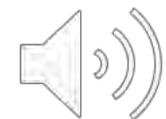
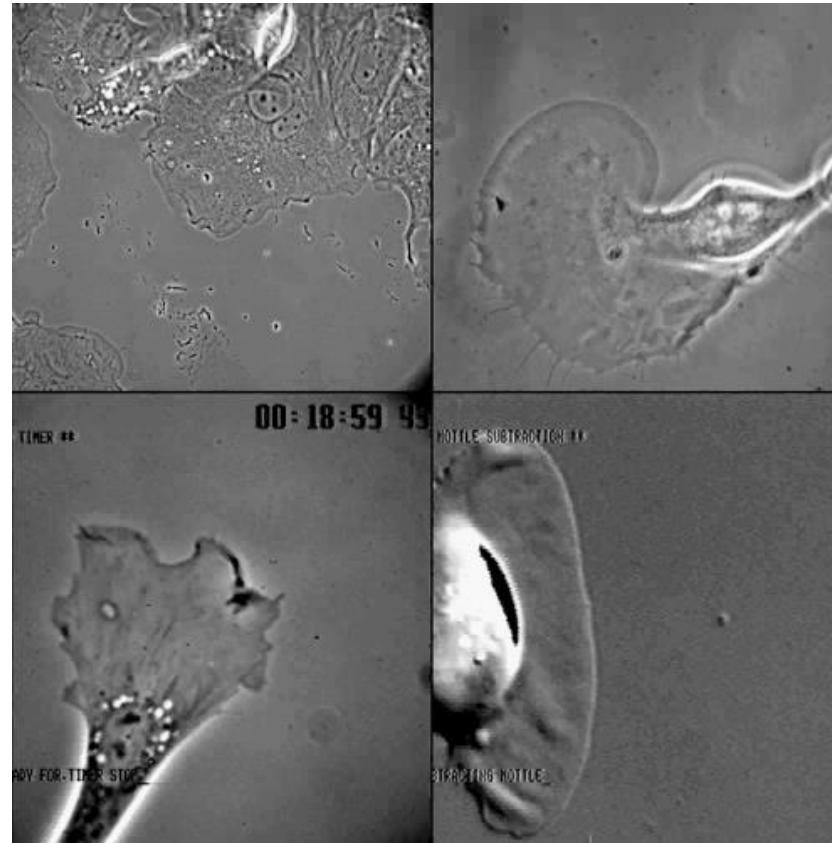


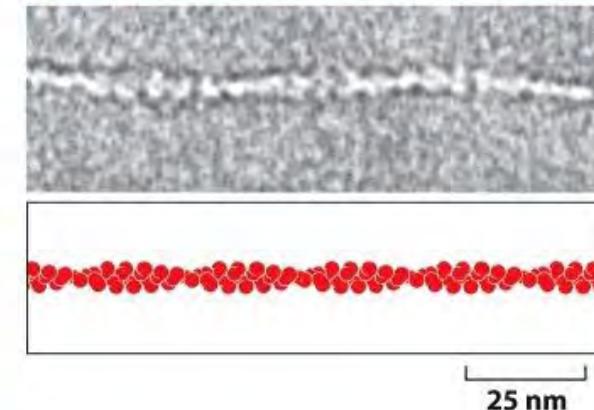
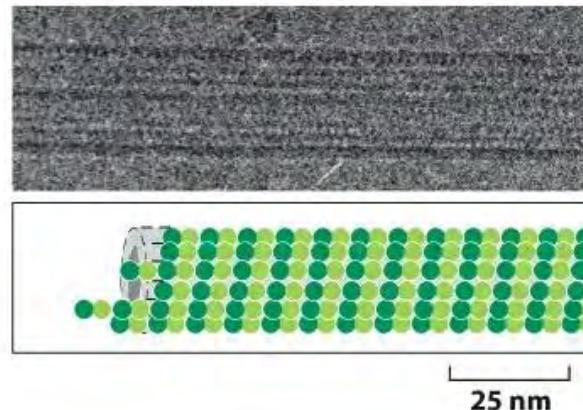
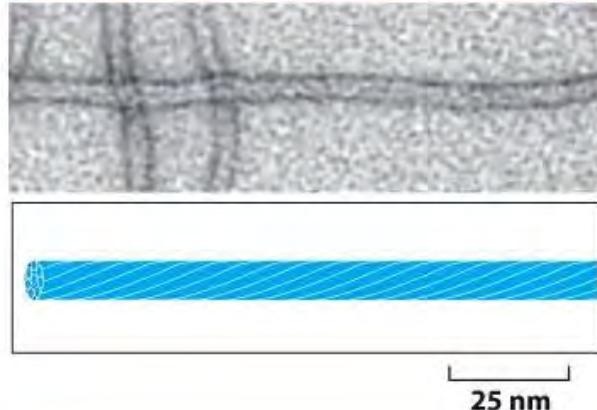
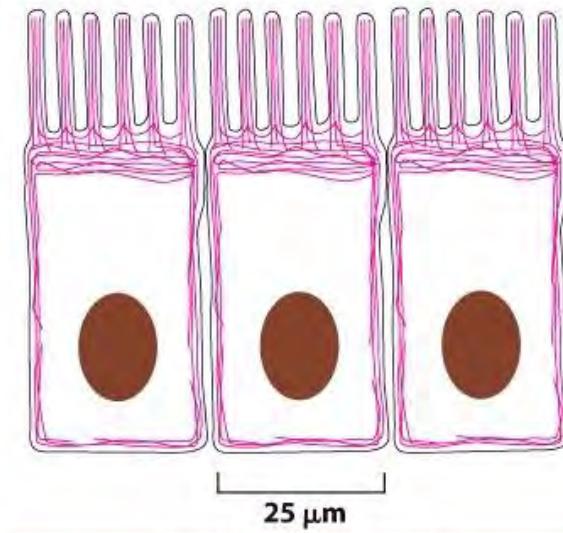
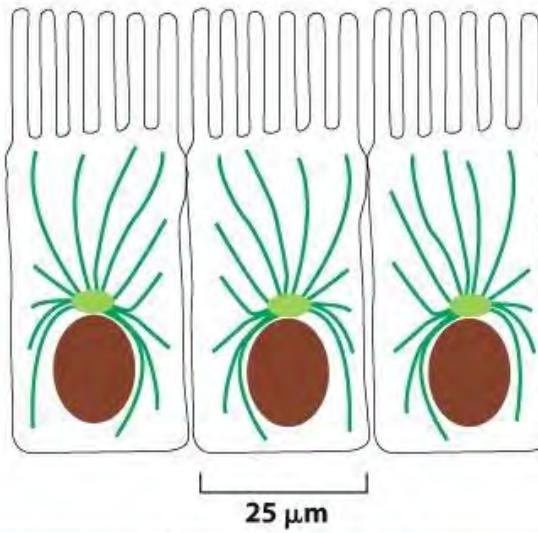
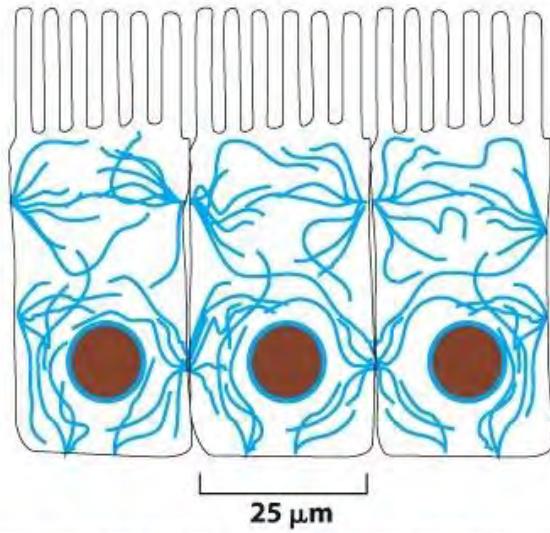
biol303 lecture 14 – cytoskeleton, actin, cell motility, muscle



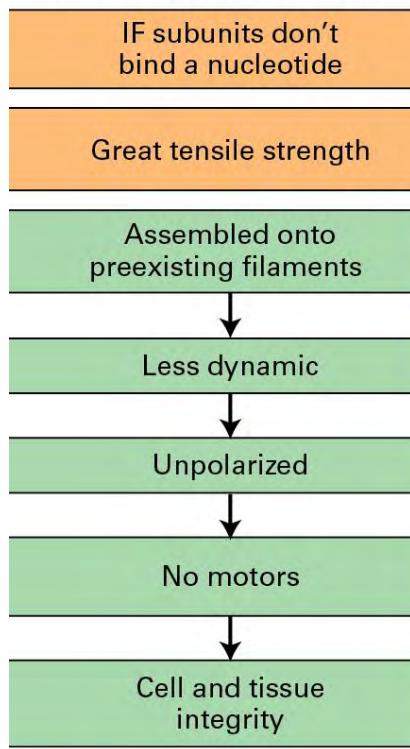
Lodish ch 18

now playing : Maroon 5 “Moves like Jagger”

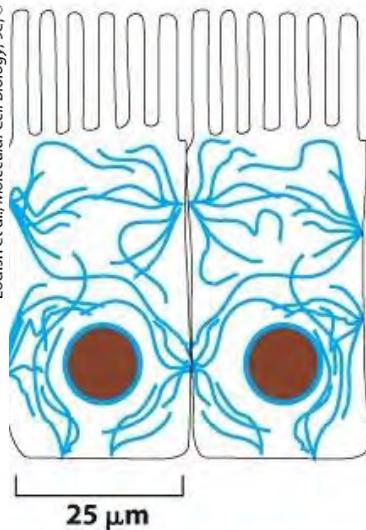
three types of cytoskeletal filaments...



Intermediate Filaments



Part (b) Courtesy of Keith Burridge. Part (c) Courtesy of William J. Brown, Cornell University. Part (d) Courtesy of Elaine Fuchs.



Microtubules

$\alpha\beta$ -Tubulin binds GTP

Rigid and not easily bent

Regulated assembly from a small number of locations

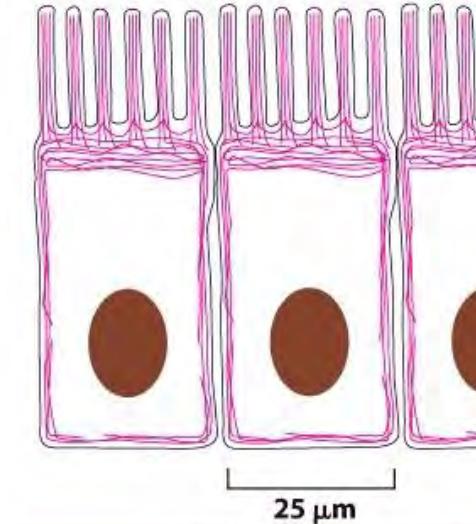
Highly dynamic

Polarized

Tracks for kinesins and dyneins

Organization and long-range transport of organelles

three types of skeletal filaments and...



(a) Microfilaments

Actin binds ATP

Form rigid gels, networks, and linear bundles

Regulated assembly from a large number of locations

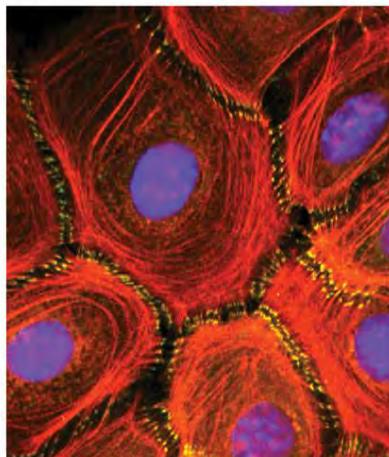
Highly dynamic

Polarized

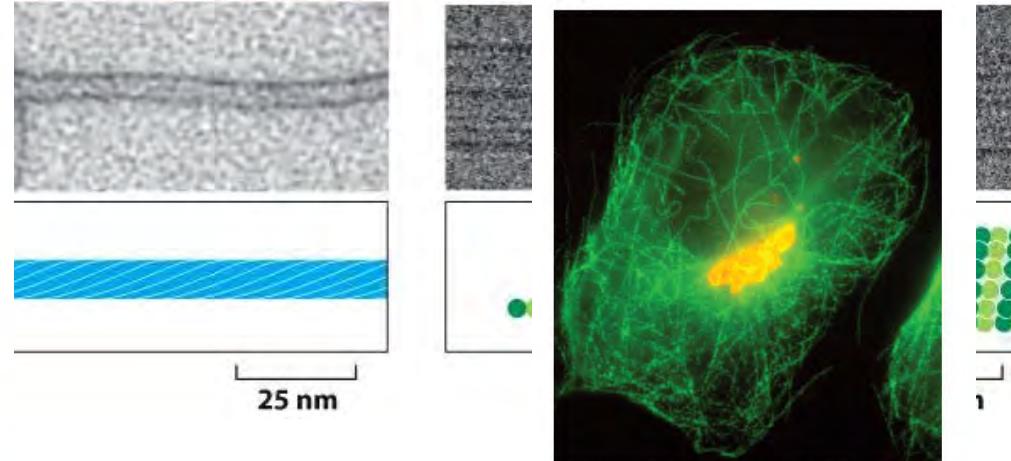
Tracks for myosins

Contractile machinery and network at the cell cortex

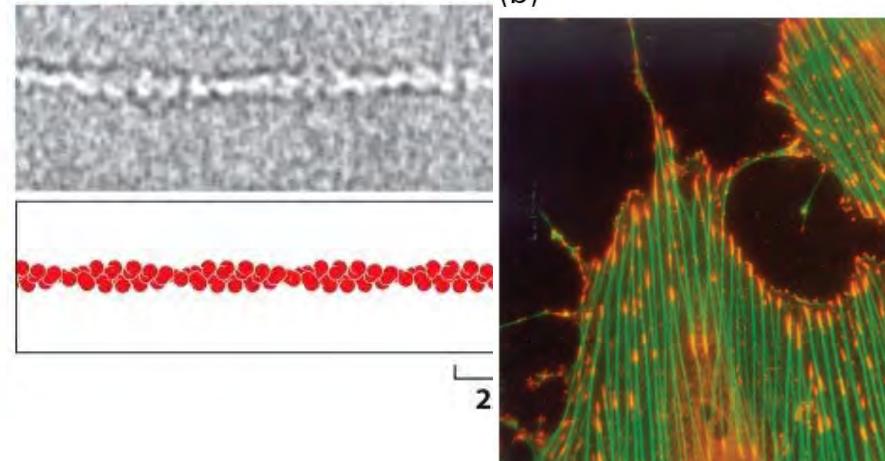
(d)



(c)



(b)



overview

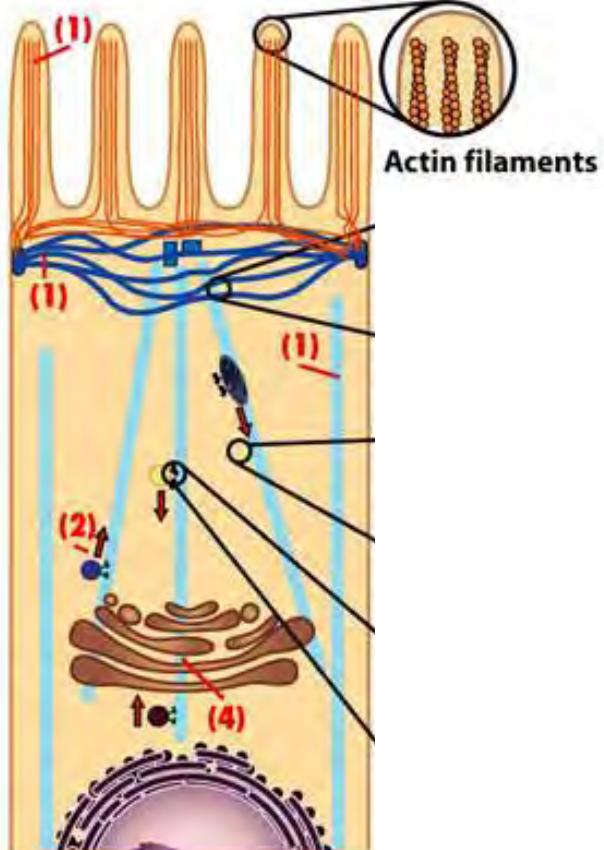
Key to Cytoskeletal Functions

(1) Structure and Support

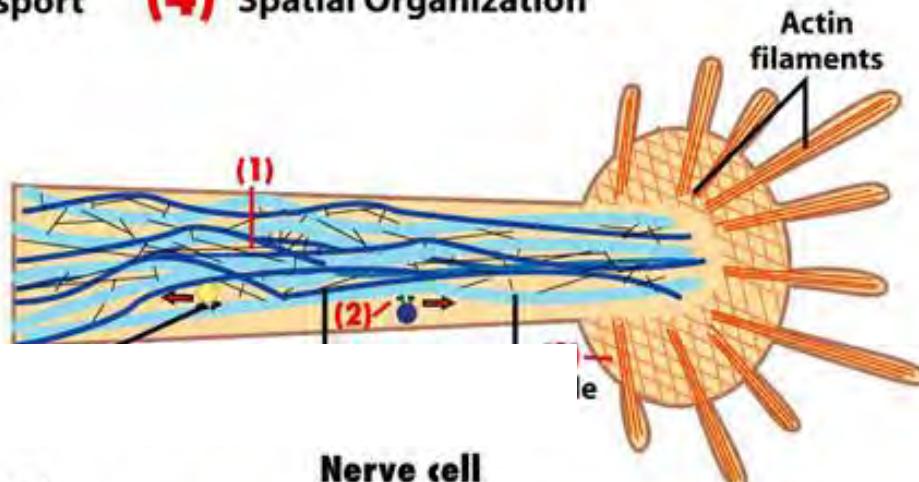
(2) Intracellular Transport

(3) Contractility and Motility

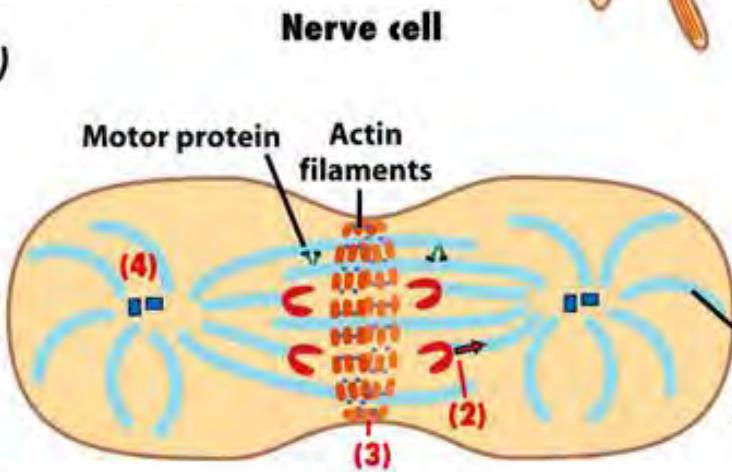
(4) Spatial Organization



(a) Epithelial cell



(b)



