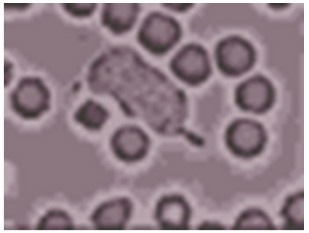
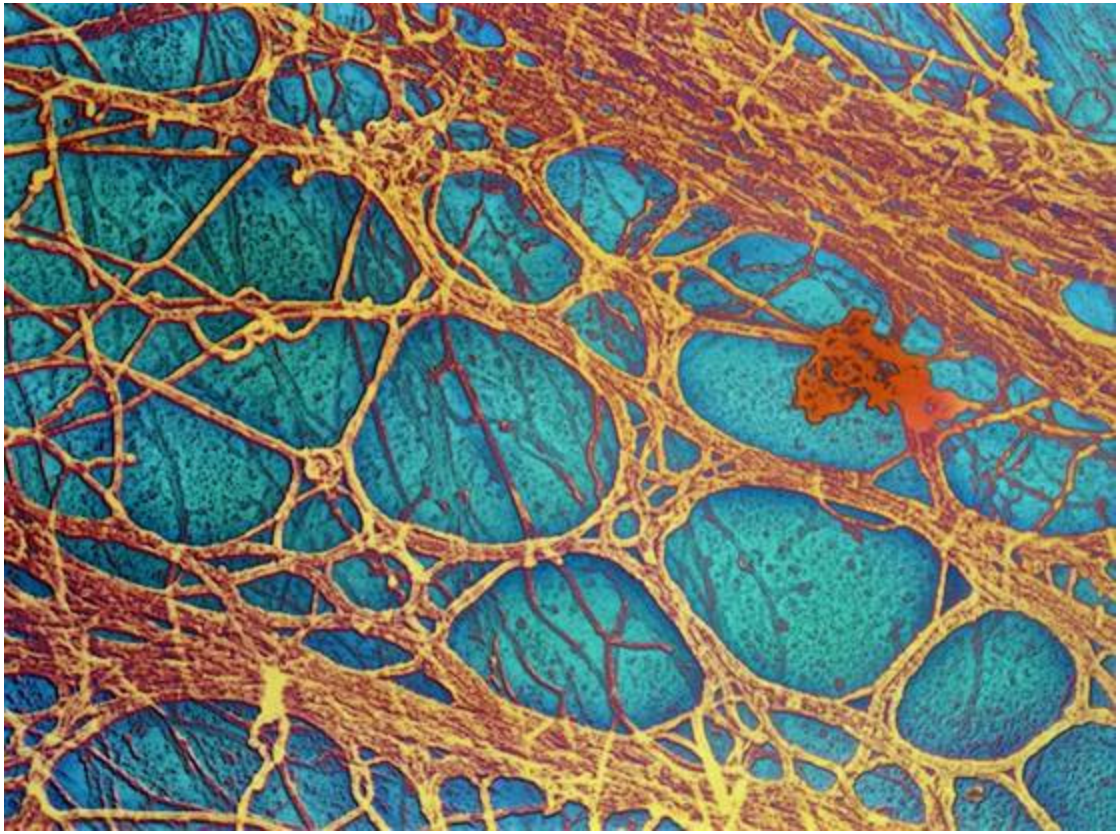
THE CYTOSKELETON



PART I: Microfilaments in cell organization and movement



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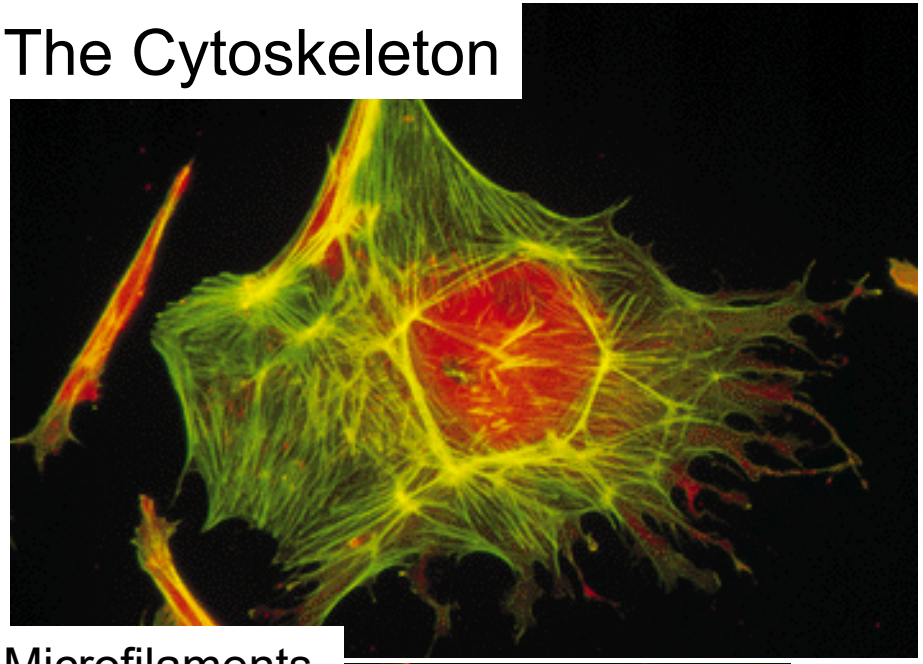
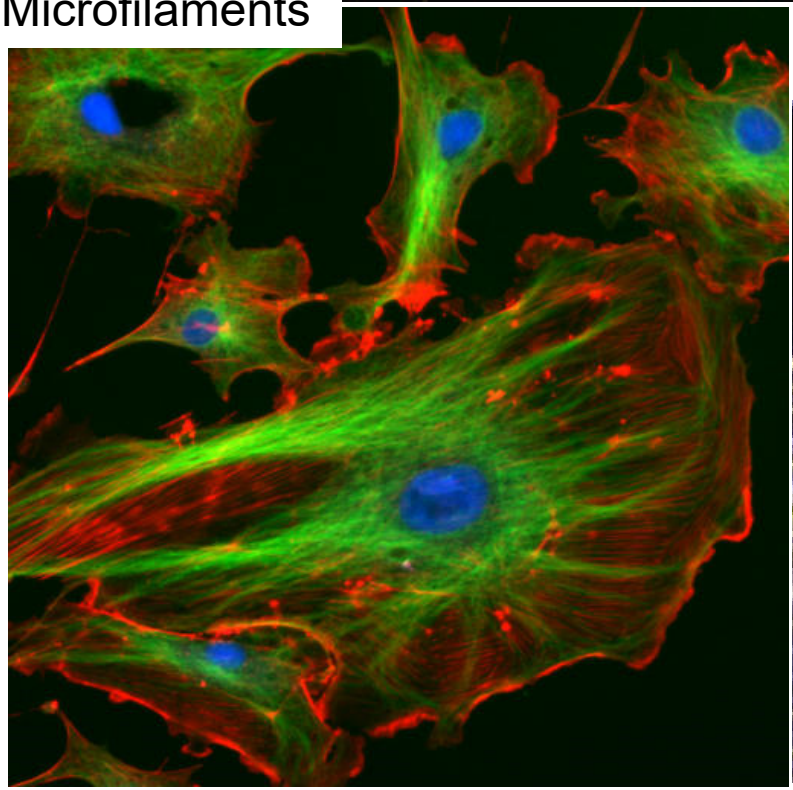
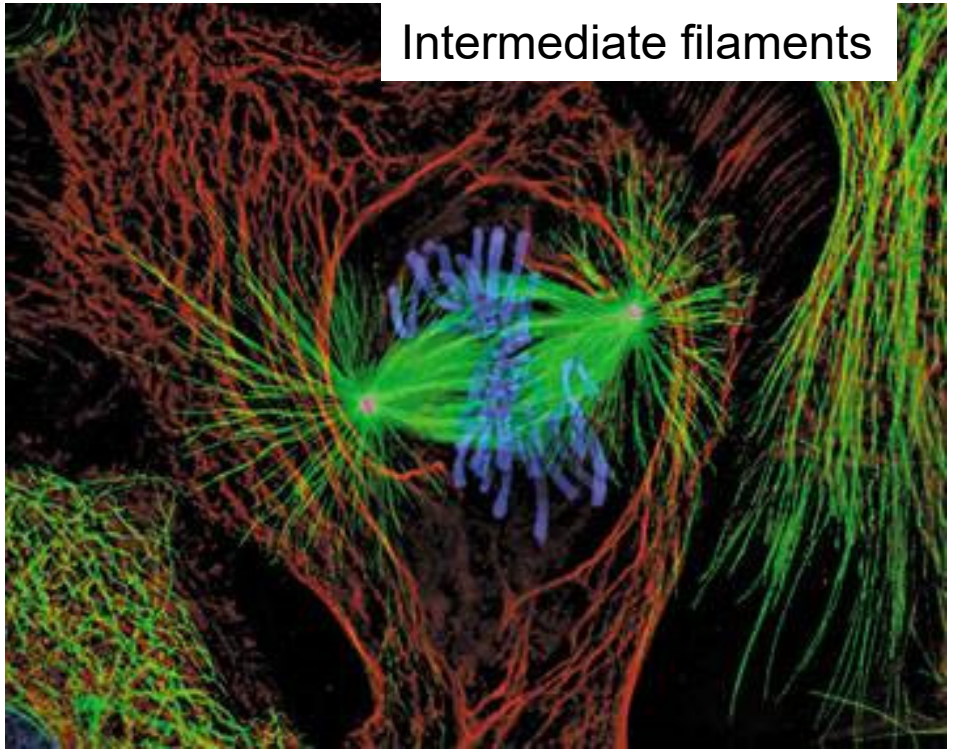
Department of Life Science

The Cytoskeleton

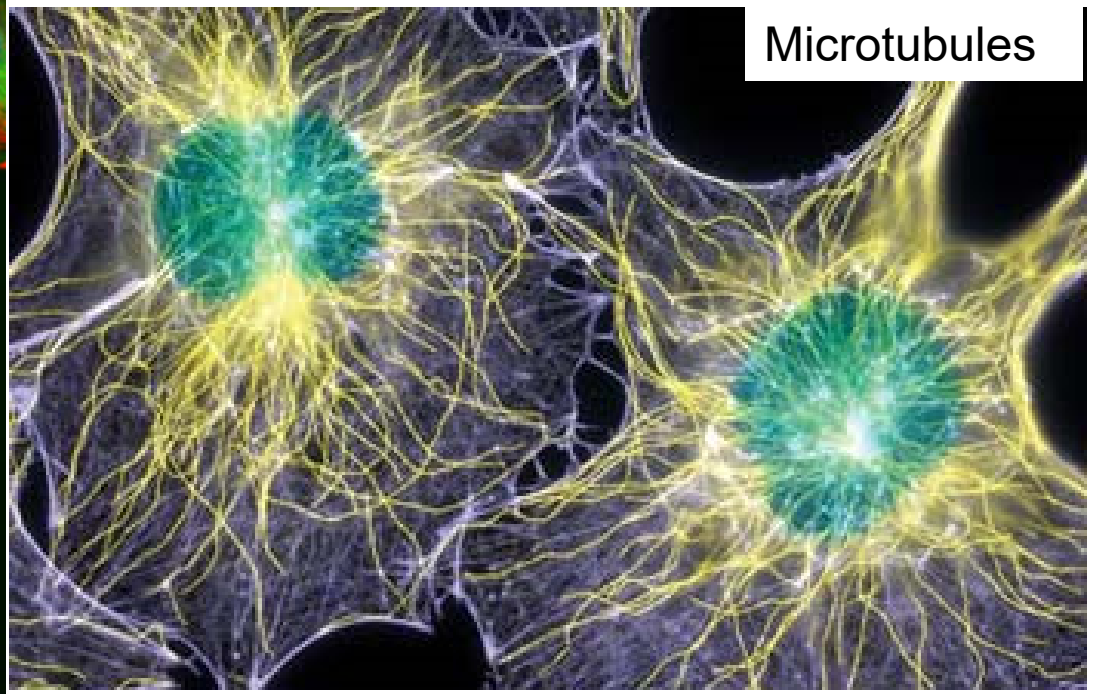


Microfilaments





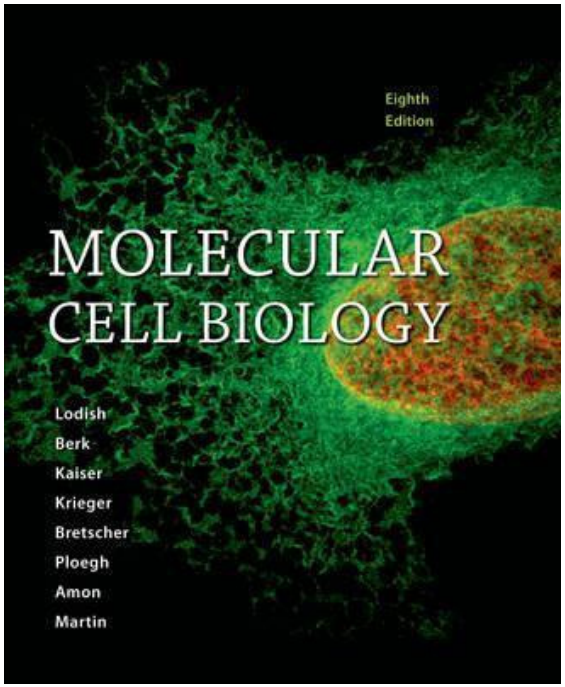
Intermediate filaments



Microtubules

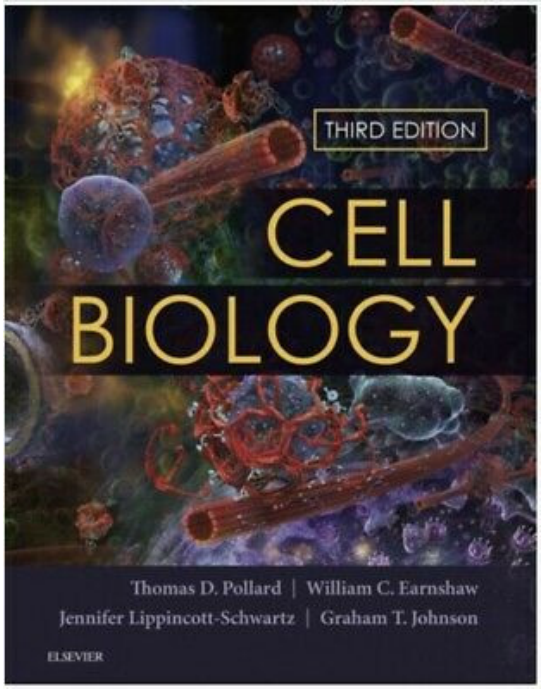
This lecture is based on the Lodish textbook but also contains material from two other important textbooks

Molecular Cell Biology, 8th Edition
by Harvey Lodish et al.



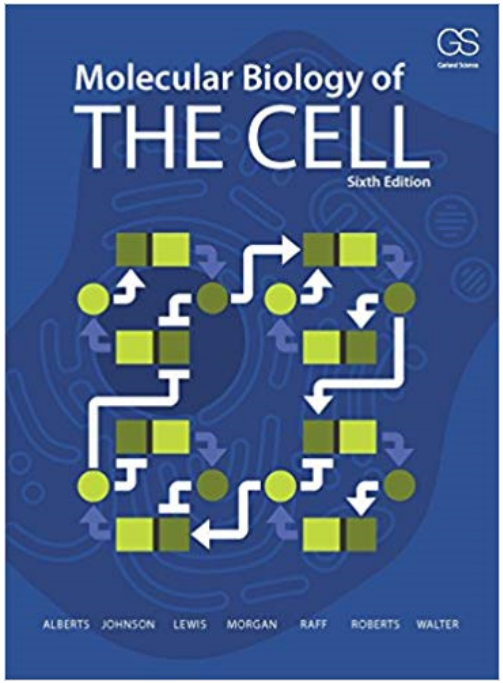
2016

Cell Biology, 3rd Edition
by Thomas D. Pollard et al.



2016

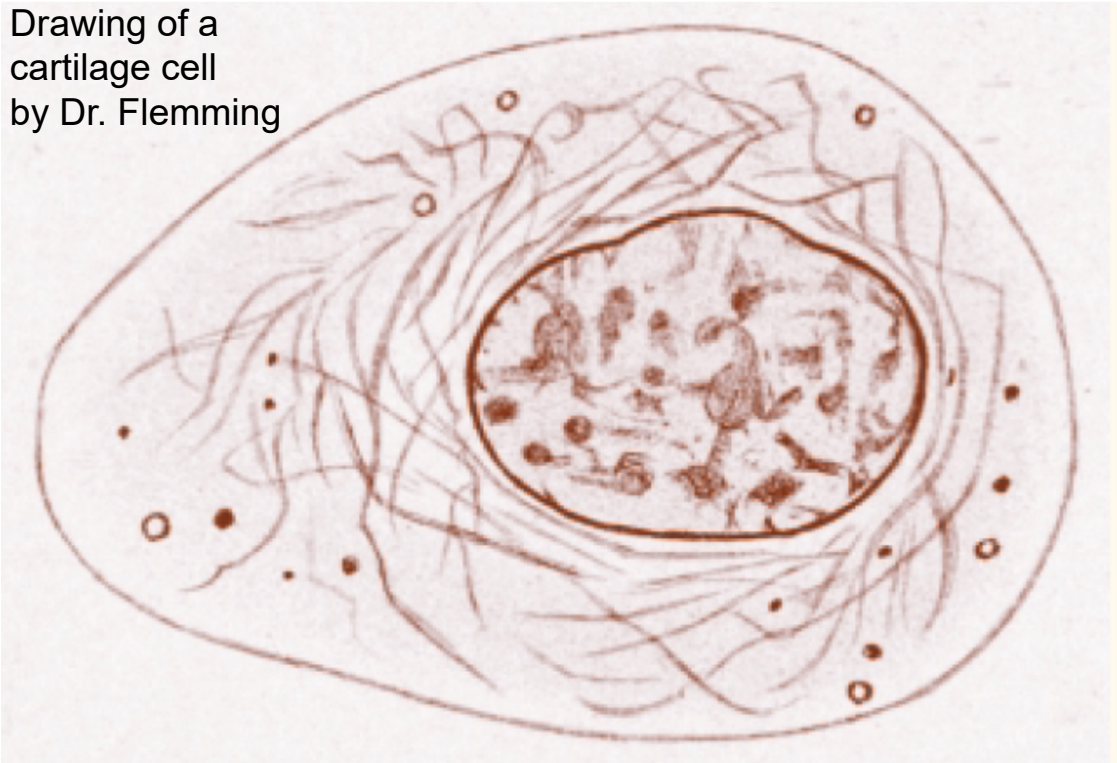
Molecular Biology of the Cell, 6th Edition
by Bruce Alberts et al.



2014

An early view of the cytoskeleton by Dr. W. Flemming (1879)

Drawing of a cartilage cell by Dr. Flemming

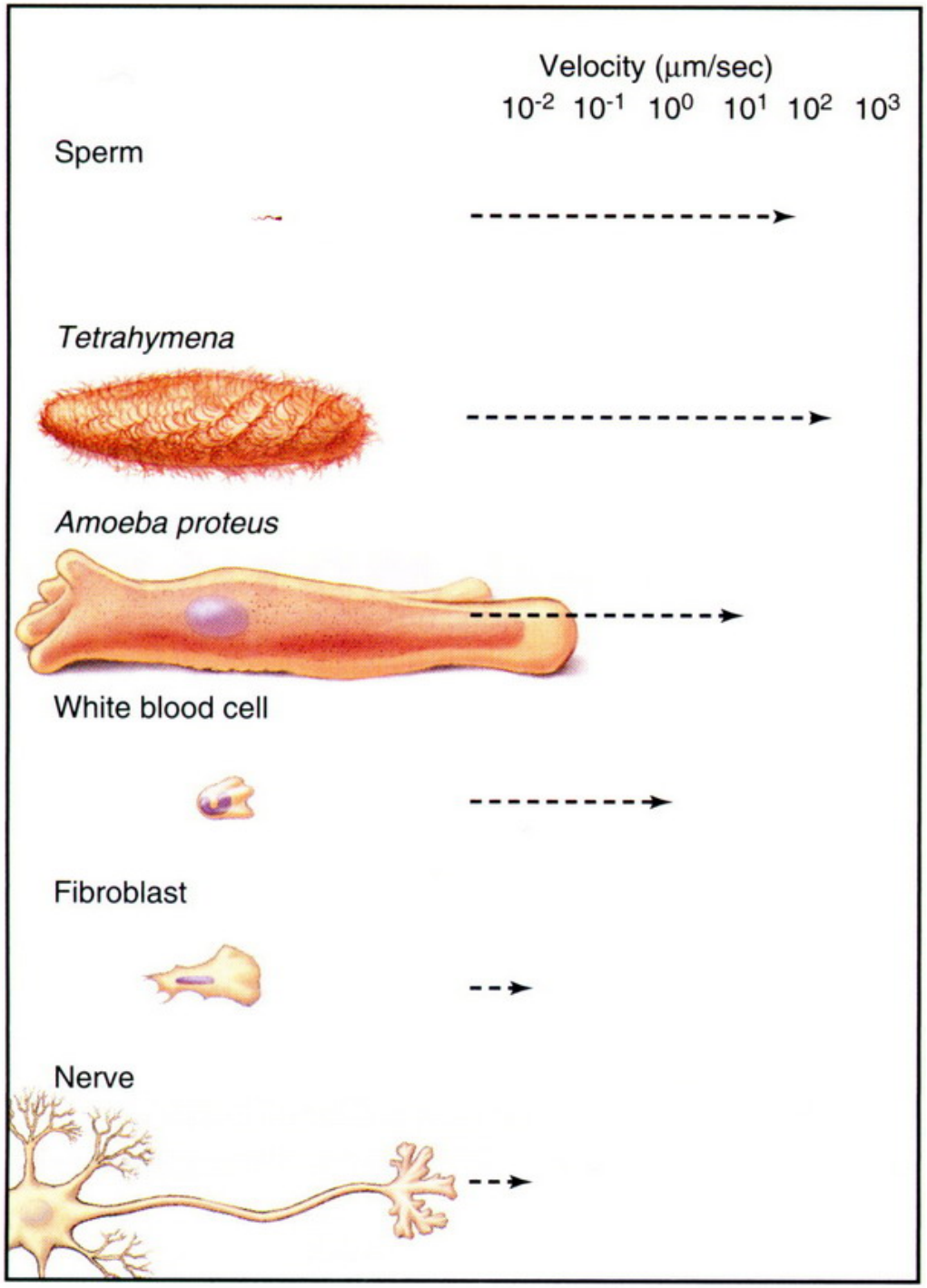


From: Flemming, W., *Arch. Mikrosk. Anat.* 16, 302–436 (1879)

Dr. Flemming: "What are those wispy [filamentous] structures in the cytoplasm?"

Later he described the cytoskeleton of **cartilage cells** as "threads" (German: "*Fäden").*

Dr. Flemming when he looked at **epithelia cells**: "**On reaching the plasma, one sees nothing at all.**"



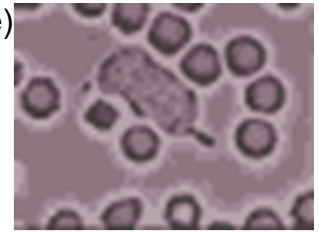
The cytoskeleton enables cells to move

- Microtubuli in flagella
- Microtubuli in cilia
- Actin polymerization and actin-myosin contraction

Pollard 3rd ed

Why cells need to be motile?

Neutrophil (macrophage) chasing bacterium

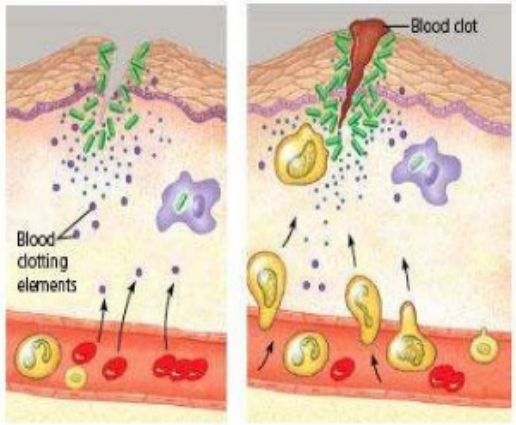


- **Development**: cells migrate inside the embryo to their defined locations
- **Host defense**: motile cells constantly **search for pathogens** inside the adult animal
- **Wound healing**: injured tissues are immediately invaded by highly motile cells to secret extracellular matrix (ECM) proteins

Why do people need to study the basics of cell movement?

Uncontrolled cell migration contributes to several pathologies:

- **Vascular diseases**
- **Chronic inflammatory diseases**
- **Cancer**: tumor formation and metastasis



What is the basis of cell migration?

A **cytoskeleton** composed of fibers which dynamically **reorient, shrink and grow**.

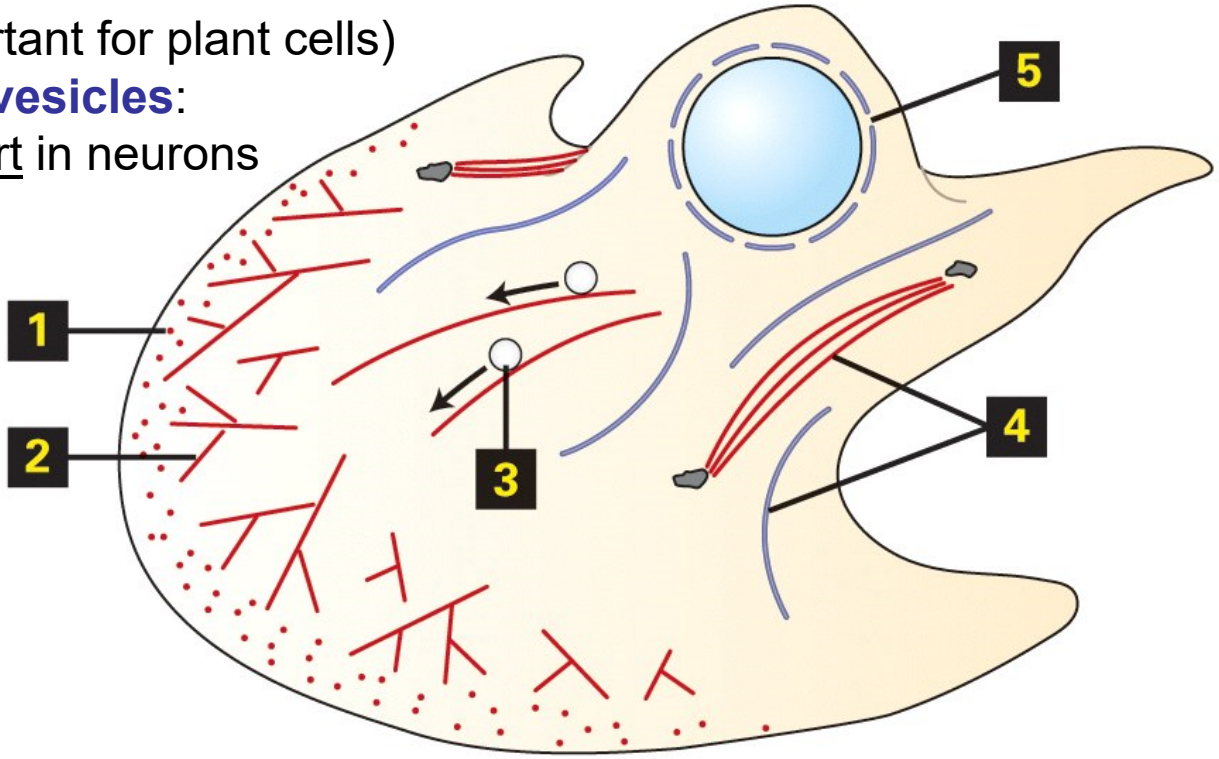
Based on this mechanism:

- axons of neurons can grow and connect to other neurons
- muscle **cells can contract** and produce force
- cells can send out small filopodia to sense their environment
- cells can divide during **mitosis**

The cytoskeleton also drives *internal* movements

- **Separation of chromosomes**
- **Streaming of cytosol** (important for plant cells)
- **Transport of membranous vesicles**:
  - Synaptic vesicle transport in neurons
  - **Endo-** and **exocytosis**
  - Membrane flow

  (recycling of membranes)



1 Cell movement: polymerization/depolymerization of actin drives membrane forward

2 **Cell shape support**: cross-linking proteins bundles and networks actin filaments

3 Tracks for motor proteins: myosin walks on actin to transport cellular cargos

4 **Form cell adhesions** & cell-cell contacts to connect to other cells and substrates

5 Lamin (an IF type) maintains structure of the nucleus

Actin (red) and **IFs** (purple)

The Nobel Prize in Physiology or Medicine 2013

Photo: © Yale University
James E. Rothman

Photo: H. Goren. © HHMI
Randy W. Schekman

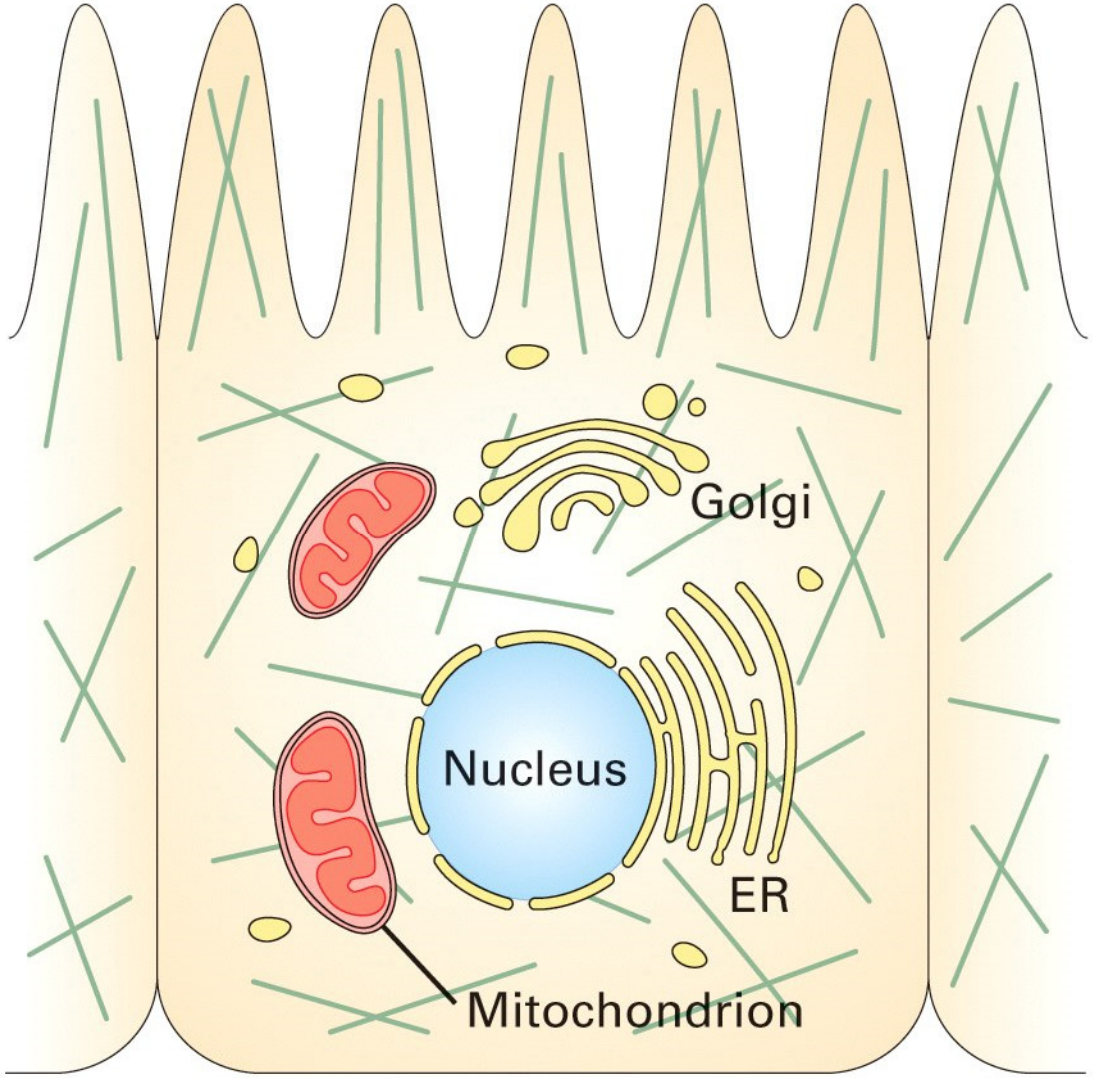
Photo: © S. Fisch
Thomas C. Südhof

The Nobel Prize in Physiology or Medicine 2013 was awarded jointly to James E. Rothman, Randy W. Schekman and Thomas C. Südhof ***"for their discoveries of machinery regulating vesicle traffic, a major transport system in our cells".***

The cytoskeleton occupies lots of space

- Cytoskeleton acts as an underlying **support** for the **cell membrane** (cell cortex)
- Organizes the cellular content (positioning of the nucleus, ER, Golgi and organelles)

