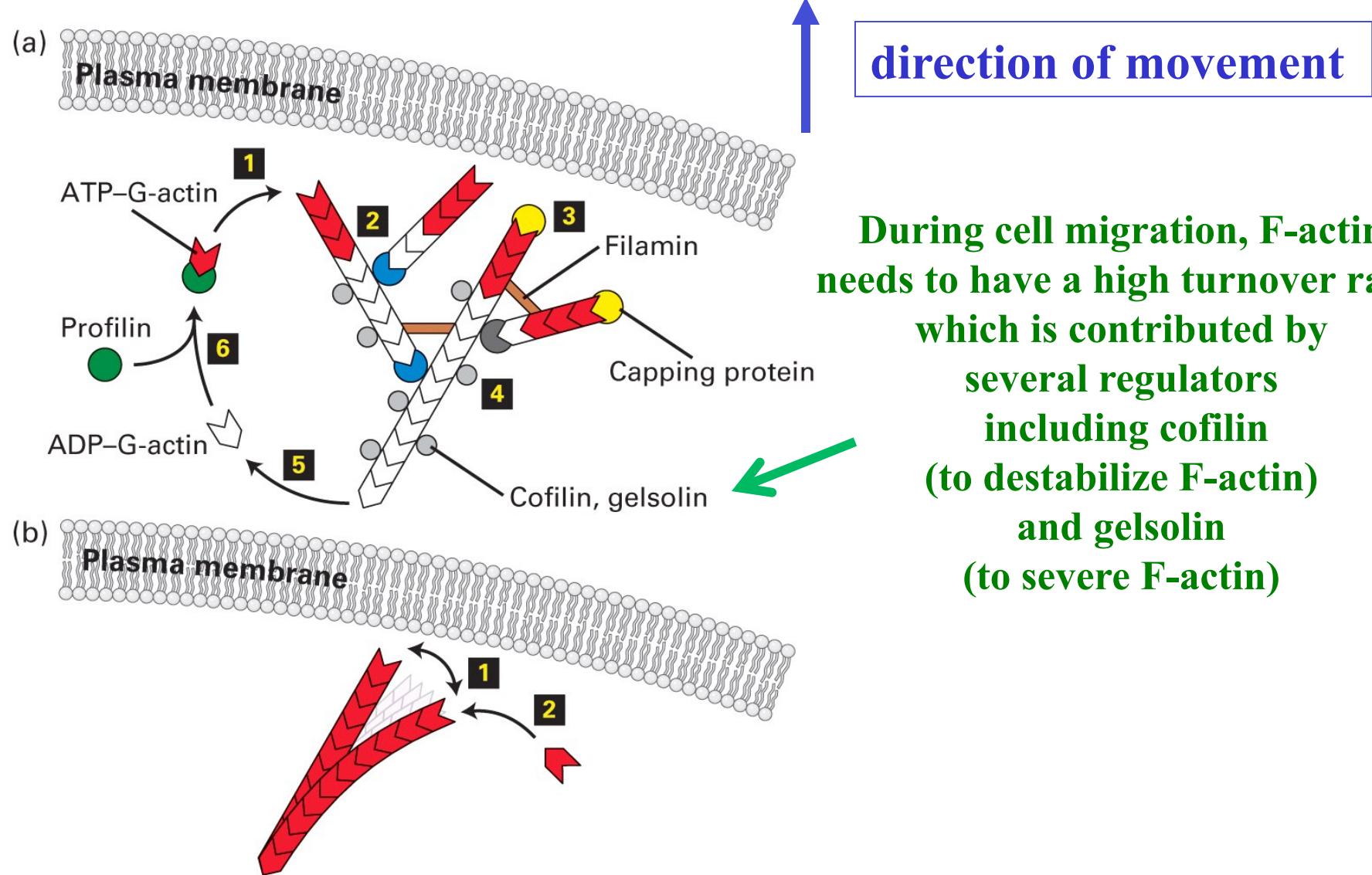


in severed population,
actin filaments grow
and shrink more rapidly

a total of 14 turnover sites

why severing? → to increase the turnover rate of F-actin and allow a quick cellular response to cytoskeletal reorganization needs

F-actin polymerization “pushes” membrane extension to cause cell movement



During cell migration, F-actin needs to have a high turnover rate, which is contributed by several regulators including cofilin (to destabilize F-actin) and gelsolin (to sever F-actin)