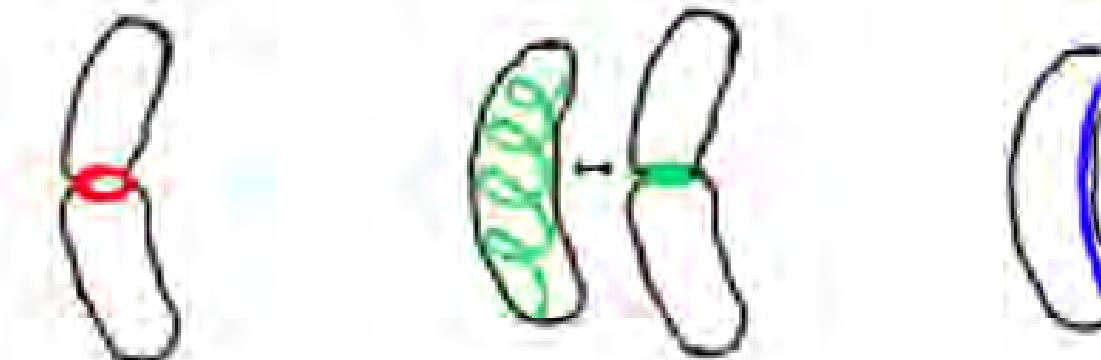
(c)

Dividing cell

prokaryotes have cytoskeletal filaments too!

	Division	Polarity	Shape
Eukaryotes	Tubulin	Actin	Intermediate filaments
Prokaryotes	FtsZ	MreB	CreS

Caulobacter localization

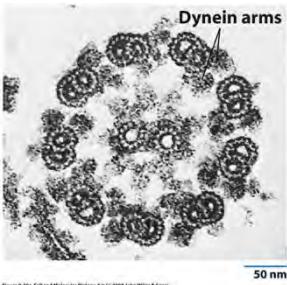


polymerizes,
uses GTP, strong
3D structural
similarity

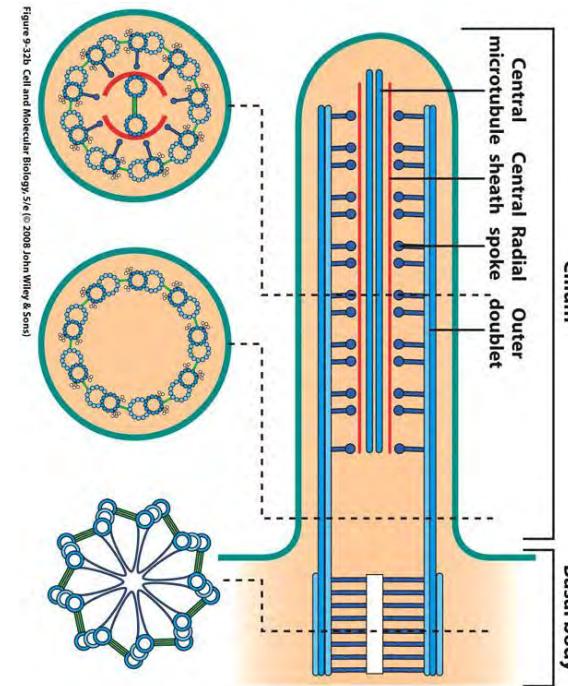
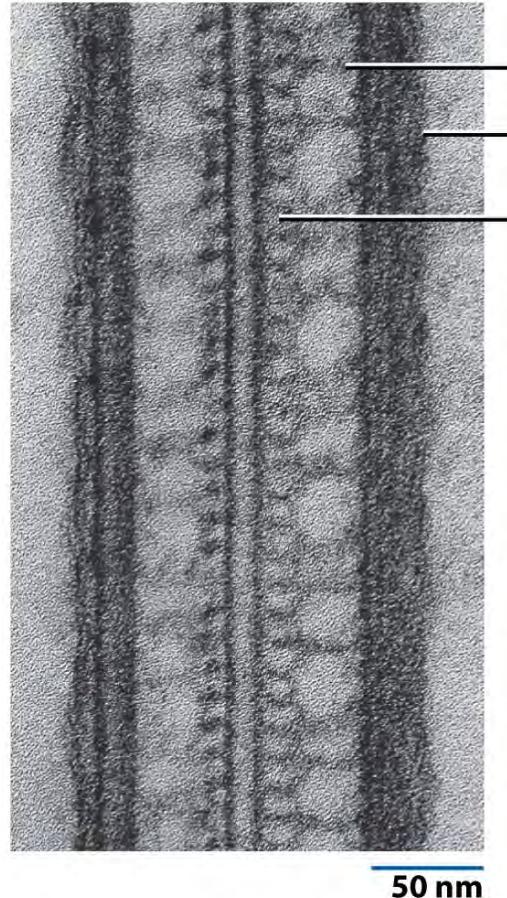
polymerizes, strong 3D
structural similarity

strong primary AND 3D
structural similarities
(nuclear lamin and cytokeratin)

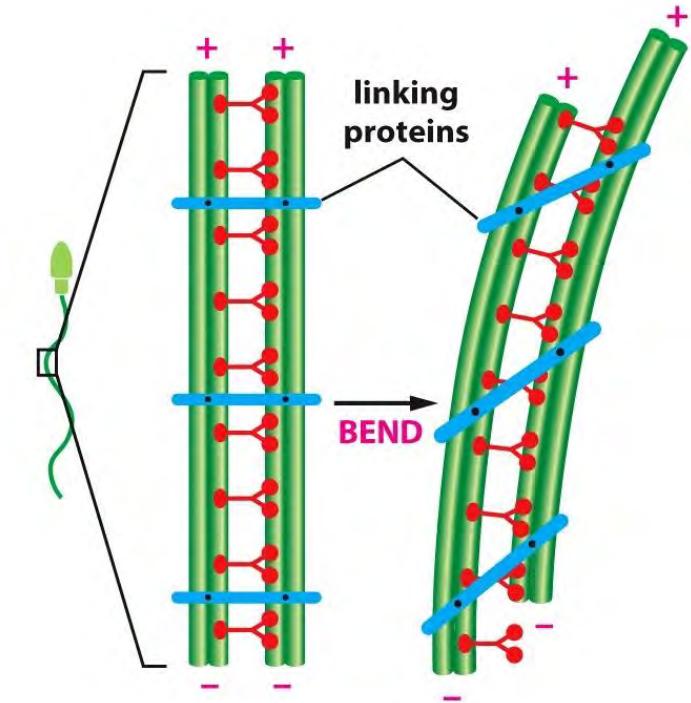
Based on - Gitai, Z. (2005). "The New Bacterial Cell Biology: Moving Parts and Subcellular Architecture". Cell 120 (5): 577-586.



flashback: the axoneme of cilia/flagella is made up of microtubules and Dynein



intraflagellar/ciliary transport also occurs - via dynein and kinesin



(B)

IN A NORMAL FLAGELLUM: DYNEIN CAUSES MICROTUBULE BENDING

From Fred D. Warner and Peter Satir, *J. Cell Biol.* 63:41, 1974; by cop

the 9 + 2 array of microtubules is very well conserved, protists to mammals

Kartagener Syndrome (ciliopathy)

cilia exist but have defective or no movement

Recall: a mutation that disrupts which of the following proteins is the most likely to result in this outcome?

- a) ~~alpha tubulin~~
- b) ciliary dynein
- c) ~~gamma tubulin~~
- d) ~~kinesin~~
- e) ~~ATP synthesizing enzymes~~

[poll](#)

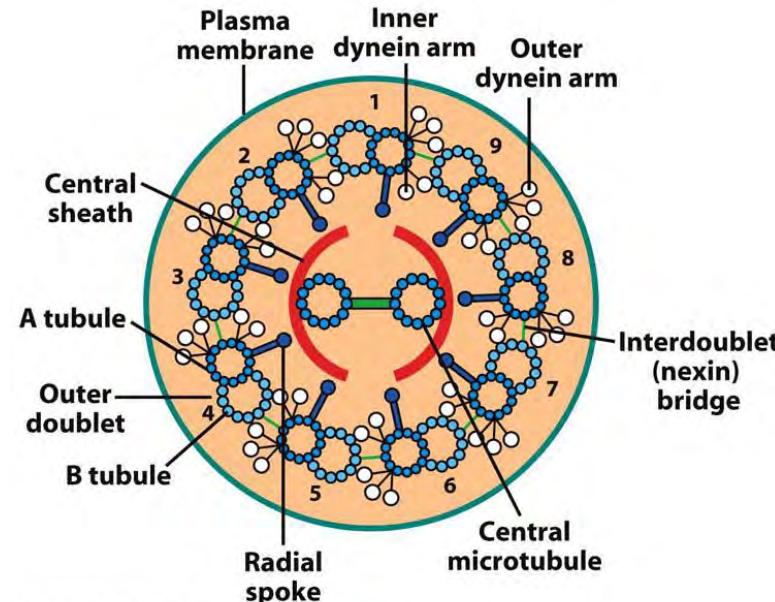


Figure 9-30b Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

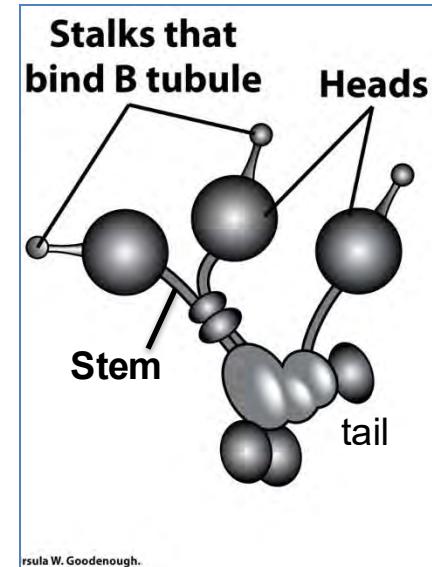
Kartagener Syndrome

cilia exist but have defective or no movement

human patients have only **small changes** in the coding region of the dynein protein, not entire loss, and you know that **cilia from these patients hydrolyze ATP** at a similar rate to normal cilia.

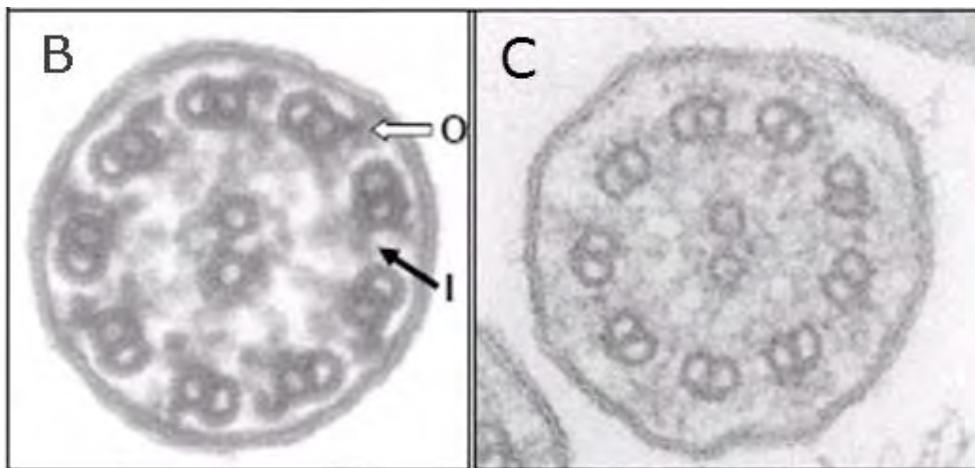
- Which region do you expect the mutation to be in?

For a mouse model of the disease, you want to make a genetic mutant with a small deletion – which part would you target?



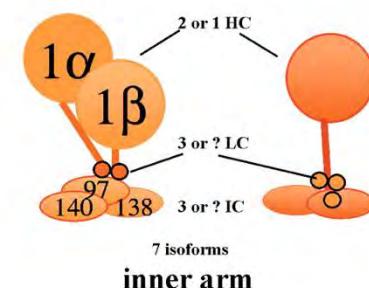
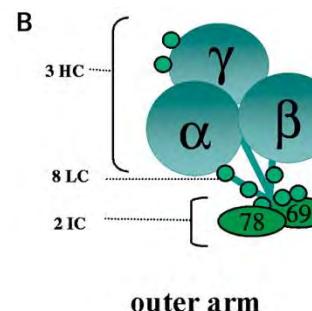
Kartagener Syndrome

- cilia have defective or no movement
- you want a mouse model of the disease, so you want to make a genetic mutant. you know that the human patients have only **small changes** in the coding region of the dynein protein, not entire loss, and you know that **cilia from these patients hydrolyze ATP** at a similar rate to normal cilia.
- so, which region of the protein(s) might be the best place to make a **small deletion** to mimic the disease?