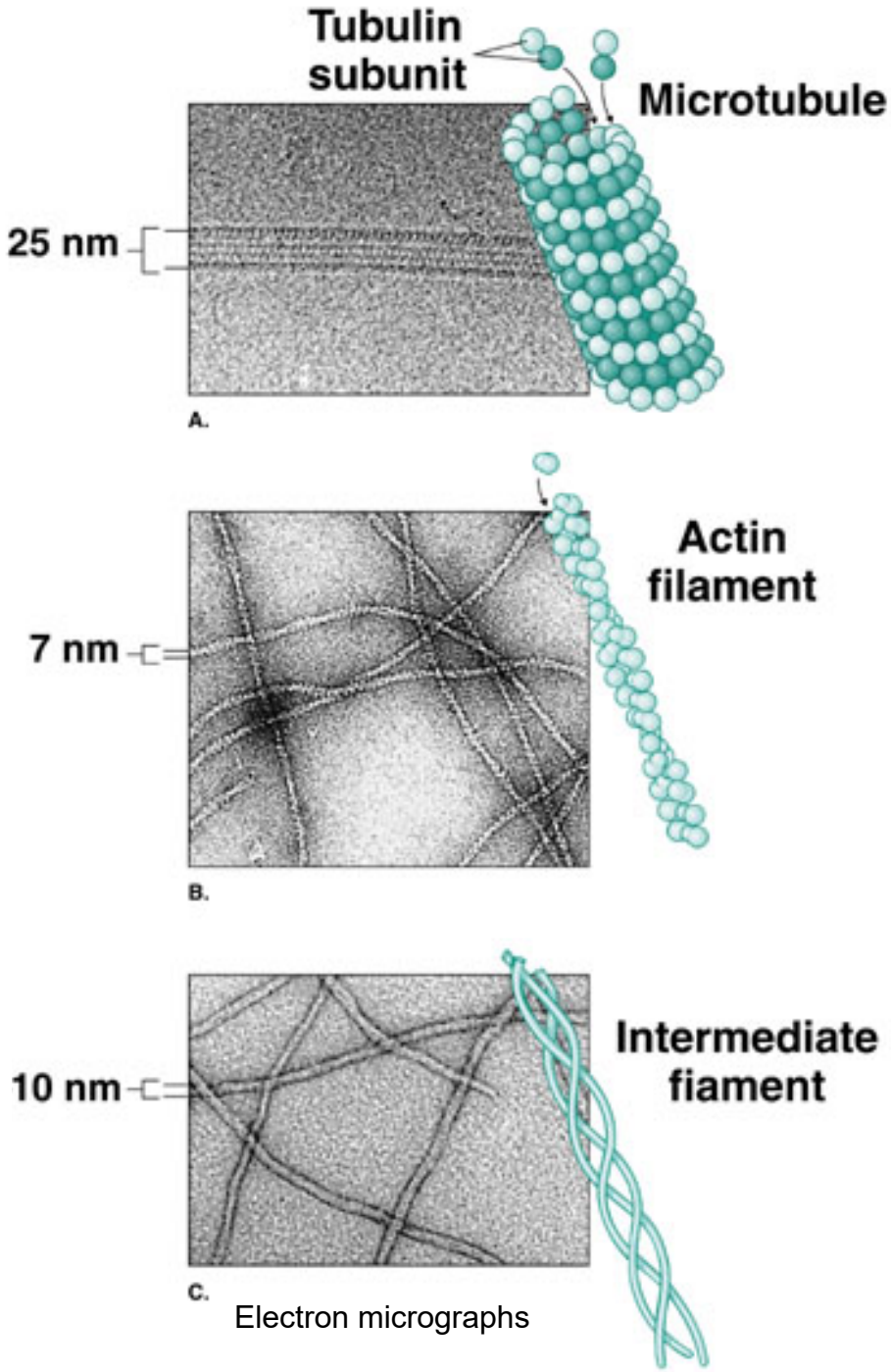
$\Rightarrow$ occupies lots of space



Plasma membrane (700 μm^2)

Internal membranes (7000 μm^2)

Cytoskeleton (94,000 μm^2)

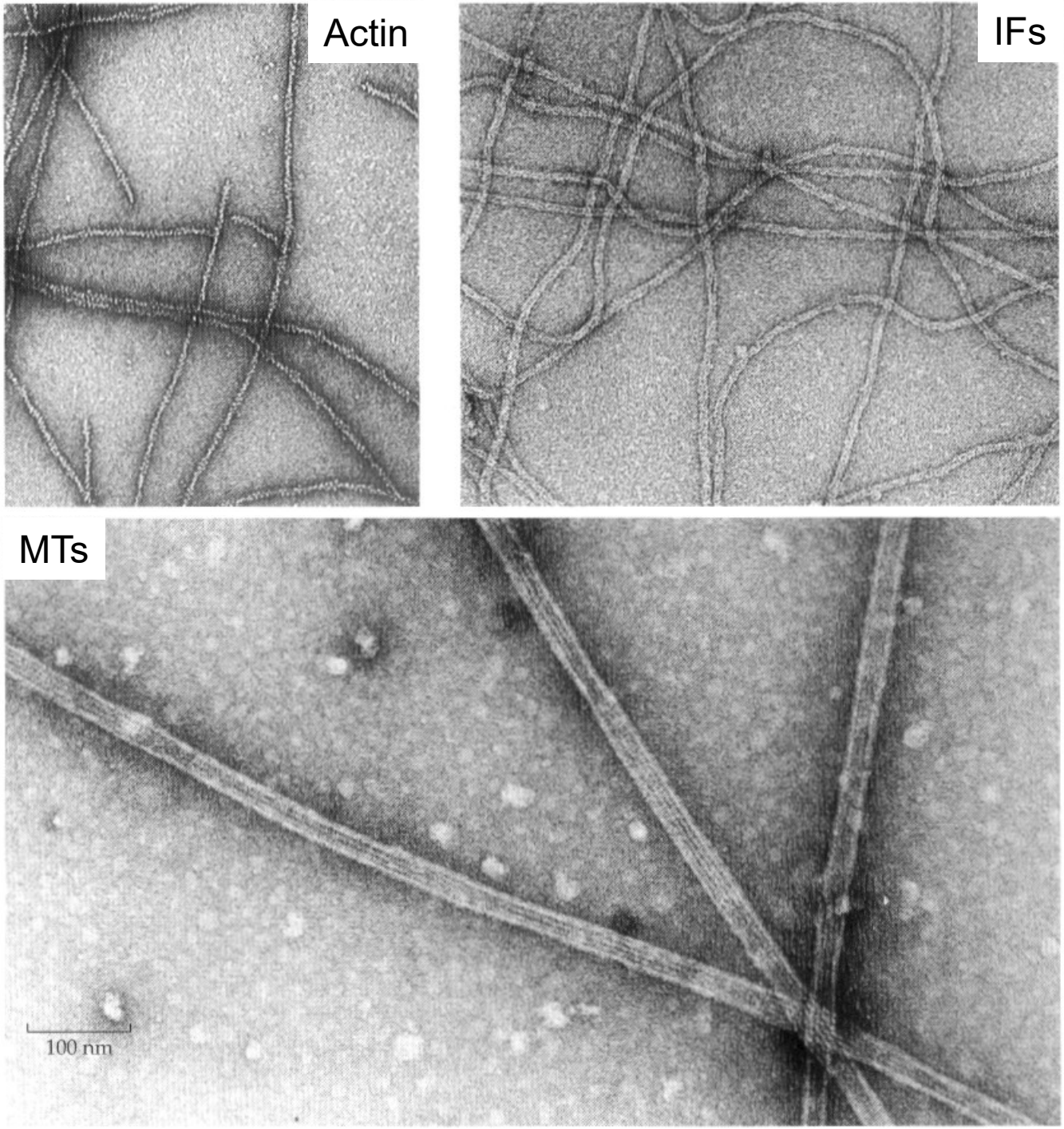


Electron micrographs

3 basic cytoskeletal elements

- Cytoskeleton is composed of **3 types of fibers** which are all polymers built from **globular protein subunits** held together by non-covalent bonds
- The fibers can be **distinguished by** their **diameter**

Actin: twisted, two-stranded (pearl-string like) structure
⇒ cell cortex, **microvilli**, stereo cilia, adherens belt, **filopodia**
MT: hollow cylinder formed by proto-filaments made of tubulin-subunits
⇒ positioning of Golgi, ER, vesicles etc.; **cilia/flagella**; chromosome separation
IFs: rope-like structure
⇒ **lamins** (nucleus support), **keratin** (in stiff epithelial cells), **vimentin** (in soft mesenchymal cells), **desmin** (bundles myofibers in muscle cells)

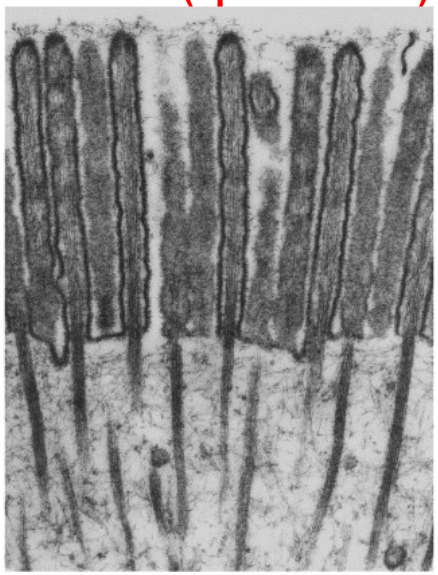


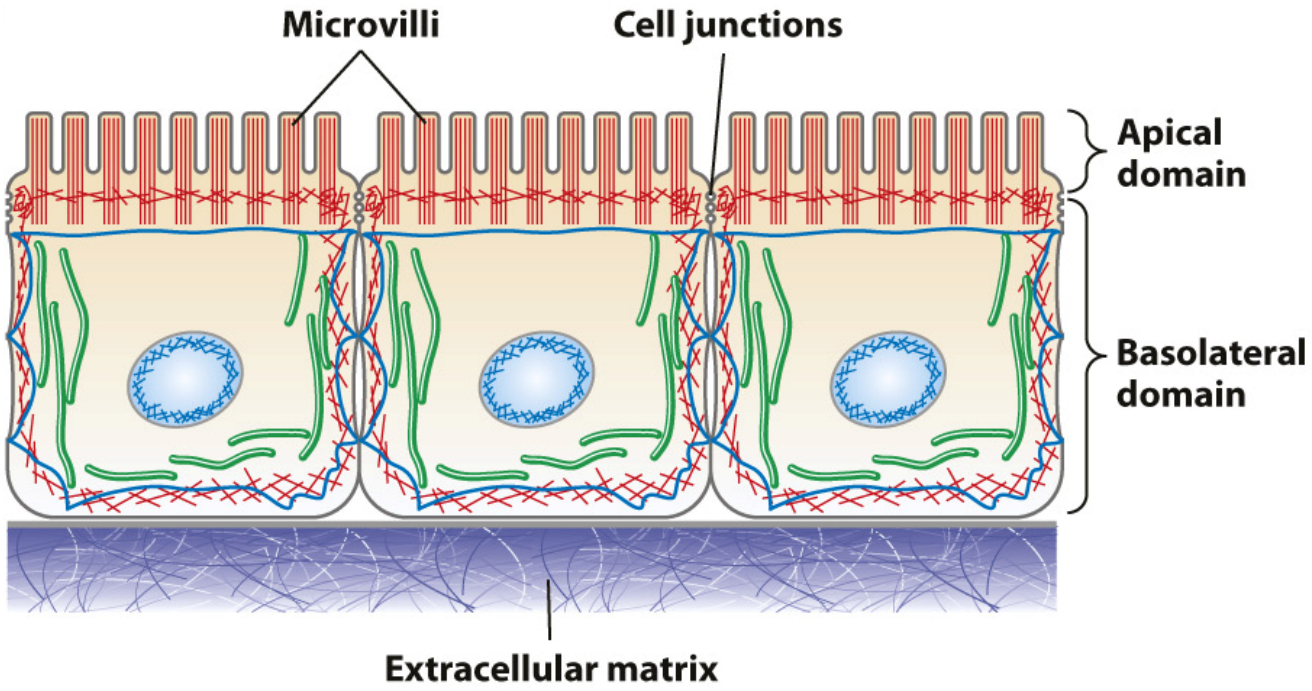
All three cytoskeletal elements drawn to scale

Howard, Mechanics of Motor Proteins, 1st Ed.

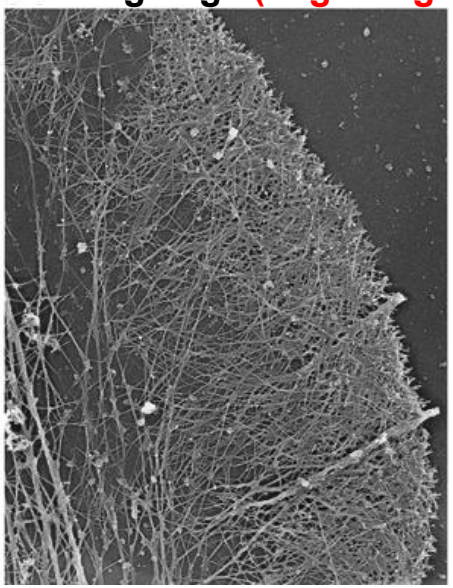
Cytoskeletal organization differs between cell types

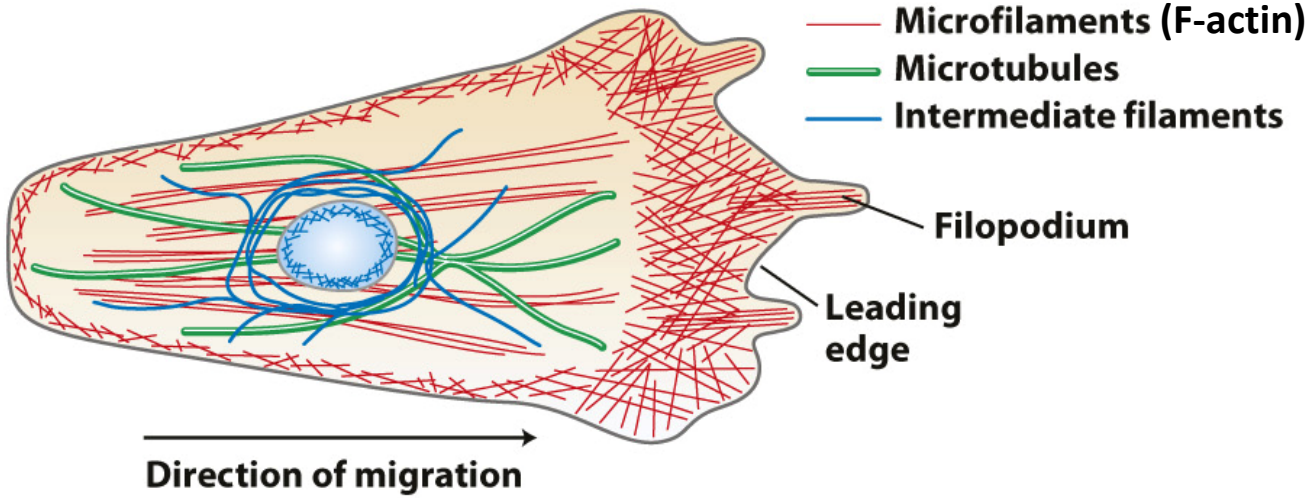
Microvilli (epithelia cell)





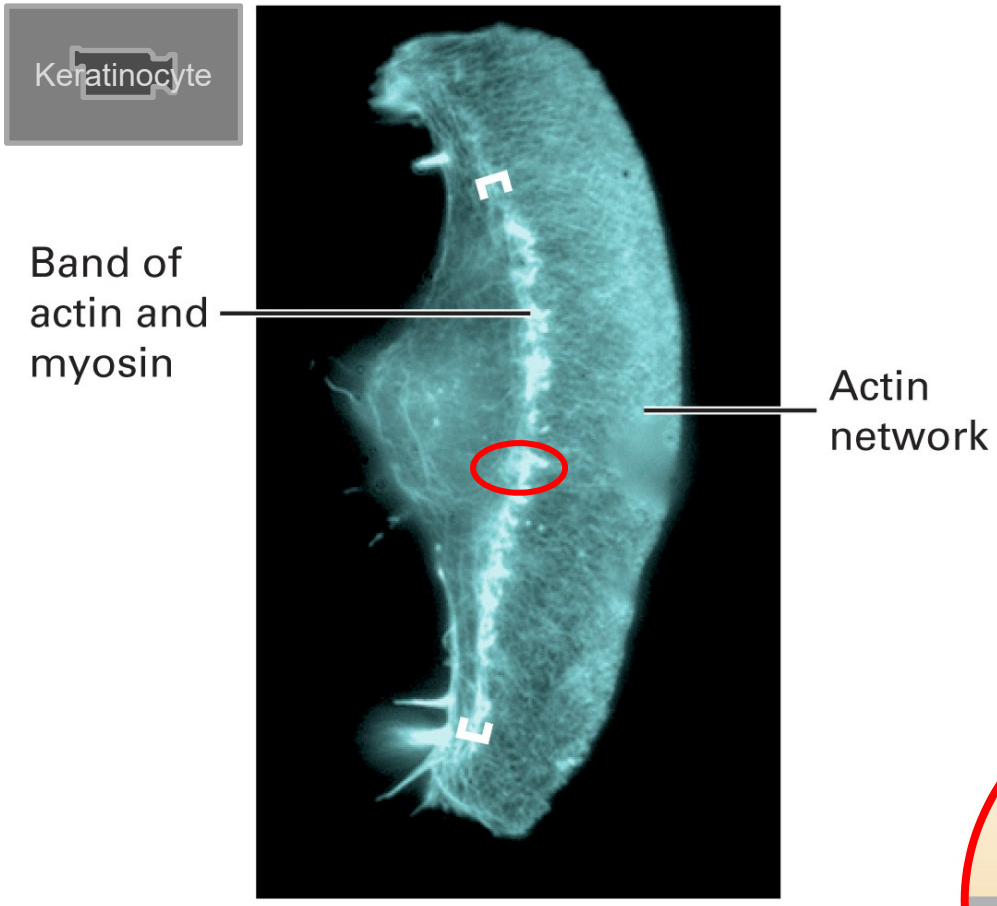
Leading edge (migrating cell)





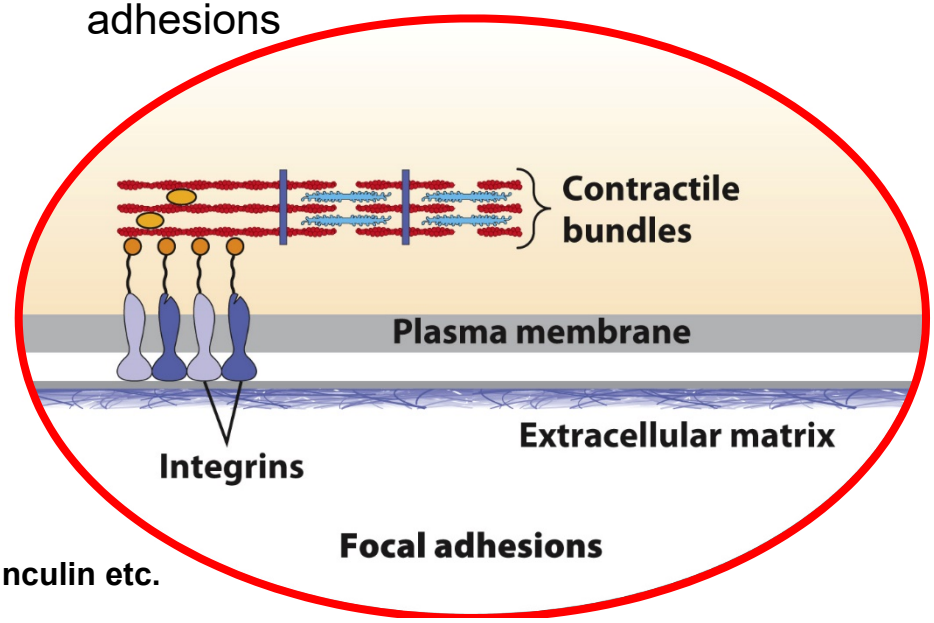
How cells move

Highly motile fish keratinocyte



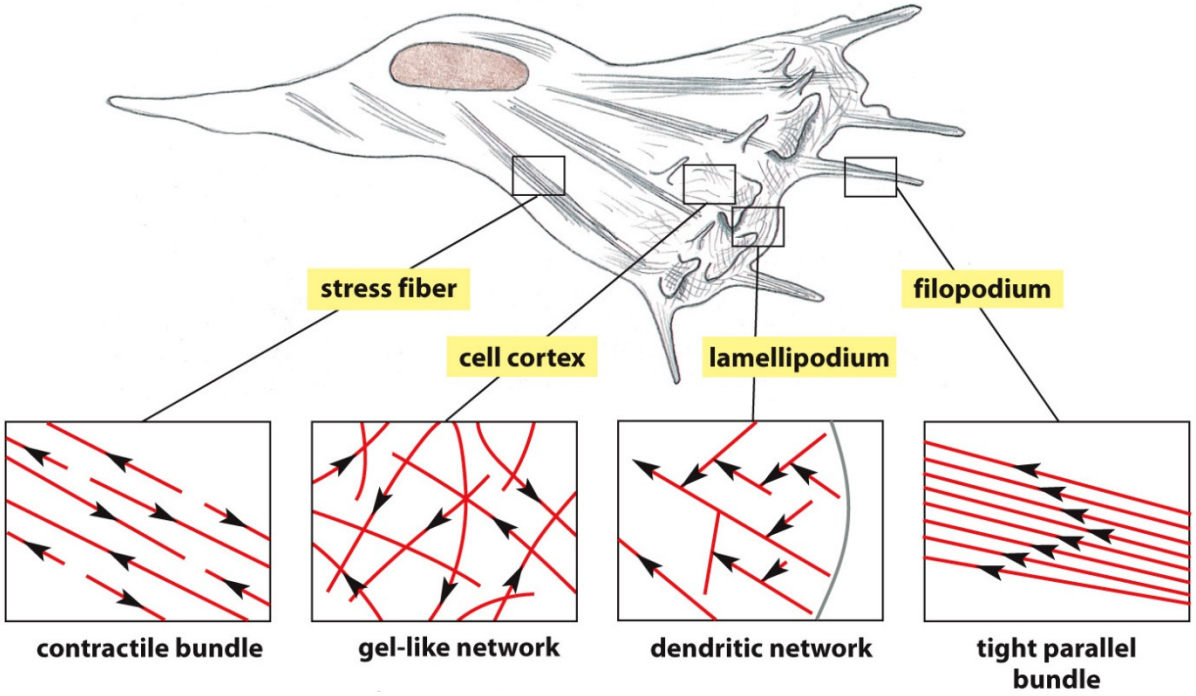
- Polymerization of actin at the "**leading edge**" expands the membrane in front of the cell
- This **tension force** and the contraction of **actin/myosin** pushes the cell forward
- The effect of the strong tension is seen in the movie when the cell becomes more elongated
- At the "**trailing edge**" of the cell all organelles are accumulated

Contractile bundles are often linked to the ECM (extracellular matrix) via focal adhesions



● = talin, vinculin etc.

Cellular actin organization

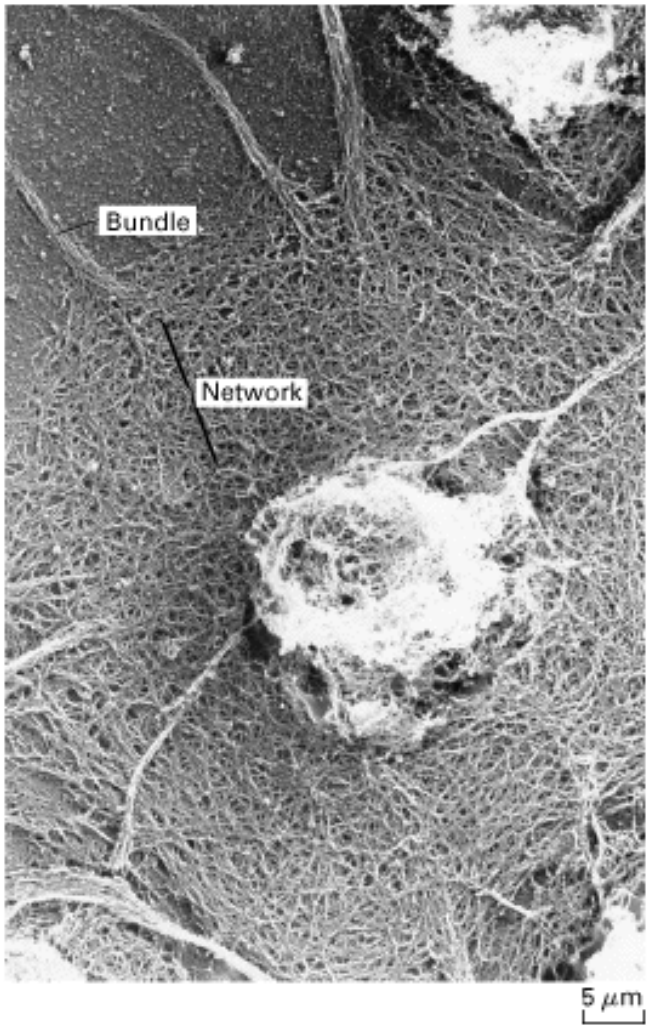


Mixed polarity

Mixed network

Uniform branches

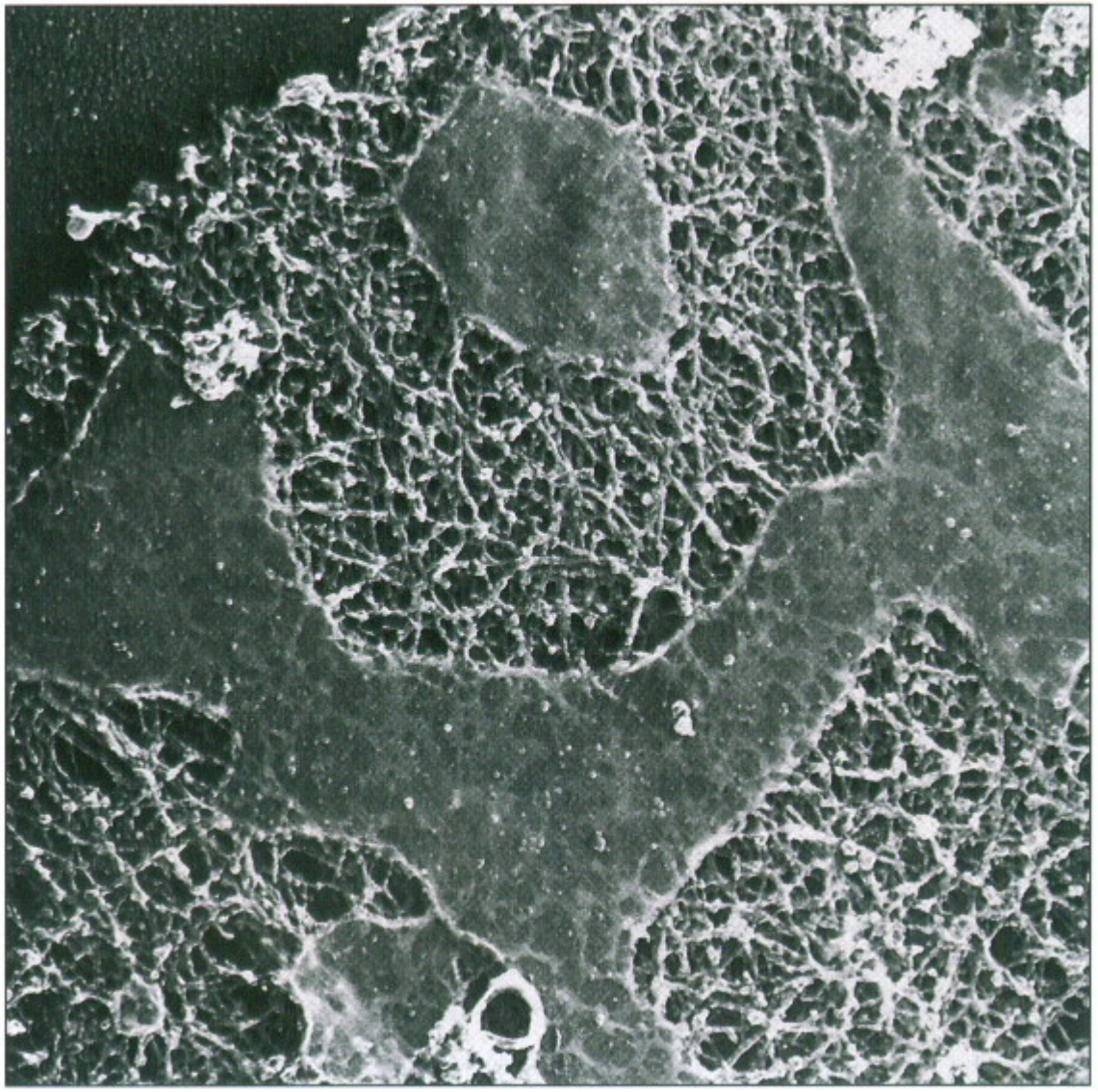
Uniform Polarity



Stress fibers: cellular support and contraction
Cell cortex: fast-acting gel-sol transition (cytosol liquefying)
Lamellipodia: leading edge (movement front)
Filopodia: sensing the environment

Alberts 6th ed

Cell cortex: elastic actin network to support the fragile plasma membrane

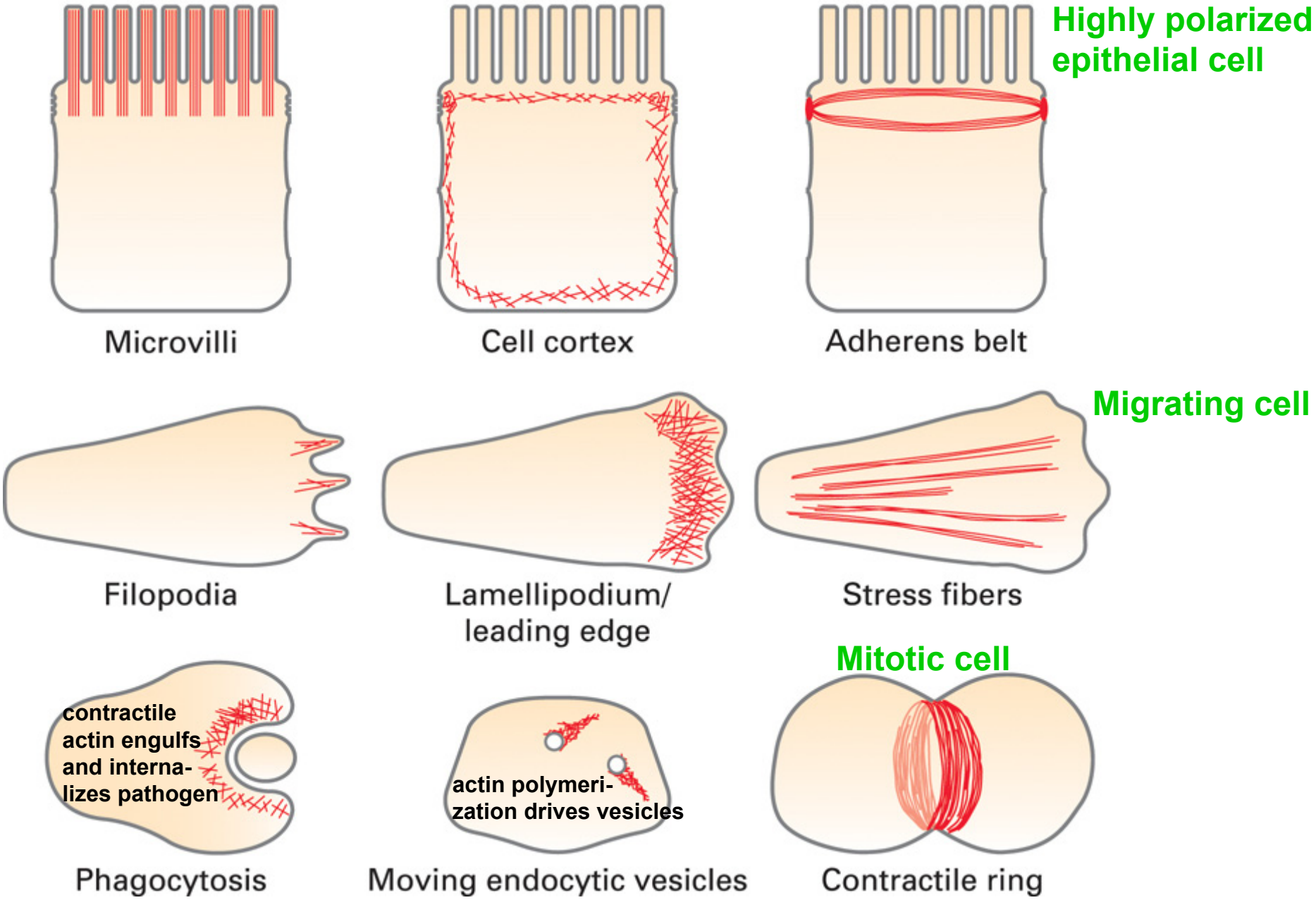


Bray, Cell Movements, 2nd Ed.