Similar to the case of F-actin, different proteins bind to the sides of MTs causing different consequences

microtubule stability: XMAP215

microtubule bundling: MAP2 and Tau

microtubule severing: Katanin

Accessory proteins: Affecting filament nucleation

Binding to the free subunits

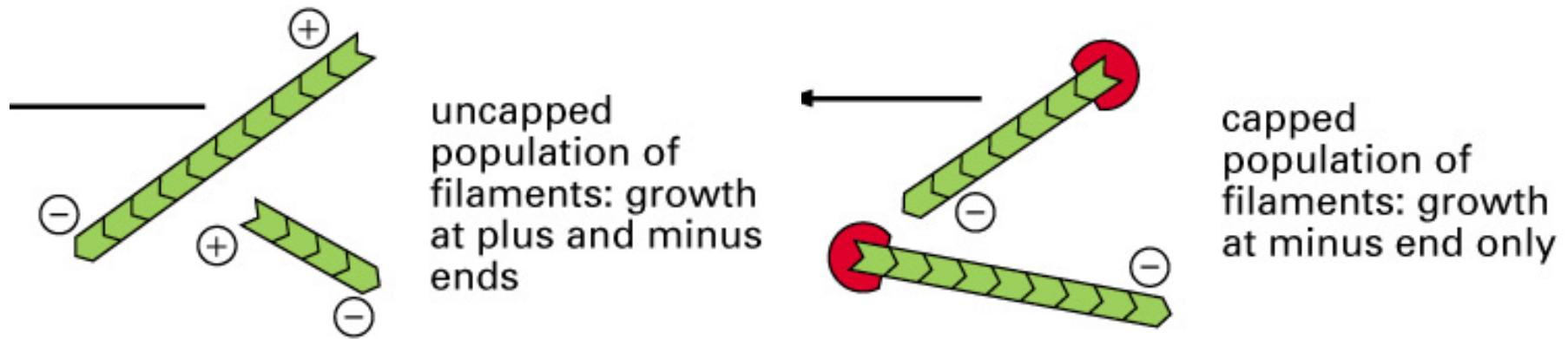
Binding along the filament side

Binding at the filament ends

- 1. A very efficient way to regulate filament dynamics
(since ends are active sites & only two ends per filament)**
- 2. Can be used to position the filament ends**

Some End-binding Proteins “Cap” the Ends of F-actin to Block its Dynamics and thus Stabilize the Filament Ends (They are called Capping Proteins)