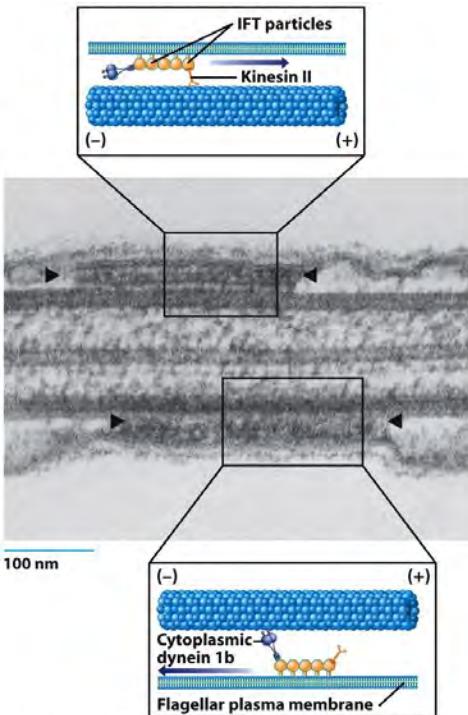
32 genes linked to this disease have been identified in humans

most common: DNAH5 – 35+ alleles
(STEM if ATP hydrolyzes- cannot make conformational change to move)



Kartagener Syndrome

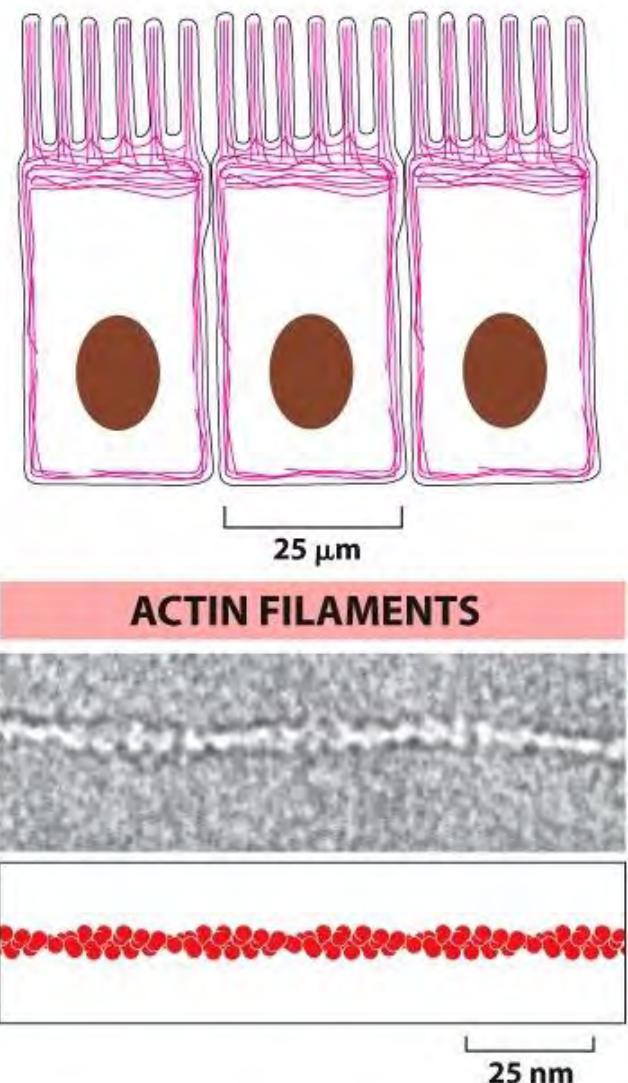
- cilia have defective or no movement
- you decide to also make this mutation in the cytoplasmic dynein that is found in the epithelial cells of the airways. do you expect to see any defect in cilia when only this dynein is mutated?
- yes



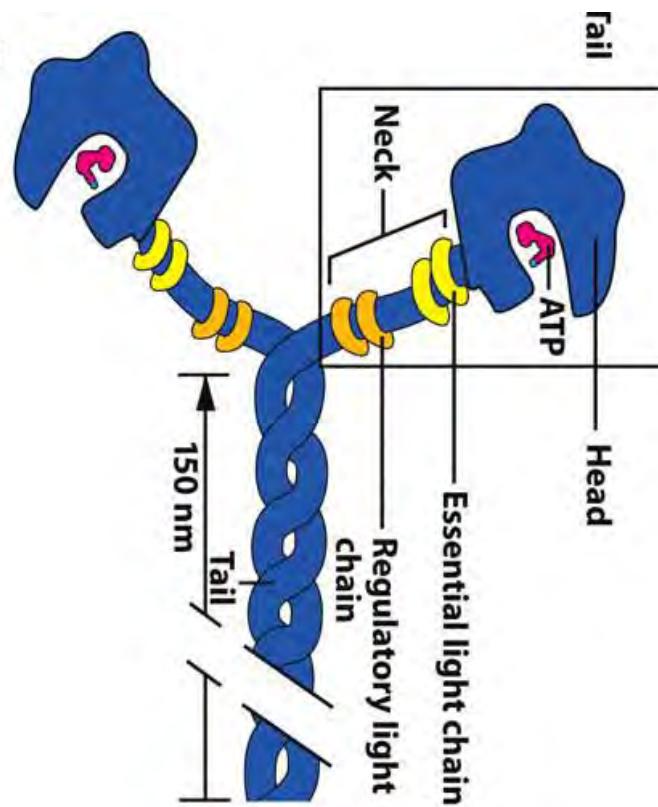
© John Wiley & Sons, Inc. All rights reserved. Micrograph from Keith G. Kozminski et al., *J. Cell Biol.* 131: 1520, 1995, courtesy of Joel L. Rosenbaum; by copyright permission of The Rockefeller University Press.

today: one filament, one motor family

actin

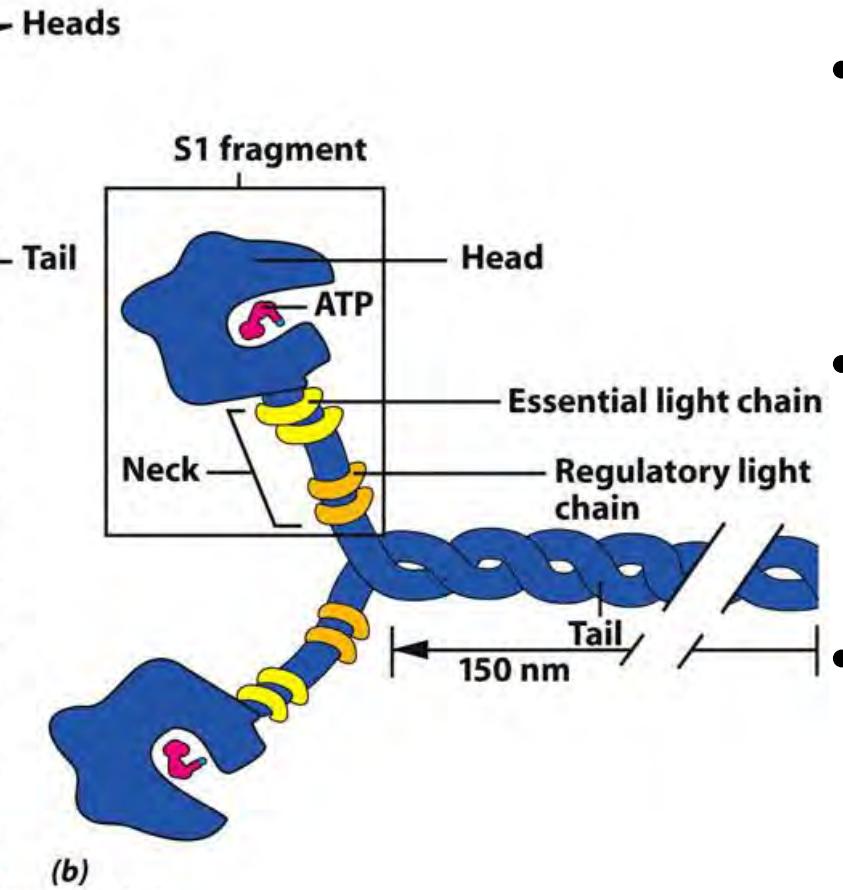


(b)
John Wiley & Sons)

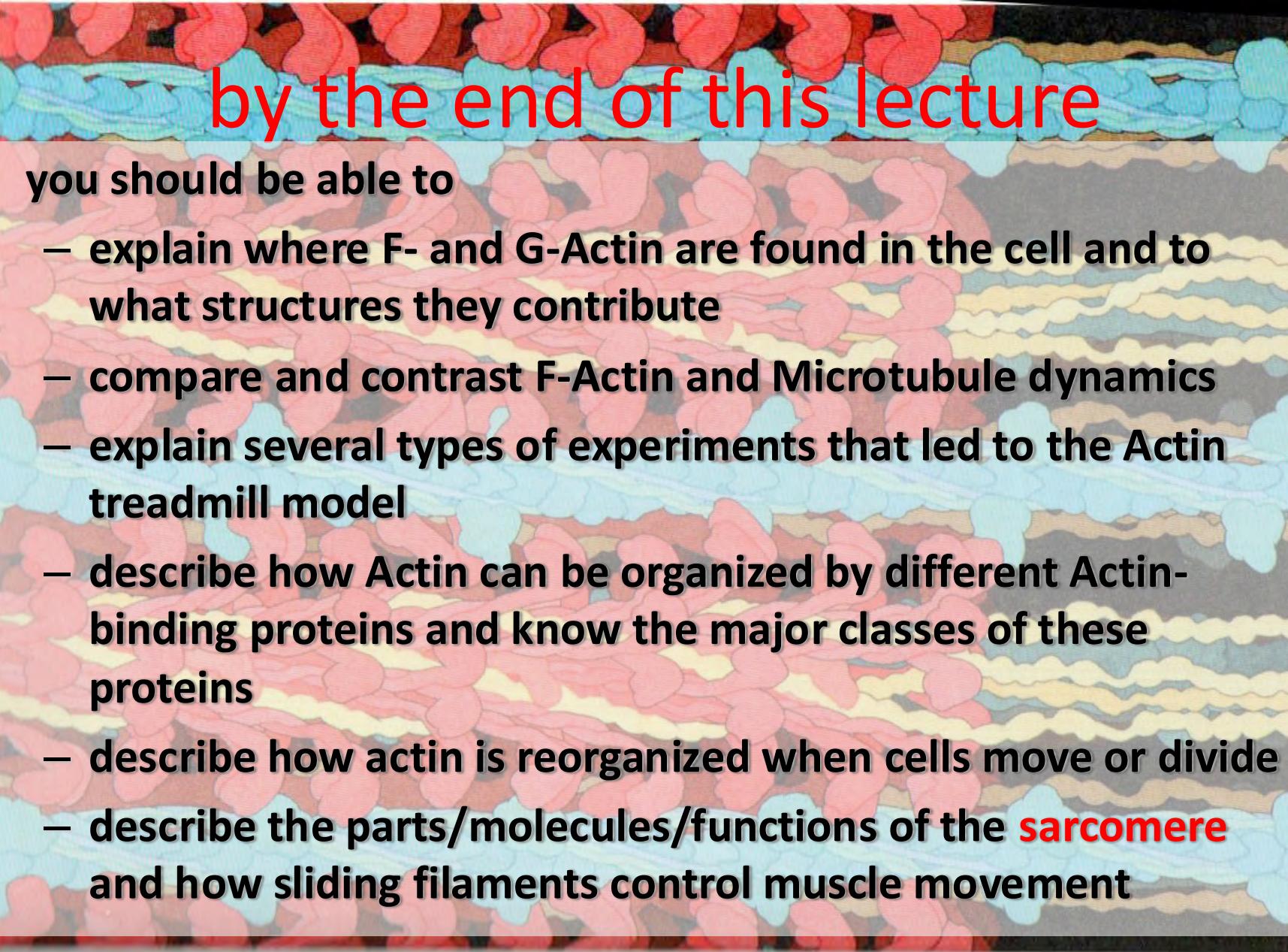


myosin

Myosin motors associate with Actin filaments



- two major classes: conventional (**type II**) primarily in **muscle**, and unconventional
- head domain binds **actin** and hydrolyzes **ATP** to move in power stroke
10nm step size



by the end of this lecture

- you should be able to
 - explain where F- and G-Actin are found in the cell and to what structures they contribute
 - compare and contrast F-Actin and Microtubule dynamics
 - explain several types of experiments that led to the Actin treadmill model
 - describe how Actin can be organized by different Actin-binding proteins and know the major classes of these proteins
 - describe how actin is reorganized when cells move or divide
 - describe the parts/molecules/functions of the **sarcomere** and how sliding filaments control muscle movement

reminders

- Exam 2 Tuesday – use your resources to study
- Bring your id and a writing implement
- my office hours this week- thurs afternoon not friday

three types of cytoskeletal filaments

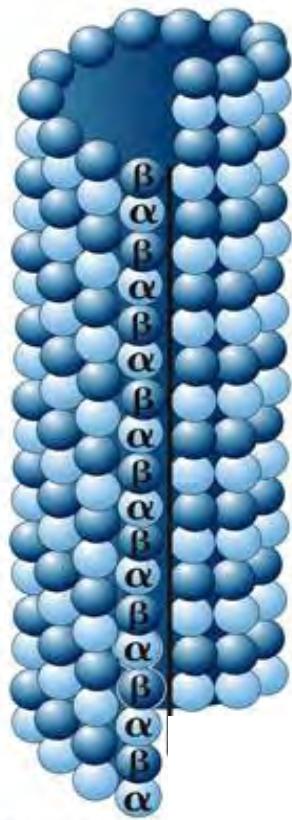
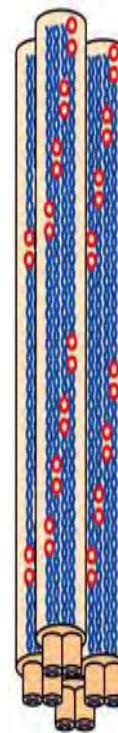
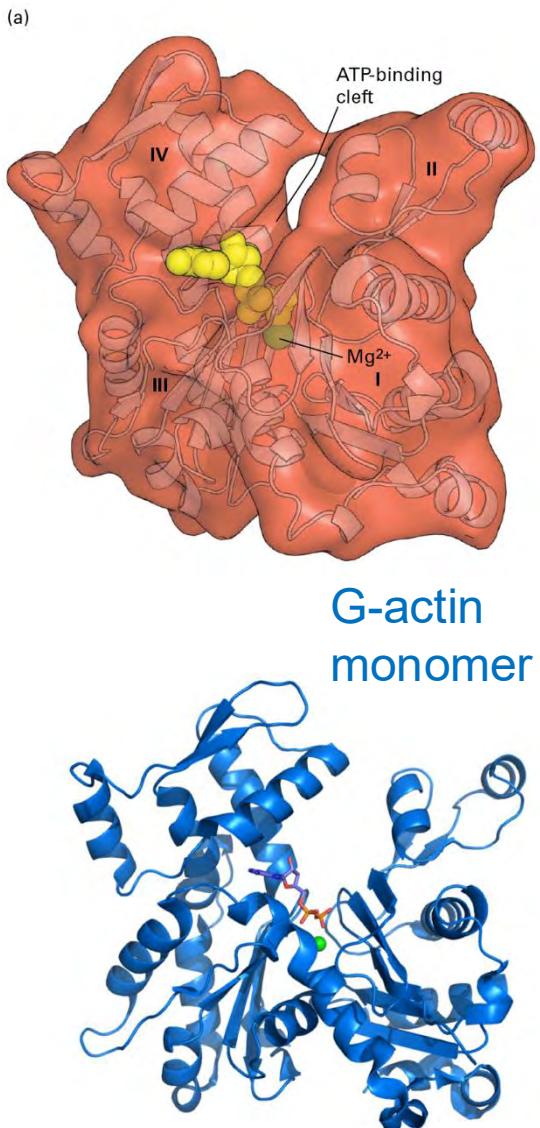


Figure 9-8d Cell and A



microfilaments = actin filaments = F-actin



(b)

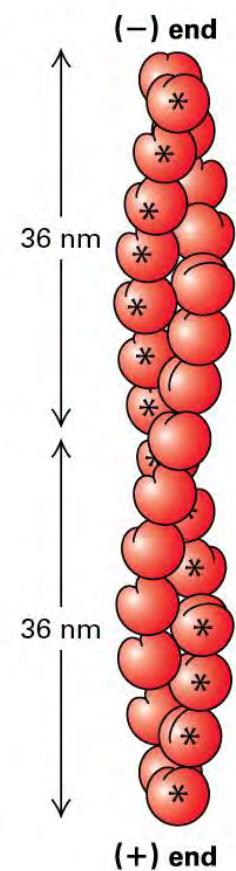
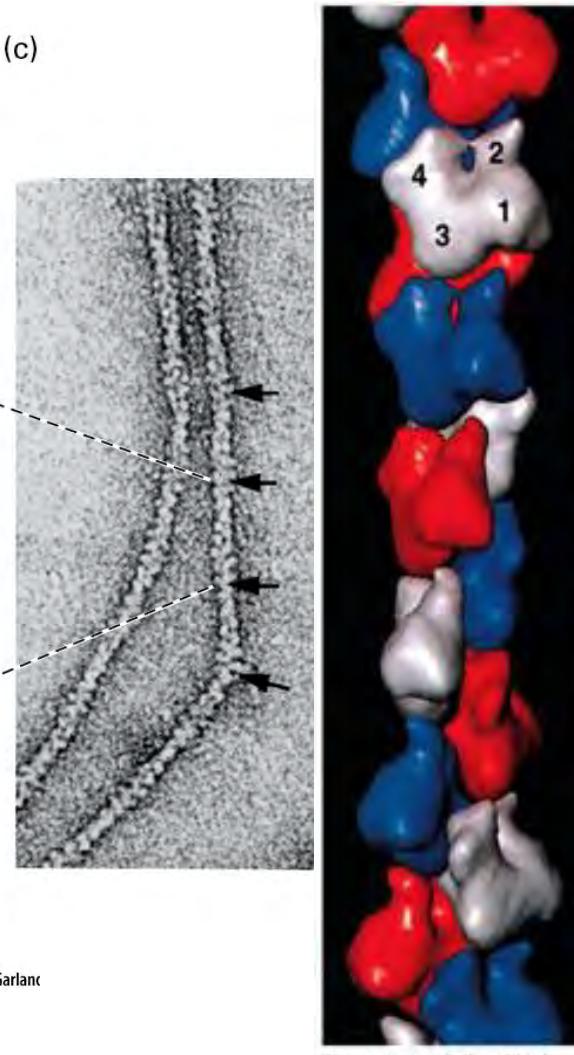


Figure 17-29abc Essential Cell Biology, 4th ed. (© Garland)

(c)



polarized polymers of actin protein monomers (G-actin) = ___ nm in diameter

required for

cell motility/dynamics (fill cell protrusions)