Fibroblast treated with **mild detergent** to partial remove plasma membrane

Fast-freeze, deep-etch electron microscopy reveals actin-network linked by filamin

Summary of cellular actin organization



Molecular basics of actin polymerization

"Understand how a cell can change its shape so quickly"

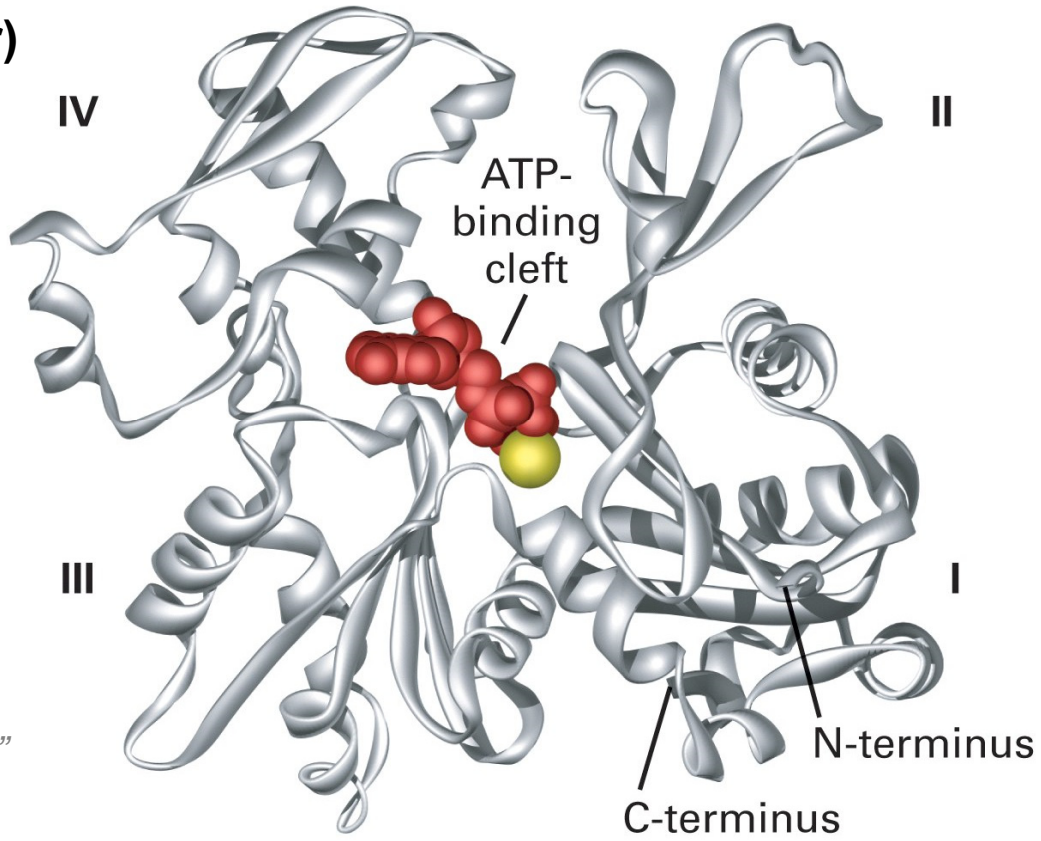
- Actin is the most abundant and one of the highest conserved protein in eukaryotic cells
- The molecular weight is around 43,000 Dalton
- Humans have **6 actin genes** encoding for different isoforms:
  - **α-actin** is found in **muscle cells** (**4 isoforms**)
  - **β- and γ-actin** in **nonmuscle cells**: β-actin = leading edge, γ-actin = stress fibers

- **G-actin** is the **monomeric (globular) subunit** of **filamentous F-actin**
- G-actin is composed of **4 subunits** which form **two** equally sized **lobes** divided by a central cleft
- The central cleft binds either **ATP** or **ADP** complexed with a **Mg^{2+} ion**

⇒ four actin states possible:

ATP-G-actin ⇔ ADP-G-actin +Pi

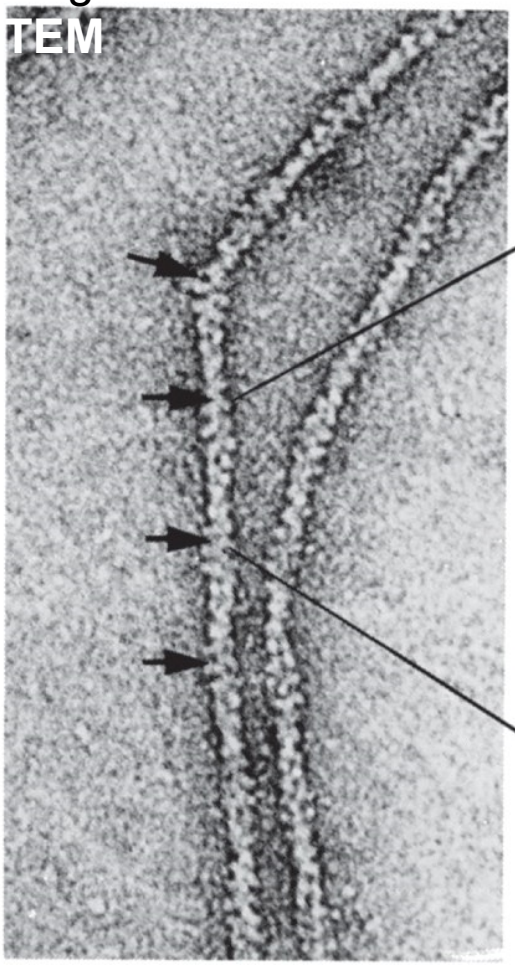
ATP-F-actin ⇔ **ADP-F-actin + Pi**

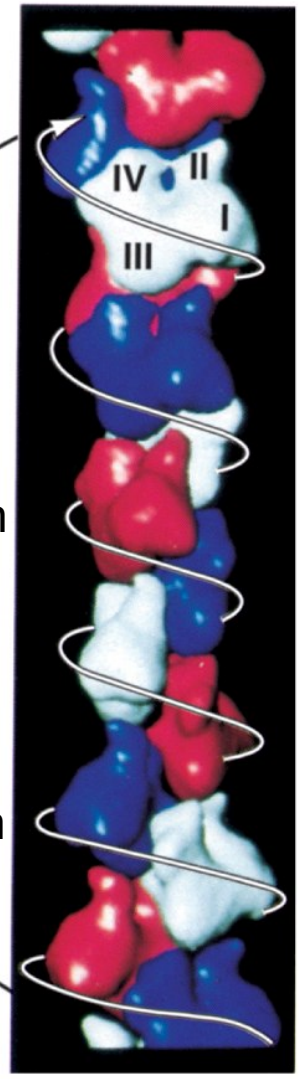


"A liver cells contains 500 million actin molecules"

Model of F-actin

"negative stained" F-actin

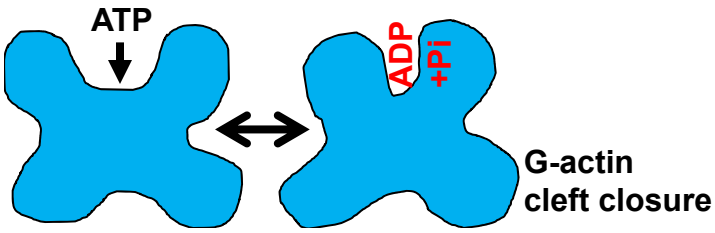




thin 7 nm

thick 9 nm

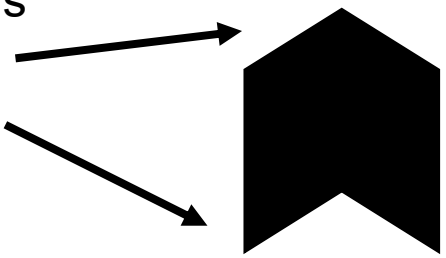
(only 14 G-actin monomers shown)

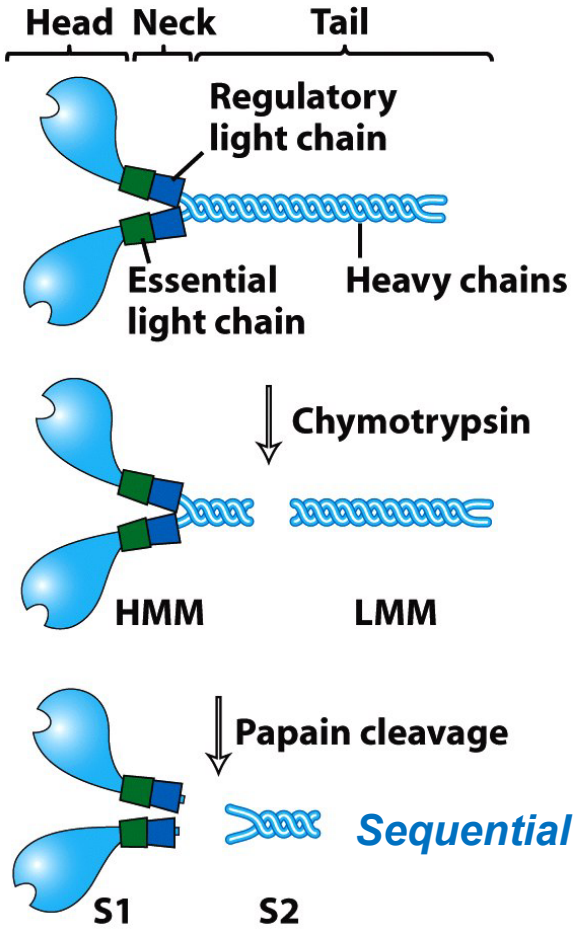


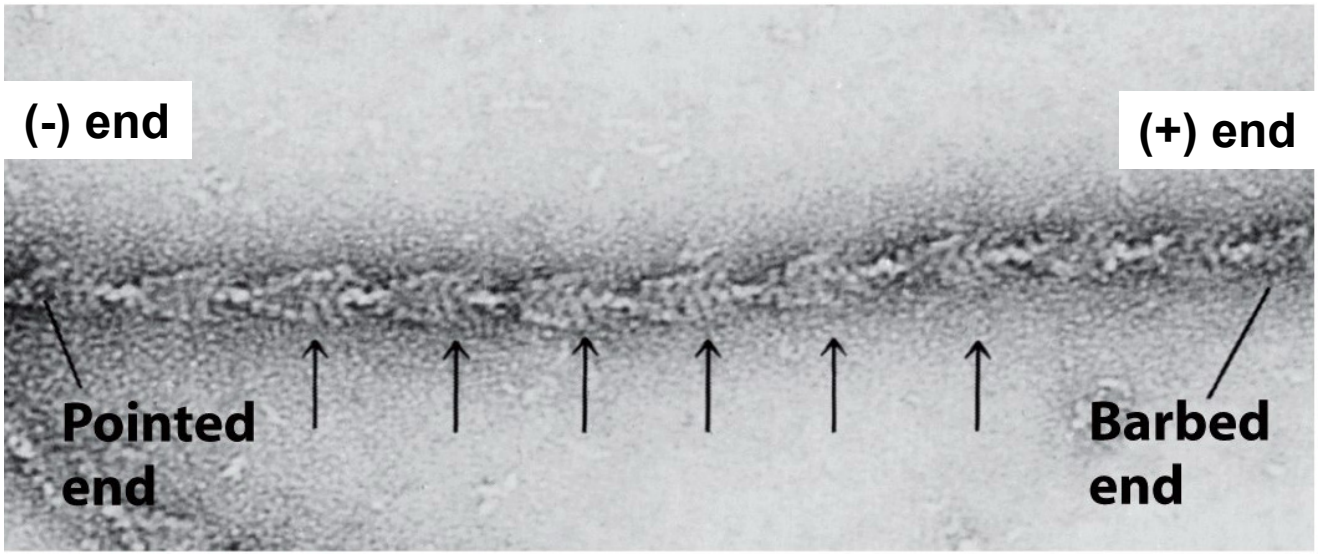
- Due to the twist of the strands, F-actin helix appears alternating thinner and thicker
- One repeating unit is **72 nm long** and consists of **28 monomers** covering **13 turns** in the helix
- The cleft acts as a hinge to bring the monomers in the appropriate position causing the helix turn
- After binding ATP or ADP the cleft closes
- Addition of ions as Mg^{2+}, K^{+} or Na^{+} can **induce polymerization of G-actin** to F-actin
- Lowering the ionic strength depolymerizes F-actin to G-actin
- During polymerization ATP is hydrolyzed => however, it affects only the kinetics of polymerization (polymerization of actin can occur *without* ATP-hydrolyzation)

The polarity of F-actin can be detected via decoration with S1

- The actin-cleft always faces to the (slow growing) minus-end of actin
- EM resolution is not high enough to see the cleft, instead we can coat F-actin with the myosin subfragment 1 (S1 = head of myosin) binding slightly tilted to actin
- S1-decorated actin looks like “arrowheads”
  - pointed end faces to the minus-end
  - **barbed end** faces to the **plus-end**



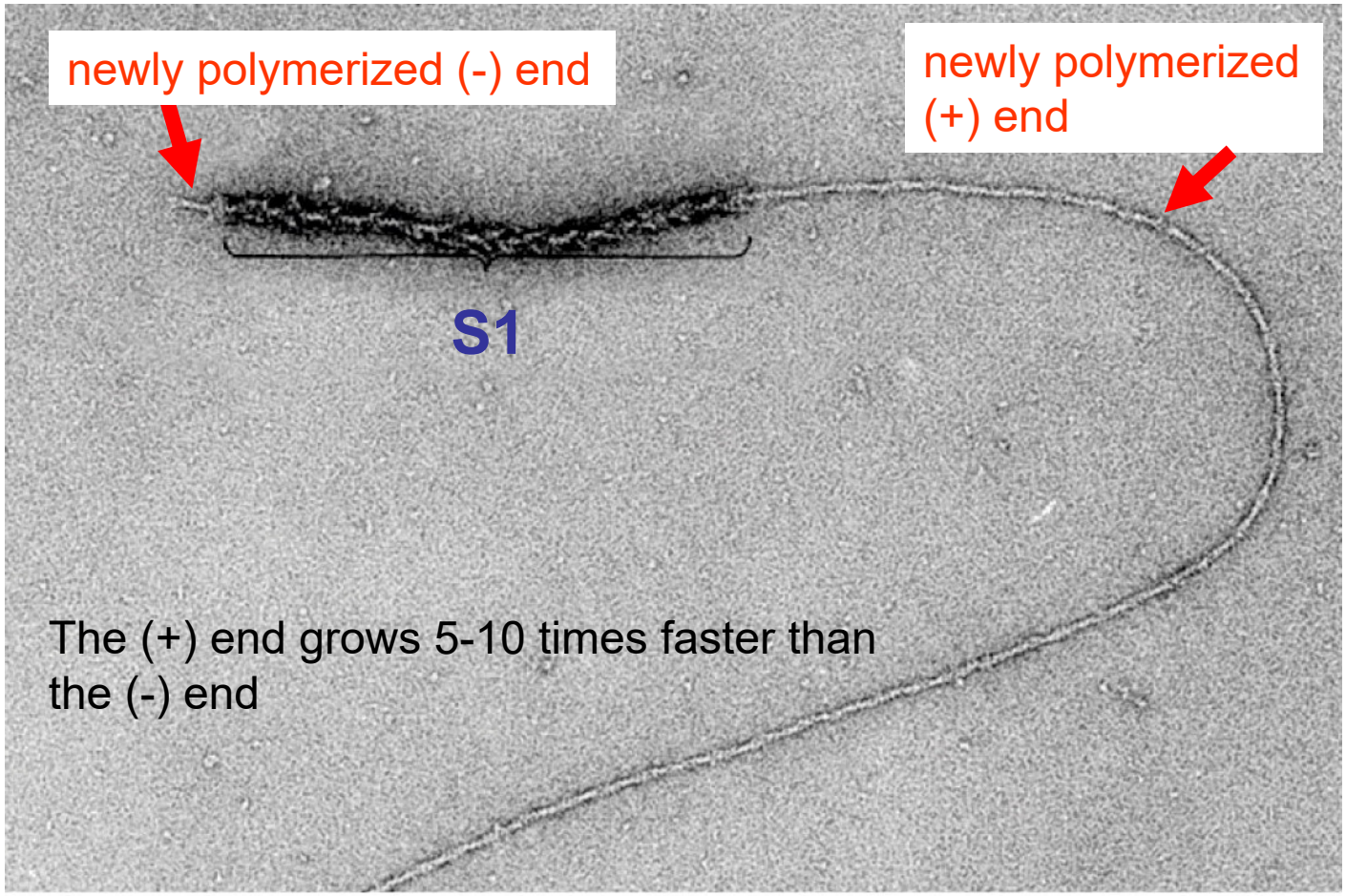


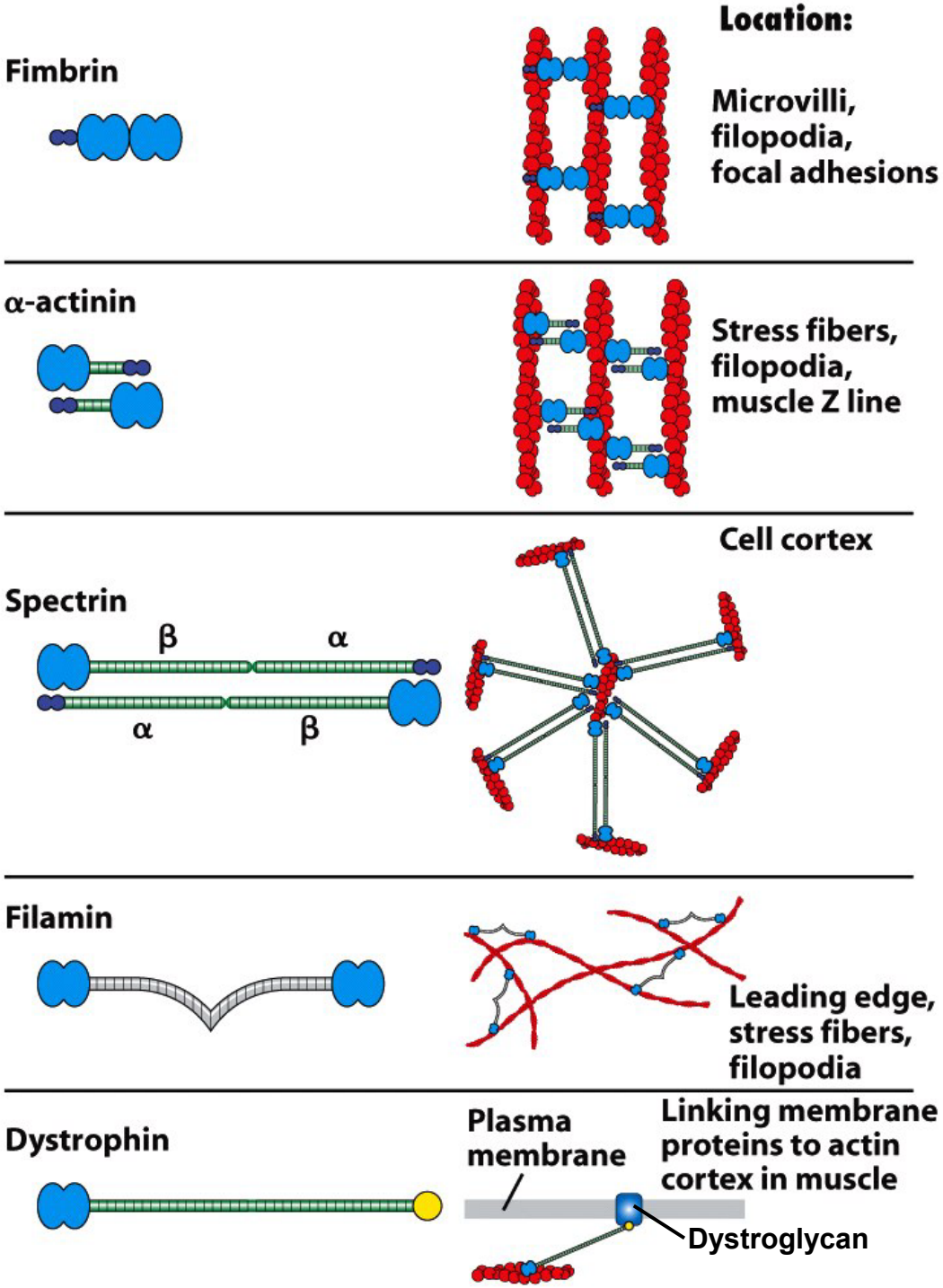


Sequential myosin cleavage by two different proteases

S1 can be used as a nuclei to boost actin polymerization

Using myosin S1 as a **nuclei** to polymerize G-actin (added in excess), the (undecorated) minus-end appears much shorter in EM as opposed to the (undecorated) plus-end

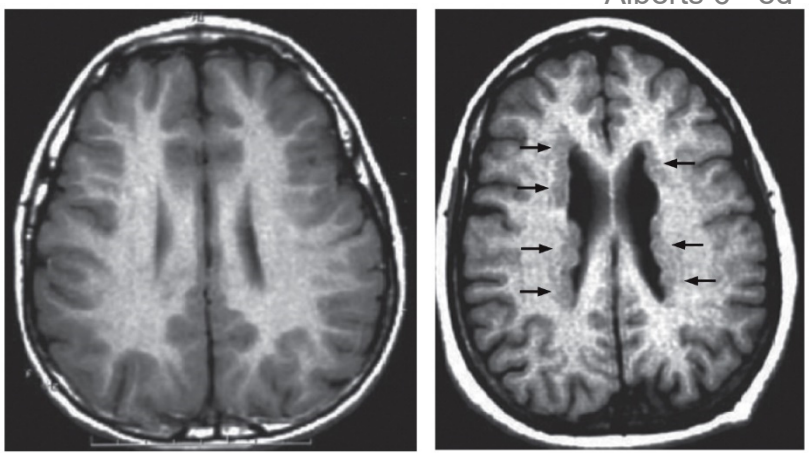




Most actin cross-linking proteins belong to the calponin homology-domain family

- Actin cross-linking proteins can either bundle actin (e.g., microvilli) or form a network of actin (e.g., cell-cortex or leading edge)
- Most of these binding proteins have **two actin binding-sites** which are **homologous to calponin** (a known smooth muscle contraction inhibitor)
- **Dystrophin** is involved in the disease ***Duchenne muscular dystrophy*** (muscles become weakened in the absence of dystrophin)

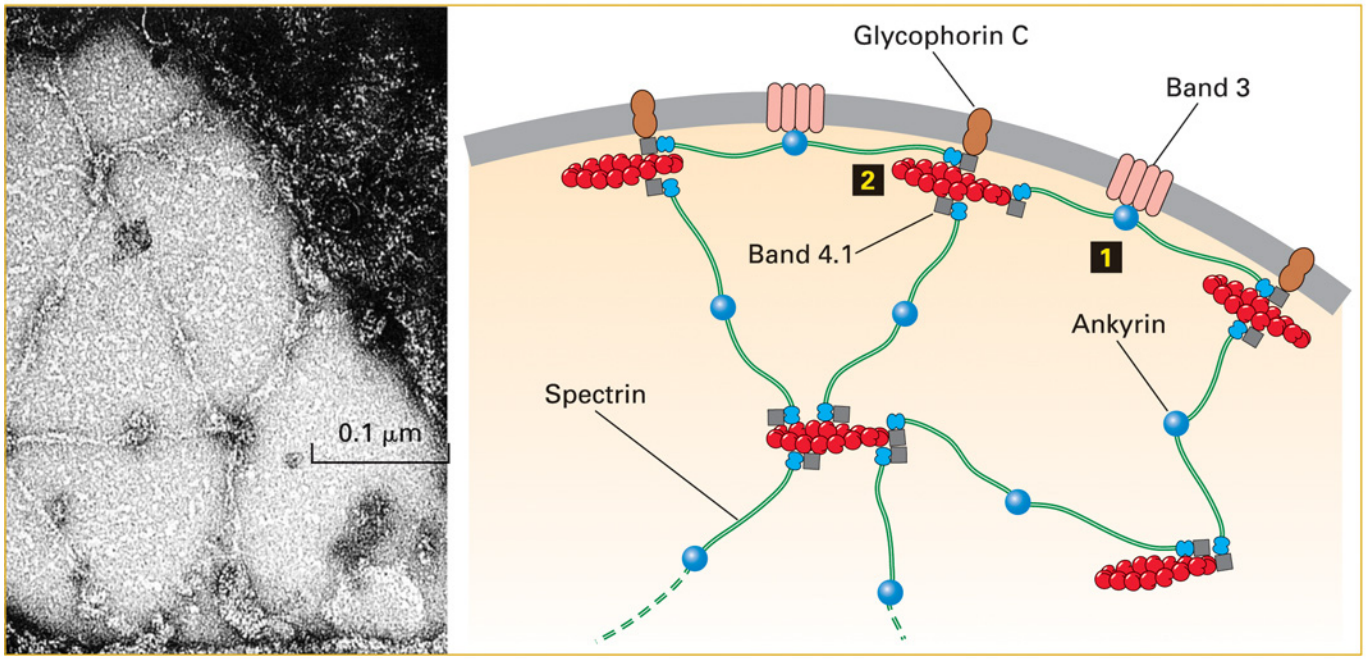
Alberts 6th ed

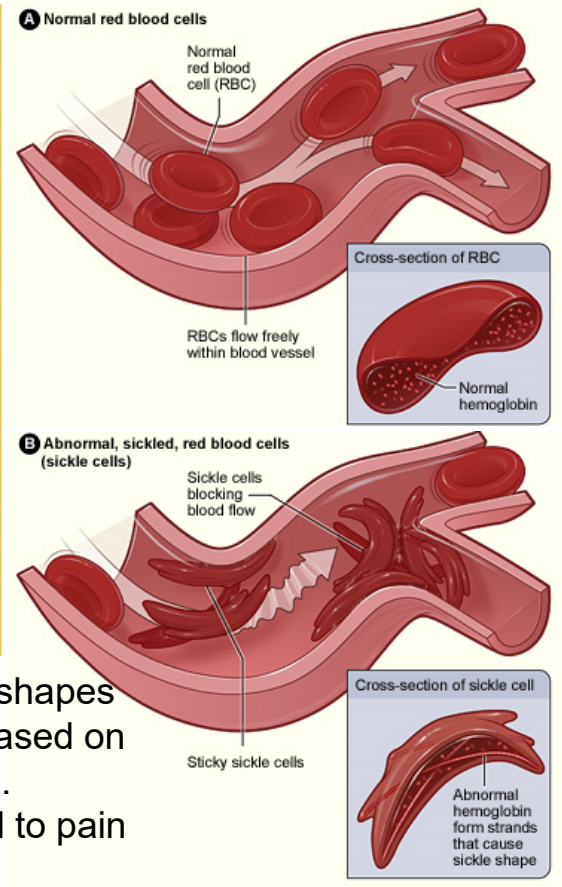


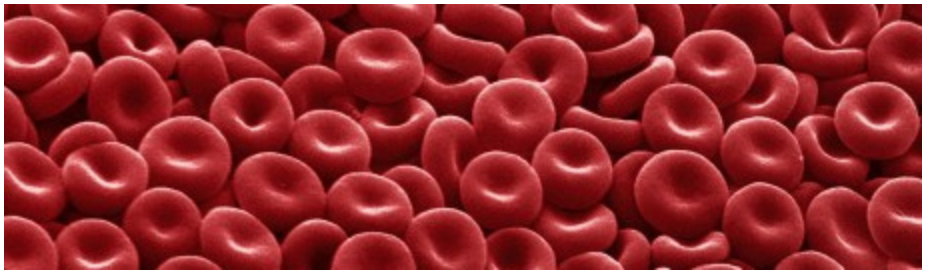
Periventricular heterotopia caused by mutation in the **filamin A** gene (brain development defects causing epilepsy)

Actin contributes to the very elastic cell membrane of red blood cells

- To be more flexible (squeezing through thin capillaries), red blood cells **lack of internal actin networks**, but contain a very flexible cell membrane supported by a fishing-net type structure
- Several "**adaptor proteins**" are needed to connect actin to the cell membrane
- Triangular pattern: small **actin trunks** bind about **six spectrin** via **band 4.1** and **ankyrin**
- 14 subunits long actin filaments are laterally stabilized by **tropomyosin** and capped at their minus-end by **tropomodulin**





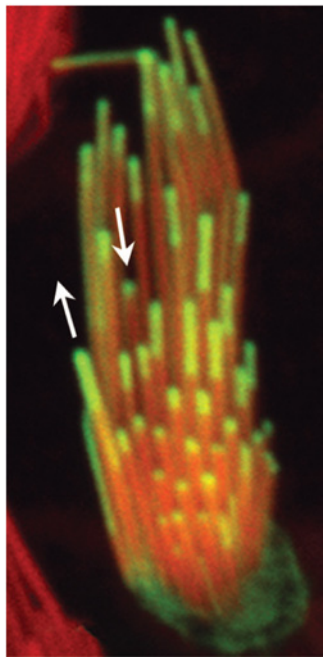


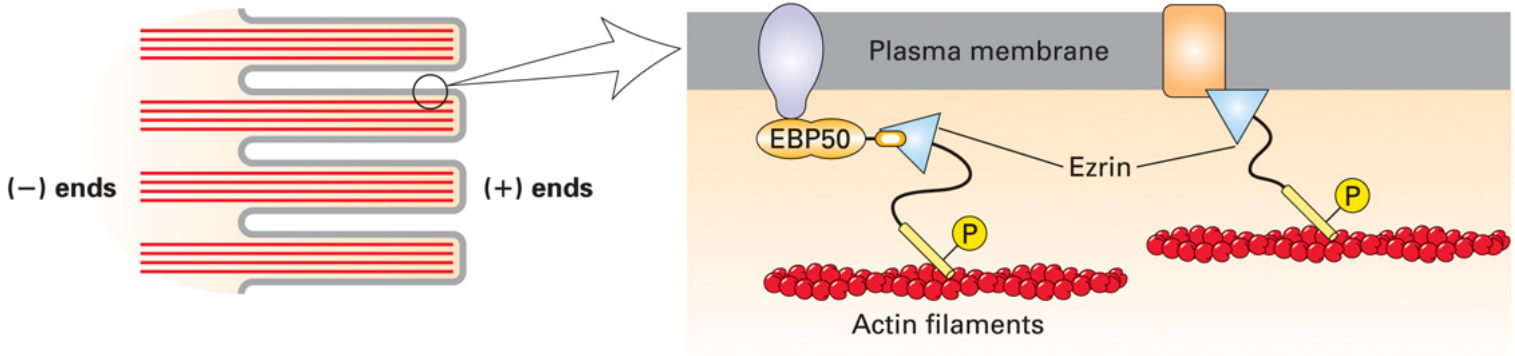
Abnormal erythrocyte shapes in sickle cell anemia based on hemoglobin misfolding.
Clogged vessels lead to pain and organ damage.

http://www.nhlbi.nih.gov/health/health-topics/topics/sca/

ERM family of proteins link actin to plasma membranes in microvilli

- Besides the dystrophin/dystroglycan complex that links actin to the muscle cell membrane, **ERM proteins** (ezrin-radixin-moesin) provide lateral connectivity of the actin/fimbrin complex to the **microvillus membrane**
- Newly incorporated GFP-actin is mostly obvious at the plus-end tips of microvilli (ABP **formin** anchors actin at the tips)
- At the sides of the microvilli actin is linked **either via ezrin directly** to the membrane **or via an ezrin/EBP50** complex
- Ezrin exist in a folded state but when phosphorylated the protein unfolds and becomes active



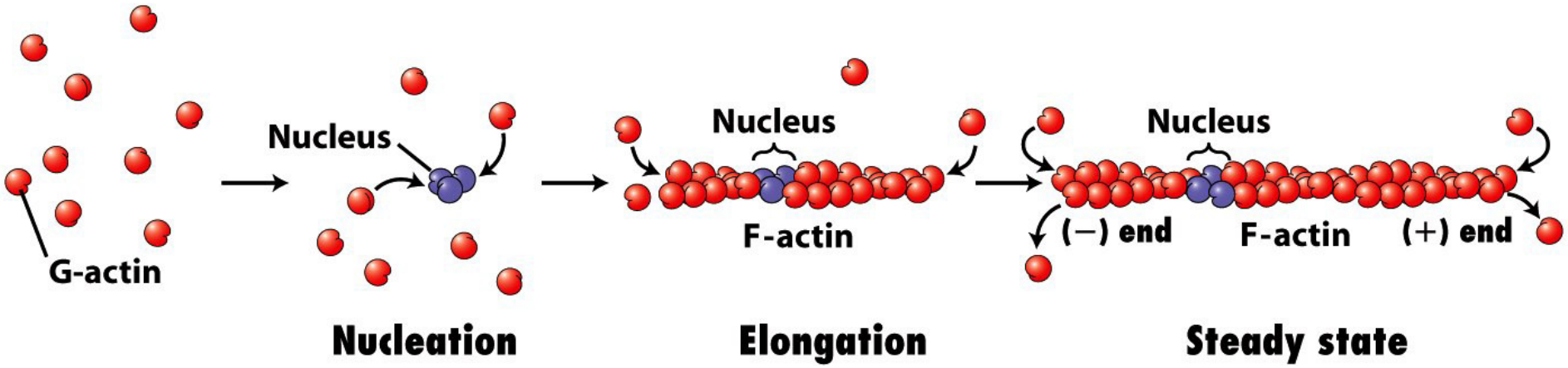


Transient expression of **GFP-actin** (**rhodamine-phalloidin** counterstaining)

"Transient expression refers to gene expression over a short period of time usually via cell transfection. Transient means the cell lost the plasmid over time mostly during mitosis"

Polymerization of actin proceeds in three steps

1) **Nucleation phase**: G-actin **slowly** aggregates into short oligomers (nuclei/seeds)
2) **Elongation phase**: To both ends of the seed, G-actin monomers **rapidly** added
3) **Steady-state phase**: Equilibrium is reached between filaments and monomers; G-actin adds and falls off on both sides of the filament but no net change in total mass of filaments



- At steady-state, the pool of non-polymerized G-actin is called the **critical-concentration** which is about 0.1 µM (= actin **dissociation constant**)