CapZ and tropomodulin cap the plus and minus ends of F-actin, respectively

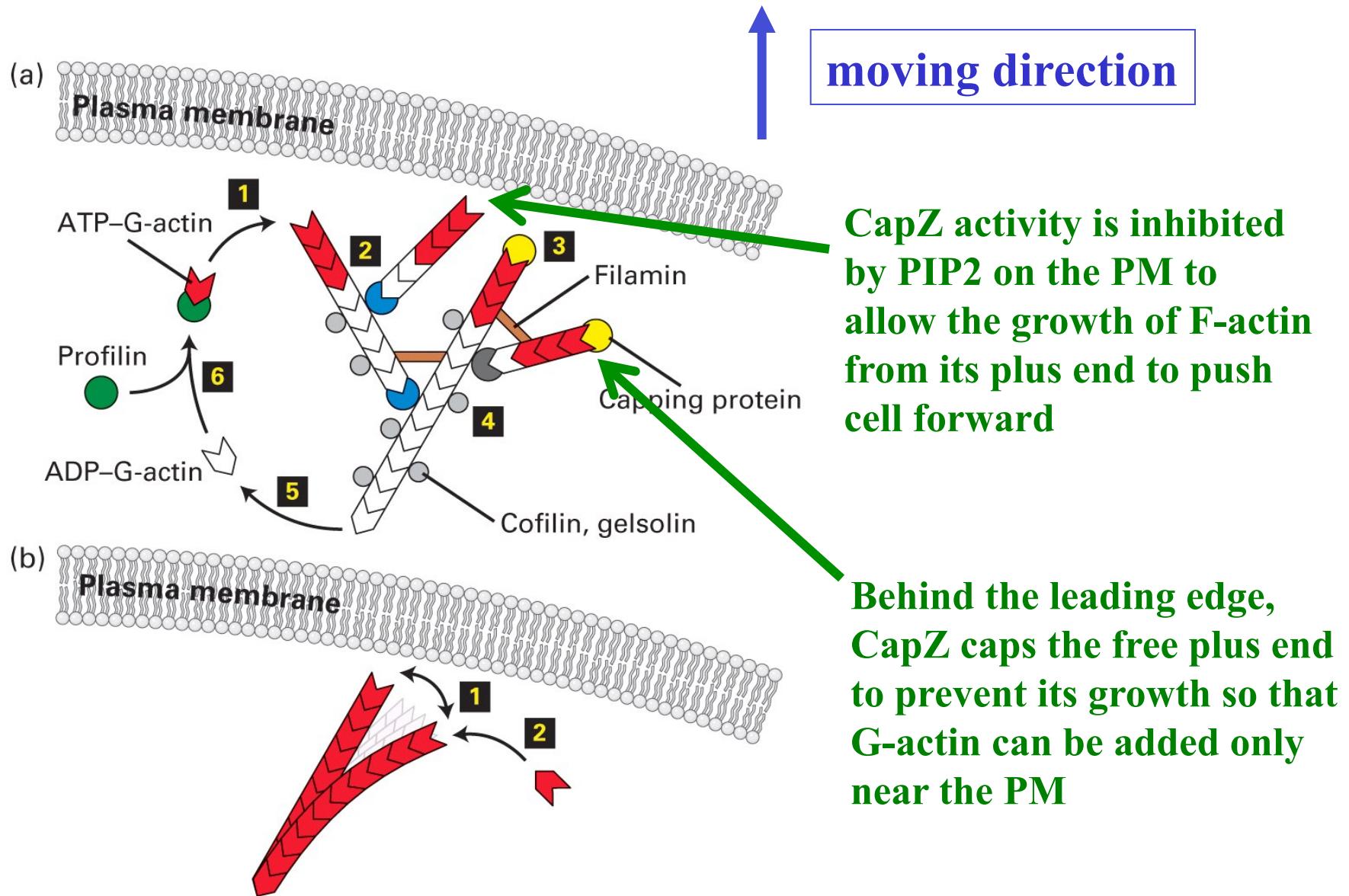


In most cases, once capped, that end of the subunit is no longer dynamic.

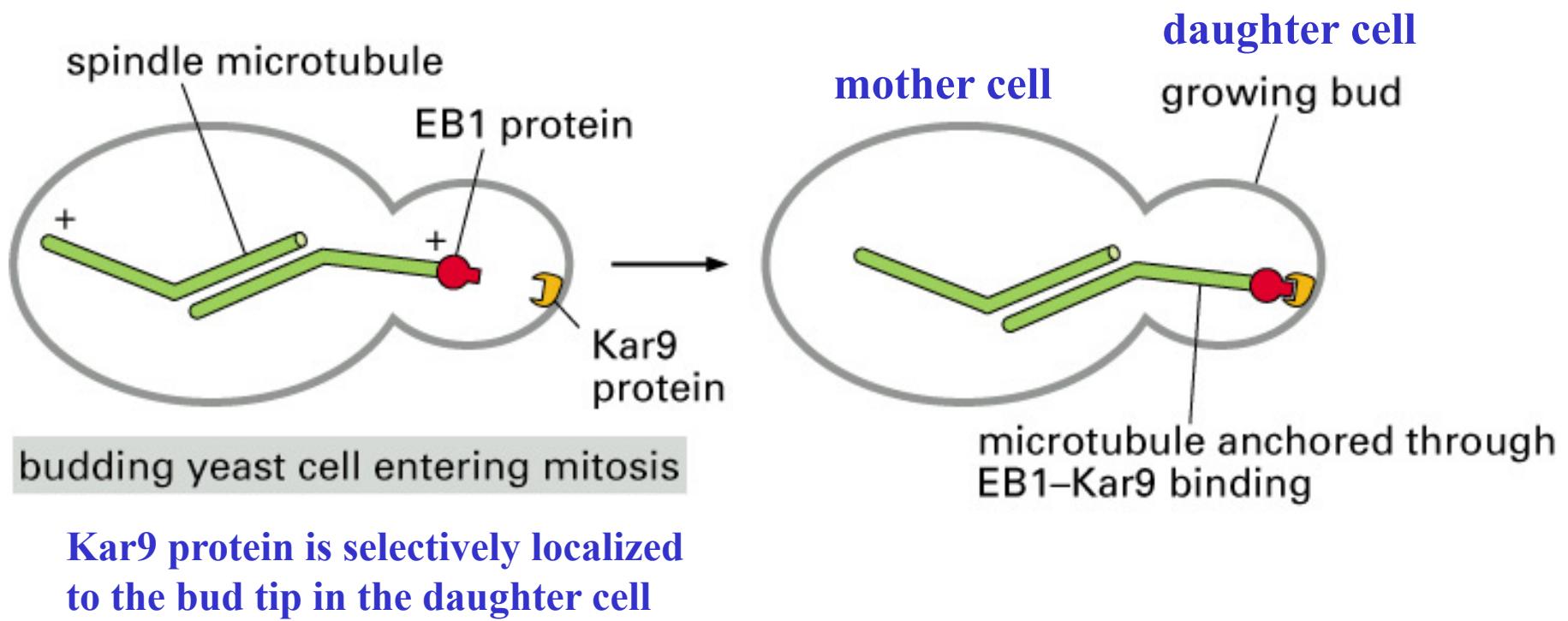
Capping proteins are very abundant in cells requiring very stable actin filaments, such as muscle cells.