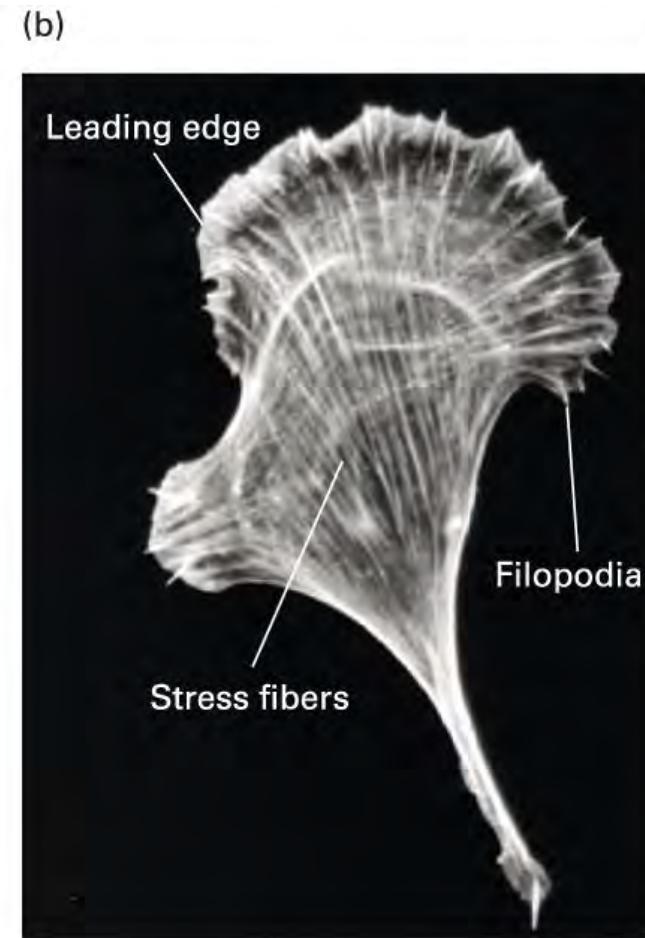
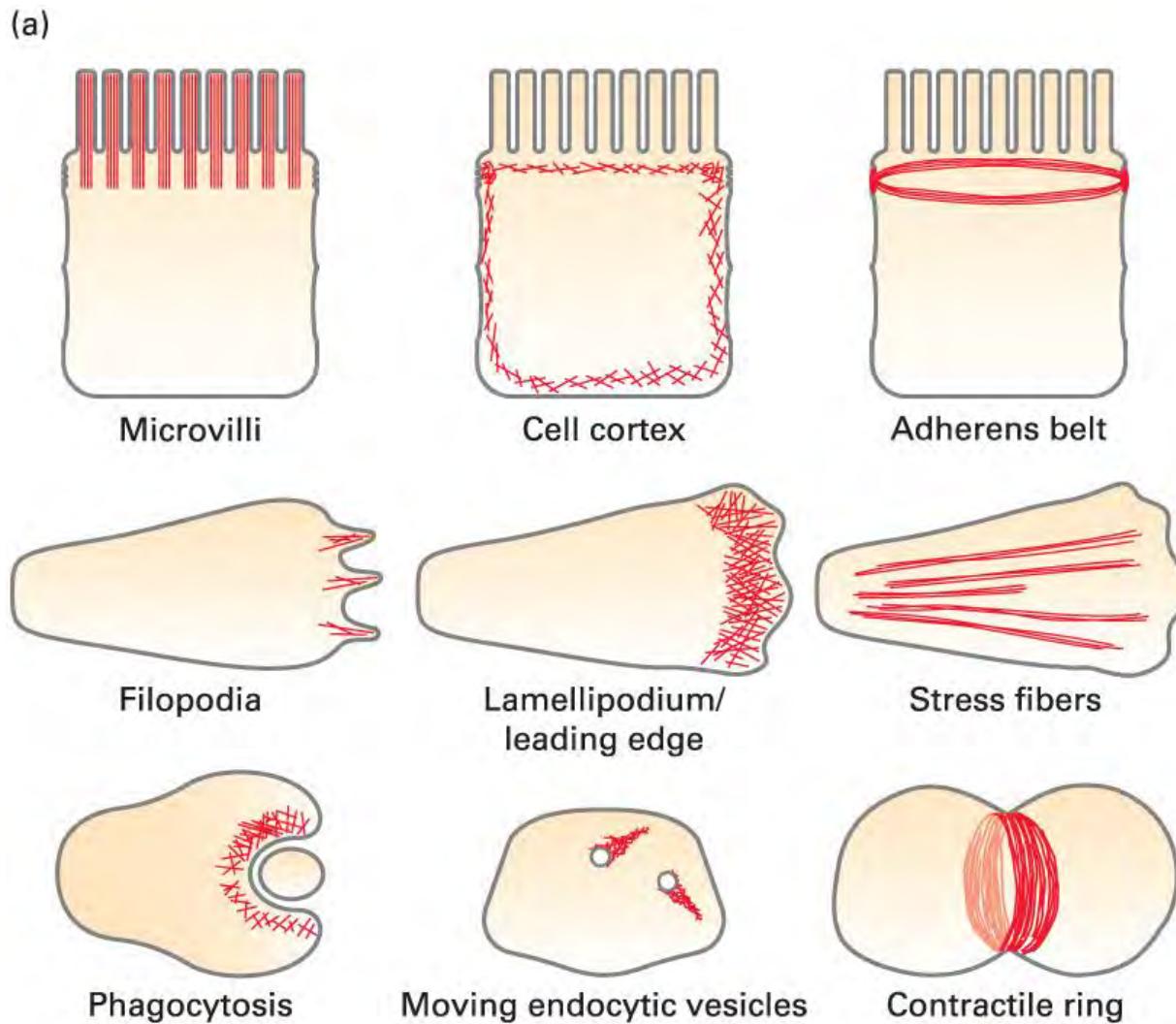
AND transport

AND structural (think: junctions)

assembly requires **ATP** – hydrolyzed within polymer (ADP bound)

can form strands, bundles, branches dependent on context and associated proteins

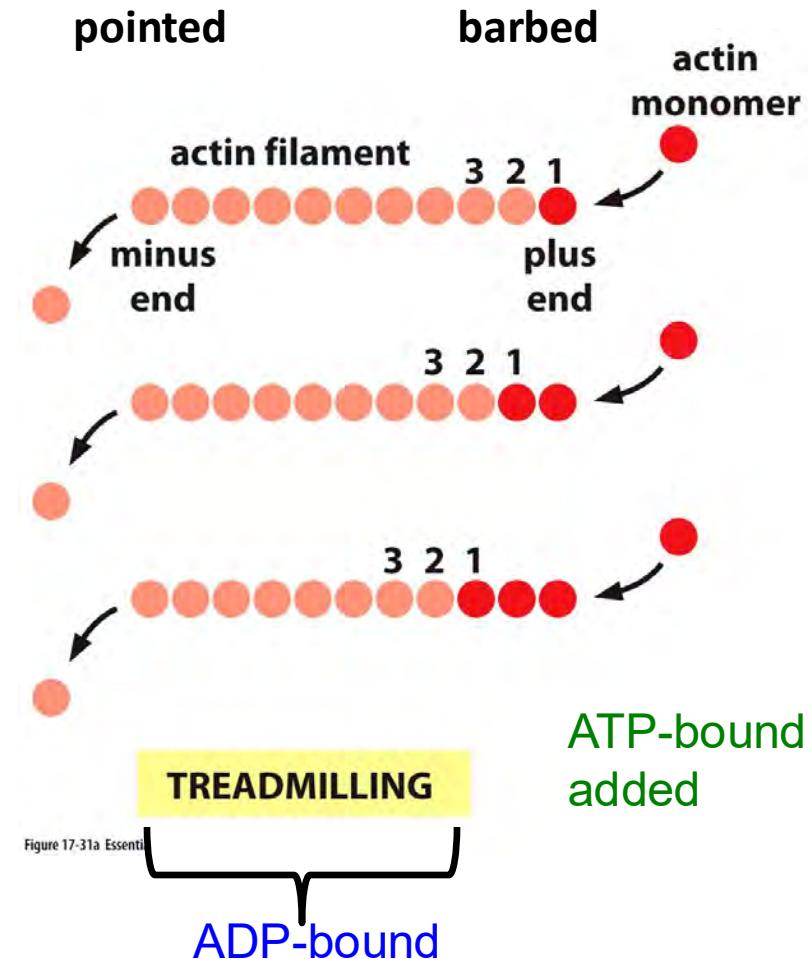
microfilament based structures



Part (b) By permission from J. Victor Small.
Lodish et al., *Molecular Cell Biology*, 9e, © 2021 W. H. Freeman and Company

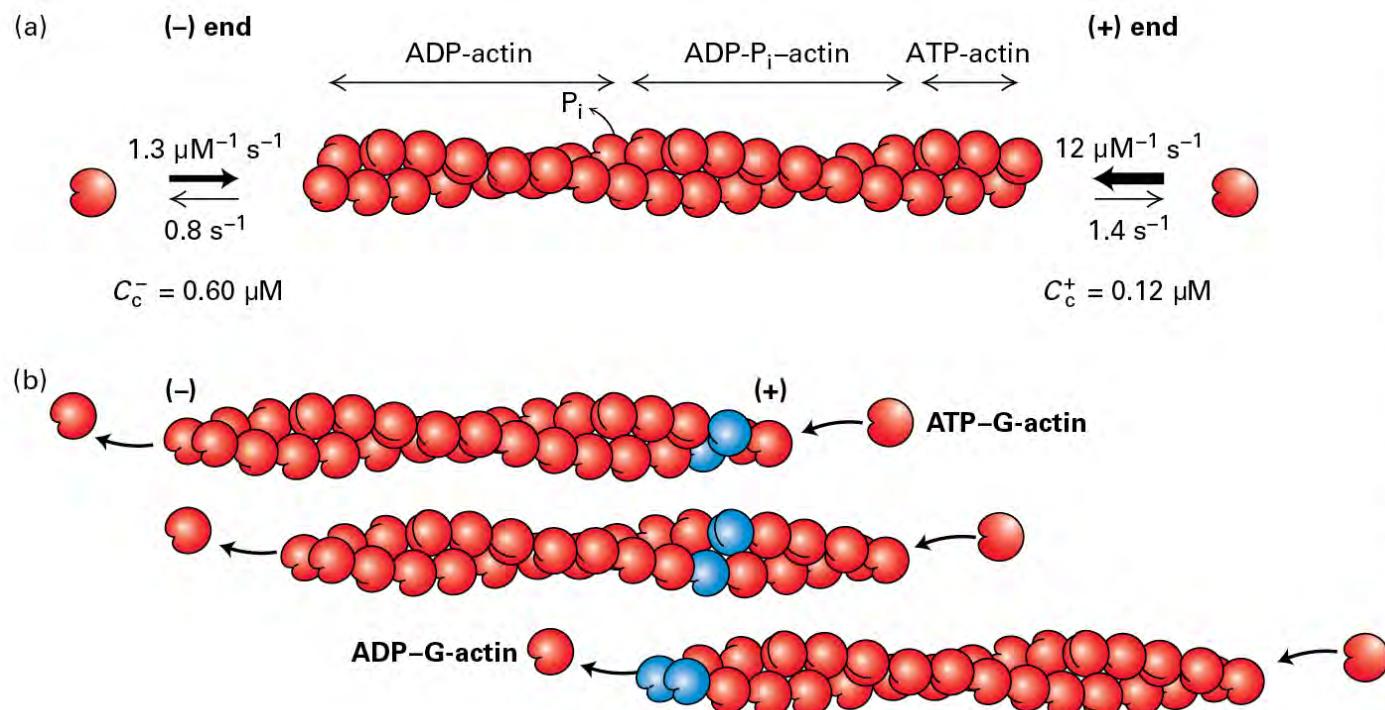
F-actin dynamics

- elongation is much faster than nucleation (like MTs) – need a “seed” or “nucleus” to start well – actin binding proteins facilitate
- subunits incorporated/released at either end, but tend to be added to the **plus** end, released from the minus end



F-actin treadmilling

- the plus end has a higher affinity for **ATP-actin** than the minus end does -> at LOW ATP-actin concentrations, growth at + end
- dynamic equilibrium between monomers and polymers***



Lodish et al., Molecular Cell Biology, 9e, © 2021 W. H. Freeman and Company

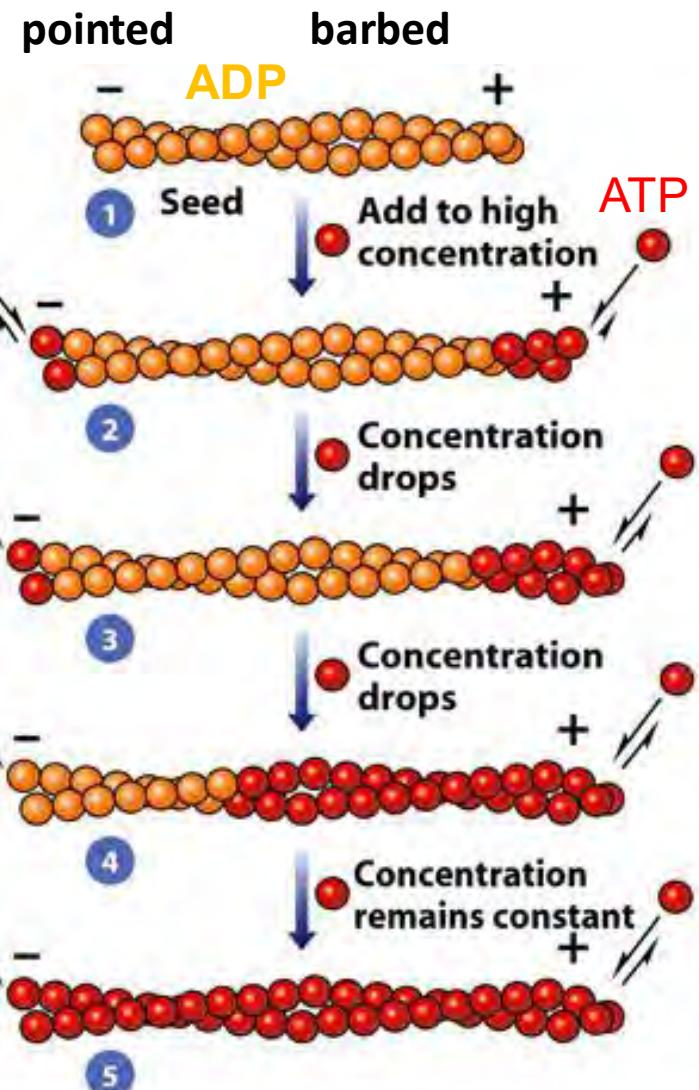


Figure 9-46b Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

Actin binding proteins can promote filament formation

- nucleation is energetically unfavored- must have **nucleating proteins**, ie, the **Arp2/3 complex** or Formins, also (*Listeria*) **ActA**