⇒ a solution of G-actin **above** 0.1 µM **polymerizes**

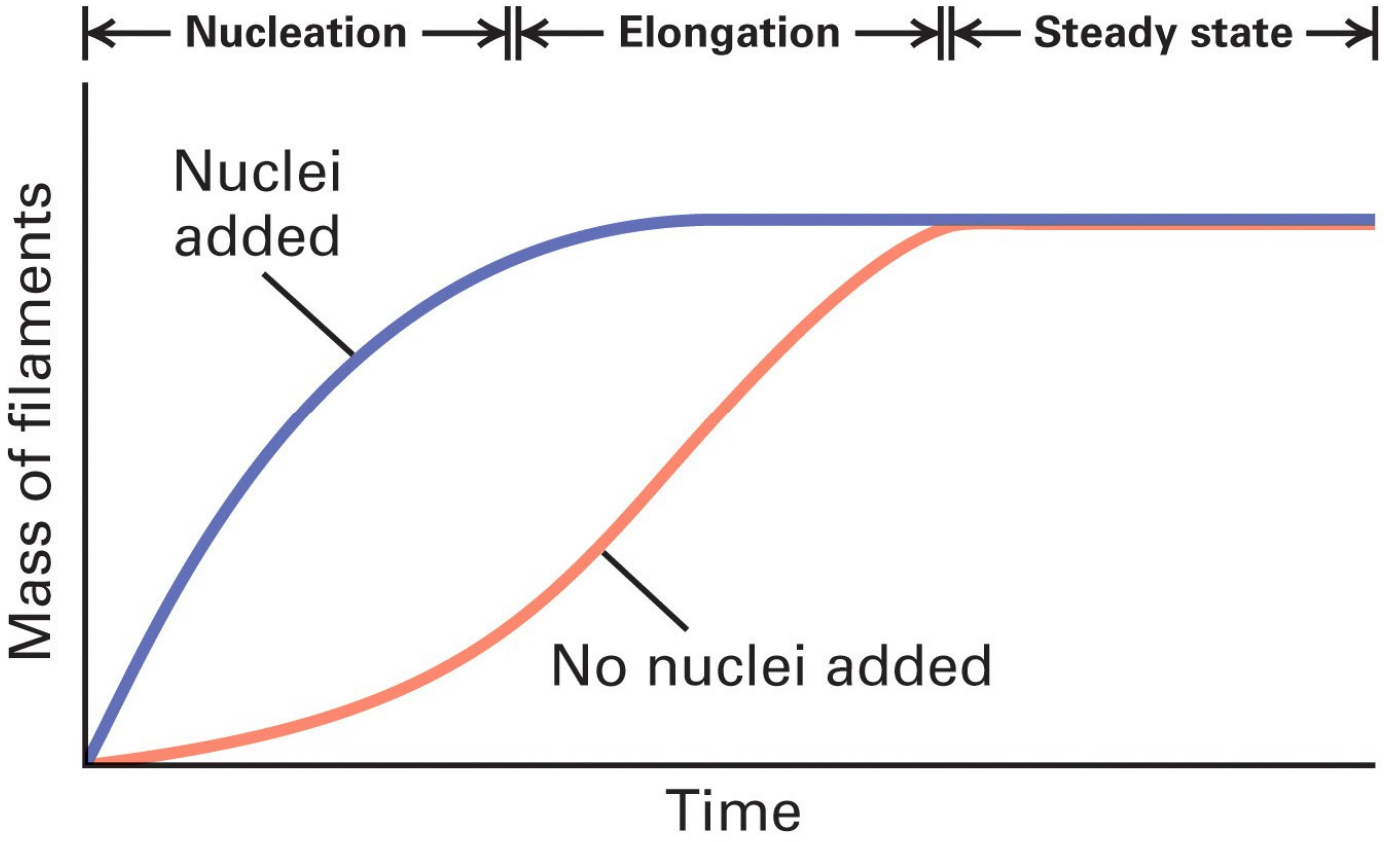
⇒ lowering the Cc **below** 0.1 µM F-actin would **depolymerize**

- At the critical concentration the "on rate" of G-actin equals the "off rate"

Actin polymerization

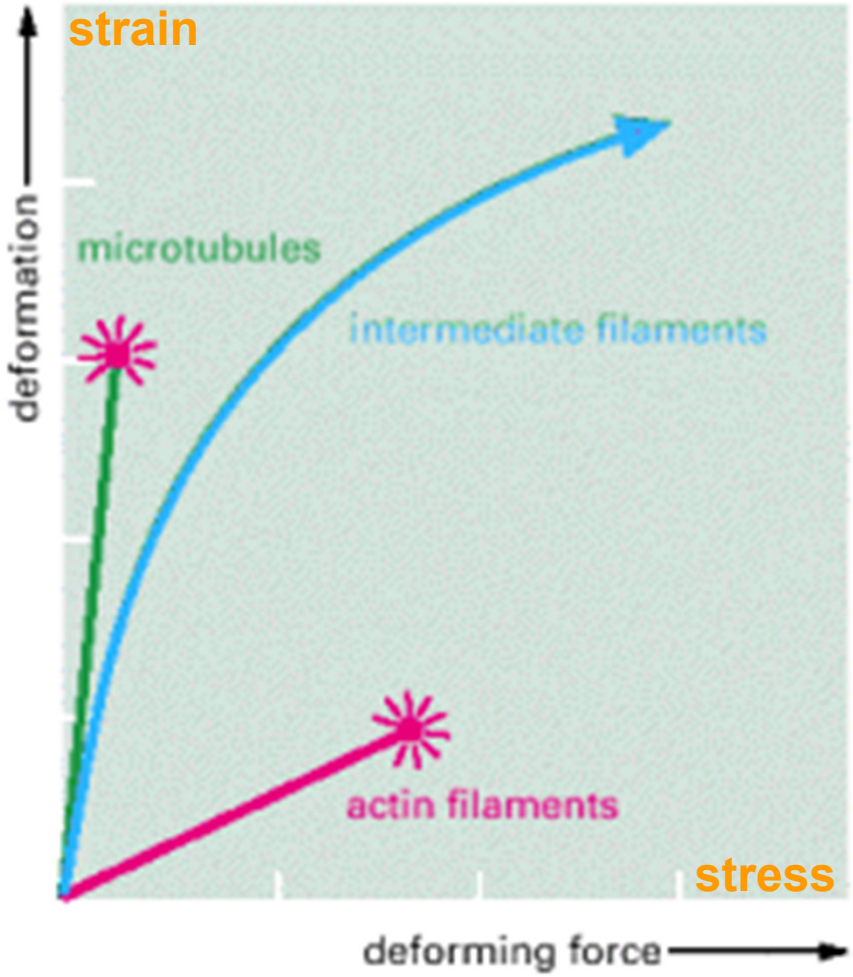
Measuring the *in vitro* polymerization of actin

1. Viscosimetry: during polymerization of actin the filaments become more entangled which *decreases* the **flow rate of the solution** (due to *increased* viscosity)
2. Sedimentation assay: **F-actin** is **pelleted** upon high centrifugation forces while **G-actin** remains in the **supernatant**
3. Fluorescence spectroscopy: the **fluorescence spectrum** of G-actin **changes during polymerization** into F-actin
4. Fluorescence microscopy: **direct observation** of the growth of labeled actin filaments



Cytoskeletal fibers also differ in their mechanical properties

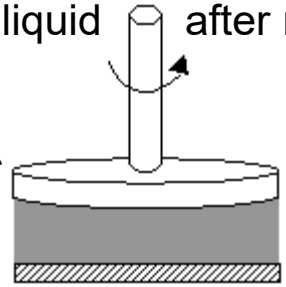
Based on their specific structures, the 3 types of cytoskeletal polymers exhibit also different elastic properties



P. Janmey, *JCB*, 1991

- **Microtubules**, **actin** and **intermediate filaments** (all the same concentrations) were exposed to *shear force* and the resulting degree of *stretchiness* was measured
- With increasing deforming force, microtubules are the first which cannot resist the stress and start to break following actin
- IFs are the most flexible filaments resisting large deforming forces

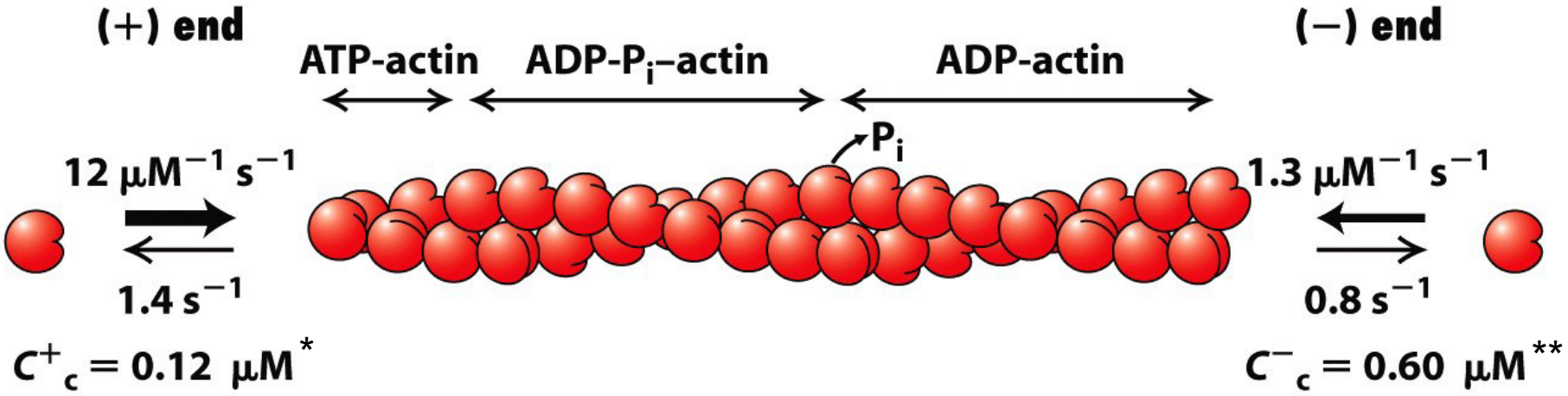
Rheometer: liquids are placed between two plates: one is fixed an the other is rotating. The rotating plate applies shear force (**stress**) to the liquid and measures the resulting deformation (**strain**) of the liquid after release of stress



Viscous liquid deforms under shear stress

ATP hydrolyzation is a slow process and an ATP cap forms

- ATP-G-actin adds faster to the plus end than ATP hydrolyzes, as a result an “ATP-cap” forms
- Rate of G-actin **addition** is about 10x <u>faster</u> at the (+) end compared to the (-) end
- Rate of G-actin **dissociation** is <u>about the same</u> at the (+) and (-) end
- <u>Result</u>: Cc(+) is lower than Cc(-)

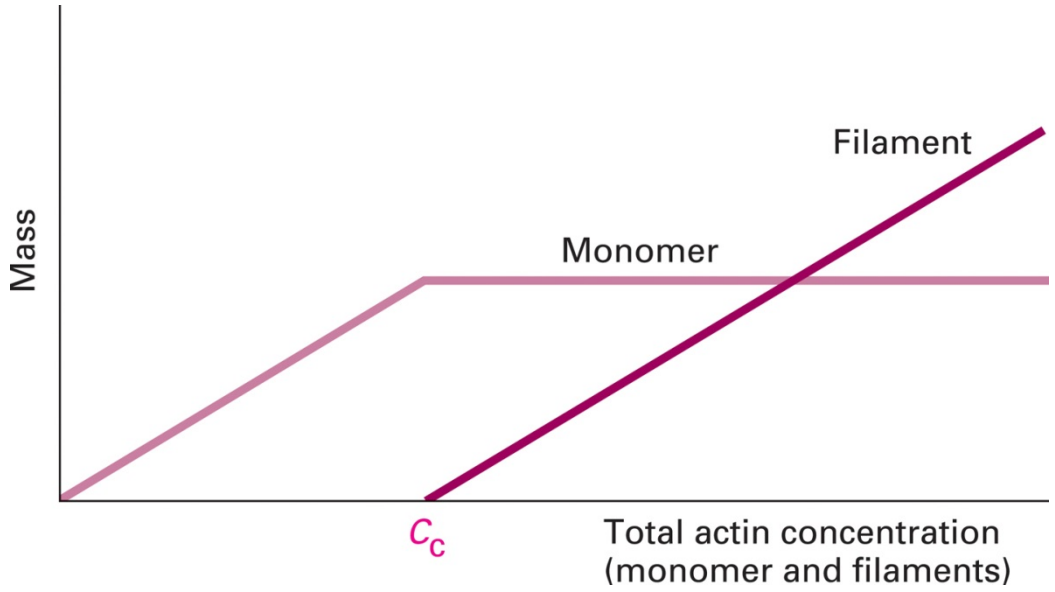


* 1.4 : 12 = 0.12

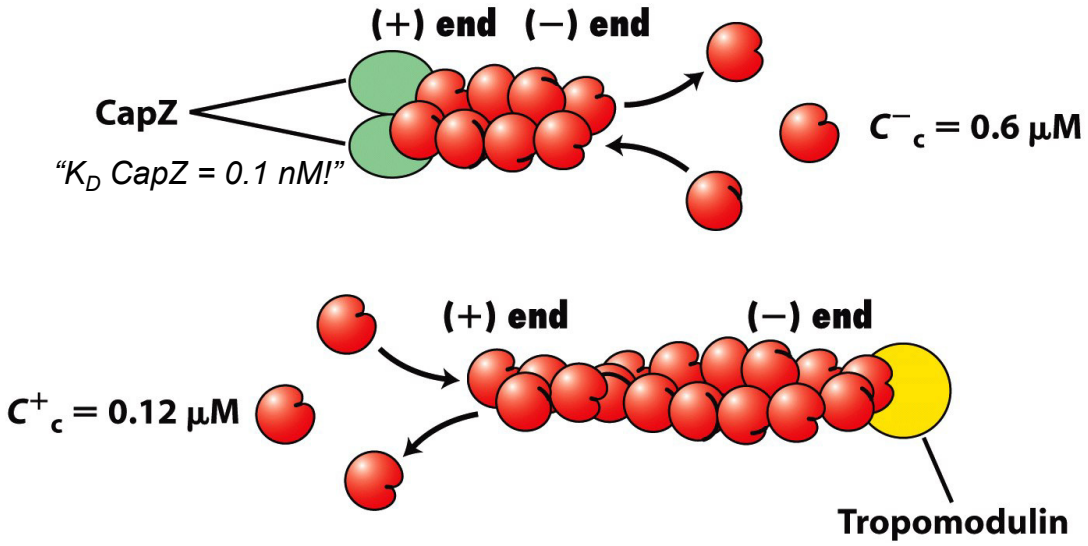
** 0.8 : 1.3 = 0.6

“A conformational change in actin during ATP hydrolysis is responsible for the different association and dissociation rates”

Manipulating the Cc is crucial for the cell to control polymerization



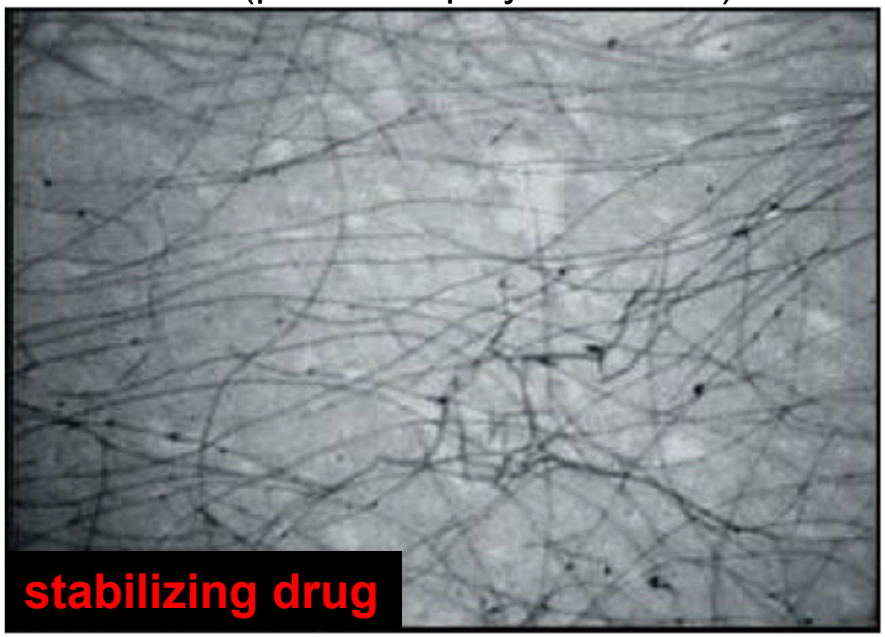
- Filament mass only effectively increases above the Cc
- Below Cc filaments depolymerize
- **Adding more monomers above Cc** won't increased the total monomer mass because they will be **immediately incorporated** into existing filaments (which are then growing and thus their mass increases)



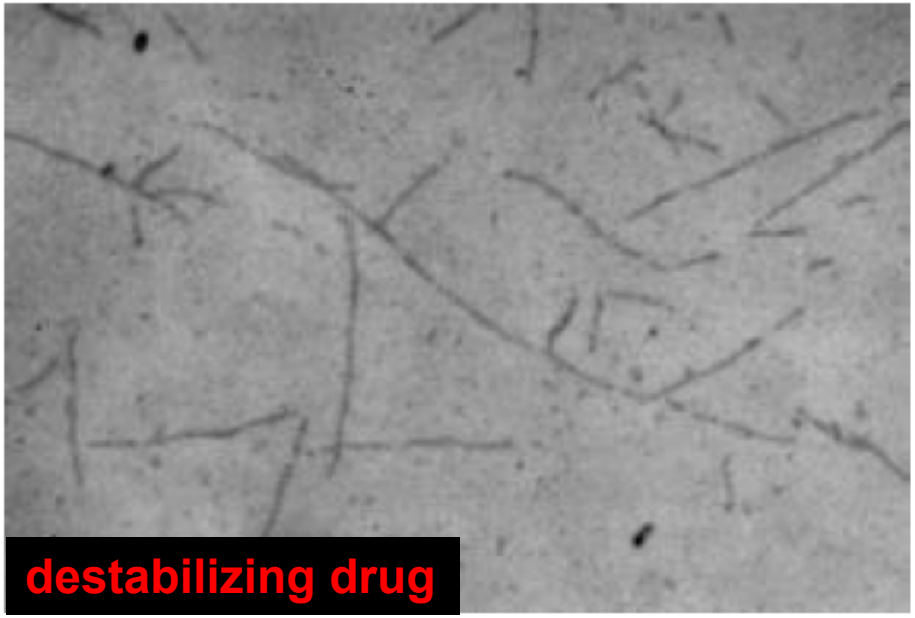
- In the cell, plus- and minus-end **capping proteins** control growth rate of filaments
- Using capping proteins, the different Cc at both ends can be determined

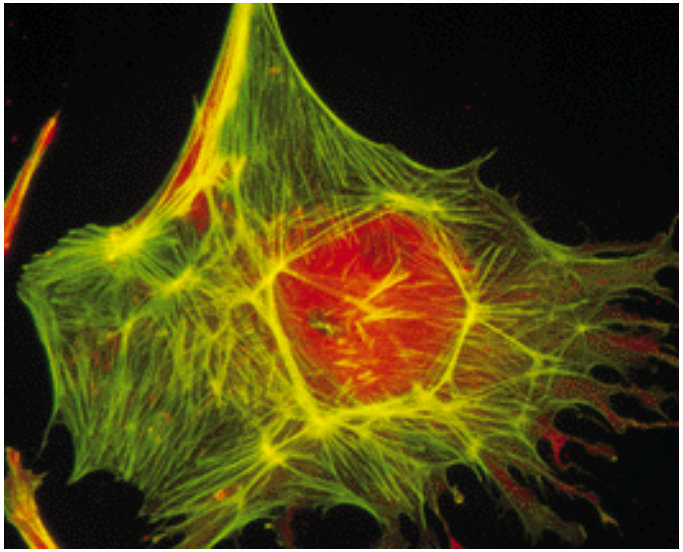
Toxins are important for basic research on the cytoskeleton

- **Phalloidin** (from fungus) stabilizes F-actin
- **Jasplakinolide** (from sponge) stabilizes actin nuclei (promotes polymerization)

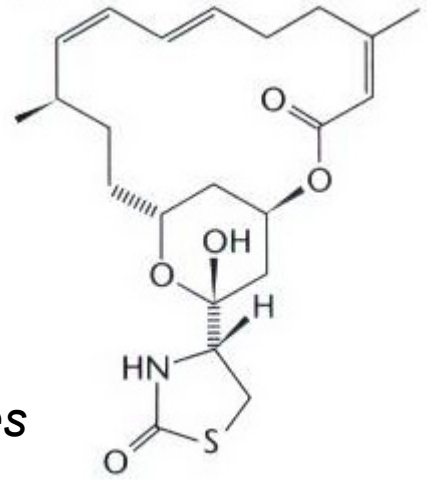


- **Cytochalasin D** (from fungus) makes a (+)-cap
- **Latrunculin** (from sponge) sequesters G-actin





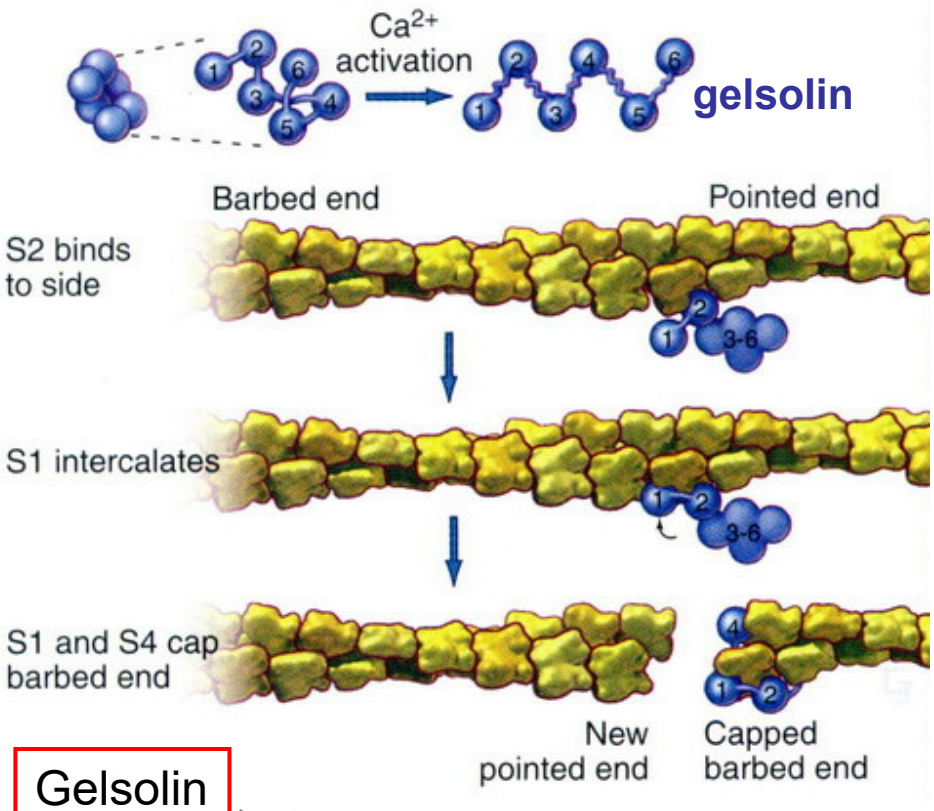
Phalloidin labeled with a green fluorophore to make the actin cytoskeleton visible
(phallodin isolated from the highly poison mushroom *Amanita phalloides* or also named “death cup”)

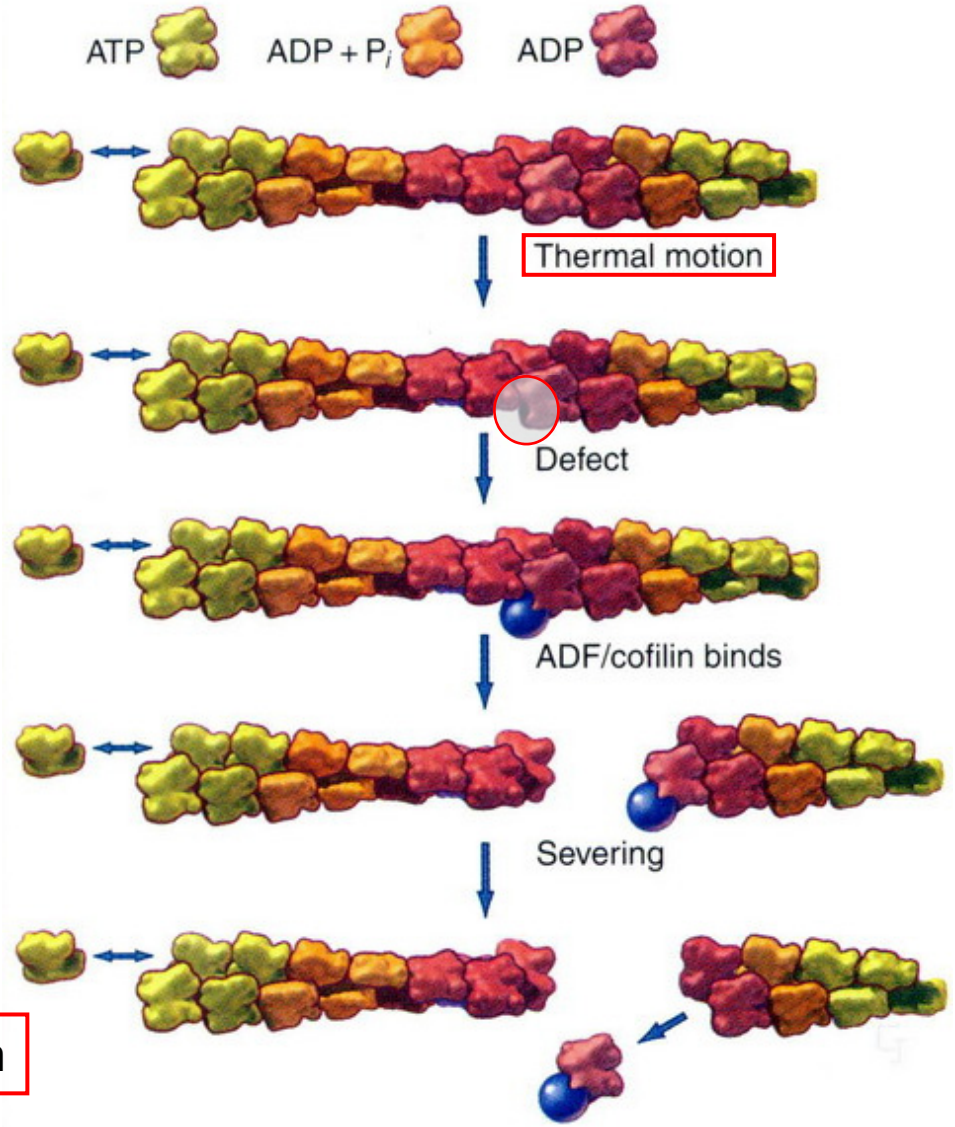


latrunculin A

Viscosity of cytoplasm is controlled by F-actin severing proteins

Severing ("cutting") proteins such as **gelsolin** and **cofilin** bind sidewise to actin filaments: induce conformational change in G-actin **=>** **strain** on the filament increases **=>** filament **breaks** **=>** a (+) **cap** remains **=>** (-) end rapidly **depolymerizes**

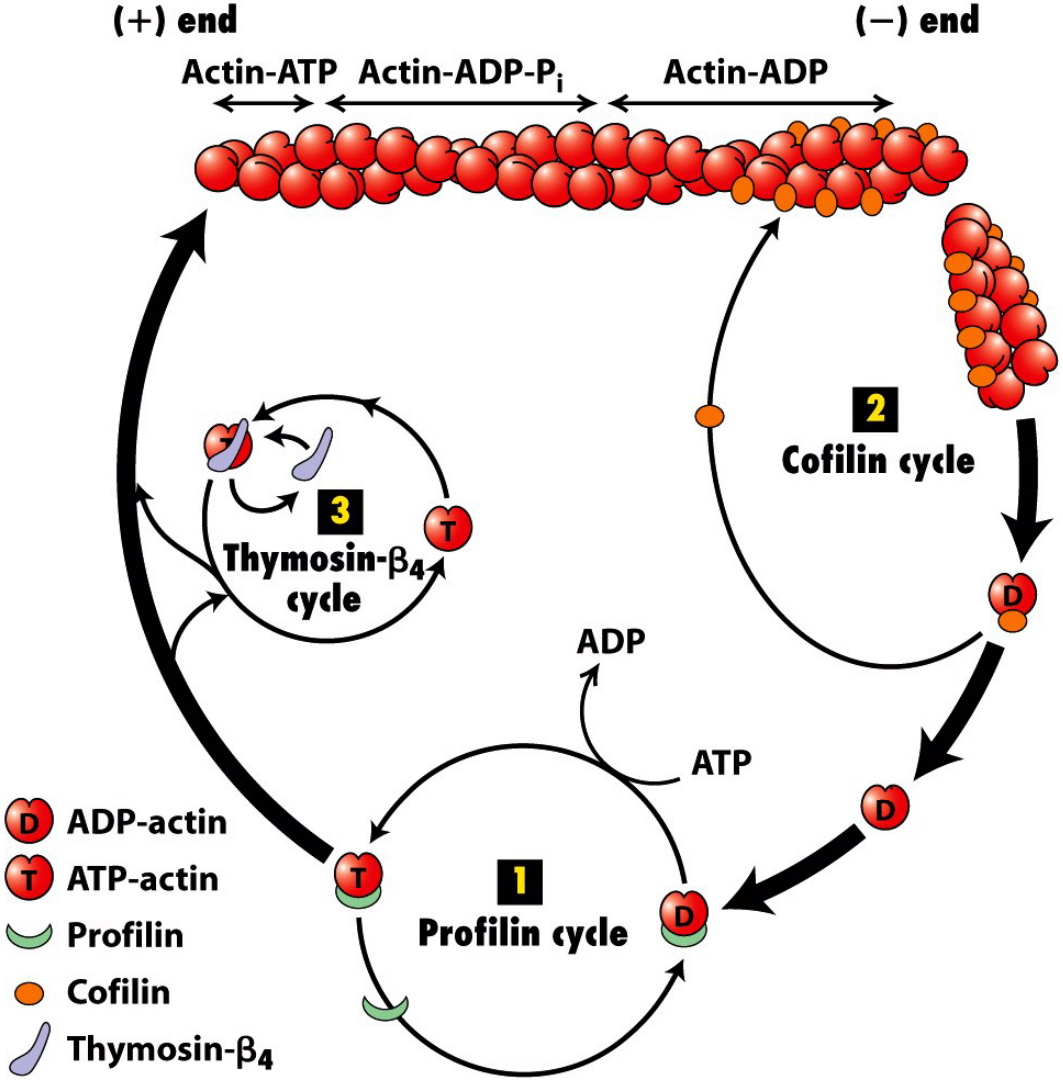




Pollard 3rd ed

In the cell, several G-actin binding proteins control actin polymerization

- Due to high salt and high actin concentration (0.5 mM) in the cell, theoretically all G-actin must be fully polymerized => still 40% of all actin is G-actin!
- G-actin pool is maintained by **G-actin sequestering proteins** such as **thymosin β4**
- **Profilin** acts as both, a G-actin buffer and promotes actin polymerization



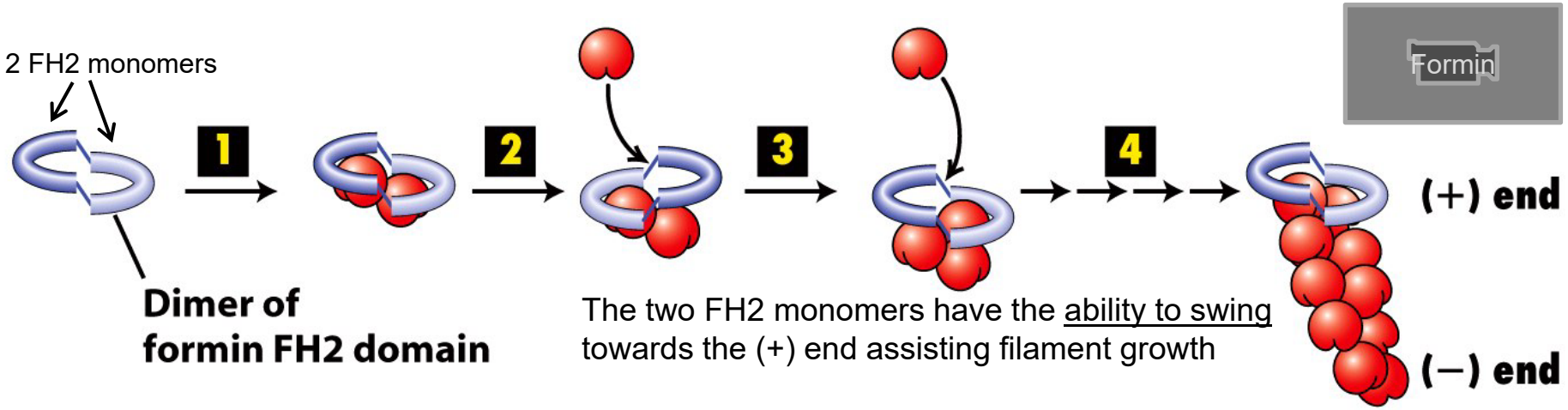
- **Cofilin** binds to ADP-F-actin and shortens filaments at the (-) end
- **Profilin** buffers dissociated ADP-G-actin and exchanges ADP to ATP
- **Profilin** then either delivers ATP-G-actin directly to the plus end or **thymosin β4** buffers ATP-G-actin

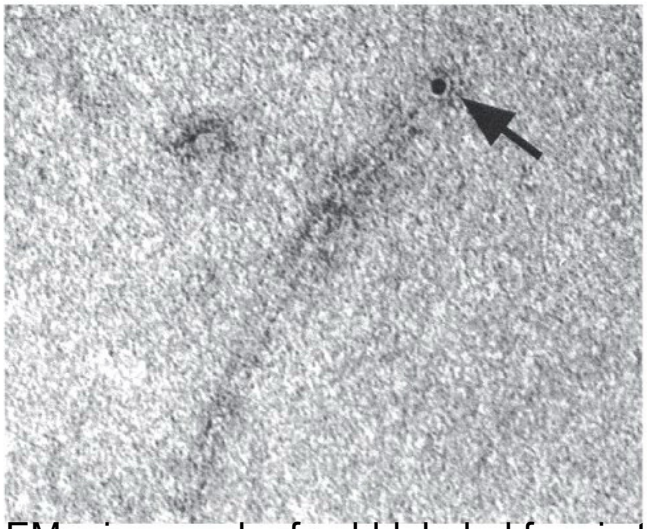
ABPs are regulated in the cell:

- **Profilin** binding to G-actin can be **inhibited by** an interaction with the phospholipid **PIP_2**
- Profilin is targeted to membranes via binding to **WASp**
- **Thymosin-β4** binds in a way to G-actin so that it cannot polymerize

ABPs that promote actin filament assembly

- Two classes of actin nucleating proteins:
  - **Formins** lead to assembling of **long actin filaments** (filopodia, stress fibers)
  - **Arp2/3** complex leads to **branched actin networks** (leading edge, cell cortex)