In cells where the actin filaments need to undergo constant assembly/disassembly, the level or activity of capping proteins must be regulated

For example, PIP2 inhibits the activity of CapZ.

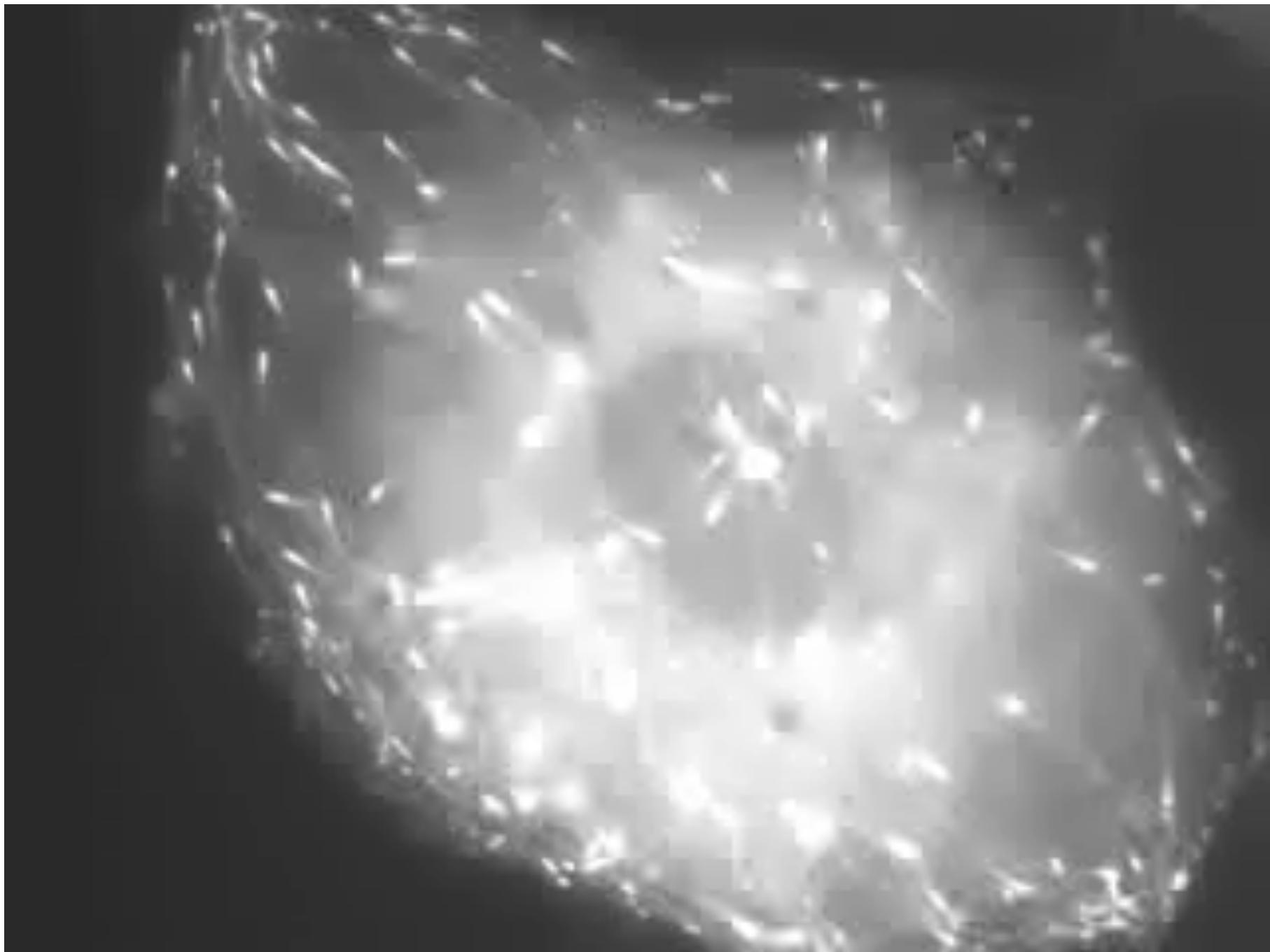


Some End-Binding Proteins Can also Position the