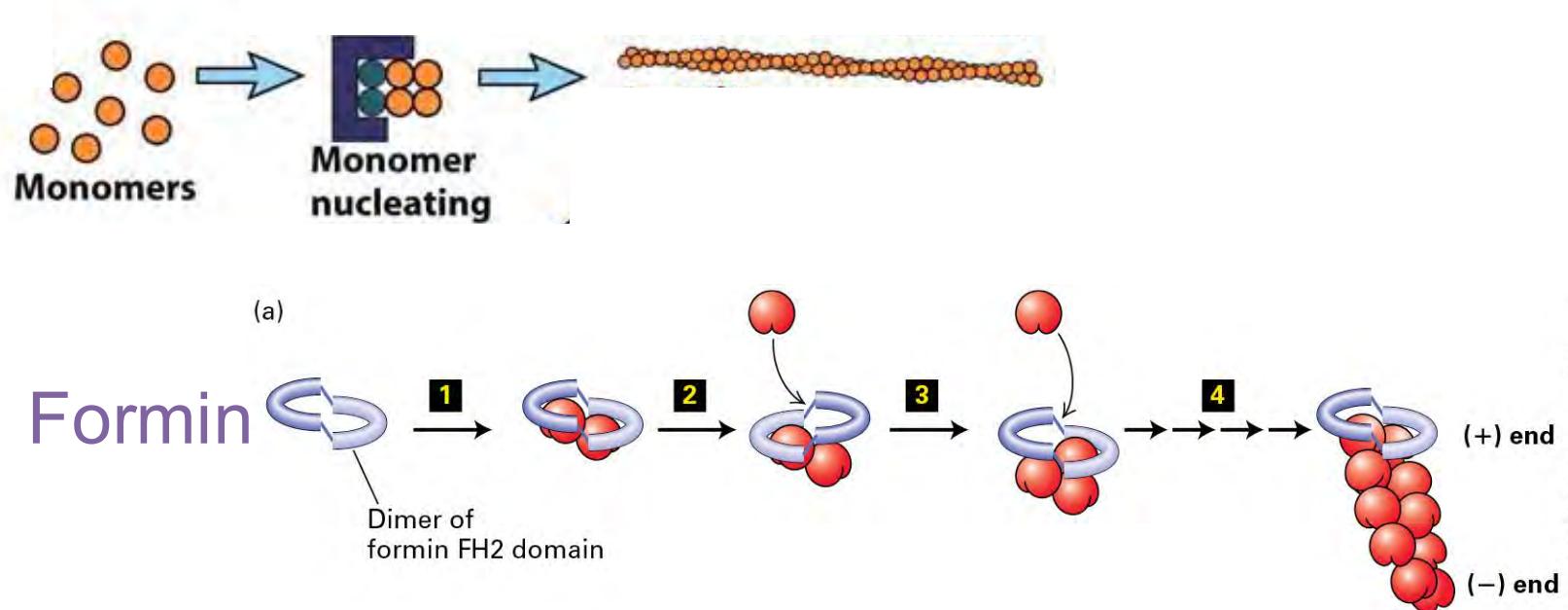
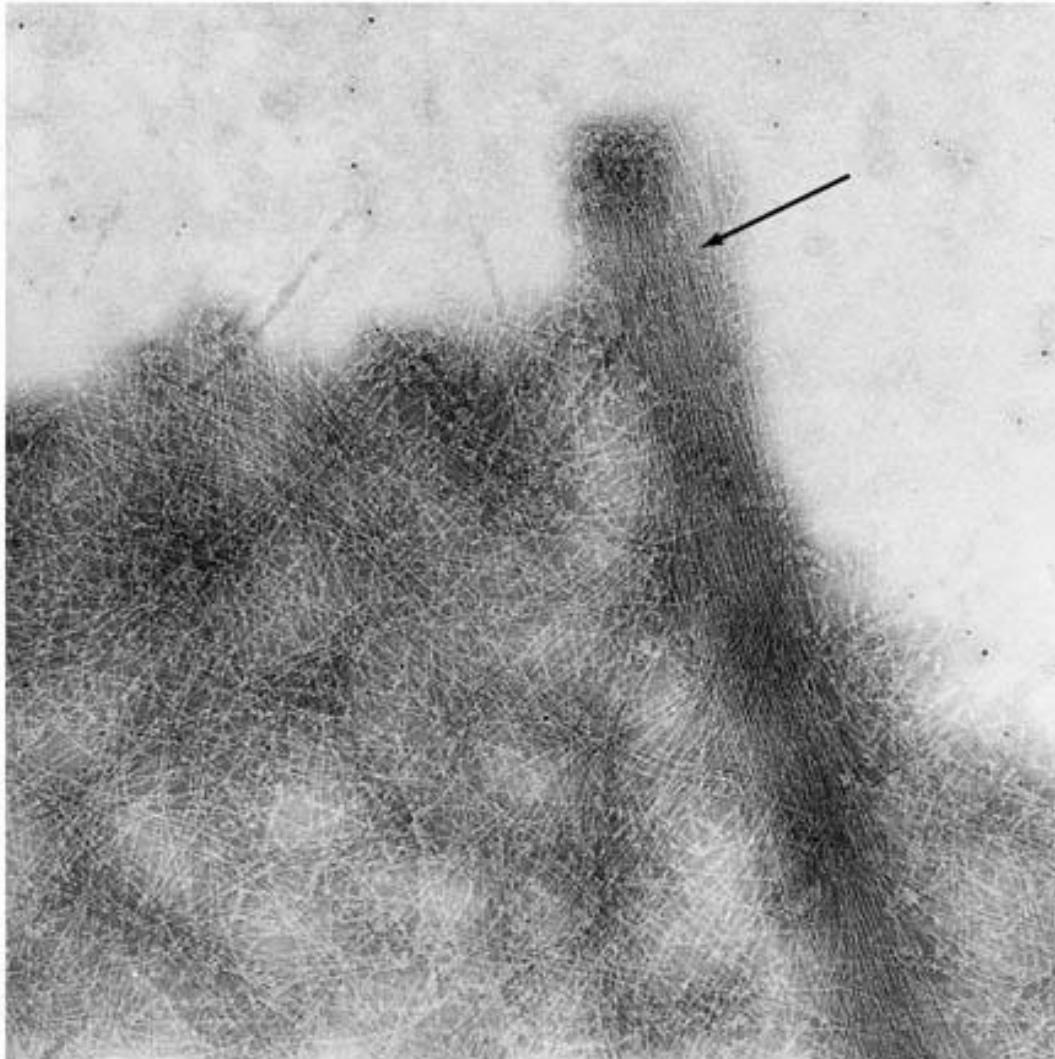


Figure 9-65 Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

Actin can be arranged differently in different situations



- commonly in a layer around the **cortex** (just inside the cell surface) as a mesh – stabilized
- cell biologists often use fluorescently labeled **phalloidin** to see the cortex
- and bundled in projections (**filopodia**) – often dynamic
- or branched in wide protrusions (**lamellopodia**) in motile cells - dynamic

Actin binding proteins have various roles that are important for quick changes in cell shape, motility (dynamics)

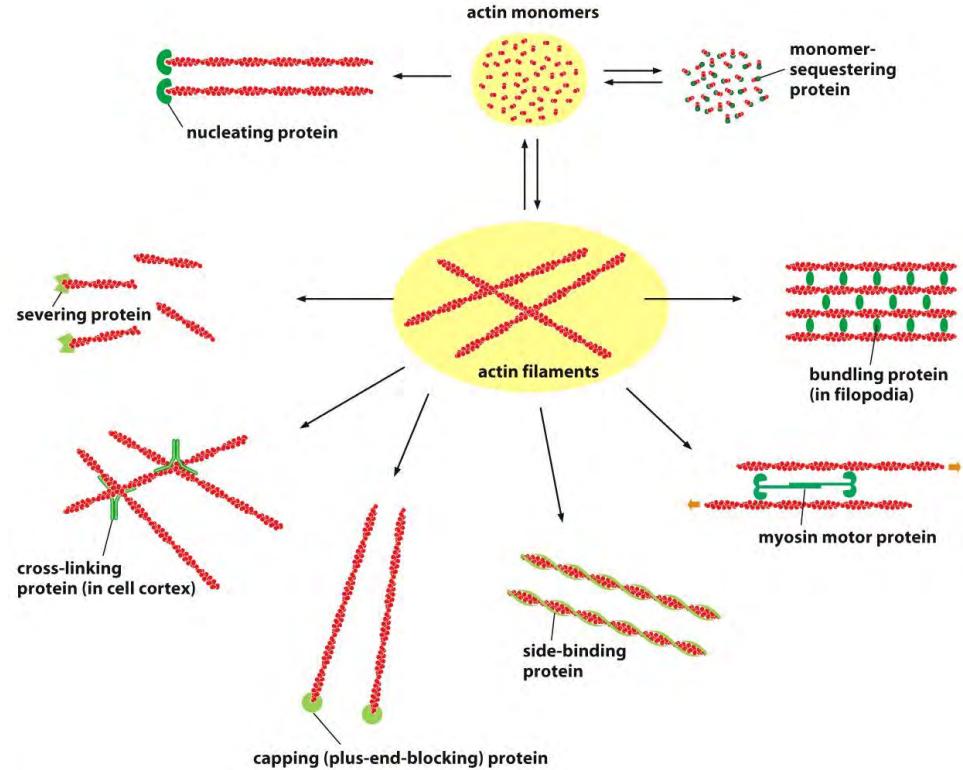
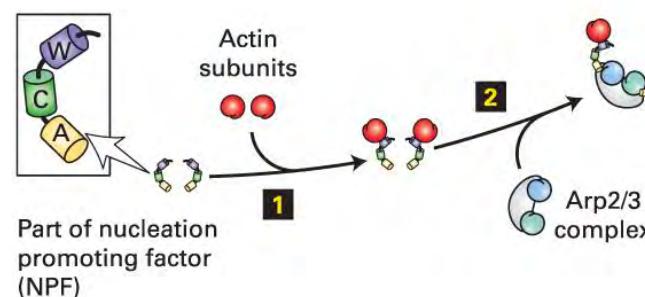
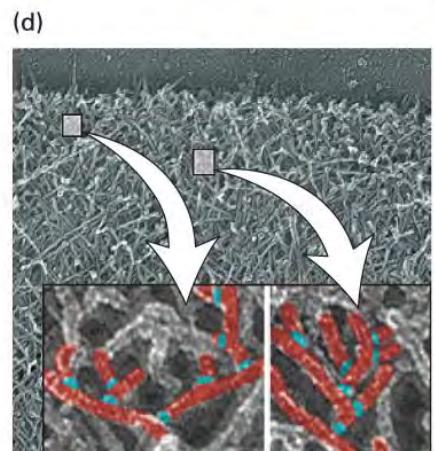
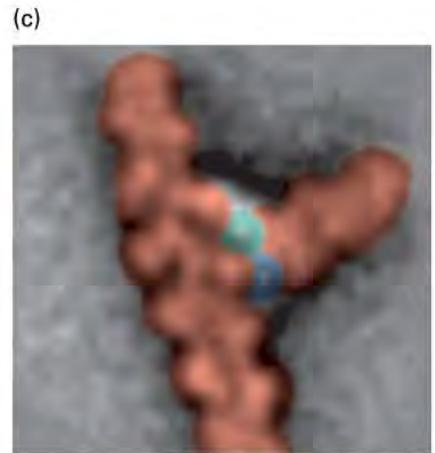


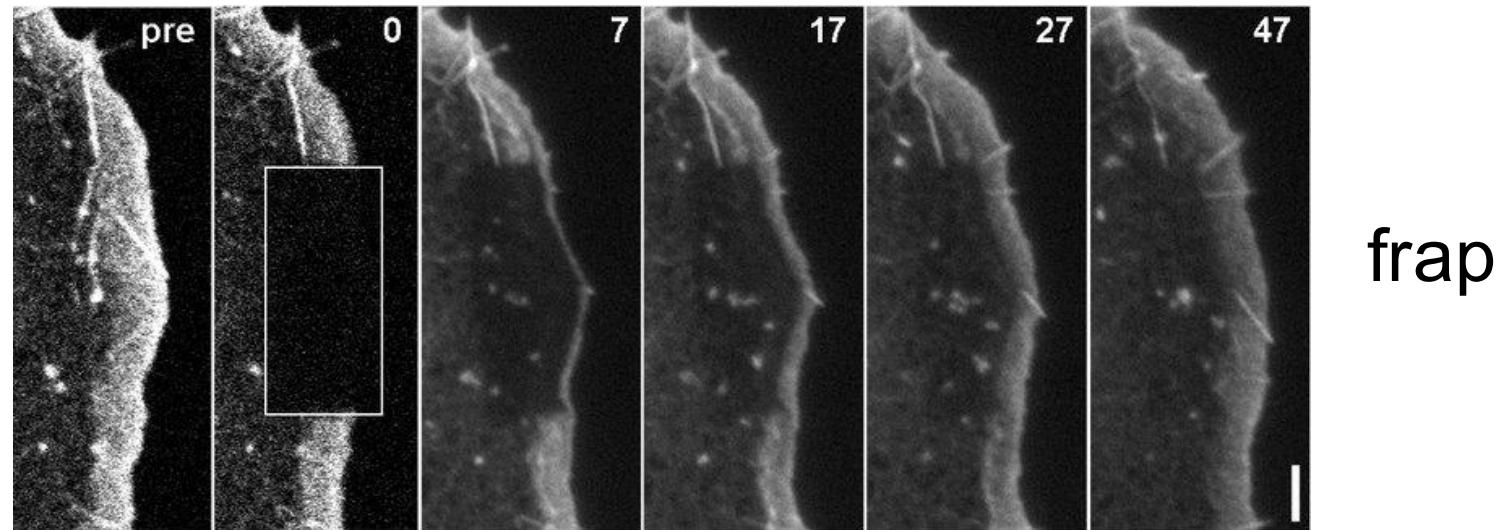
Figure 17-32 Essential Cell Biology, 4th ed. (© Garland Science 2014)



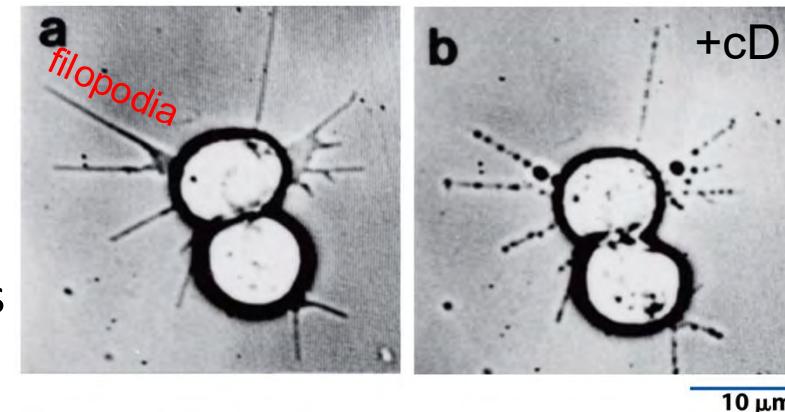
branching



F-actin dynamics and experiments



- other evidence for treadmilling:
 - **important drugs:**
 - cytochalasin D blocks plus ends;
- ★ phalloidin binds filaments and prevents breakdown;
latrunculin binds/sequesters free monomers



From Gerald Karp and Michael Solursh, Dev. Biol. 112:281, 1985.

cytochalasin treated cells – predictions

<http://www.youtube.com/watch?v=NgsECmGSkCM>

after treatment, what will the cells do?

PollEv.com/msg303

Send msg303 to 22333

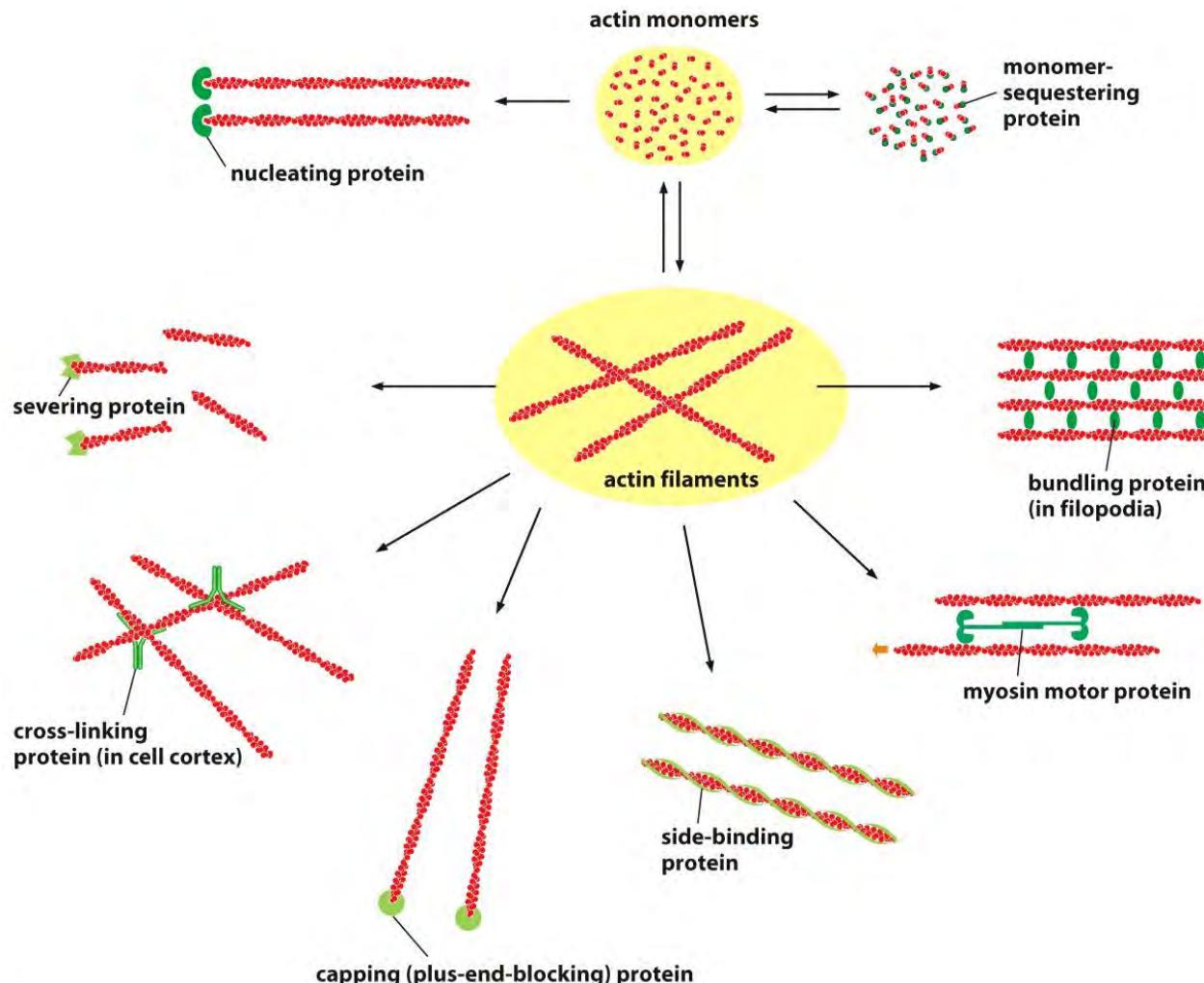
cytochalasin treated cells – predictions – poll