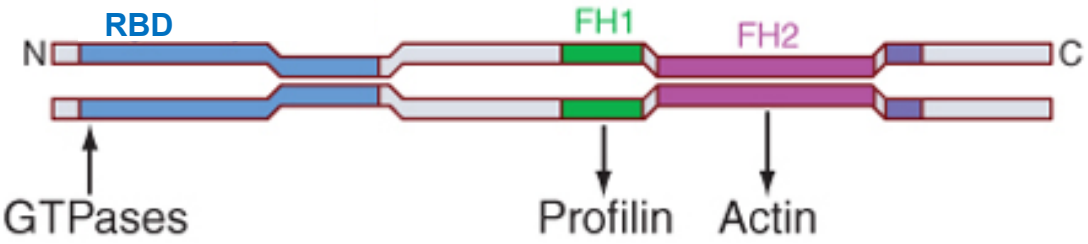




EM micrograph of gold-labeled formin that promoted growth of a long actin filament

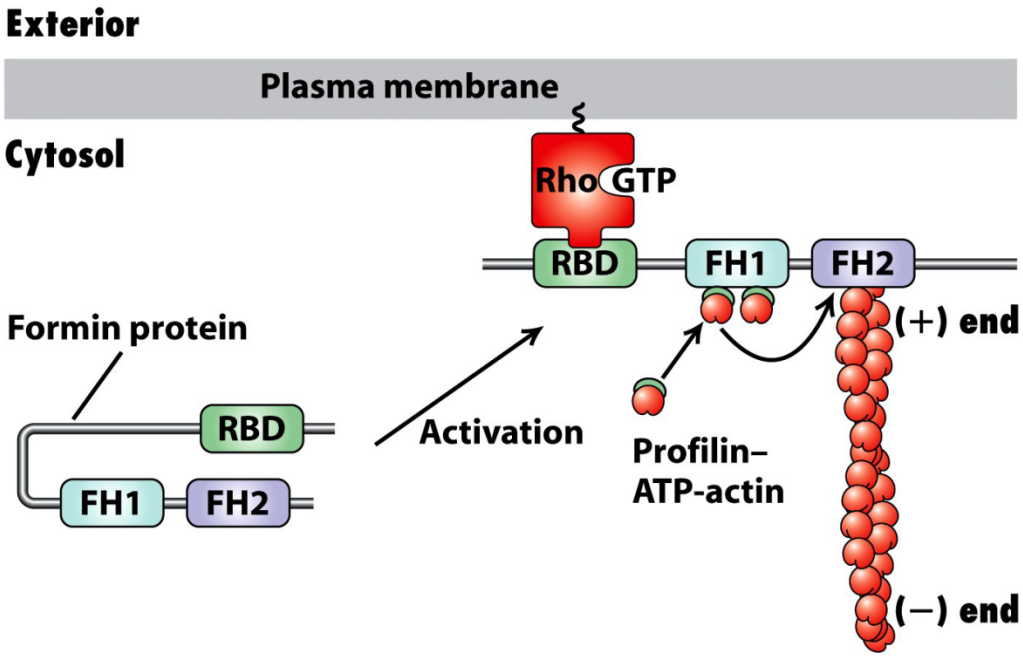
Formin is a dimer with several regulatory domains:

- **RBD** (**r**ho-**b**inding **d**omain) binds **G proteins**
- **FH1** (FH = formin homology domain) has prolin-rich domains that can be recognized by **profilin**
- **FH2** nucleates and binds **actin**

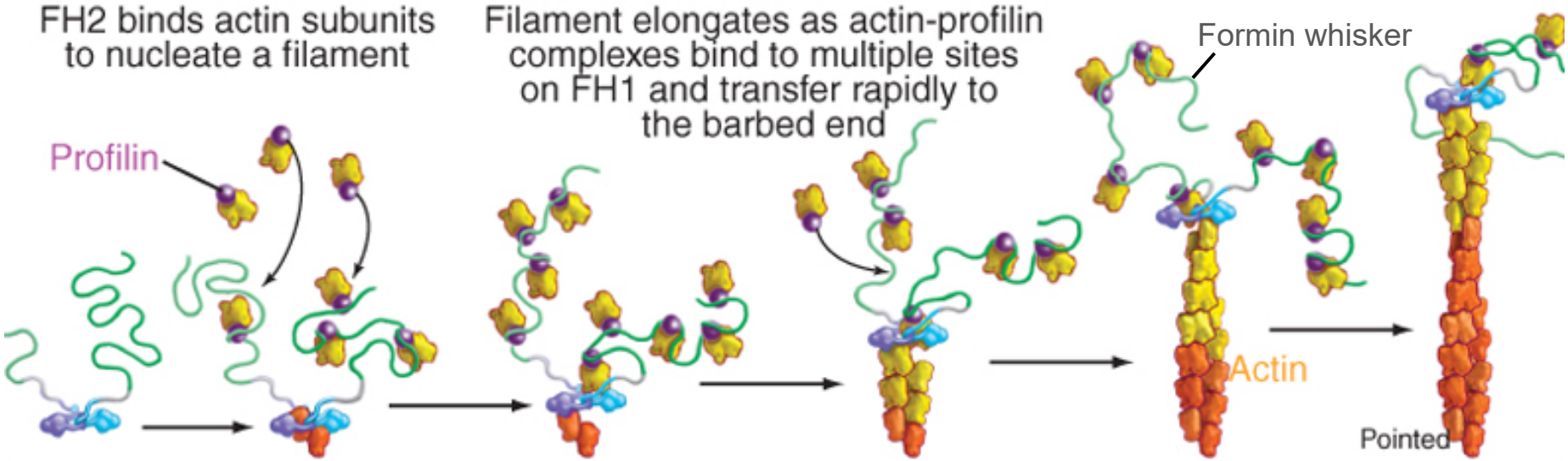


Pollard 3rd ed

Formins are controlled by a Rho-pathway



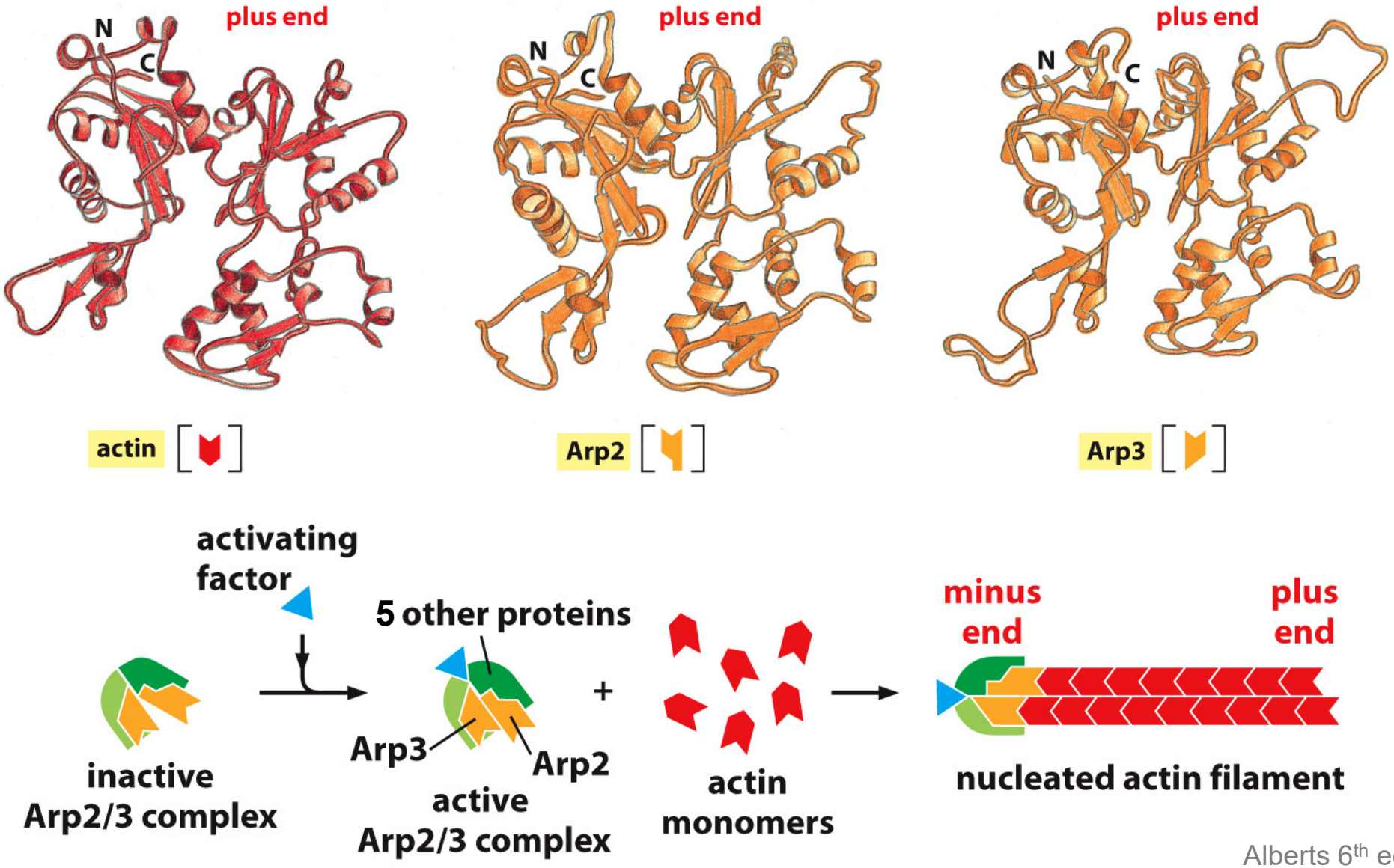
- Formins are <u>inactivated by</u> an <u>intramolecular interaction</u> between the **FH2** and **RBD** domain
- <u>RBD binds to activated **Rho**</u> and formin unfolds (now activated)
- Formins are found in **stress-fibers** and the **contractile ring** (mitosis)
- Formins <u>inhibit binding of CapZ</u> to F-actin
- Formin exhibits **whiskers** that can bind profilin-ATP-G-actin



Pollard 3rd ed

Arp2/3 binds to the minus ends of actin filaments

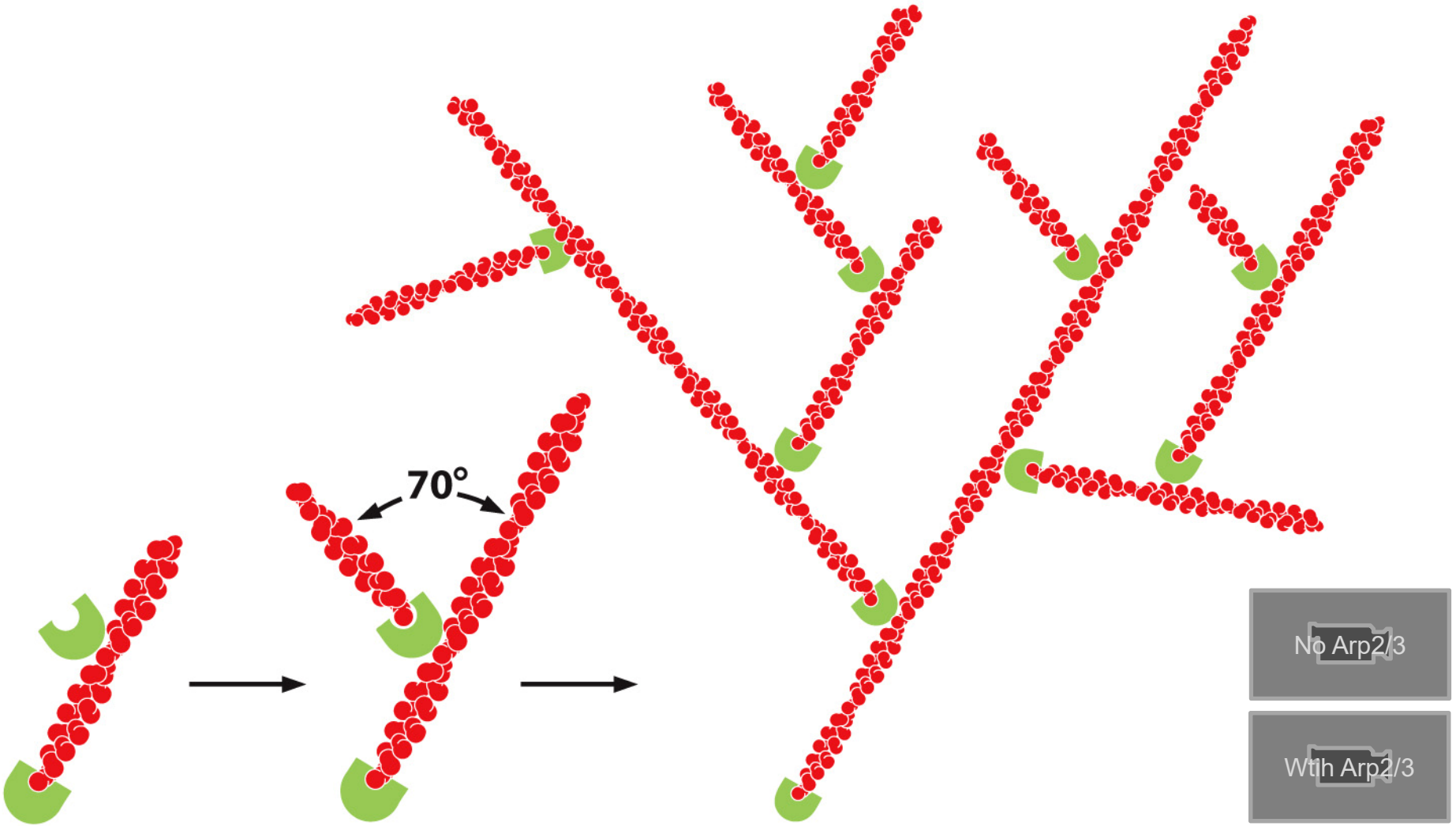
- Arp = actin-related protein: has 50% similarity to actin
- Arp 2/3 is a **minus-end capping** protein and **nucleates polymerization**



Alberts 6th ed

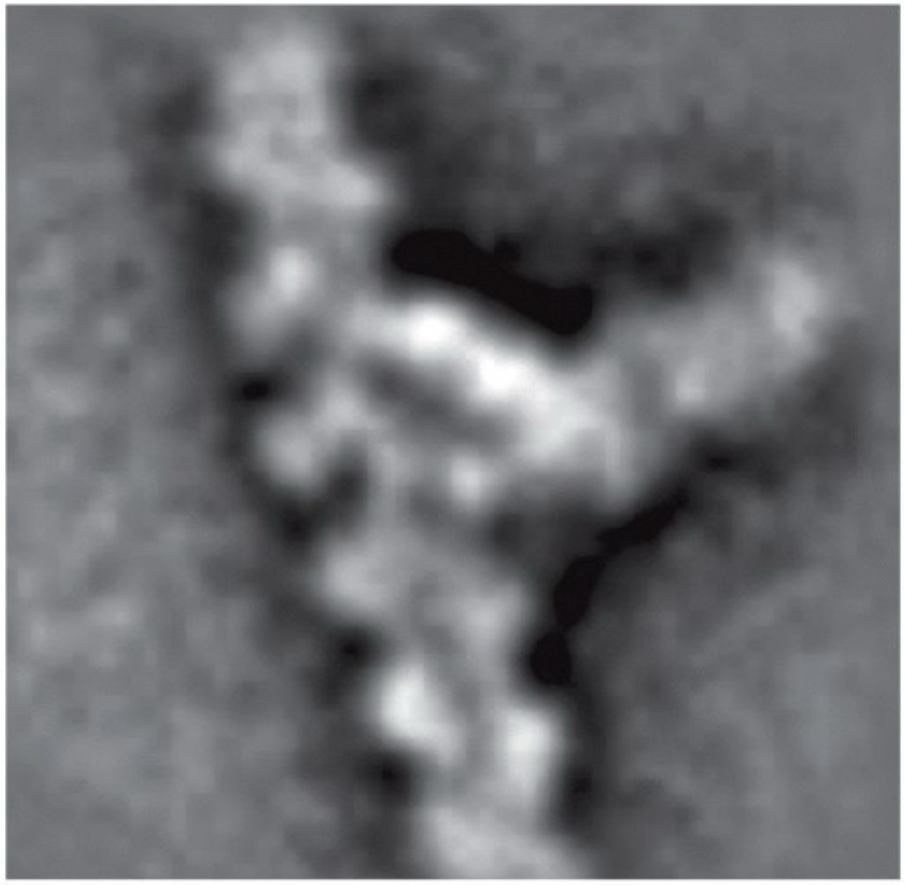
Arp2/3 branches actin filaments at a defined angle

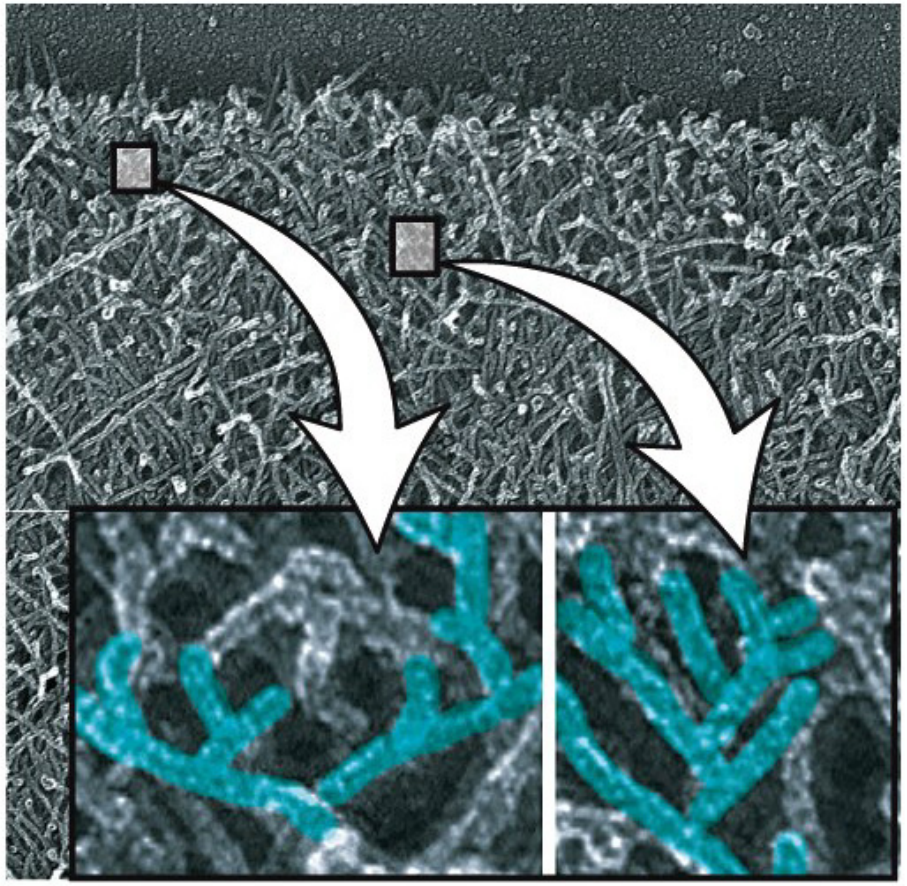
Arp 2/3 **binds to the sides** an actin filament and **branches** them at an angle of 70°



Alberts 6th ed

Arp2/3 branches actin filaments at cell cortex and lamellipodia

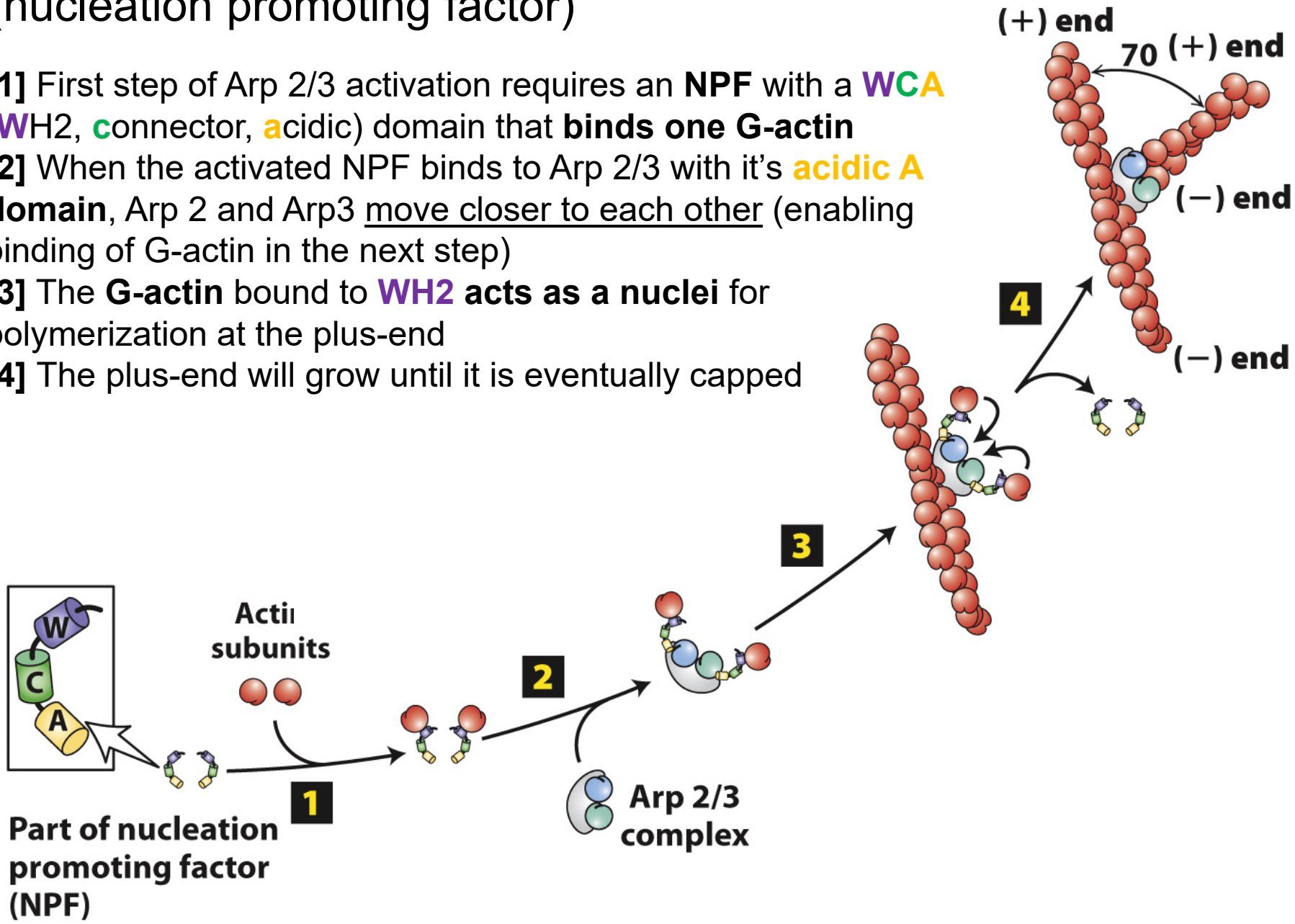




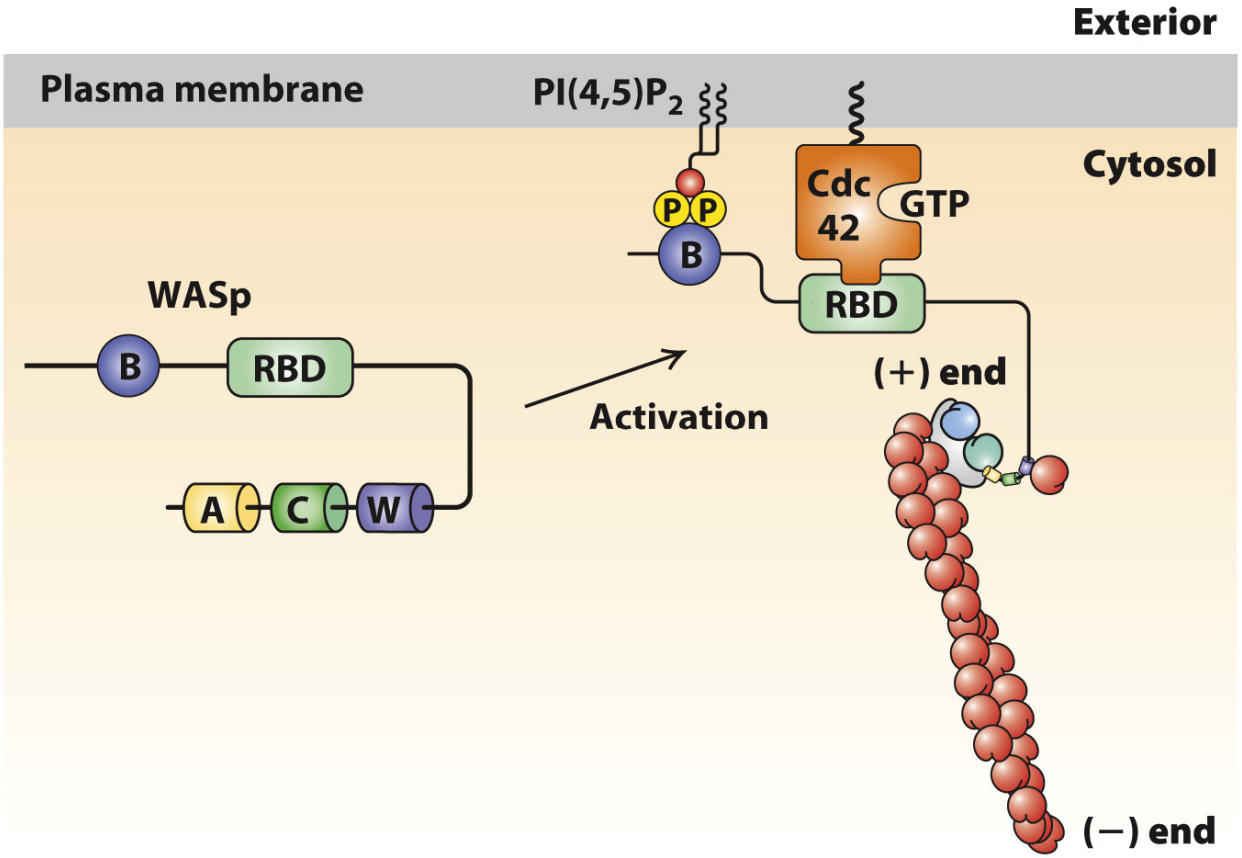
• Arp2/3 promotes fast growing of (+) ends pushing the membrane forward at the leading edge
• S1 decoration: filaments face with **(+) end towards the leading edge**
• Filament density, branching and cross-linking (via filamin) become is reduced towards the cell body

Arp 2/3 action requires WCA domains that are part of NPF (nucleation promoting factor)

[1] First step of Arp 2/3 activation requires an **NPF** with a **WCA** (**W**H2, **c**onnector, **a**cidic) domain that **binds one G-actin**
[2] When the activated NPF binds to Arp 2/3 with it's **acidic A domain**, Arp 2 and Arp3 move closer to each other (enabling binding of G-actin in the next step)
[3] The **G-actin** bound to **WH2 acts as a nuclei** for polymerization at the plus-end
[4] The plus-end will grow until it is eventually capped



WASp is an NPF that is regulated by the small G protein Cdc42

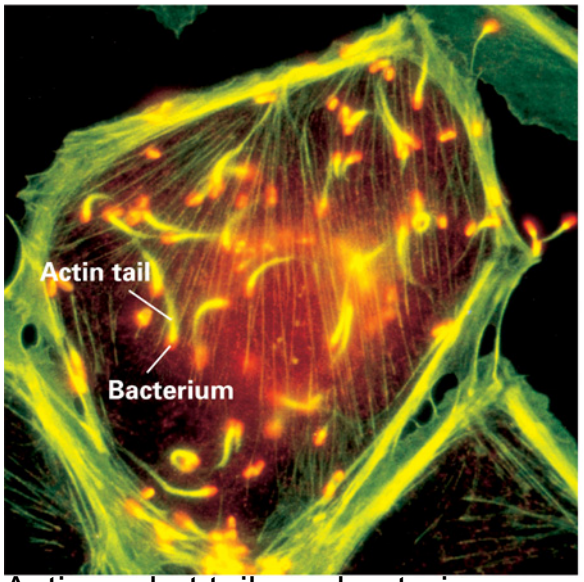


- **WASp** is <u>inactivated</u> by an <u>intramolecular folding</u> (**WH2** domain not accessible for G-actin)
- To become activated (and unfolded) WASp's **RBD** domain needs to bind to **activated Cdc42** as well as (via its **basic domain**) to **phospholipids**
- WASp primarily regulates **filopodia** formation

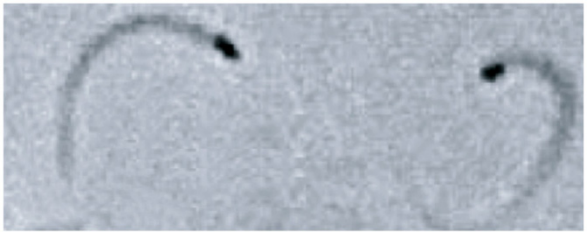
WASp = *Wiskott-Aldrich syndrome* protein (X-linked disease characterized by eczema, low platelet count and immune deficiency)

Arp2/3 is needed for *Listeria* movement in infected cells

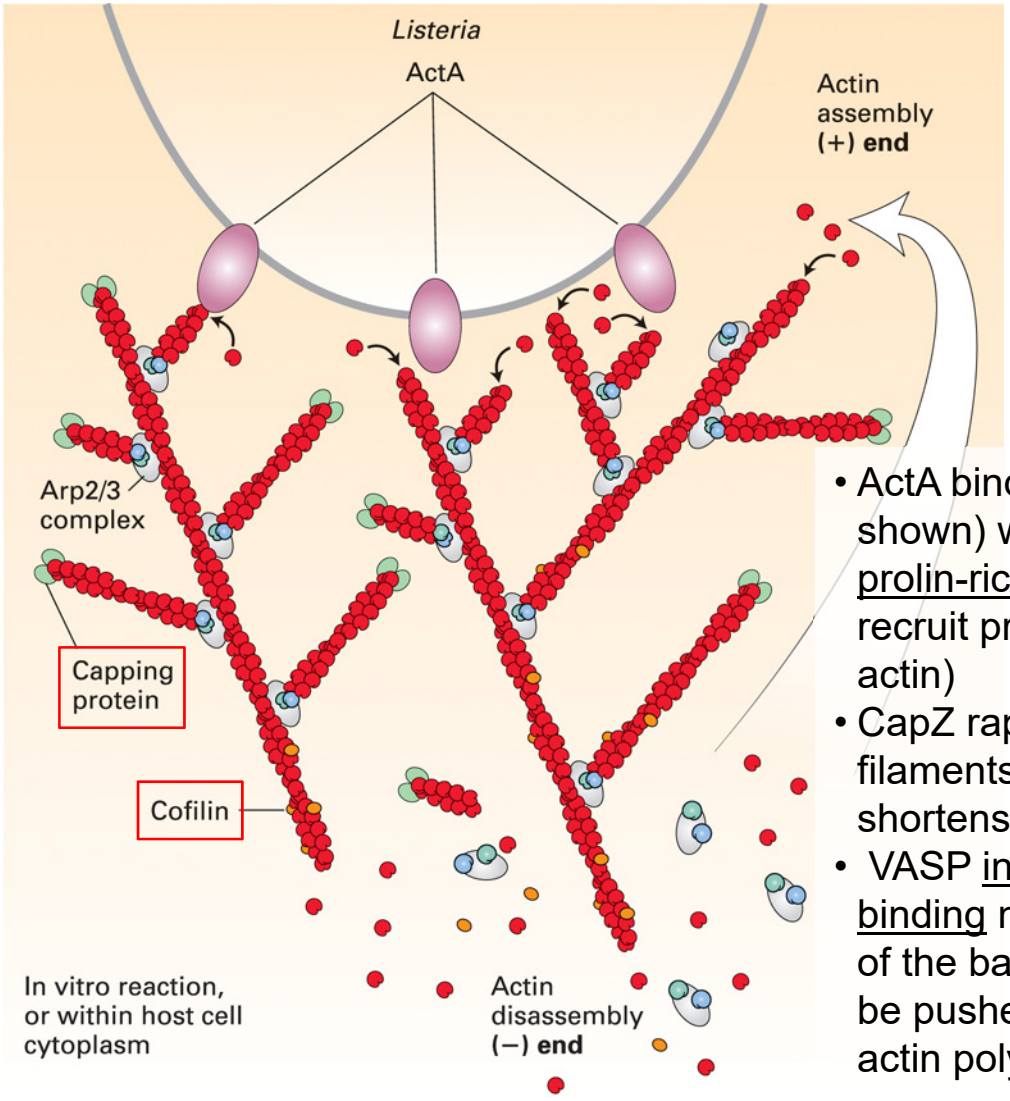
- *Listeria monocytogenes* (found in rotten food) is a bacterium which propels thru the cytoplasm using the **power of actin polymerization** stimulated by Arp2/3
- Actin polymerizes into filaments at the base of the bacterium pushing it forward
- Similar to an NPF, **ActA** has an **actin binding site** and an **acidic region** to activate Arp2/3