Ends of Cytoskeletal Filaments to the Cell Cortex



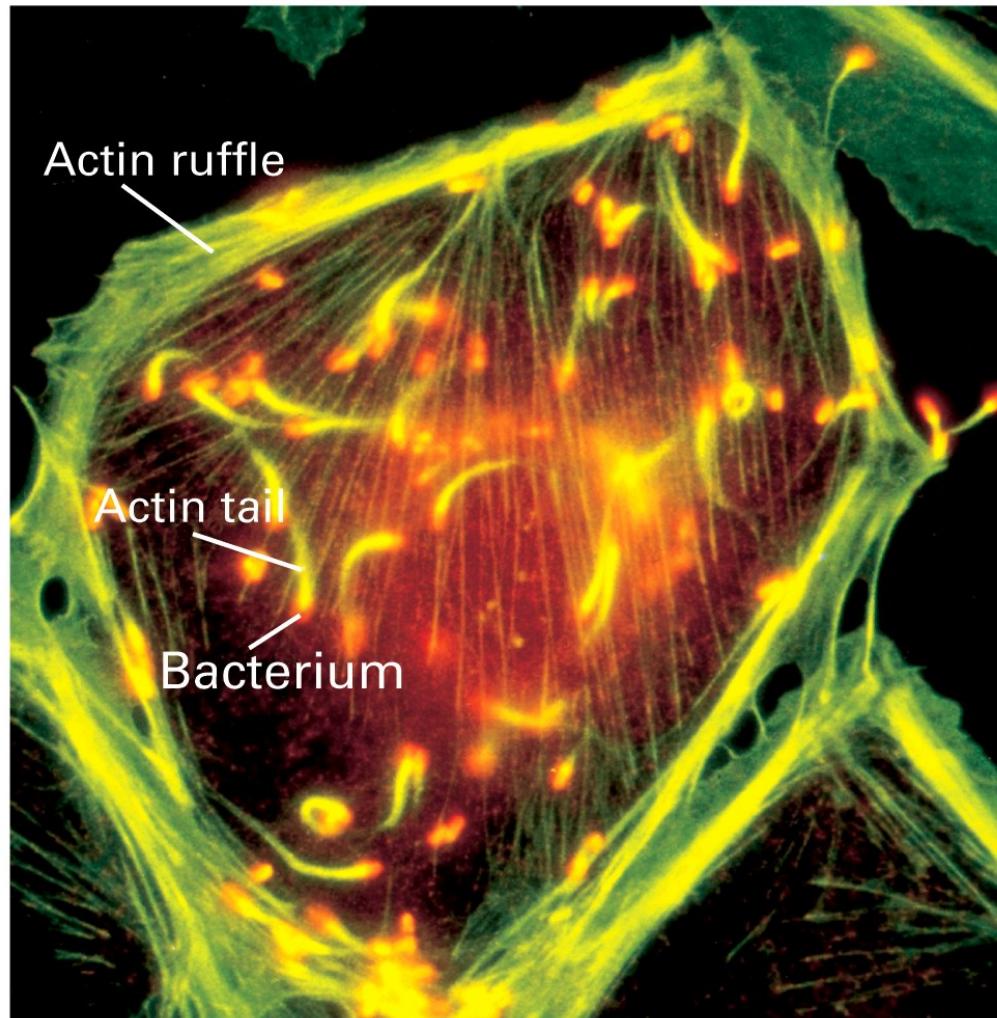
The plus end-binding protein EB1 interacts with Kar9 and directs the microtubule spindle to the growing bud tip during mitosis, which facilitates the segregation of replicated chromosomes.