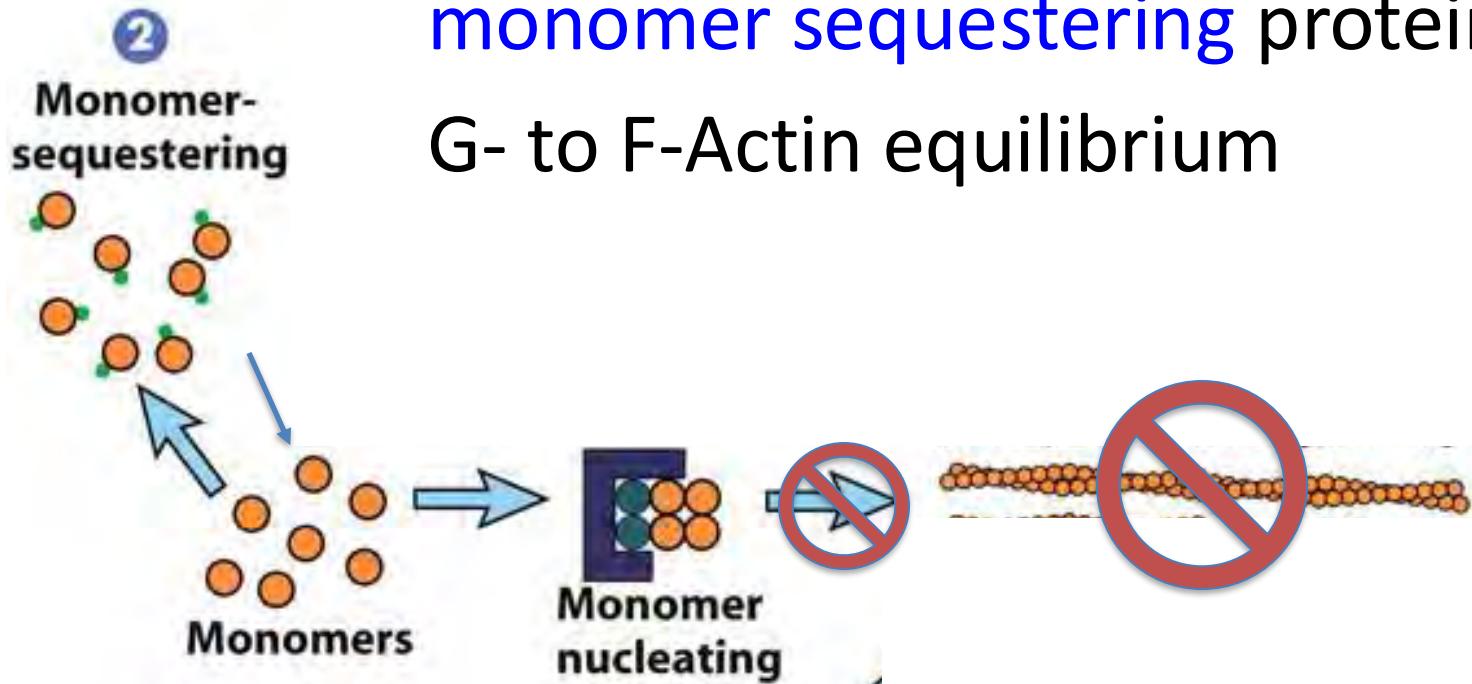
**what aspect of actin dynamics helps
to explain this result?**

- a) elongation depends on “seeds”
because nucleation is slow
- b)treadmilling of subunit
addition/release
- c) stabilization of F-actin by
bundling disrupted
- d)branching prevented by
disruption of Arp2/3

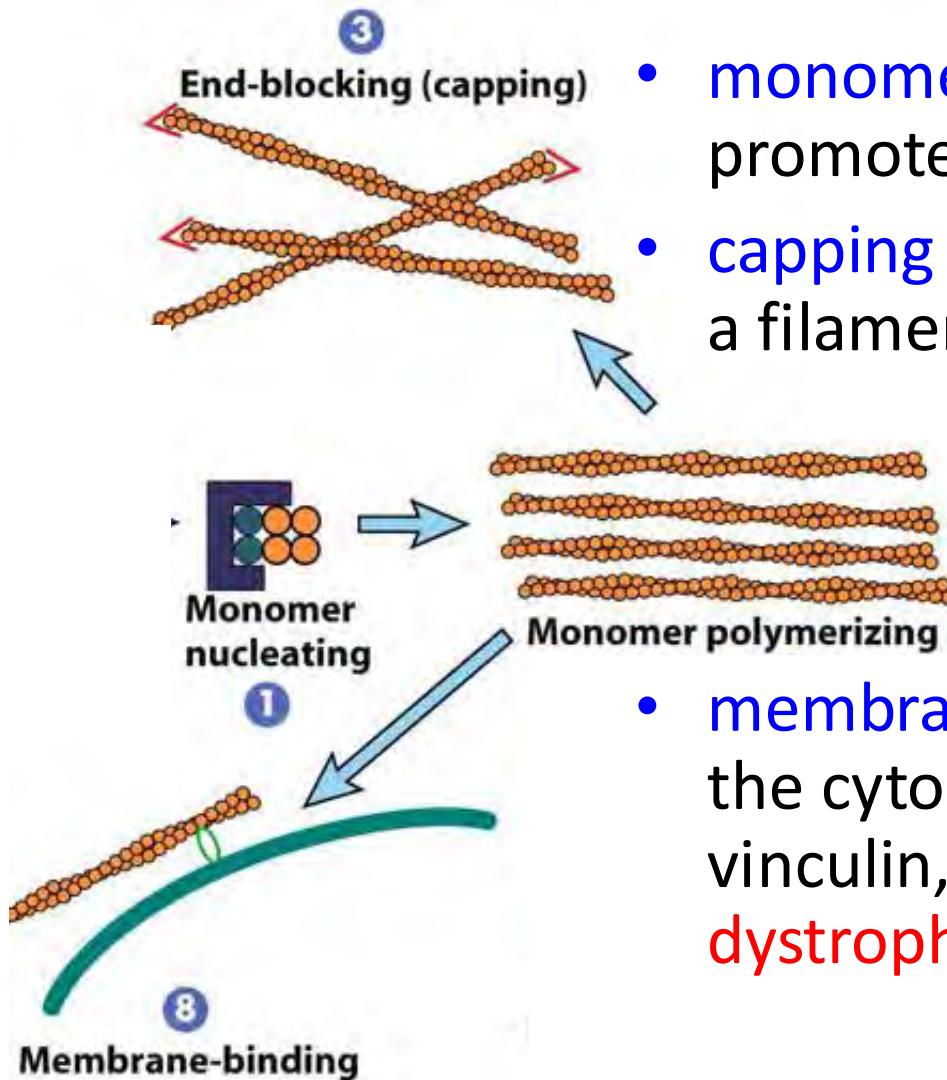
Actin binding proteins have various roles that are important for quick changes in cell shape, motility (dynamics)



Actin binding proteins can reduce filament formation

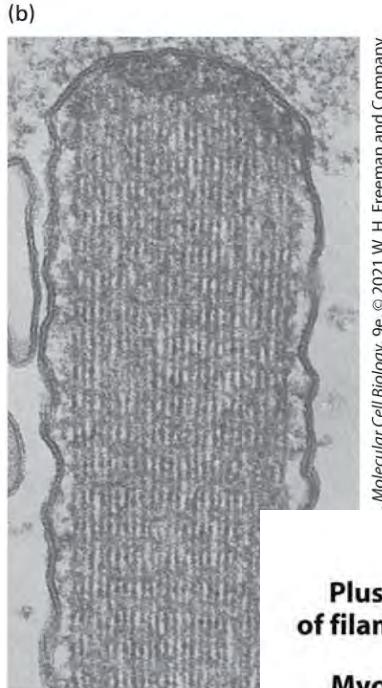
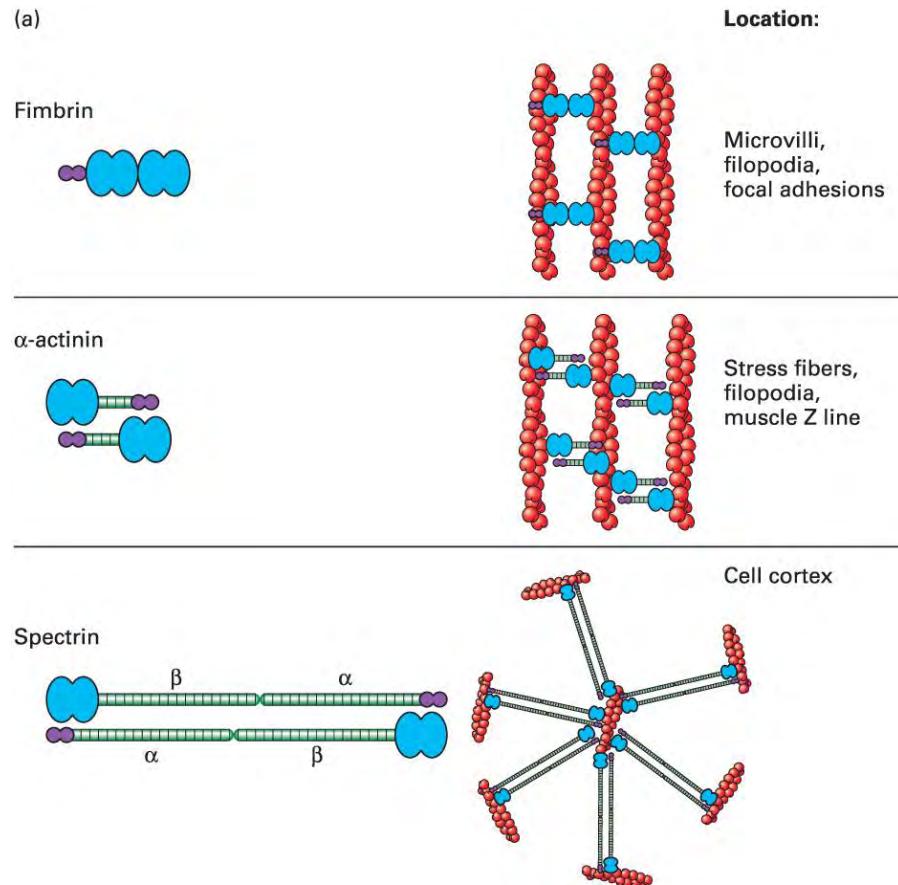


Actin binding proteins can stabilize filaments or link them



- monomer polymerizing proteins promote growth – **Profilin** (activates)
- capping proteins can stabilize length of a filament
- membrane-binding proteins connect the cytoskeleton to the cell surface – vinculin, ERM family members, spectrin, **dystrophin** (muscular dystrophy)

Actin filaments are linked into 3-D structures



cross-linking and
bundling proteins
create strong structures
(microvilli, stereocilia)

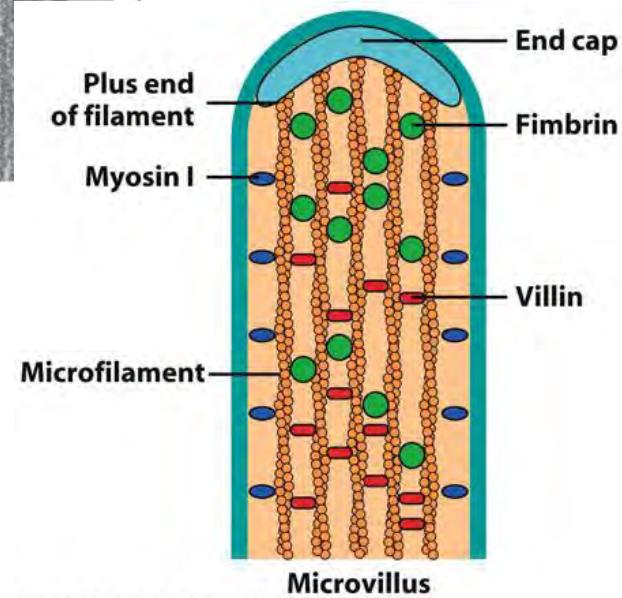
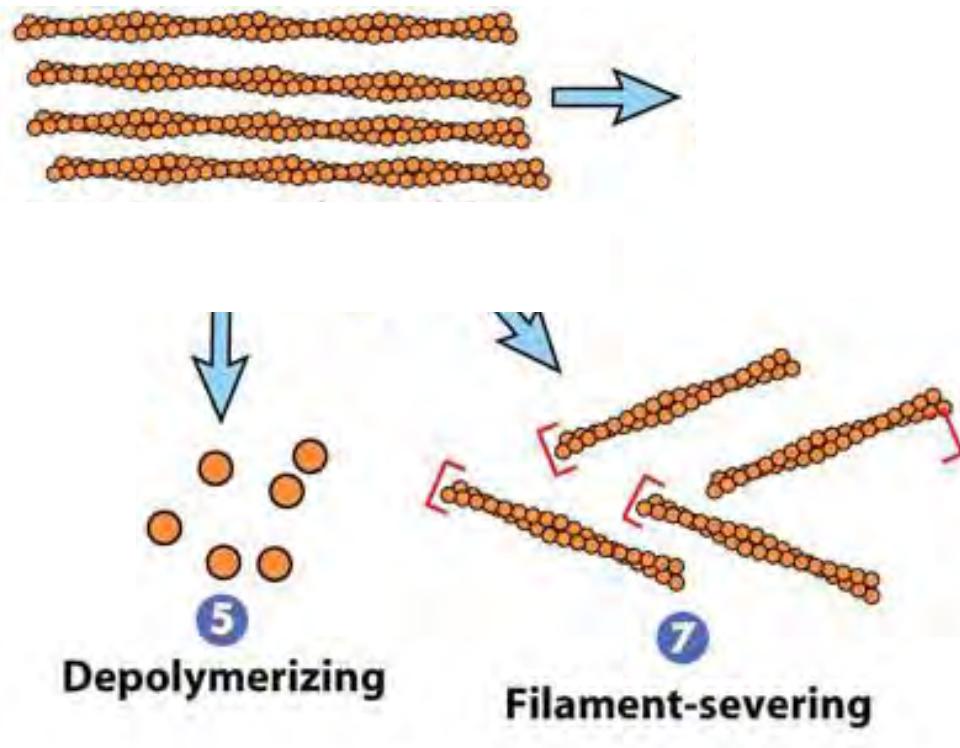


Figure 9-66 Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

Actin binding proteins can destabilize filaments



- filament-severing proteins can break filaments into smaller filaments (**cofilin**, gelsolin)
- depolymerizing proteins (also cofilin)

Listeria capitalizes on host Actin

- Listeria are Gram-positive bacteria that can cause infection
- (*Shigella flexineri* also)
- *Listeria monocytogenes* moves through a cell by polymerizing Actin via ActA
- *movie*
- <https://www.youtube.com/watch?v=6EAAvwTaHKE>