Actin rocket tails on bacterium

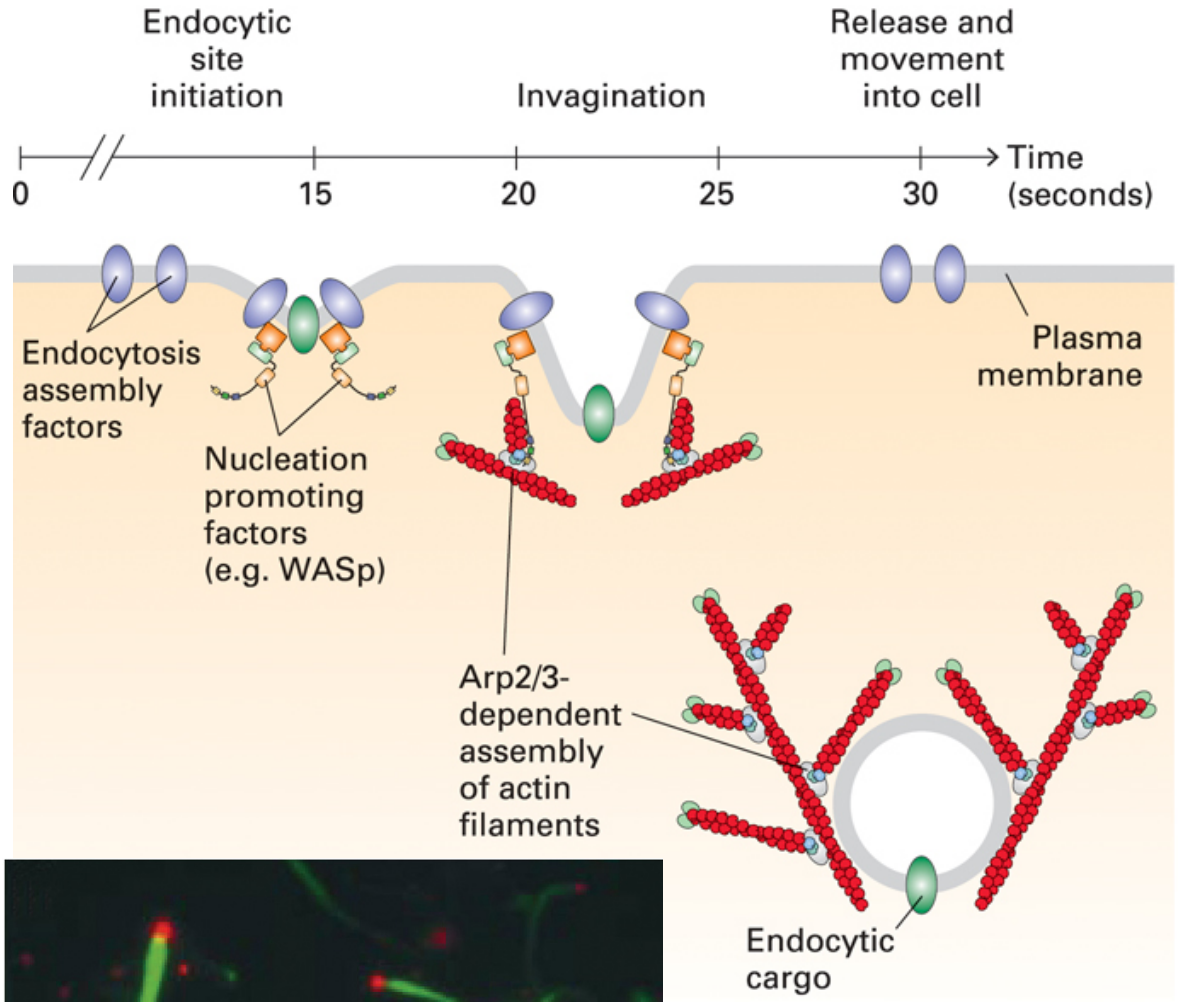

TEM of comet tails

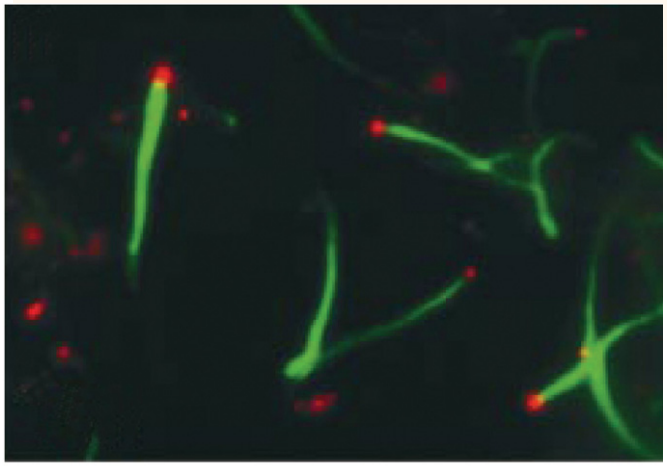
Lysteria infection



- ActA binds **VASP** (not shown) which has <u>prolin-rich regions</u> (to recruit profilin-ATP-actin)
- CapZ rapidly caps new filaments and cofilin shortens old filaments
- VASP <u>inhibits CapZ binding</u> near the base of the bacteria (so it can be pushed forward by actin polymerization)

Actin rocket tails also push endosomes forward

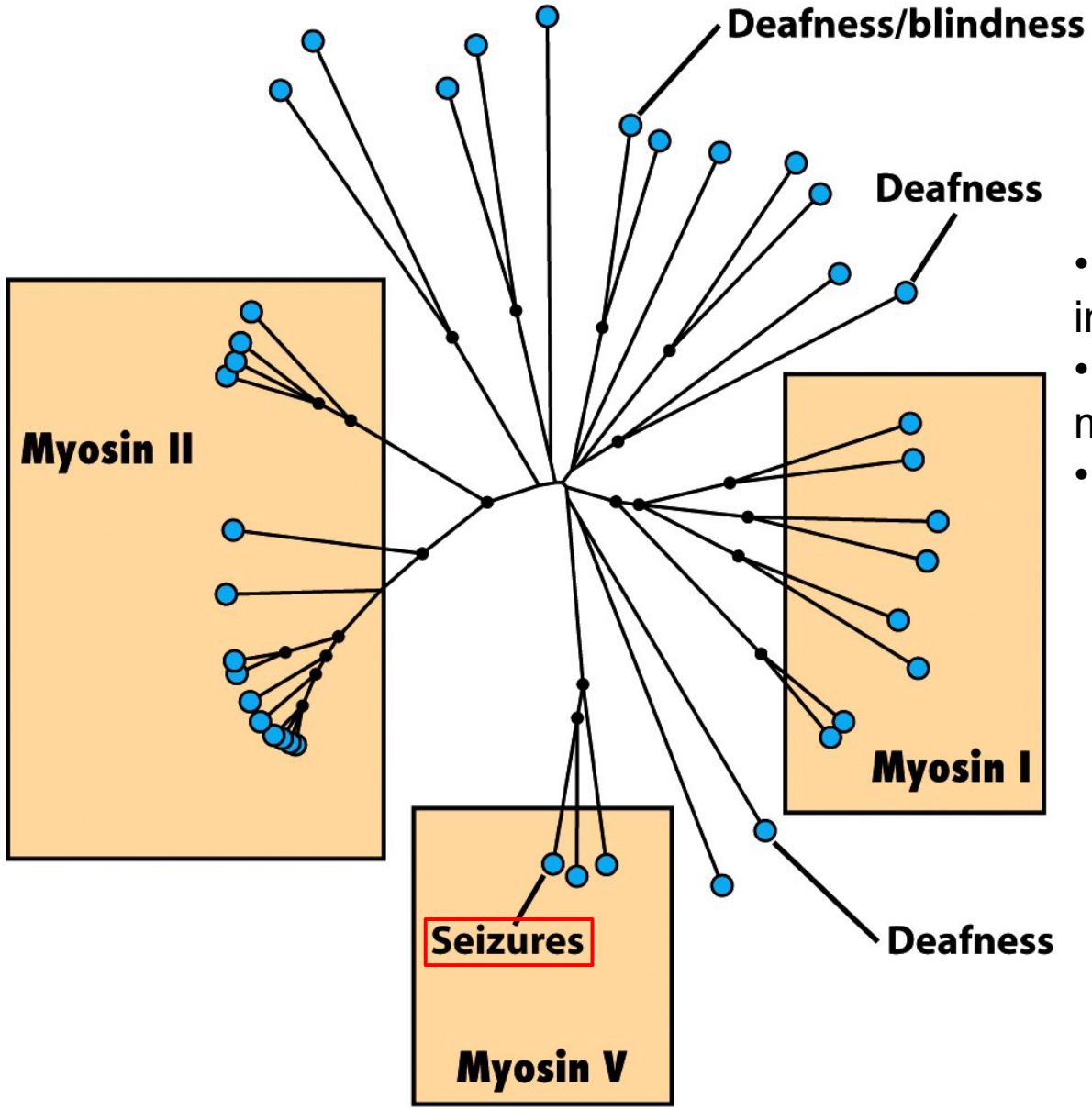




Visualization of **actin comet tails** (green) on endocytic **endosomes** (red)

- Clathrin-mediated endocytosis is a process that involves the uptake of molecules or liquid
- **Endocytosis assembly factors** recruit **WASp** initiating **Arp2/3** dependent actin assembly
- After invagination and the endosome has pinched off, a short **burst of branched-actin polymerization** drives the endosome forward

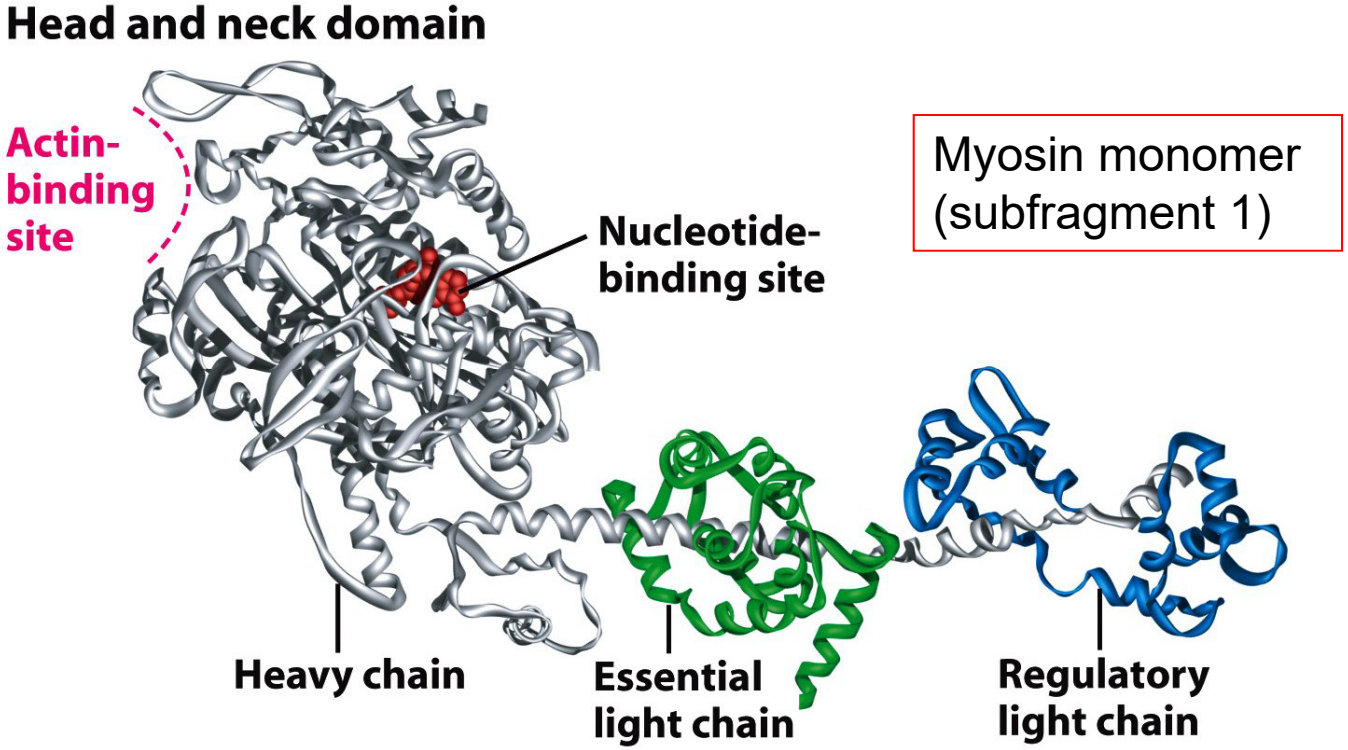
Myosins make up a large family of actin-based motor proteins



- **40 myosin genes** found in the human genome
- Loss of specific myosins may cause a particular disease
- 3 important classes:
  - Myosin I (**endocytosis**)
  - Myosin II (**muscle contract.**)
  - Myosin V (**cargo transport**)

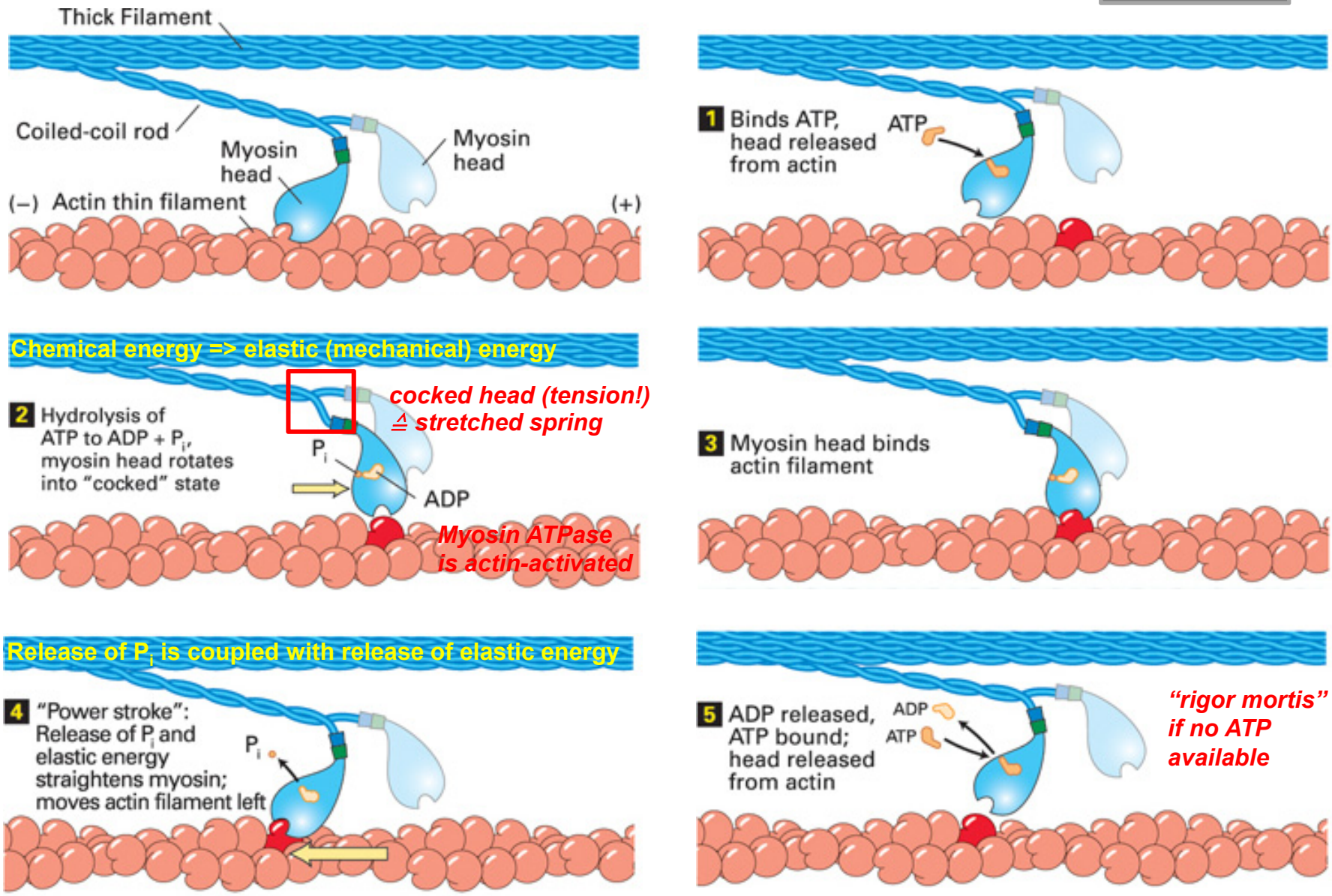
Basic structure of myosins

• All myosins consists of **several light** and one or two **heavy chains**
⇒ **light chains** have regulatory function (myosins are regulated by Ca^{2+})
⇒ **heavy chains** consist of a motor head (**actin-binding** and **ATP-binding**), a stiff neck and a tail domain (with "cargo-binding" function)
• Tail consists of **α-helical coiled coils** which **forms** (the rod-like) myosin **dimer**
• Tail differs and defines the function of myosin (e.g. some can bind to membranes)
• **Regulatory light chains** in myosin I and II are **calmodulin** with Ca^{2+}-binding sites
• Myosin II contains **essential** and **regulatory** light chains: **essential light chains** stiffens the neck so it can act as a **lever arm**



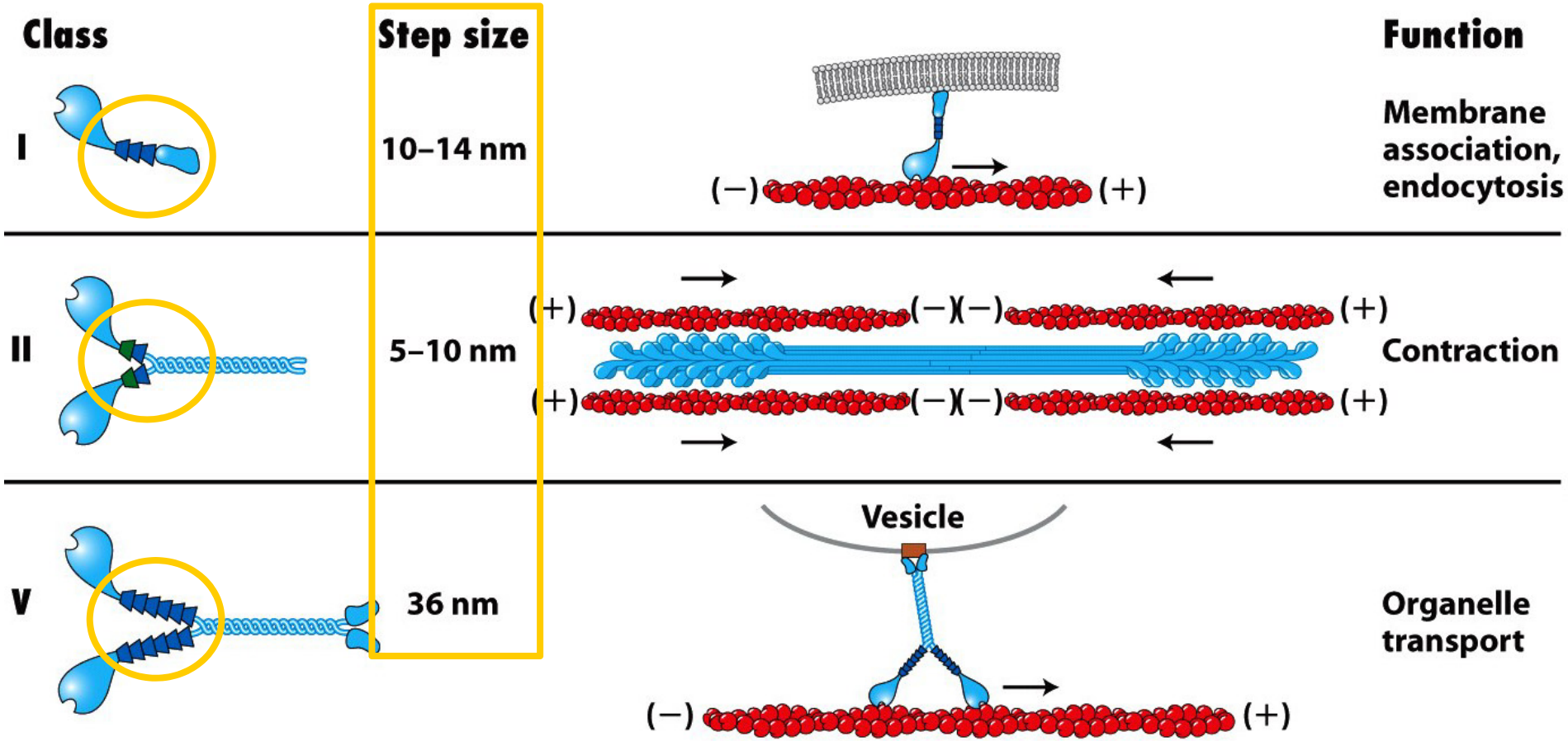
The myosin-actin cross-bridge cycle

Myosin-Actin interactions



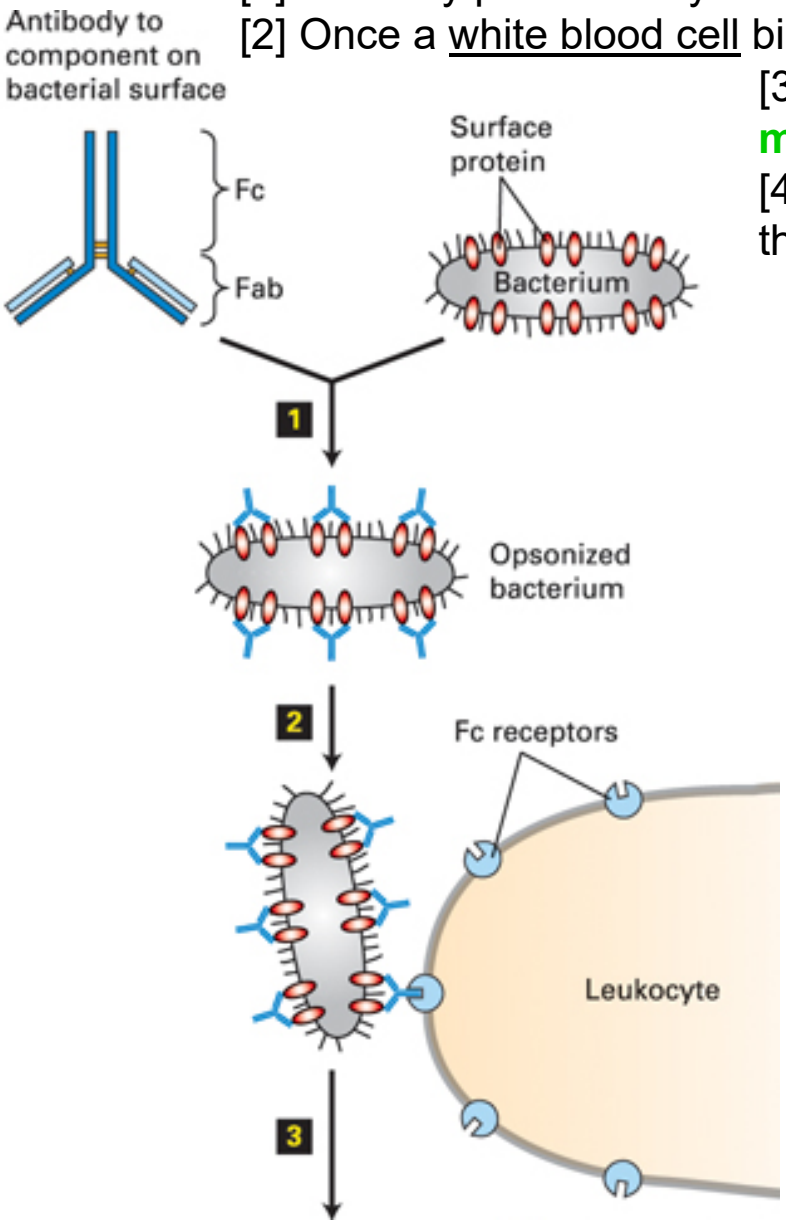
Cellular and intracellular movements depend on myosins

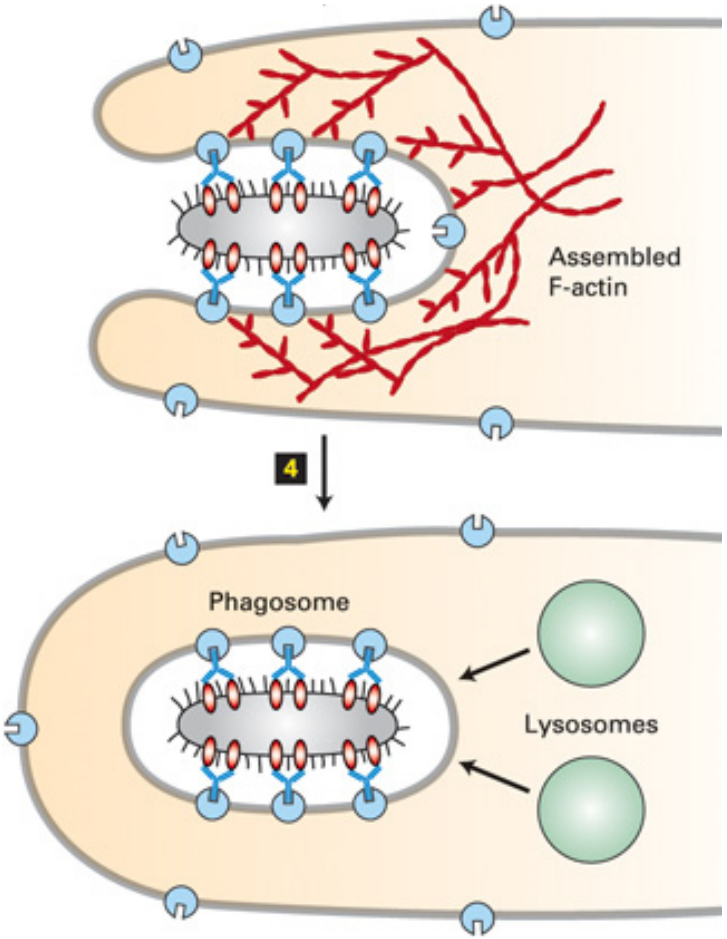
- **Endocytosis**: monomeric **myosin I** (**3 regulatory** light chains)
- **Muscle cells**: contraction based on filament-sliding between F-actin and **muscle myosin II** (**1 essential** and **1 regulatory** light chain)
- **Non-muscle cells**: **non-muscle myosin II** (same structure but differently regulated)
- **Vesicle transport**: major vesicle transporter **myosin V** (**6 regulatory** light chains)



Phagocytosis involves branched actin filaments and myosin

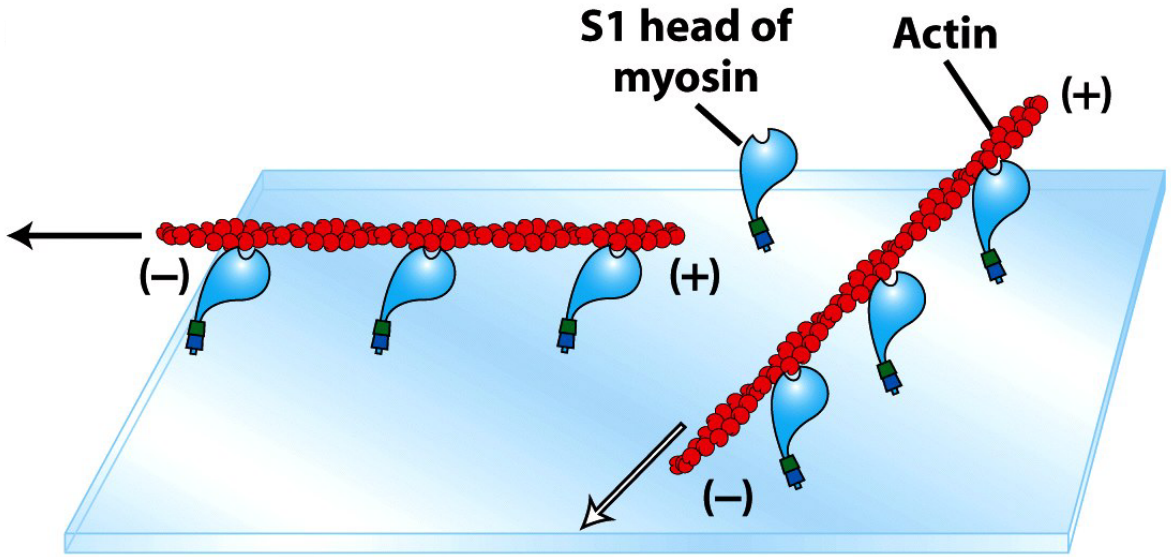
Phagocytosis is an endocytotic process for the **uptake of large particles** (> 0.8 µm)
[1] Antibody produced by the immune system targets bacterium (opsonization)
[2] Once a white blood cell binds AB with its Fc receptors **internalization starts**
[3] Assembled actin filaments together with **backward moving myosin VI** power the engulfment
[4] **Lysosomes** fuse with the phagosome and **digest** the **particle**



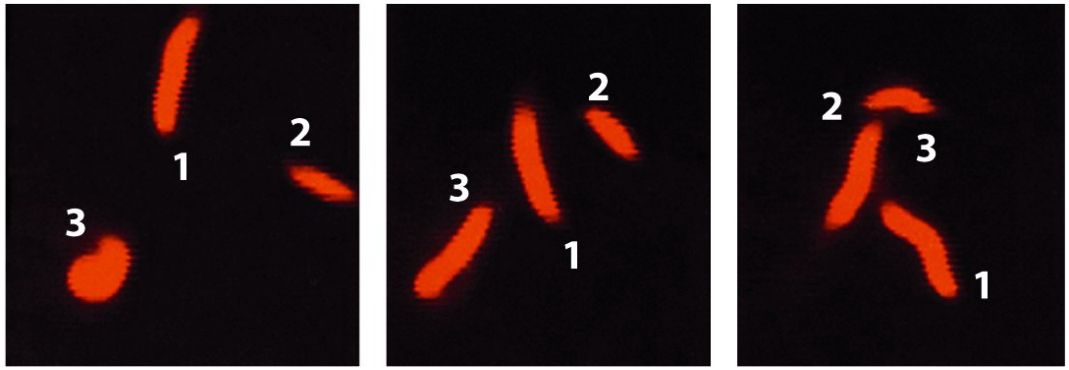


In vitro motility assay

• Movement of fluorescently-labeled **actin filaments** on immobilized **myosin heads** on a cover glass is observed using a fluorescence microscope (TIRF type)
• Addition of ATP triggers the **movement of actin filaments** along the fixed myosins
• Upon ATP-binding myosin heads dissociate from the filament while the **head tilts towards the (+) end** (no power-stroke yet)
• The **upcoming power-stroke** pushes the filament **with the (-) end in the lead**



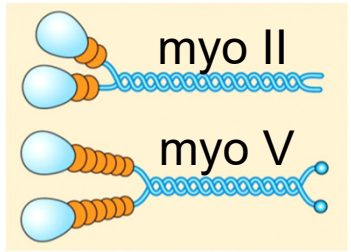
Myosin heads spent only 10% of their time attached to actin: **short duty cycle**

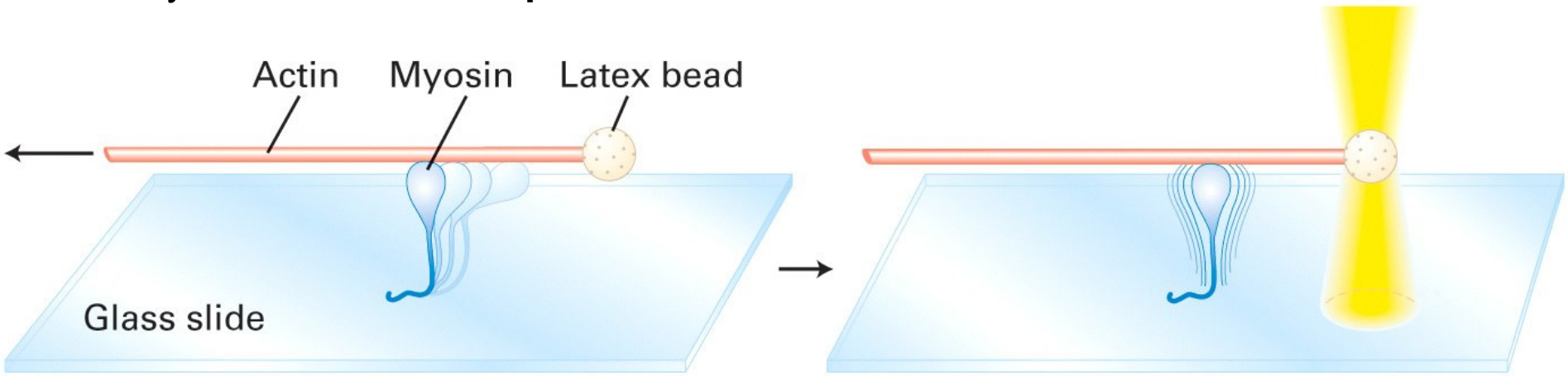


Rhodamine-phalloidin labeled actin filaments. One frame each 30s.

Measuring the force generated by single myosin heads using the optical trap

- With a **highly focused** (divergent) **infrared laser beam** we can induce so called differential forces (light pressure) able to trap small particles
- This **optical tweezer** can, e.g., immobilize a bead attached to an **actin filament**
- Upon the myosin's **power stroke** the actin filament is hold in position
- The force generated by the myosin head is determined by measuring the bead displacement => for myosin II about **3-5 pN** (piconewton)
- **Step-size**, **generated force** and **velocity** depends on the length of the **lever arm**:
  - myosin II = **8 nm step size**
  - myosin V = **36 nm step size**





The Nobel Prize in Physics 2018

Ill. Niklas Elmehed. © Nobel Media

Arthur Ashkin

Prize share: 1/2

Ill. Niklas Elmehed. © Nobel Media

Gérard Mourou

Prize share: 1/4

Ill. Niklas Elmehed. © Nobel Media

Donna Strickland

Prize share: 1/4

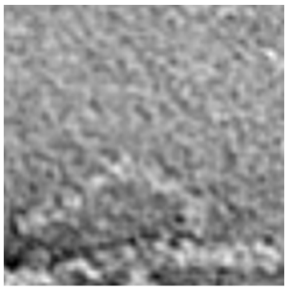
The Nobel Prize in Physics 2018 was awarded "for groundbreaking inventions in the field of laser physics" with one half to **Arthur Ashkin "for the optical tweezers and their application to biological systems"**, the other half jointly to Gérard Mourou and Donna Strickland "for their method of generating high-intensity, ultra-short optical pulses" ."