Microtubule dynamics: movie



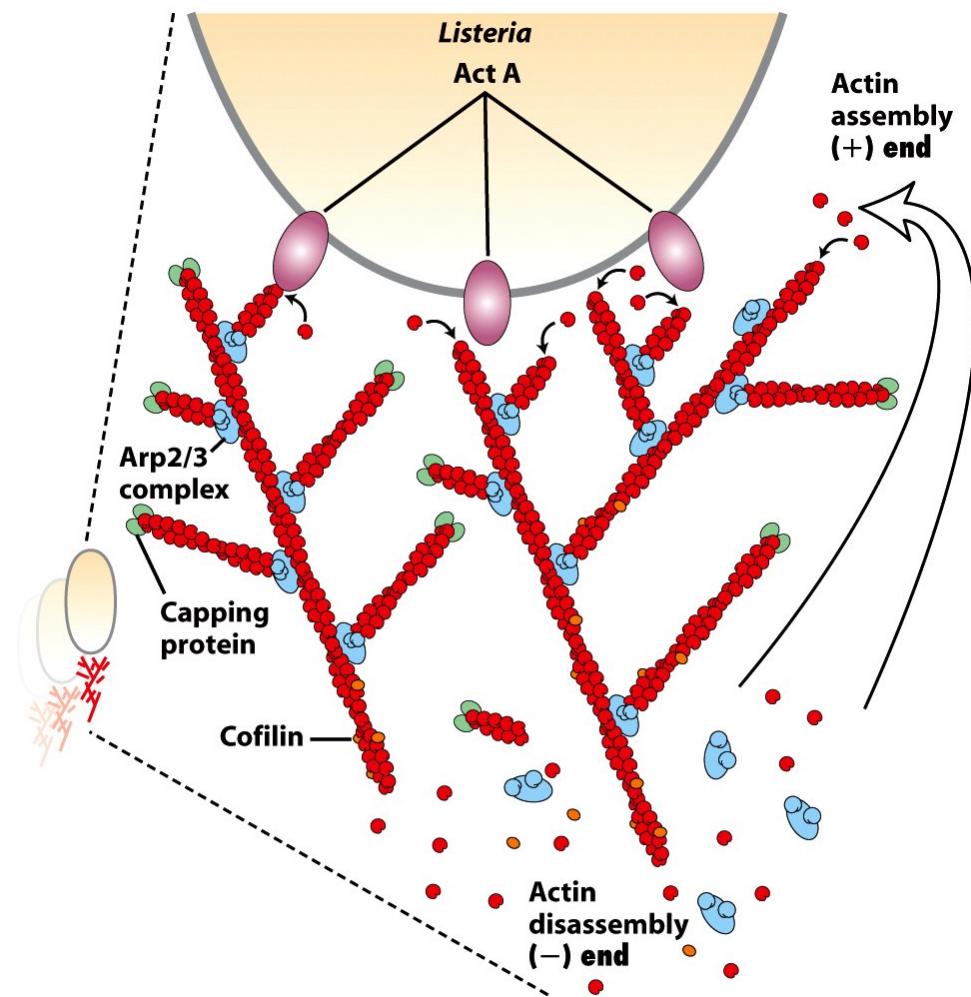
Coordination of Different Actin-Binding Proteins

Listeria utilizes the power of actin polymerization for its intracellular movement



Coordination of Different Actin-Binding Proteins

Listeria utilizes the power of actin polymerization for its intracellular movement