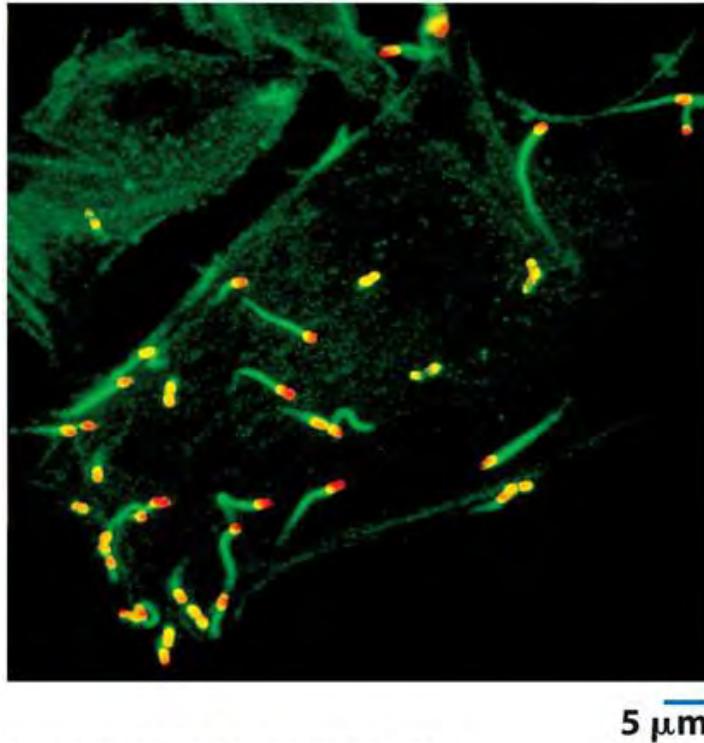
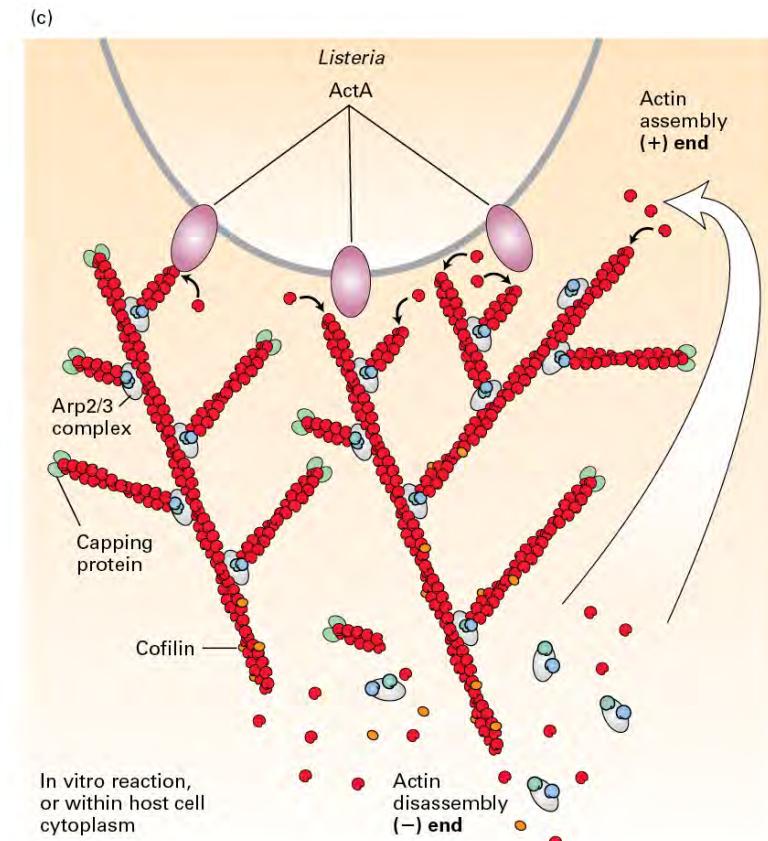
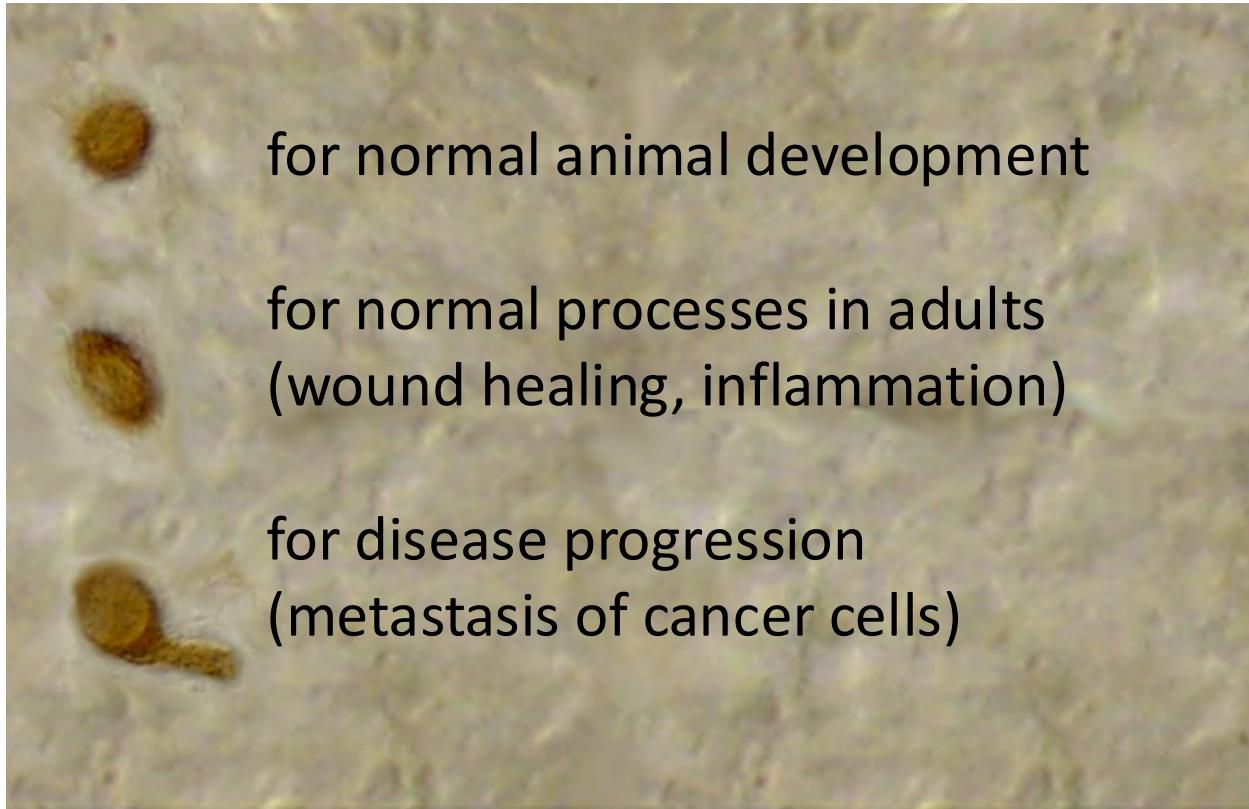


Figure 9-67a Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)



eukaryotic cell migration is essential to biology



for normal animal development

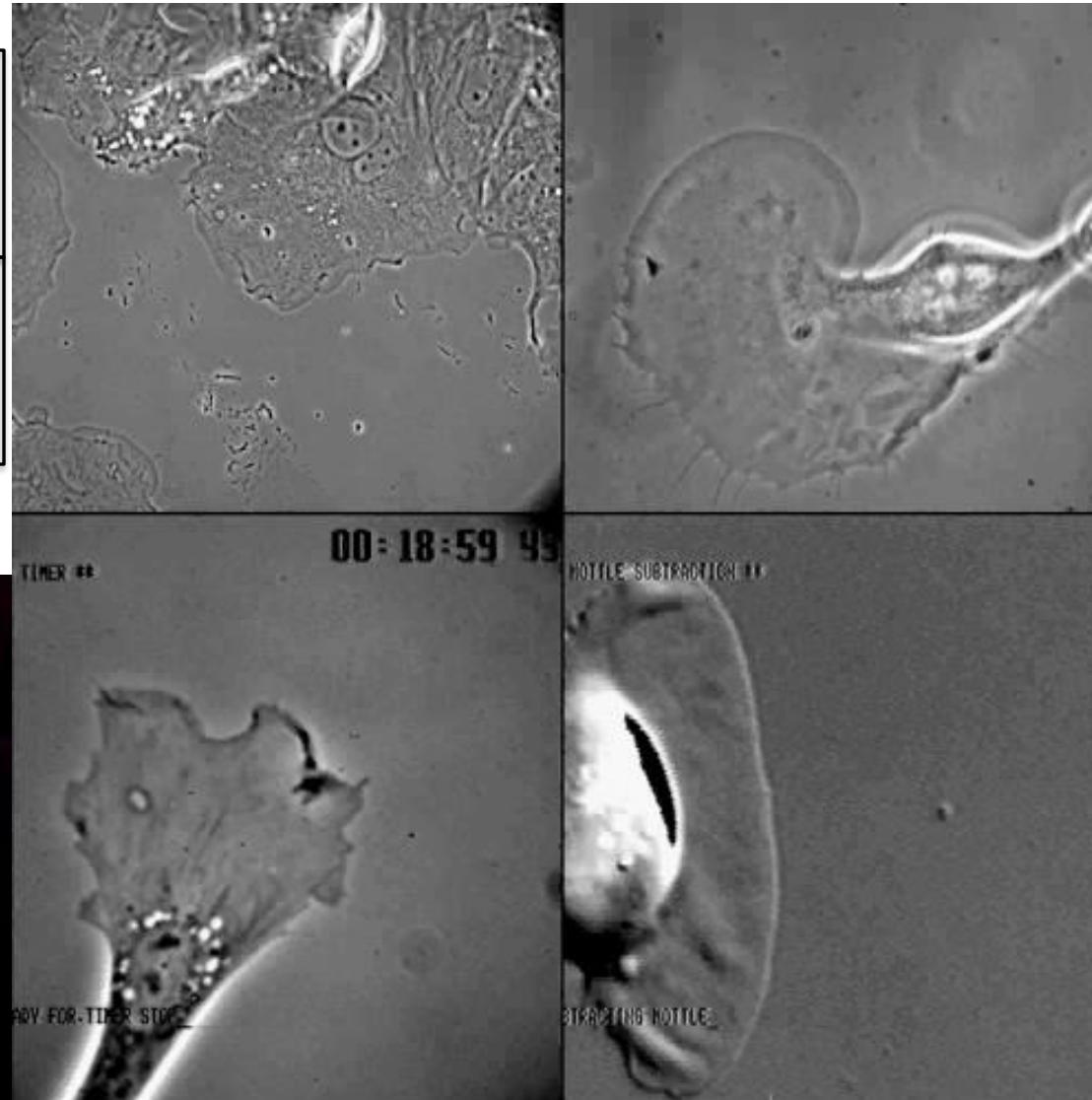
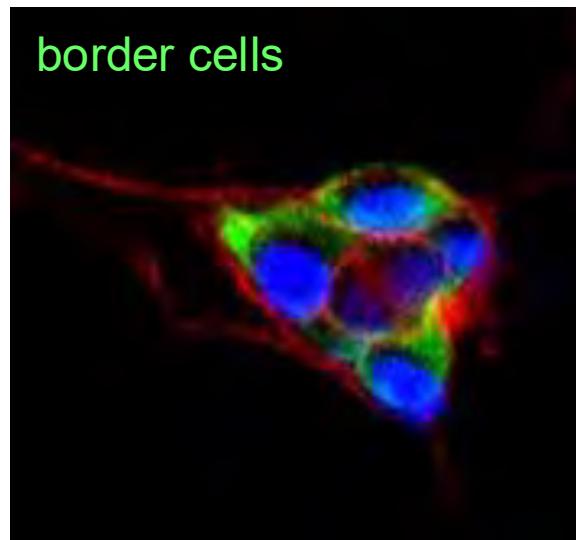
for normal processes in adults
(wound healing, inflammation)

for disease progression
(metastasis of cancer cells)

"It is not birth, marriage, or death, but gastrulation which is truly the most important time in your life." Louis Wolpert