Myosin V stepping

TIRFM (total internal reflection microscopy) revealed the stepping mechanisms of myosin V: each head spent >50% of their time on actin => **long duty cycles**

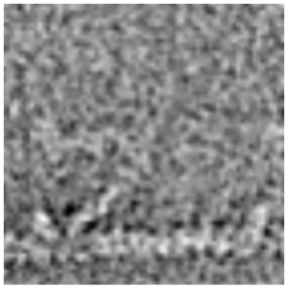
Myosin V **head and neck** on F-actin (visualized by EM)

(animation)



Pre-power stroke

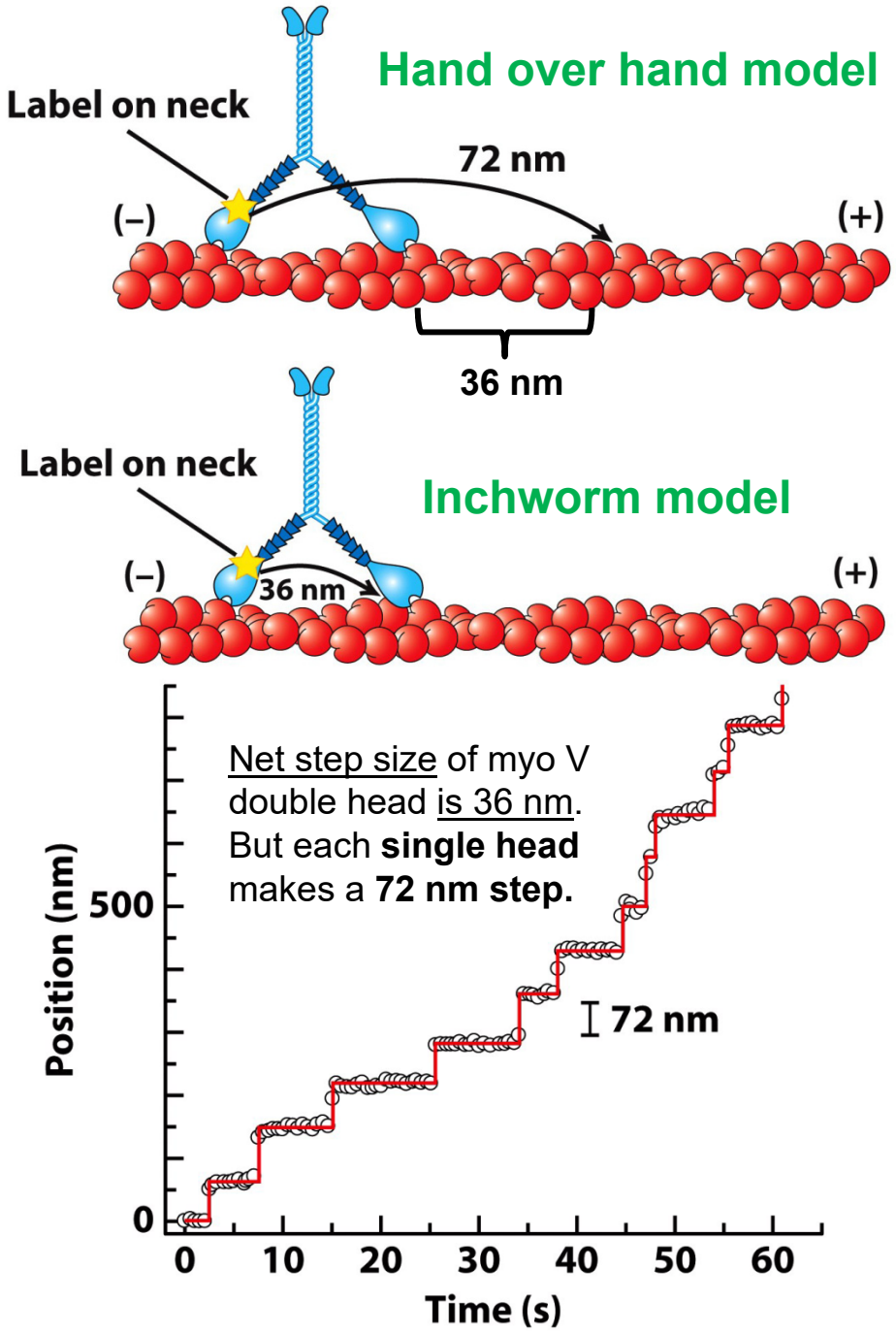
(animation)



Post-power stroke

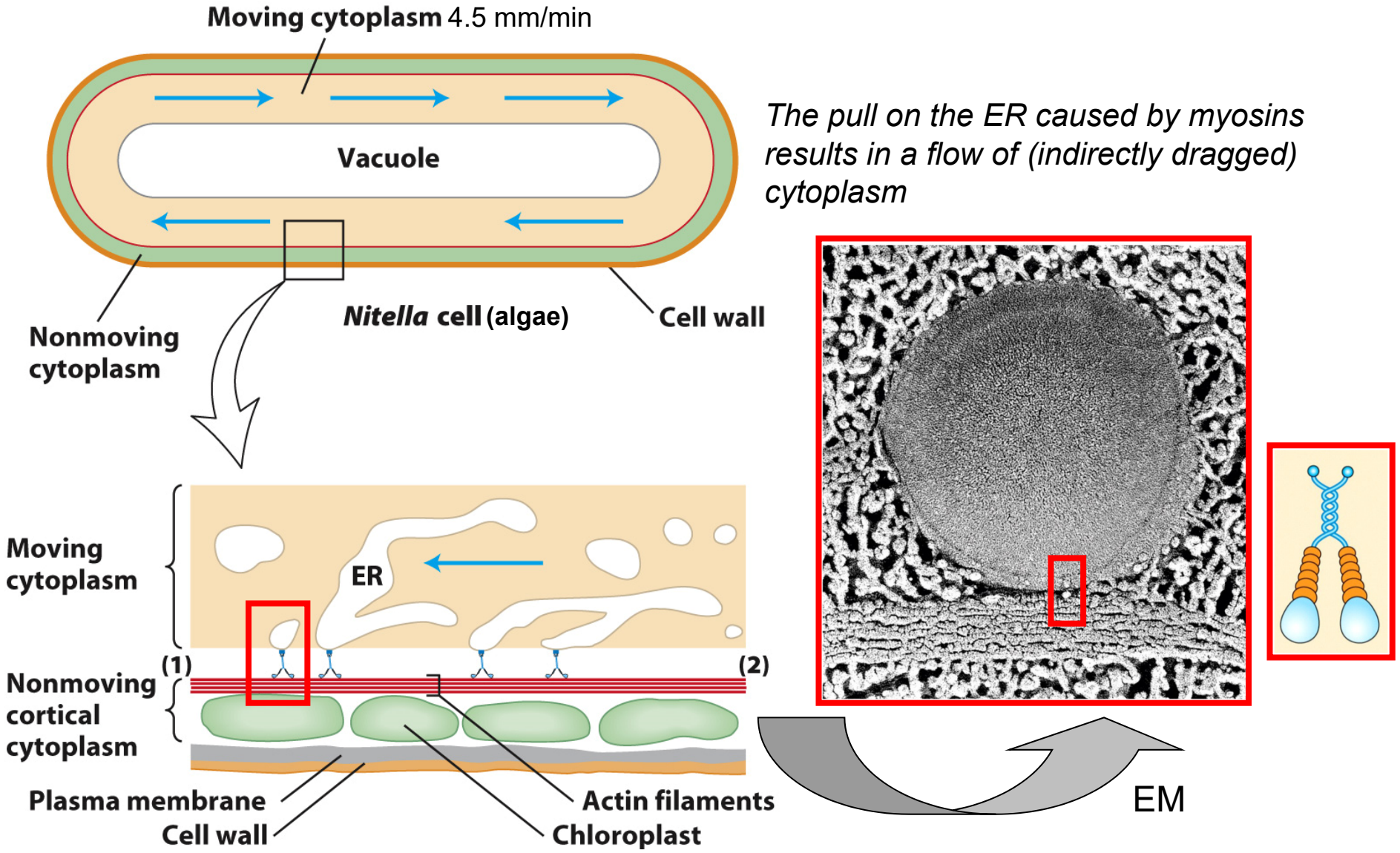
(animation)

Walker et al., Nature, 2000



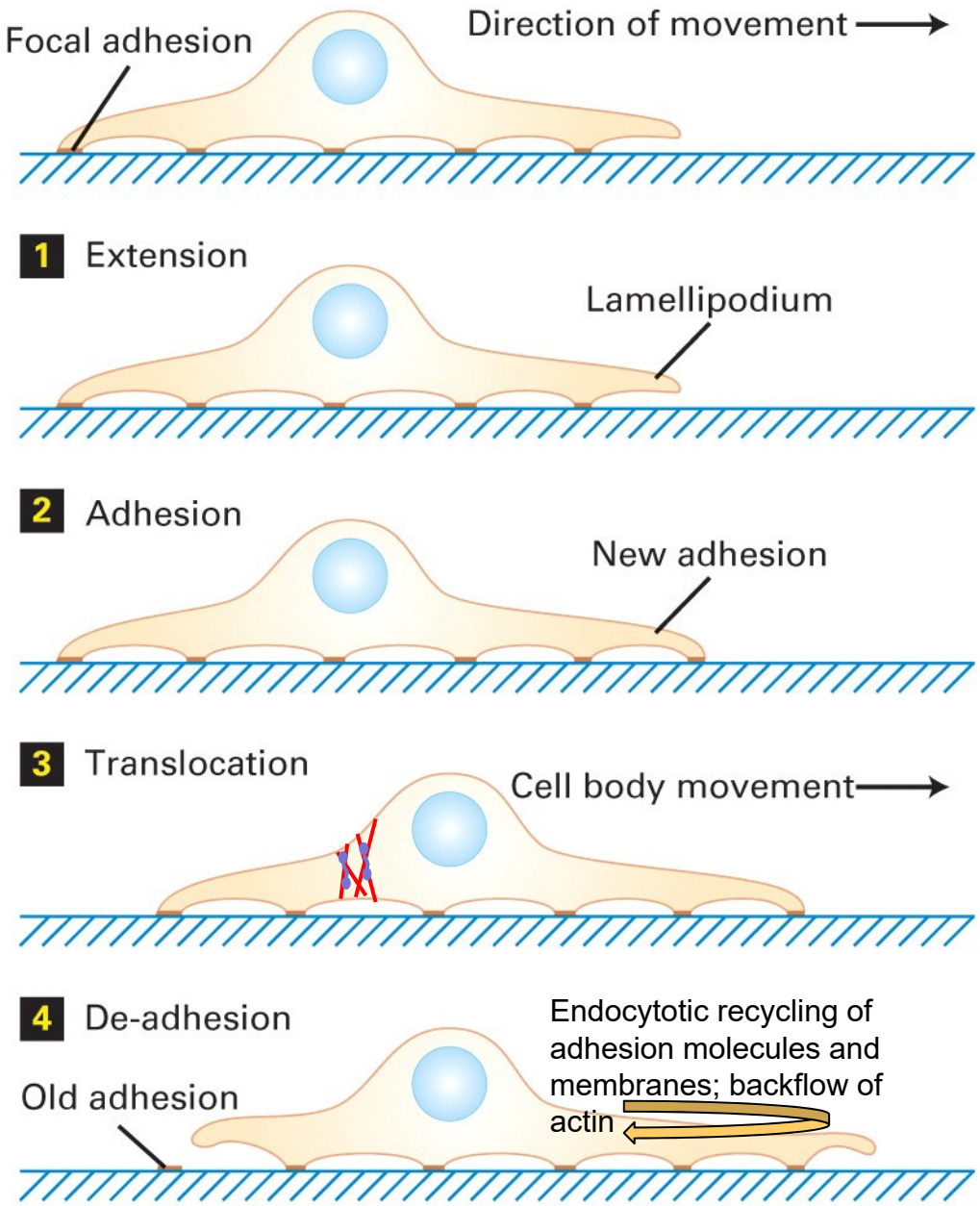
Cytoplasmic streaming in *nitella* plant cells

- Near the cell wall **actin** tracks are immobilized on **chloroplasts** while **myosins** are free to move
- Myosin V tails bind to the endoplasmic reticulum (myosin V also named myosin XI in plant cells)



The pull on the ER caused by myosins results in a flow of (indirectly dragged) cytoplasm

Cell movement requires contractile bundles and cell adhesions



Cell movement occurs in 4 discrete steps

Branched actin polymerization (powered by Arp2/3) and **cross linking** of new filaments (by filamin) forms a lamellipodium at the *leading edge*

The lamellipodium (or filopodia) form new **focal adhesions** to fix the leading edge to the substratum (interaction between the extracellular matrix and focal adhesion is mediated by **integrins**)

Contraction of the **actin-myosin** cortex (near the rear of the cell) leads to translocation of the tail

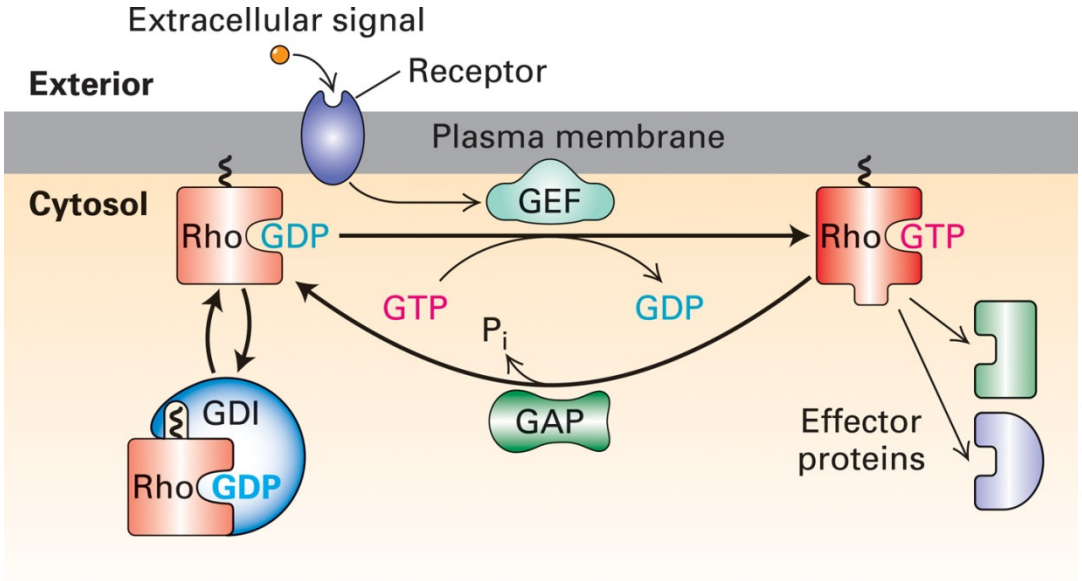
De-adhesion of focal adhesions at the tail releases the stress caused by contraction of the actin-myosin network

Cell motility animation

Macrophage movement

Signal transduction pathways control cell movements

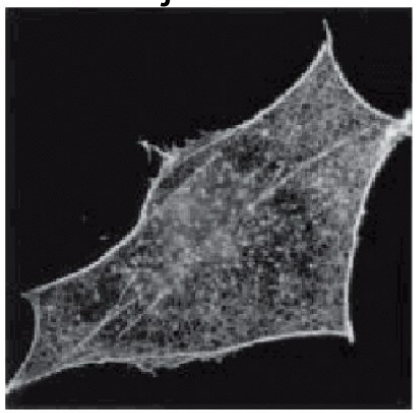
- **G proteins** (Ras-like small GTP-binding proteins) mediate cell motility
- G proteins (for example Rho) act as **molecular switches** which are active in their GTP-bound state and inactive in their GDP-bound state



G proteins are regulated by specific inhibitors (**GDI**), exchange factors (**GEF**) or GTPase activity stimulators (**GAP**)

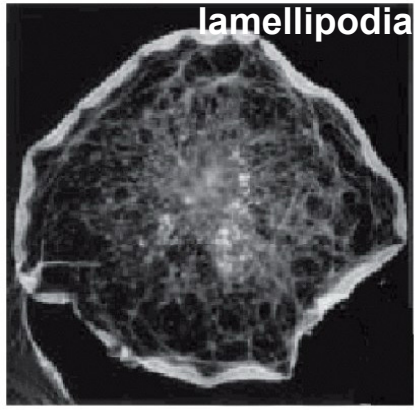
GEF = guanine nucleotide exchange factor
GAP = GTPase-activating proteins
GDI = guanine nucleotide dissociation inhibitor

Microinjection of constitutively active G proteins in growth-factor starved fibroblasts:

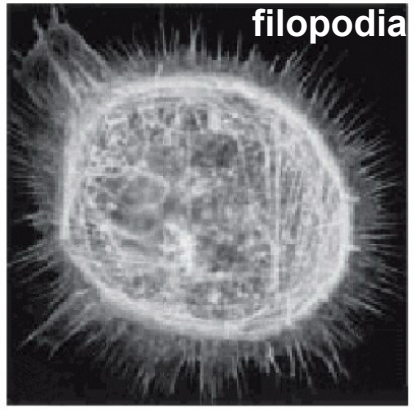


Control

Dominant active Rho



Dominant active Rac



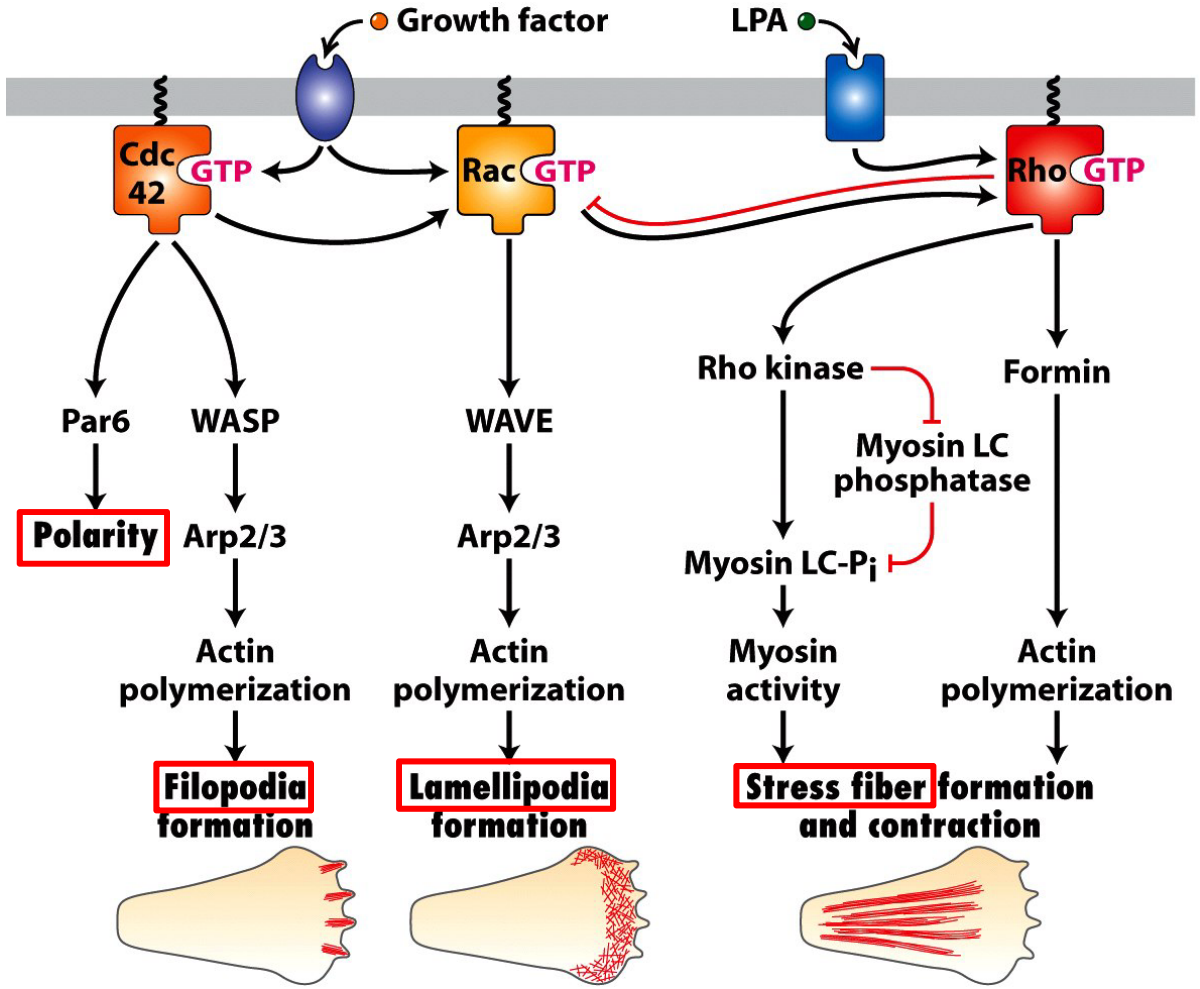
Dominant active Cdc42

Signal transduction pathways control cell movements

Activation of the different actin structures follows a strict hierarchy:

• **First** event is **Cdc42**-activated filopodia formation **following** **Rac**-activated lamellipodia formation and the **last** is the **Rho**-activated stress fiber formation.

• However, activated Rho pathways also **inhibits** Rac pathway (to ensure the asymmetry of this process)

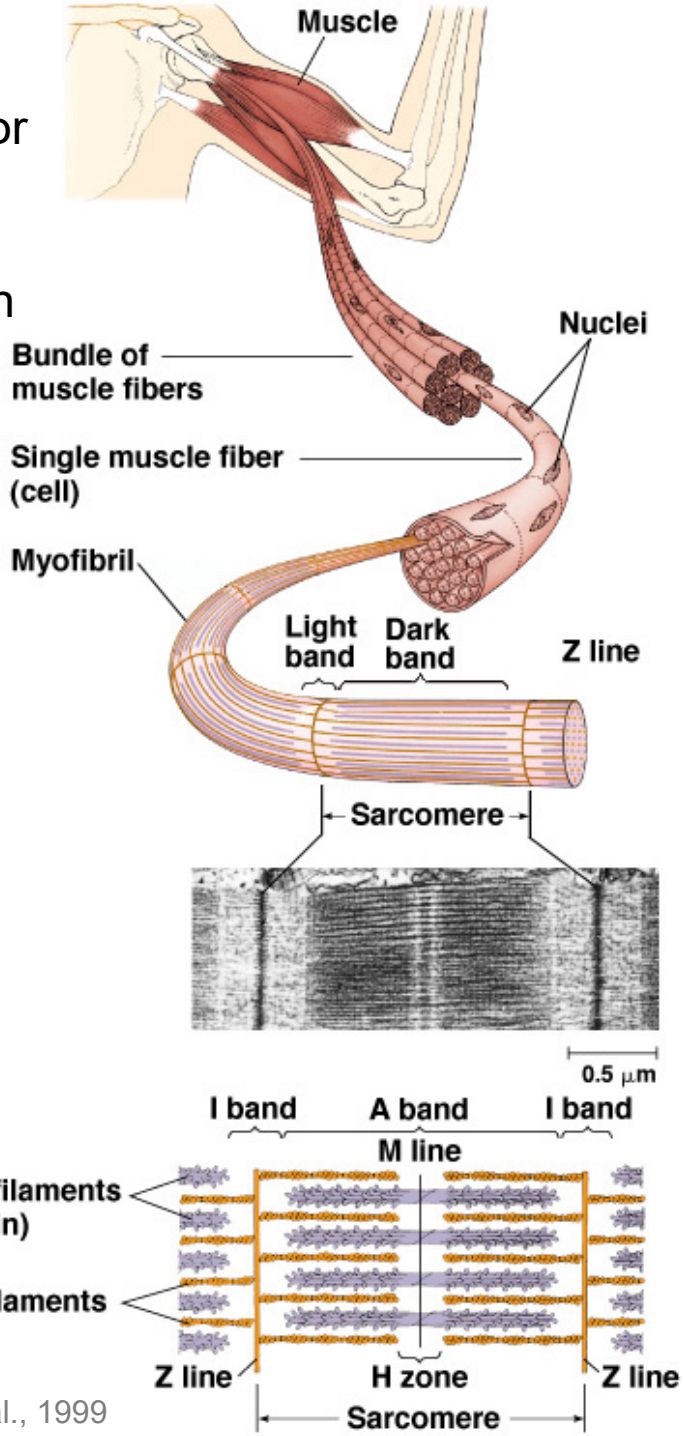


MOLECULAR BASIS OF MUSCLE FUNCTION

Structure of striated muscle cells

• Muscle = bundle of **muscle fibers** (aka **muscle cells** or **myofibers**): 1-40 mm long **syncytium** of >100 nuclei
• **Sarcomere** is a specialized 2 µm long cytoplasmic structure in muscle cells containing densely packed actin and myosin filaments
• Bundled chains of sarcomers form the **myofibril** (and many myofibrils can be found in a muscle cell)
• Upon contraction, the sarcomer shortens to about 70% of its resting length
• The bright I band is entirely composed of thin **F-actin**
• The dark A band reflects the **thick myosin filaments**
• The H zone is entirely composed of myosin
• Actin filaments are attached with their (+) ends to the Z disks

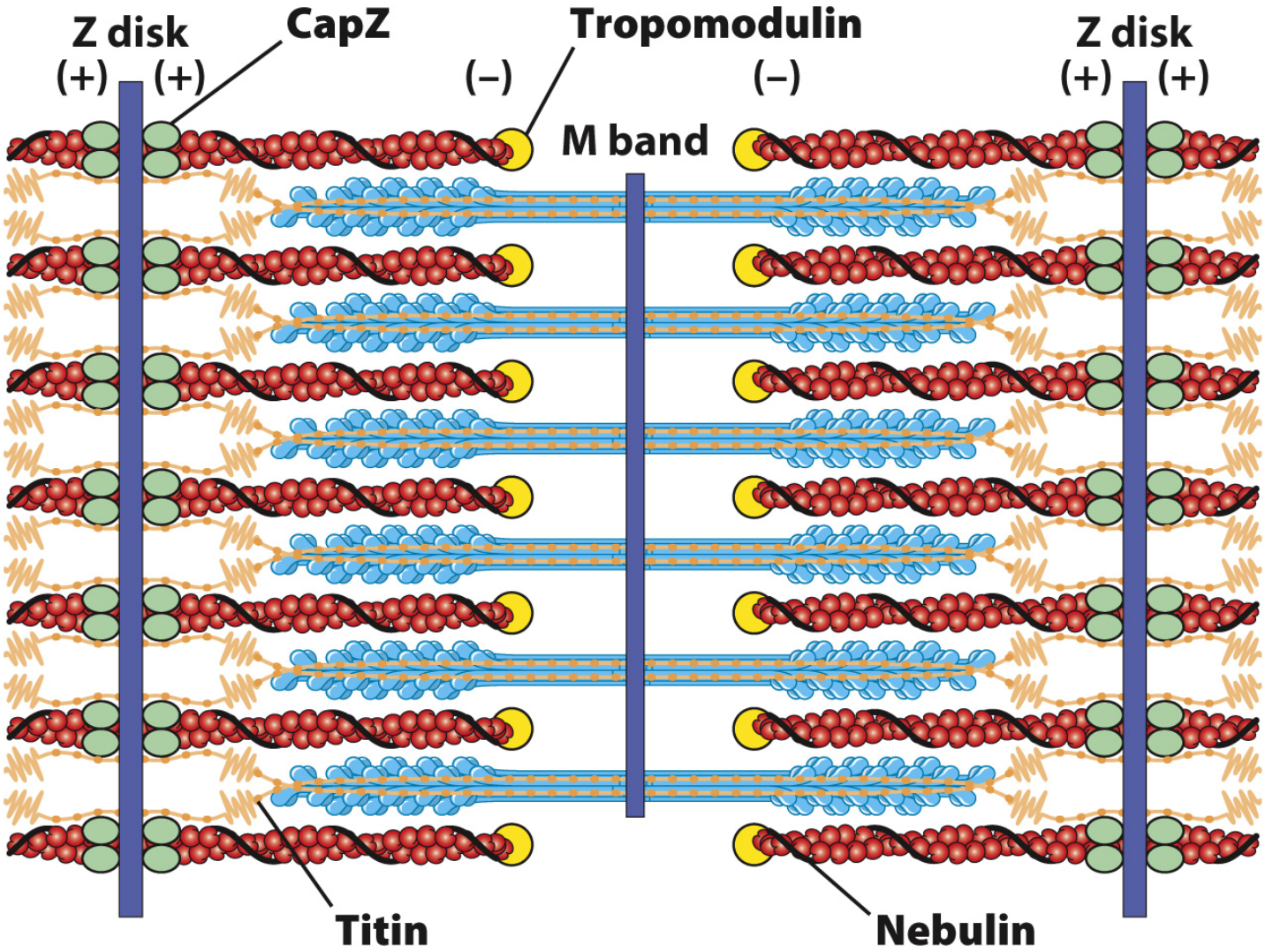
Most of the names for the sarcomere bands derived from early observation with an polarized microscope:
I = **i**sotropic
A = **a**nisotropic
M = **m**iddle line
Z = "**Z**wischenscheibe" (German: disks)
H = "**h**eller" (German: brighter)



Addison et al., 1999

Sarcomere accessory proteins

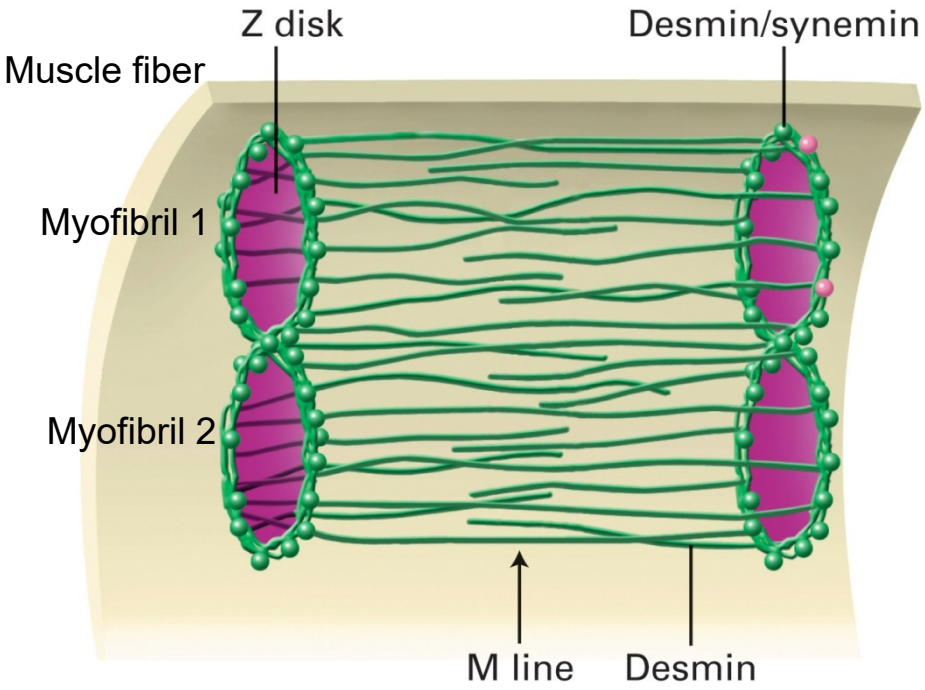
- Actin filaments are stabilized by **CapZ** at their plus-ends and **tropomodulin** at their minus-ends
- The thin filaments are also laterally wrapped by the giant protein **nebulin** (900 kDa)
- Similarly, thick filaments are mechanically supported and fixed to the Z disks by giant **protein titin** (largest known protein with 3800 kDa)

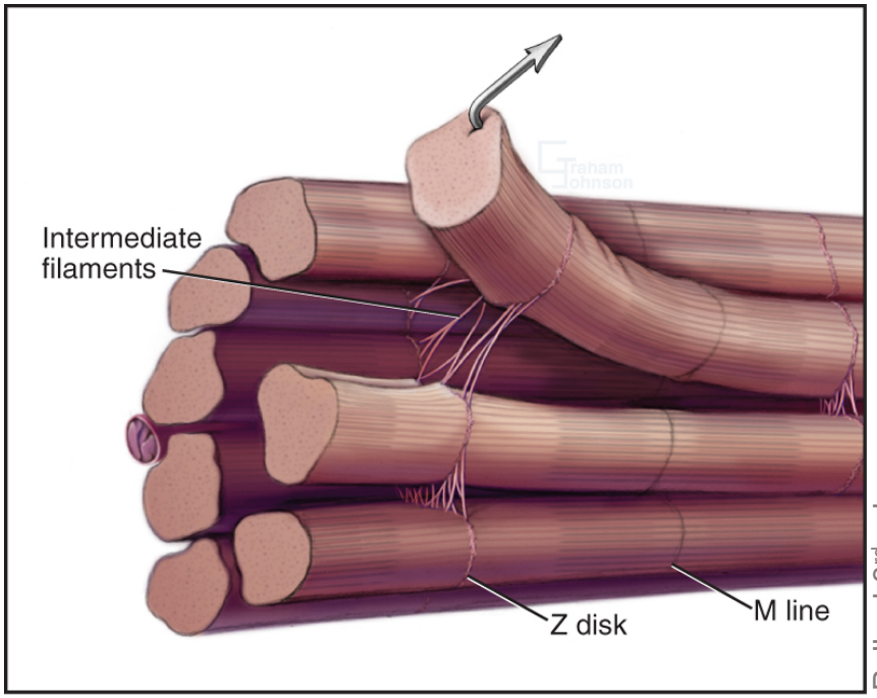


youtube.com/watch?v=GZ-fgWXPOBY

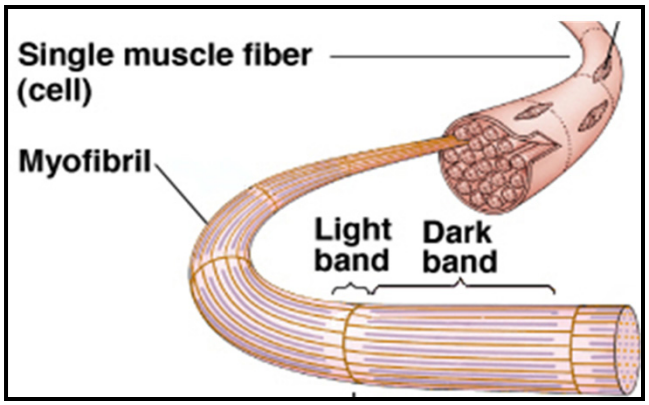
Desmin interconnects and bundles the sarcomeres

- **Desmin** surrounds the Z disk and connects sarcomeres to the plasma membrane
- The longitudinal **desmin filaments** interconnect neighboring myo*fibrils* and bundle them into a muscle *fiber*