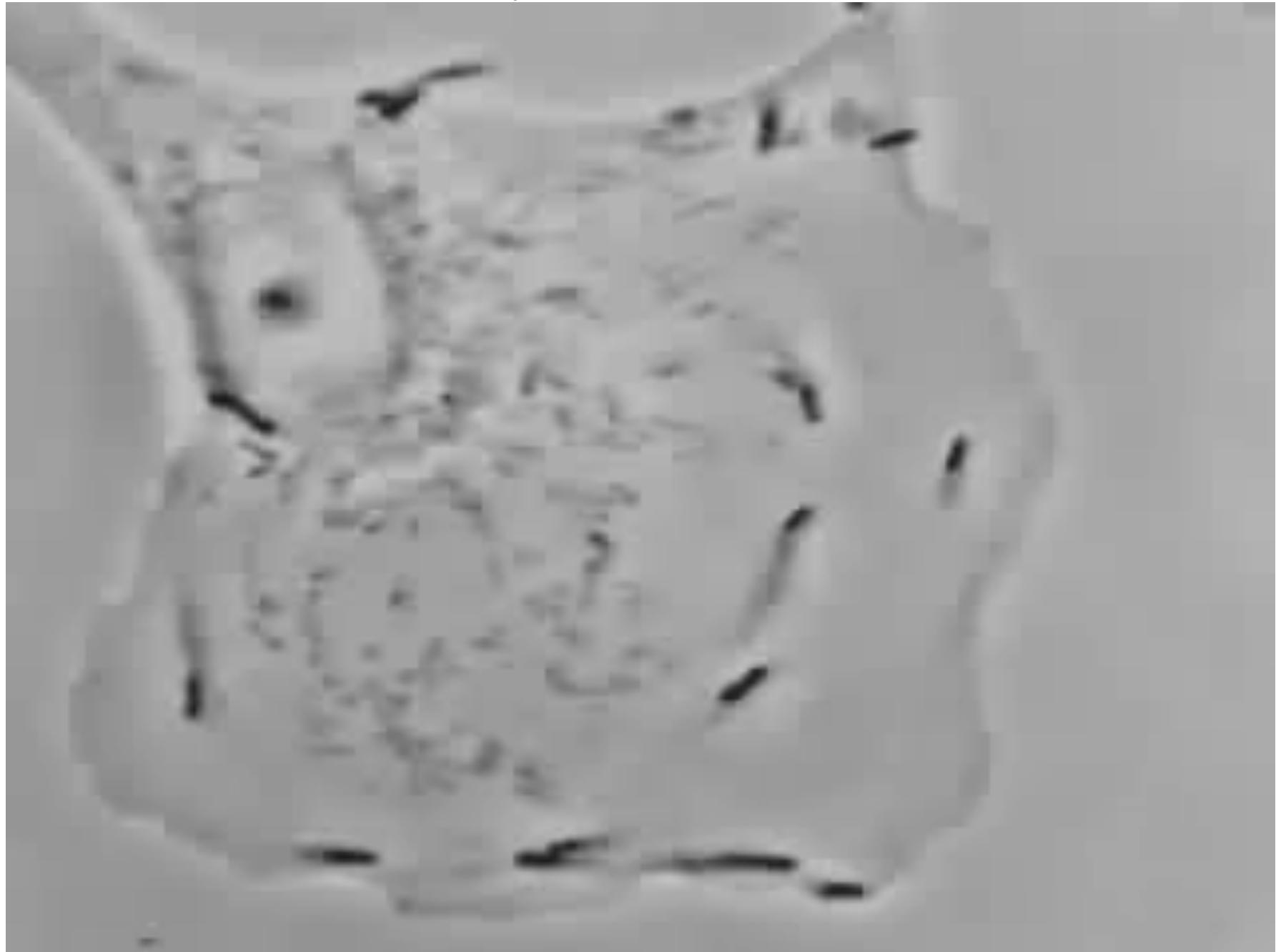
The ActA protein on the bacterium surface activates the ARP complex

New filaments are assembled from pre-existing F-actin until they are capped by CapZ

Cofilin disassembles the minus end of the filament and recycles the G-actin for polymerization at the plus end

The polymerization at the plus end of the filament propels the bacterium forward.

Listeria Uses Actin Polymerization for Movement: Movie



filament proteins:
(intrinsic factors)

plus end more dynamic than minus end
ATP/GTP cap decreases Cc
covalent modifications of subunits

accessory proteins:
(extrinsic factors)

affecting filament nucleation
binding to free subunits
binding along the filament side
binding at the filament ends

Overall dynamics is determined by the combinatorial effects of both types of proteins and regulation.