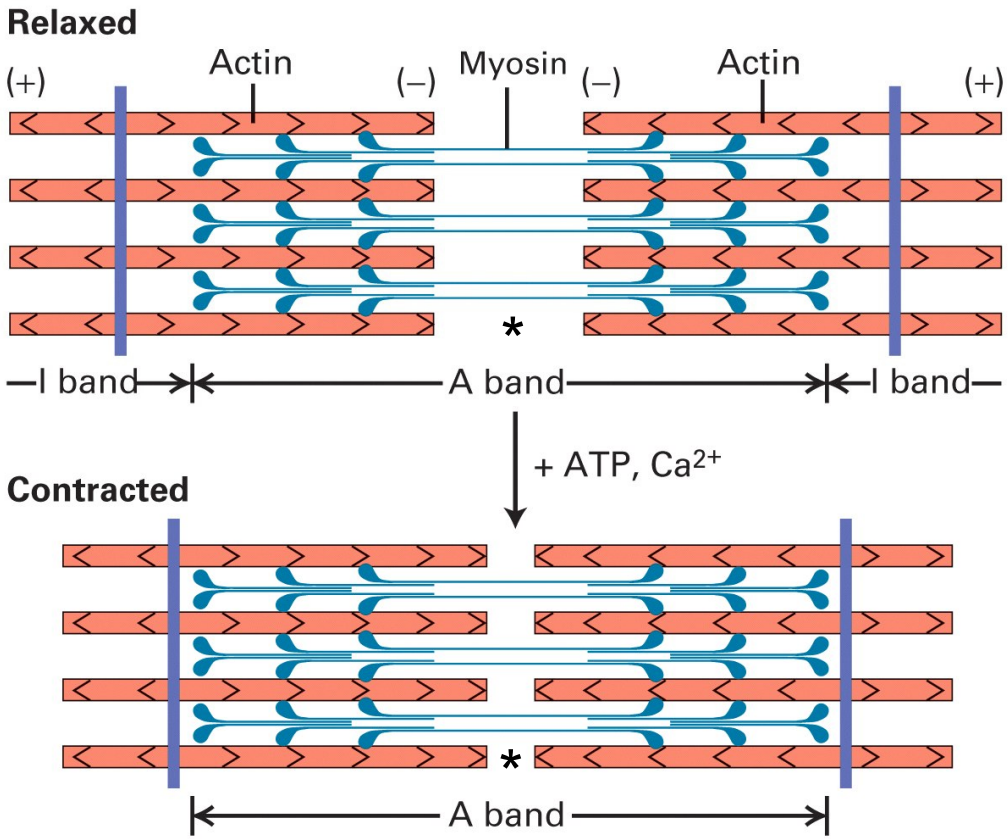
Desmin filaments lie outside the sarcomere and do not participate in muscle contraction

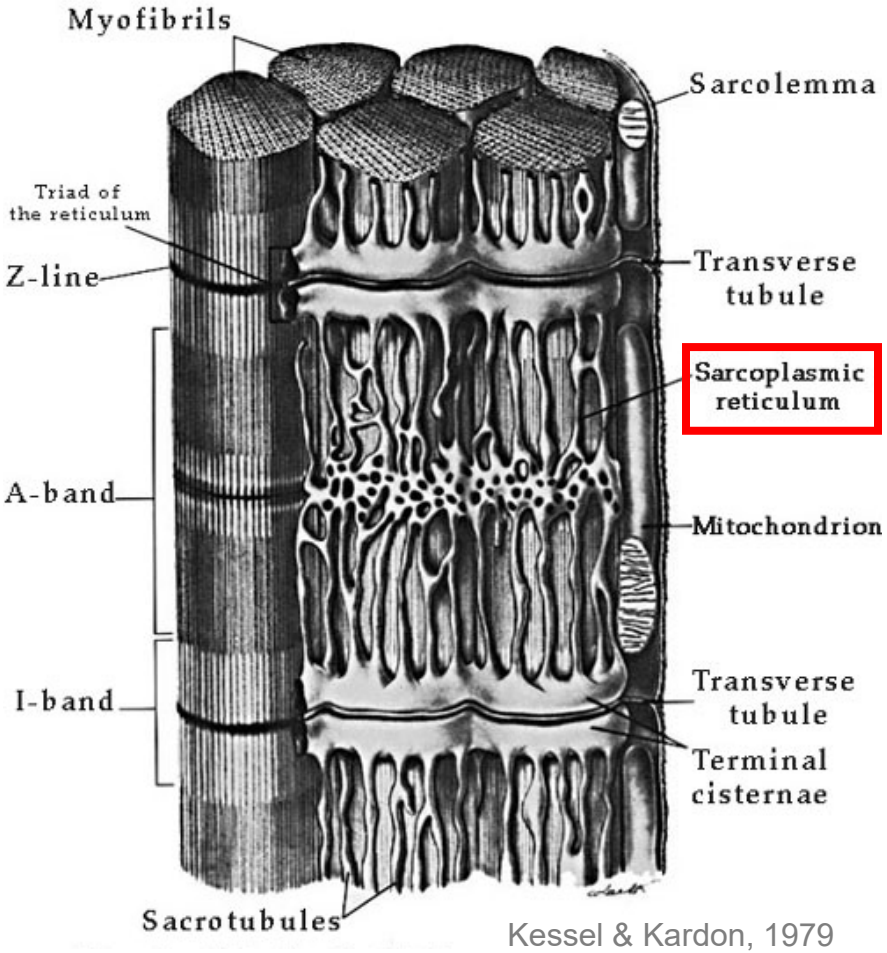


A chain of sarcomers form the myofibrils and many myofibrils can be found in a single muscle cell (muscle fiber)

Contraction of the sarcomere shortens the H zone*

- During the cross-bridge cycle (ATP-hydrolysis), myosin heads move the actin filaments from each side of the sarcomere until their (-) ends overlap
- Contraction requires Ca^{2+} which is pumped by Ca^{2+}-ATPases into the **sarcoplasmic reticulum (SR)**
- An incoming nerve impuls at the **neuromuscular junction** triggers voltage-gated Ca^{2+} channels raising the cytoplasmic Ca^{2+} level from 0.1 µM to above 1 µM

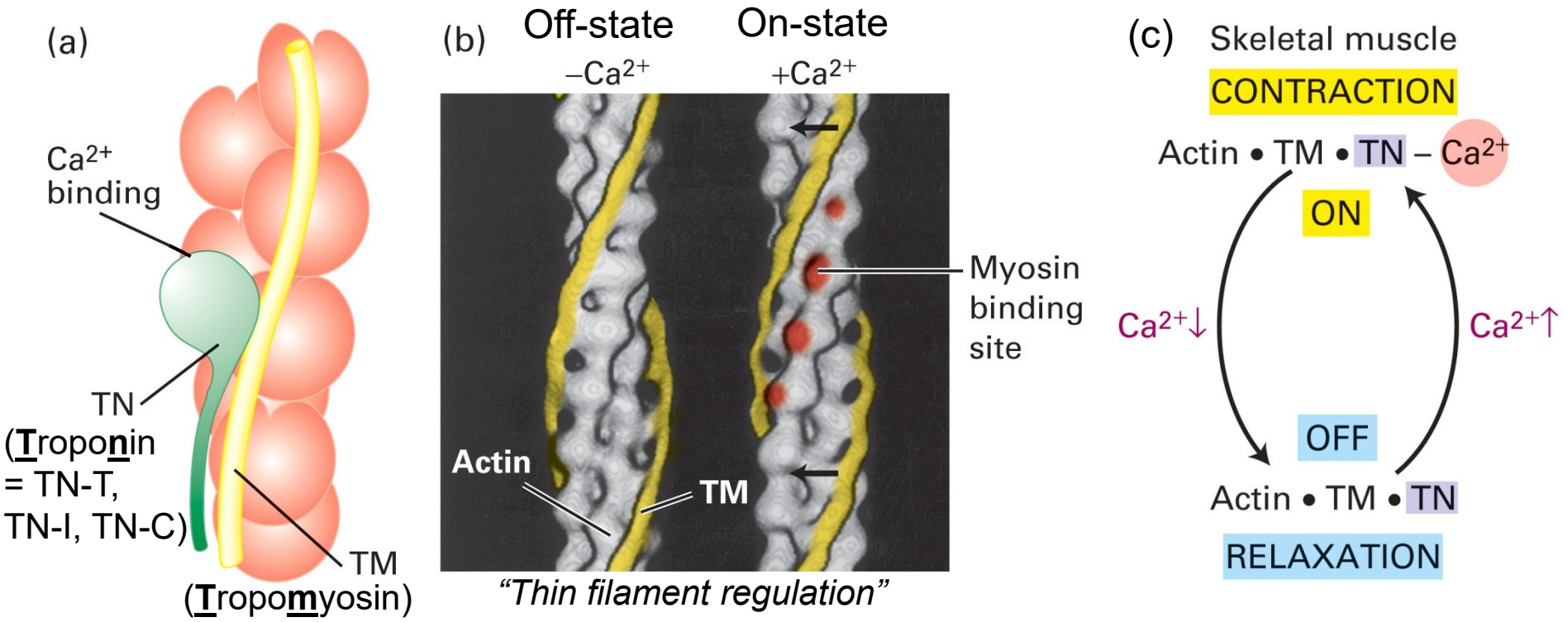




Kessel & Kardon, 1979

The cross-bridge cycle is controlled by actin binding proteins

- **Troponin** regulates muscle contraction. It consists of three subunits TN-T, TN-I and TN-C (**c**almodulin homology). Binding of **Ca^{2+}** to TN-C triggers the movement of **tropomyosin** => myosin-binding sites on **F-actin** are now exposed
- In the *off state* myosin still can bind actin, but it cannot move (no power-stroke)



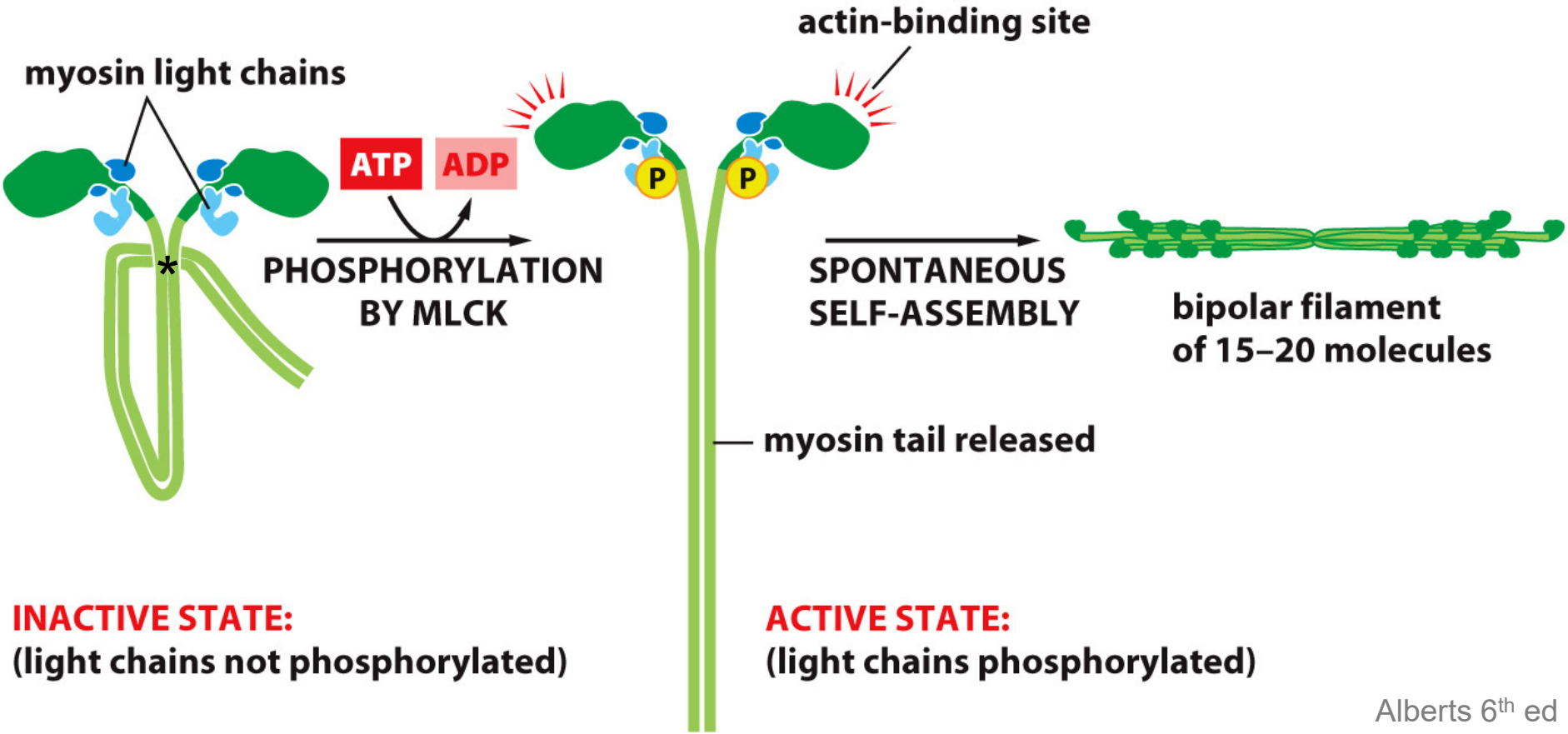
Thin filament regulation

Ca^{2+} = yellow sphere
Mg^{2+} = white cone

Actin-myosin contraction in non-muscle or smooth muscle cells

- **Smooth muscle** cells are present in the gut, respiratory tract, blood vessels, uterus
- Smooth muscle and non-muscle cell contraction is regulated by **turning myosin on and off** (compare *striated muscle cell*: actin is turned on and off by tropomyosin!)
- In smooth muscle cells loosely aligned actin-myosin bundles contract when **myosin light chains (LC)** are **phosphorylated by** myosin LC kinase (**MLCK**)

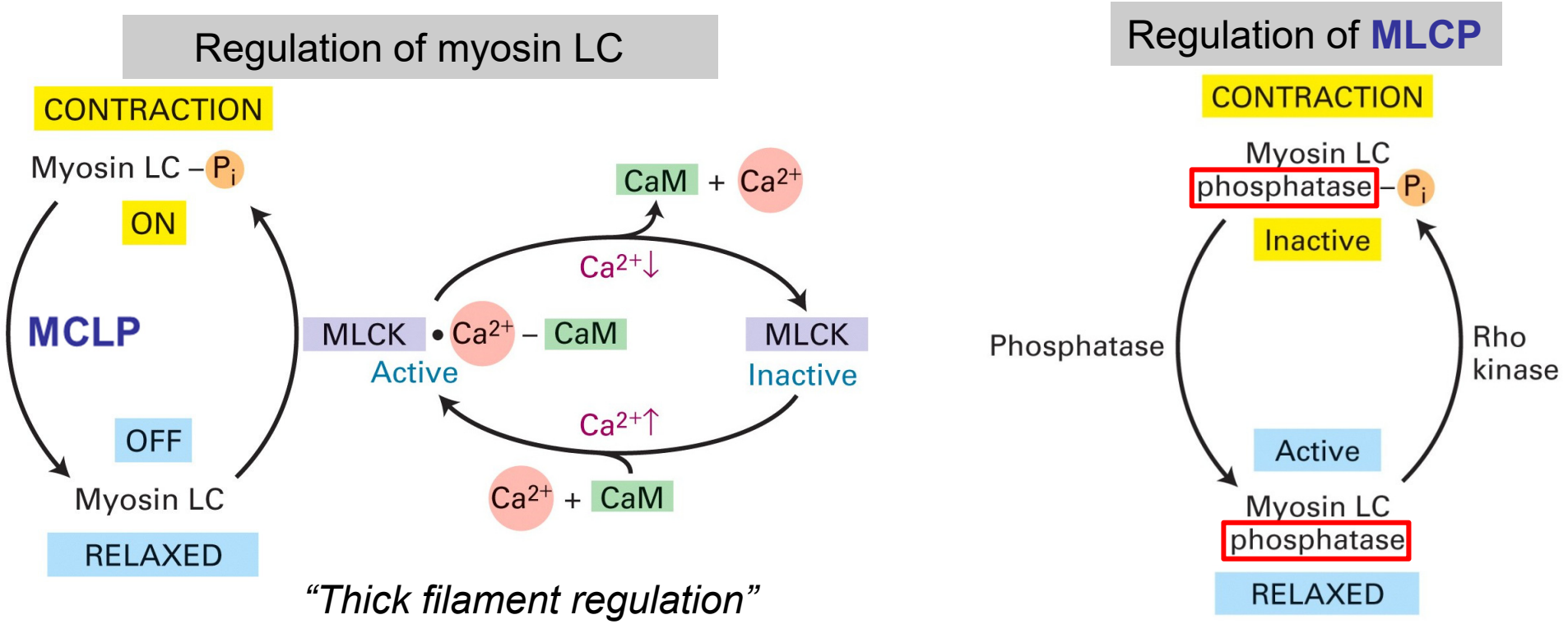
Phosphorylation of myosin LC releases the myosin tail from its "sticky patch"* allowing the myosin molecule to assemble into thick filaments



Alberts 6th ed

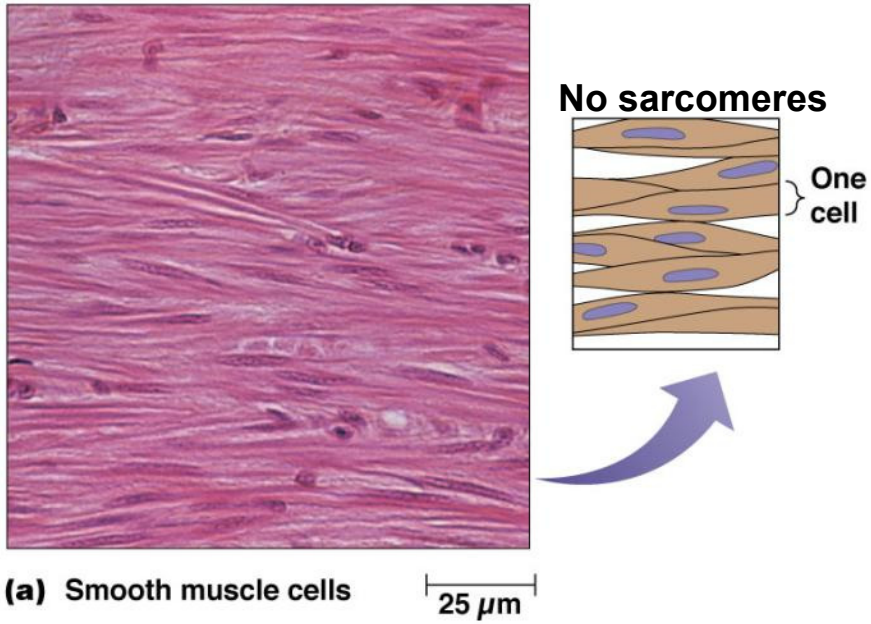
The MLCK has to be activated by Ca-Calmodulin

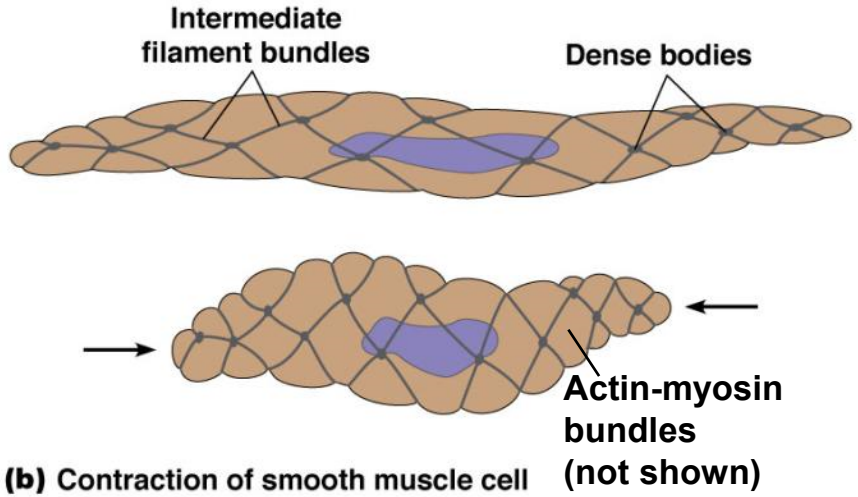
- Upon Ca^{2+} conc. > 1 µM **Ca^{2+}** binds to **Calmodulin** (CaM) which activates **MLCK**
- A **myosin light chain phosphatase** (**MLCP**) can remove the P_i from the myosin LC
- MLCP can be *inactivated* by **phosphorylation** via **Rho kinase** (ROCK) (thus, myosin LC keeps being phosphorylated preventing relaxation)
- Why is smooth muscle contraction so slow?
  a) More proteins involved (calmodulin, MLCK, MLCP, ROCK)
  b) Phosphorylation is a slow process (of both myosin LC and MLCP)
  c) Calcium needs to diffuse over long distances (not stored in near compartments)
  d) Hormones controlled by external signals (much slower as fast action potentials)



"Thick filament regulation"

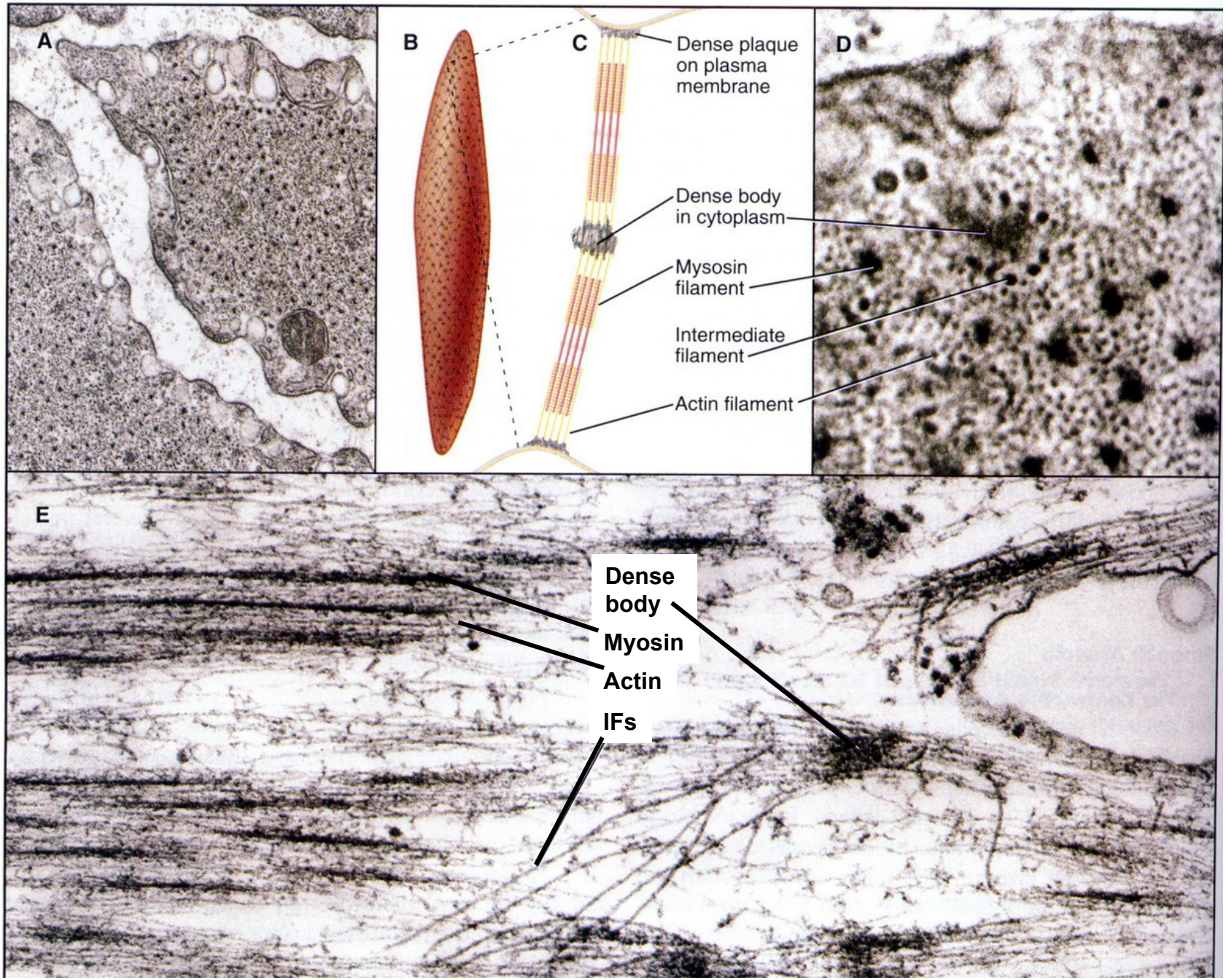
Smooth muscle cells are not striated and contract independently from our free will





- Smooth muscle cells are **not striated**, instead they have a **dotted appearance** in TEM reflecting dense bodies
- **Dense bodies** are the anchoring points for contractility units (comparable to the function of Z discs in skeletal muscle cells) composed of actin and myosin bundles
- Actin/myosin units are also cross-linked and stabilized by **intermediate filaments**
- When actin/myosin units contract they pull on the intermediate filaments and the cell contracts

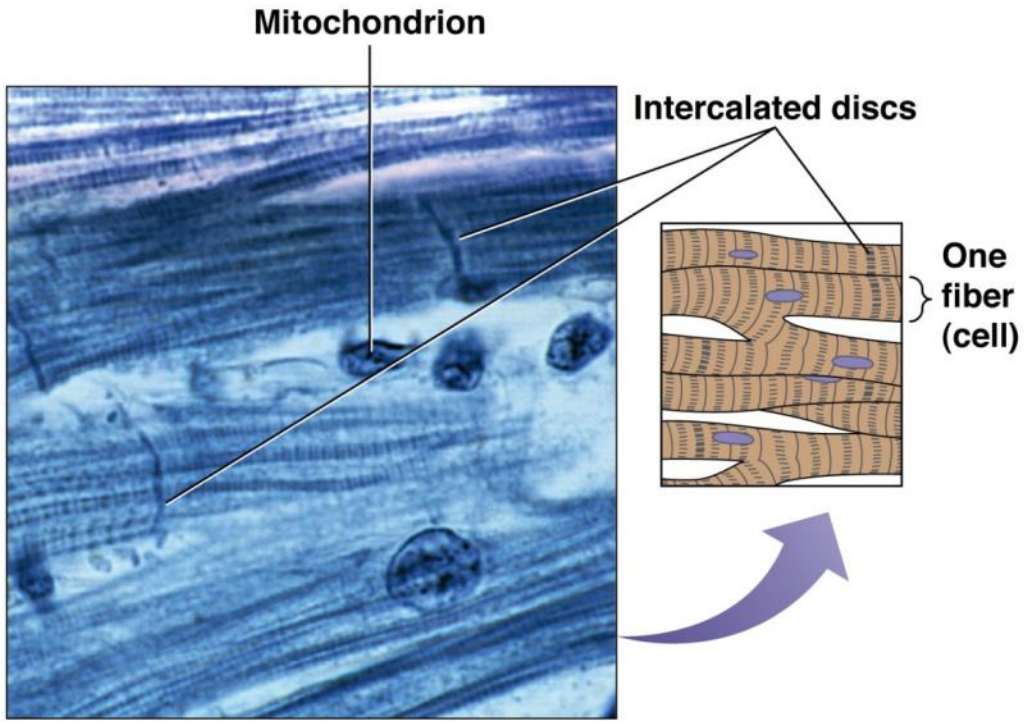
Dense bodies and dense plaques anchor actin-myosin filaments in smooth muscle cells

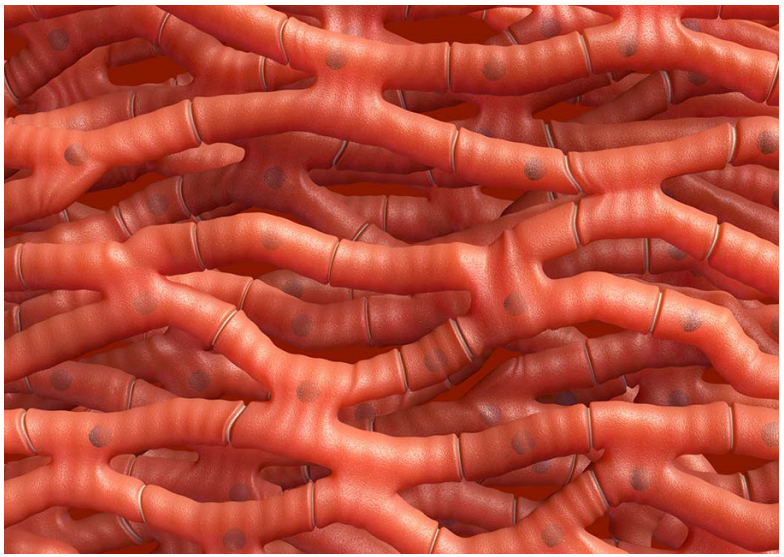


Smooth muscle cells have a spindle-like appearance

Pollard 3rd ed

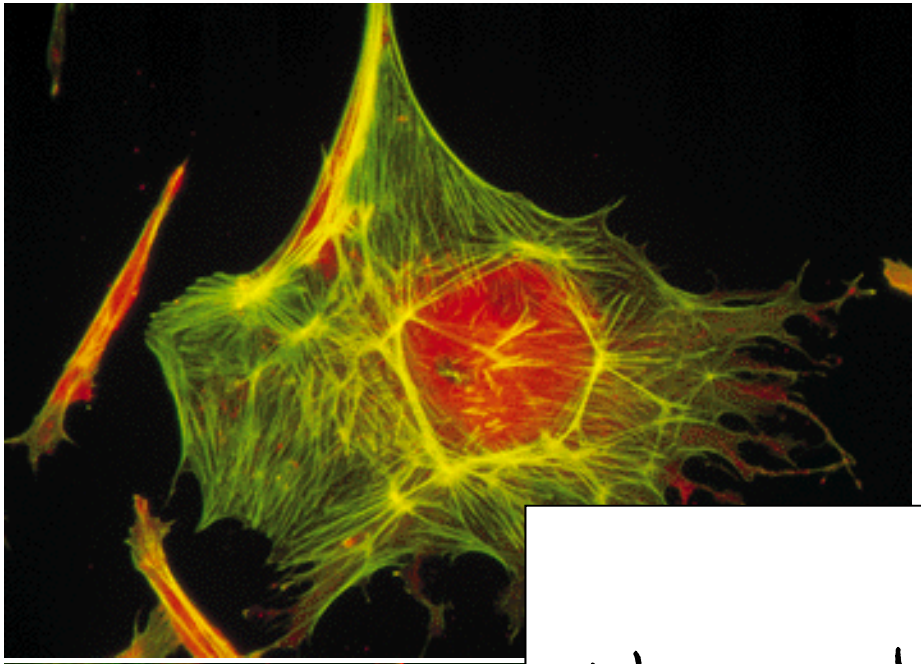
Cardiac muscle cells also appear with striated pattern

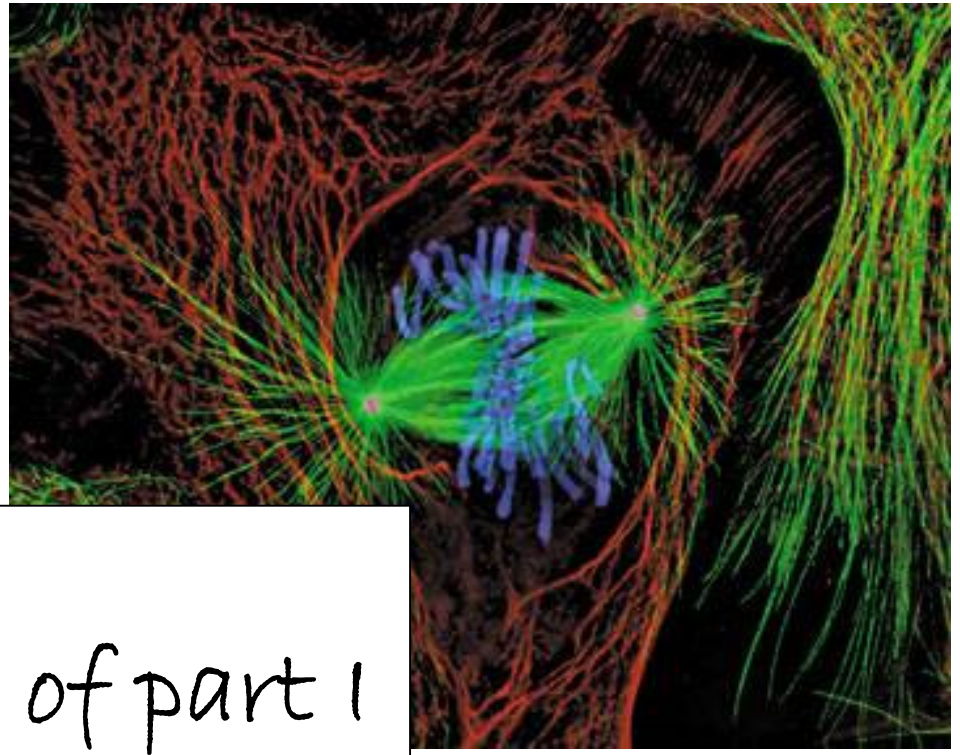




Myosin::GFP

- Cardiac (heart) muscle cells also have striated pattern based on I bands, A bands and Z disks
- The difference to striated skeletal muscle is that cardiac muscle cells are **not multinucleate**
- Mono- or bi-nucleated cells are connected to each other (linear or in branches) via **intercalated disks**
- Intercalated disks are rich of **desmosomes** and **gap junctions**
- The energy used for contraction comes from **fat metabolism** rather than from glucose metabolism (skeletal muscle)
- **Heart attack** happen when blood flow to cardiac muscle cells is disturbed and cells die. Permanent heart dysfunction is the result because **heart cells do not regenerate** (stem cell therapy is thought to partially cure dysfunction)





The end of part 1