Drugs Affecting F-Actin and Microtubules

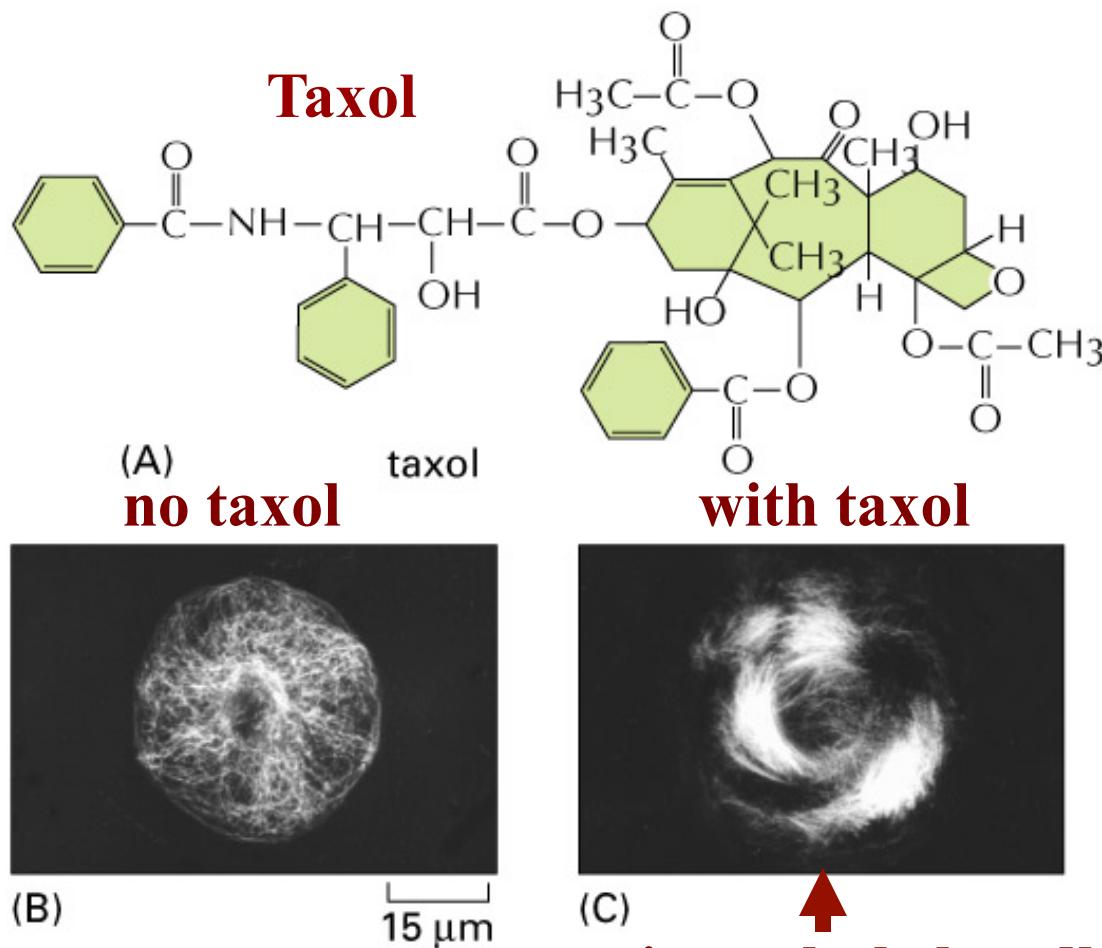
Effects on filaments:
S (stabilize), D (disrupt)

Drugs That Affect Actin Filaments and Microtubules

| ACTIN-SPECIFIC DRUGS | | |
|----------------------------|--|---|
| Phalloidin | binds and stabilizes filaments | S |
| Cytochalasin | caps filament plus ends | D |
| Swinholide | severs filaments | D |
| Latrunculin | binds subunits and prevents their polymerization | D |
| MICROTUBULE-SPECIFIC DRUGS | | |
| Taxol | binds and stabilizes microtubules | S |
| Colchicine, colcemid | binds subunits and prevents their polymerization | D |
| Vinblastine, vincristine | binds subunits and prevents their polymerization | D |
| Nocodazole | binds subunits and prevents their polymerization | D |

No need to memorize drugs and effects.
Instead, understand the cause-consequence relationships.

Drugs Affecting Microtubules and Cancer Treatment



Pacific Yew tree



Proper microtubule assembly/disassembly is required for the correct function of the mitotic spindles. Thus, rapidly growing cells are most sensitive to drugs such as taxol.