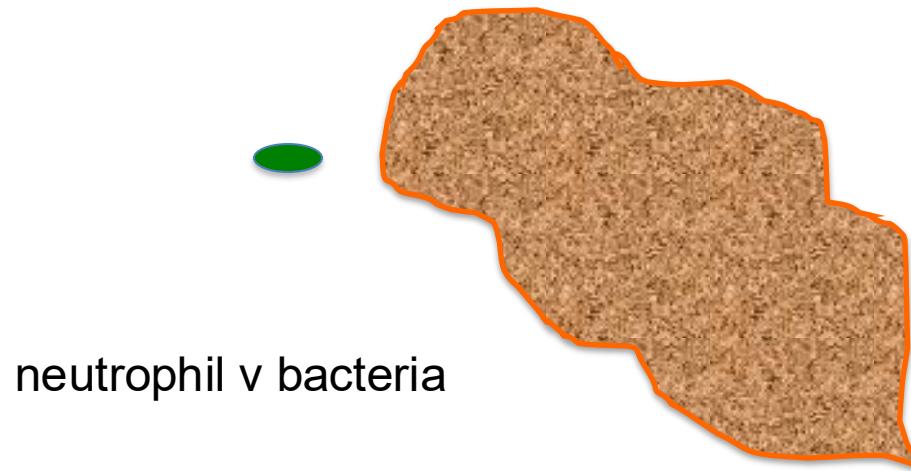
eukaryotic cell migration and motility

mouse fibroblasts	mouse melanoma cell
chick fibroblast	fish keratocyte



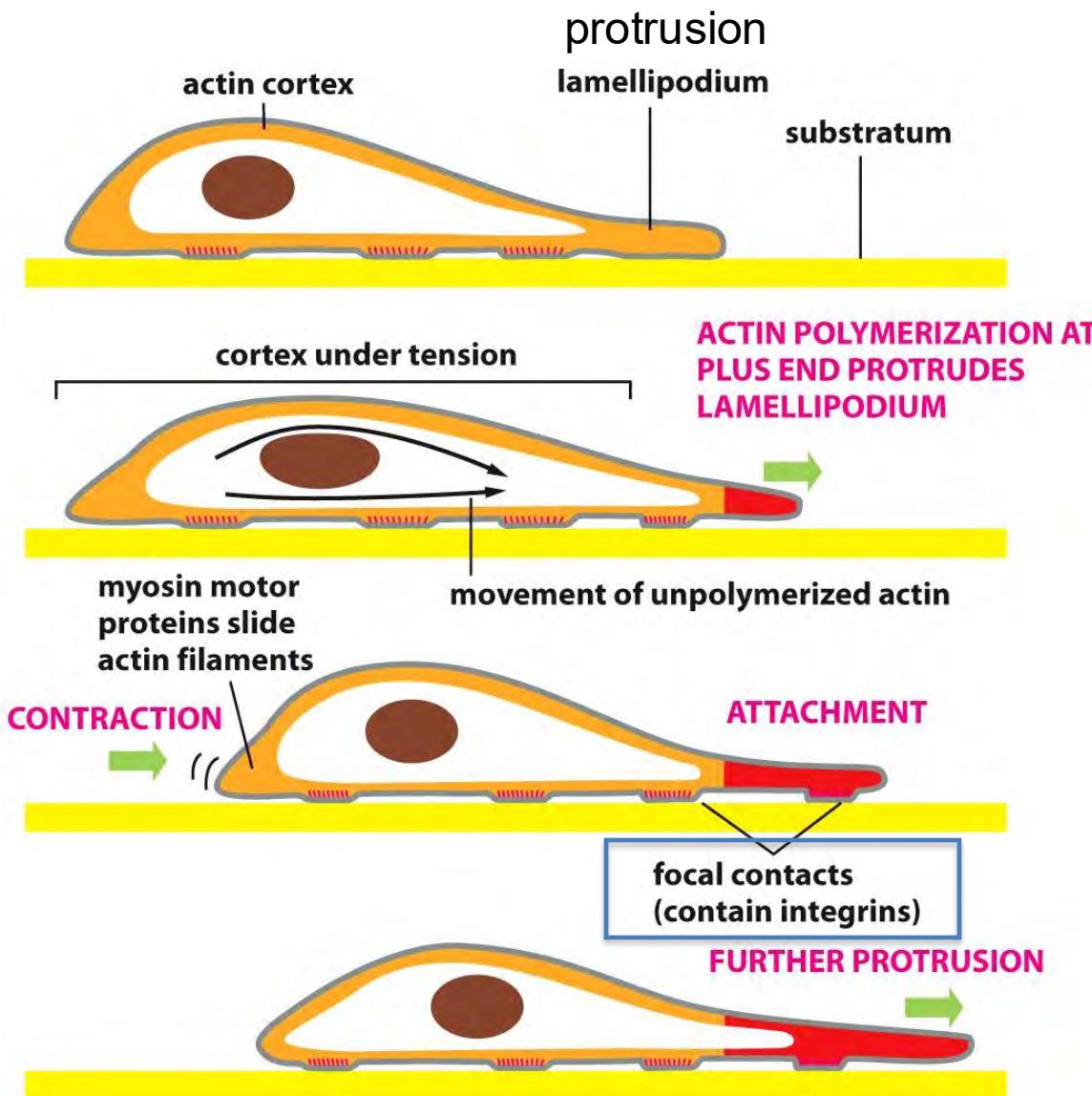
a classic film

- <https://www.youtube.com/watch?v=VAhM9OxZDkU>



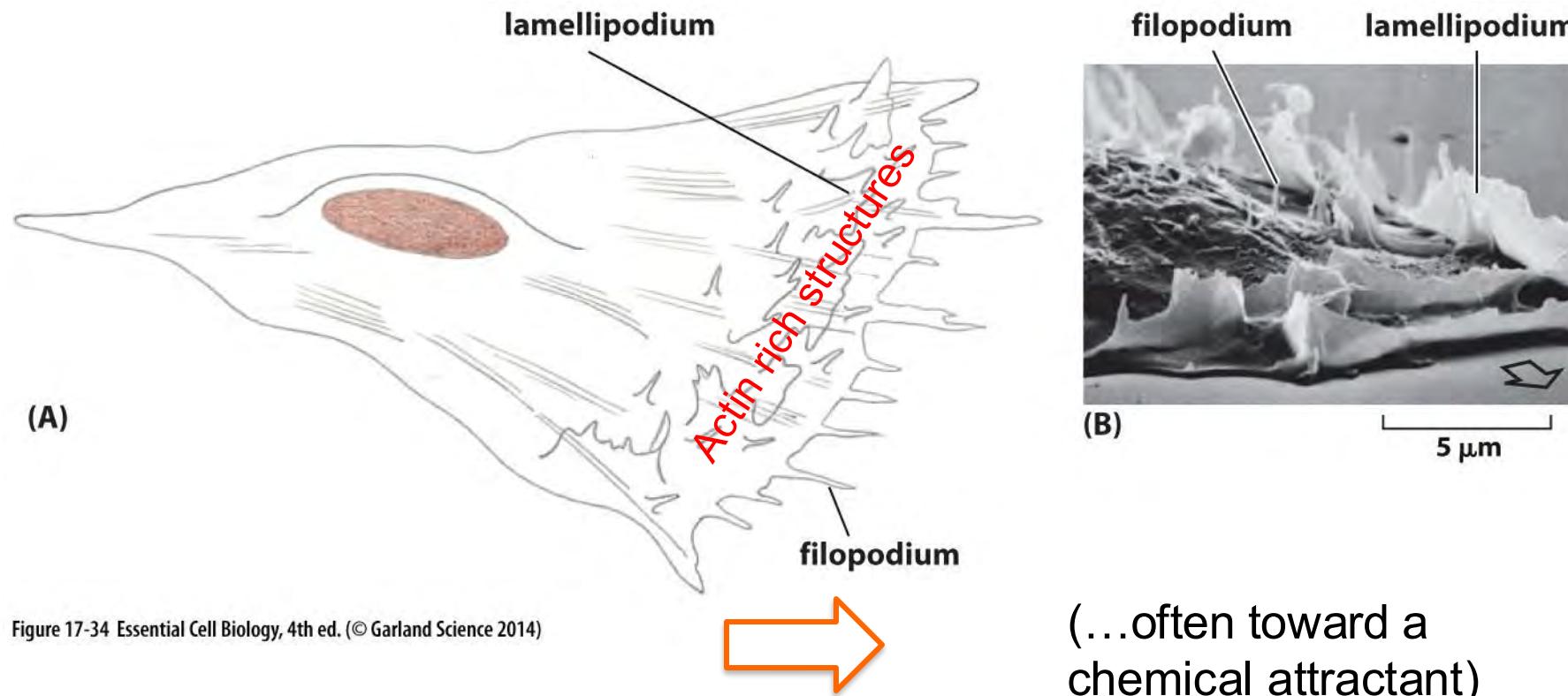
neutrophil v bacteria

the process of a migrating cell



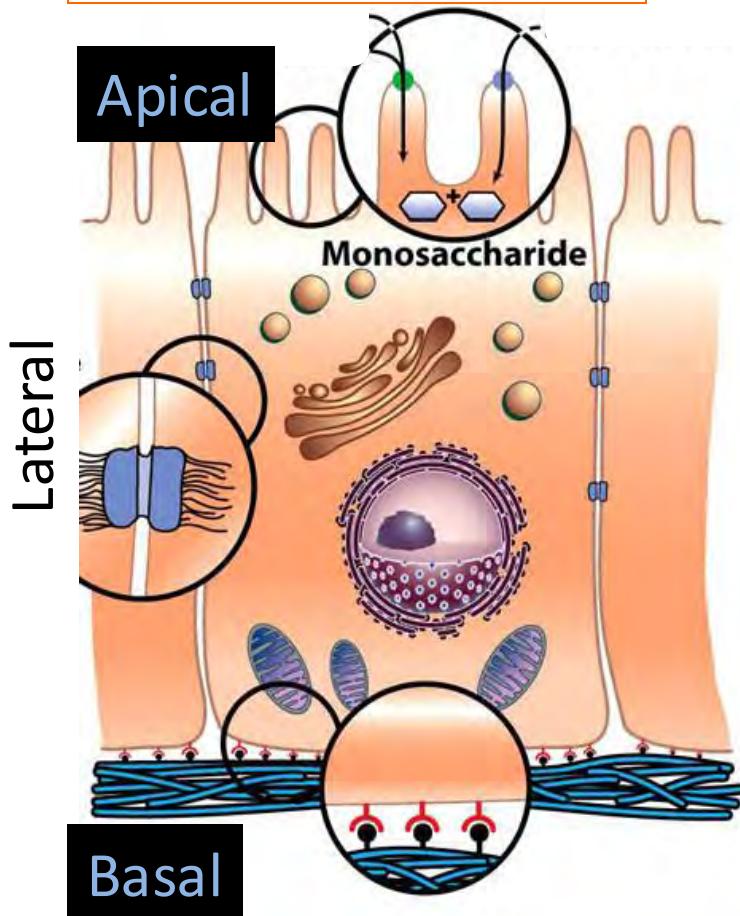
re-assess/repeat

a migrating cell makes forward protrusions and moves directionally

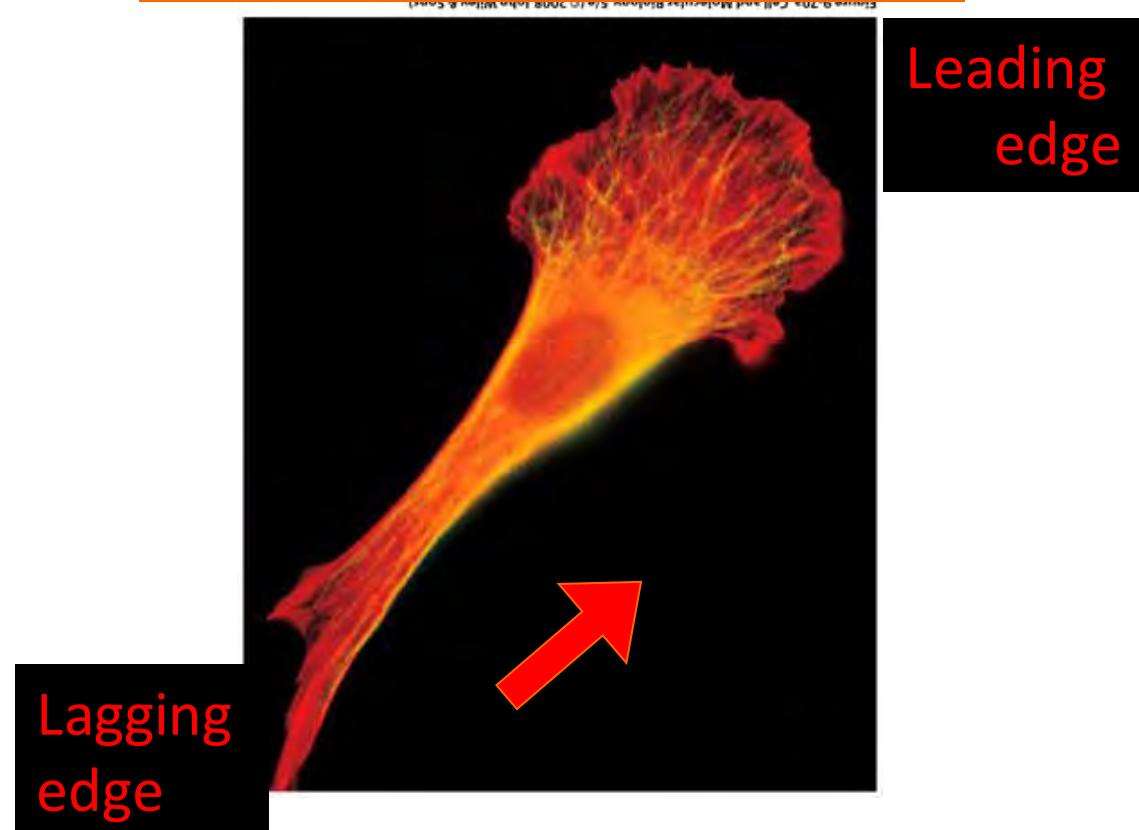


cell polarity

an epithelial cell has constant polarity

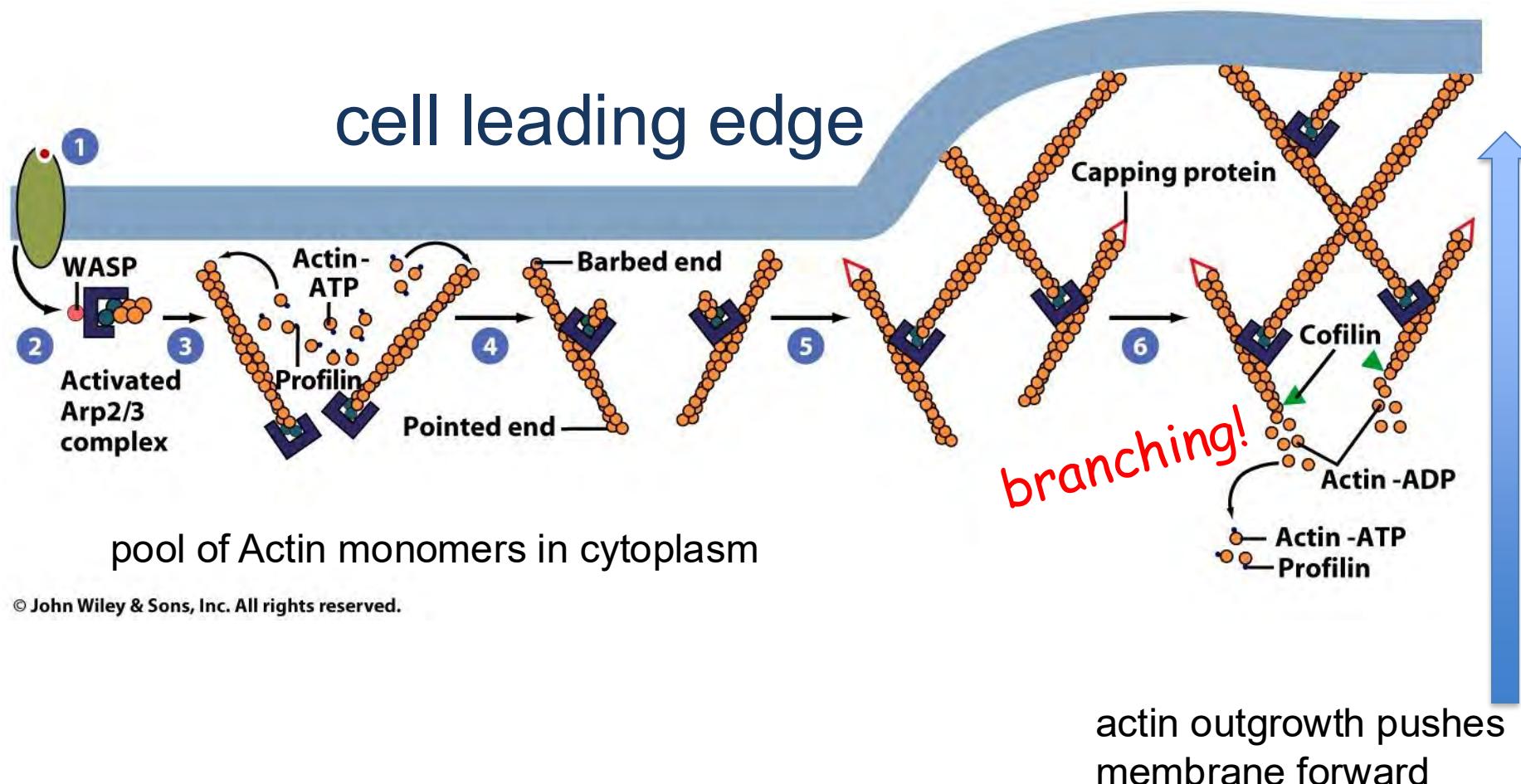


a migrating cell polarizes in response to the environment



relocalization of Golgi, nucleus, MTOC towards leading edge (probably cell type dependent)

organized changes in actin structures coordinate cell motility



the Rho family of monomeric GTPases promote actin reorganization

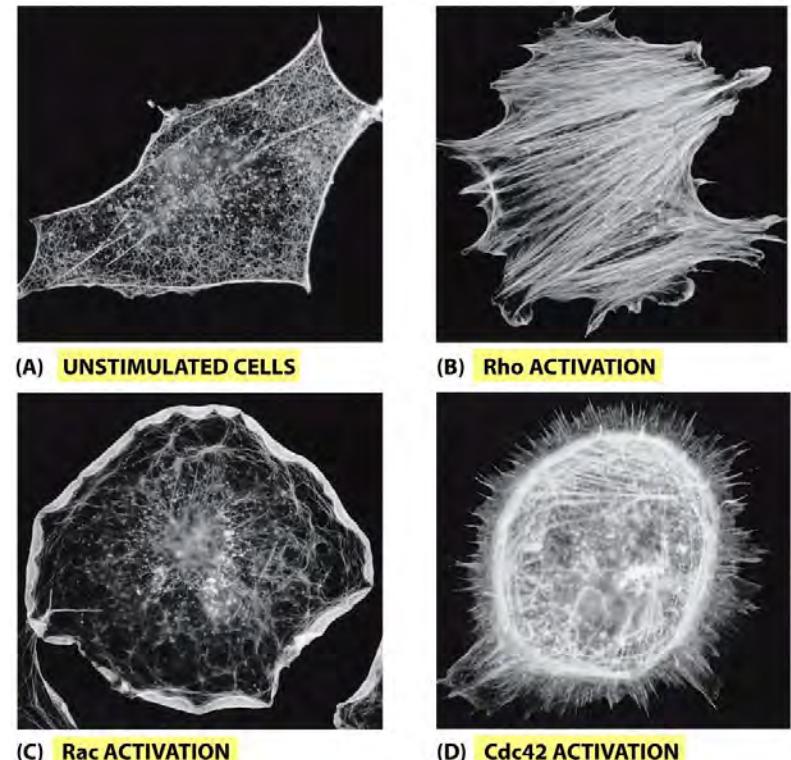
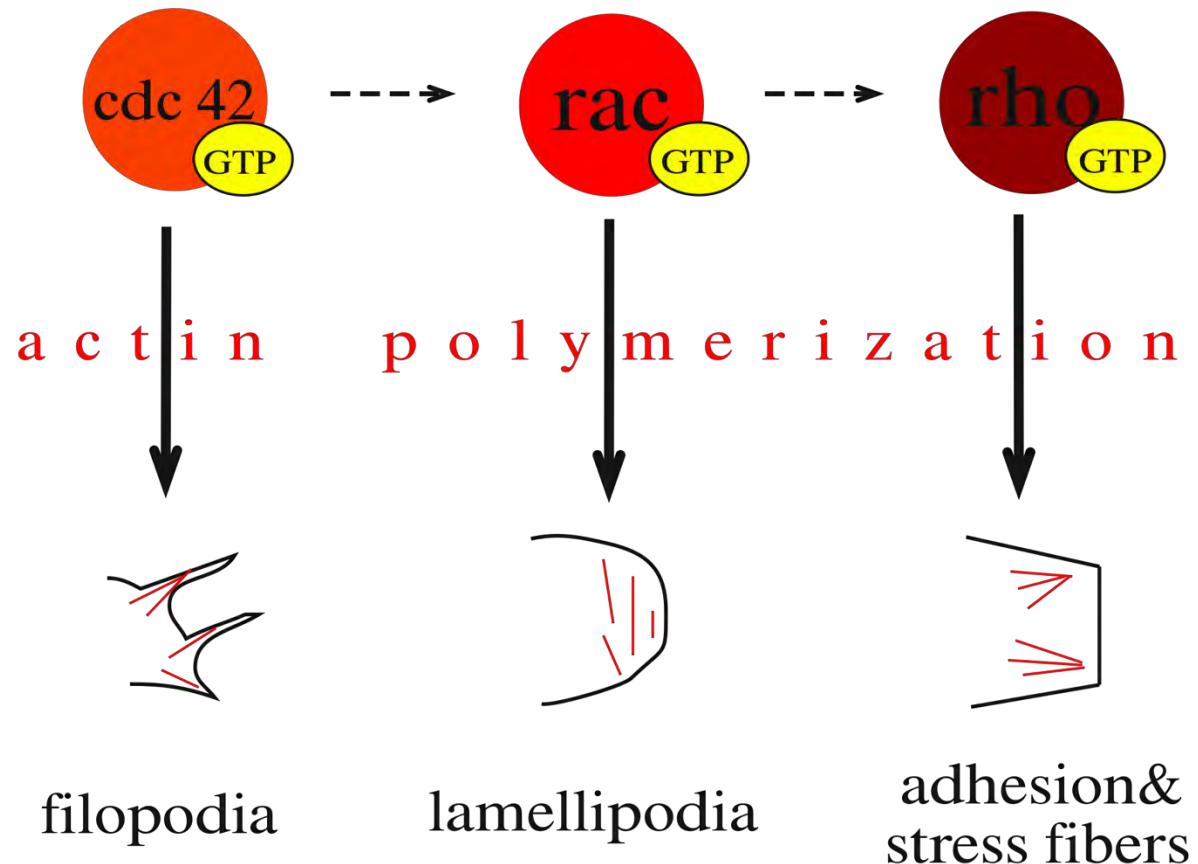
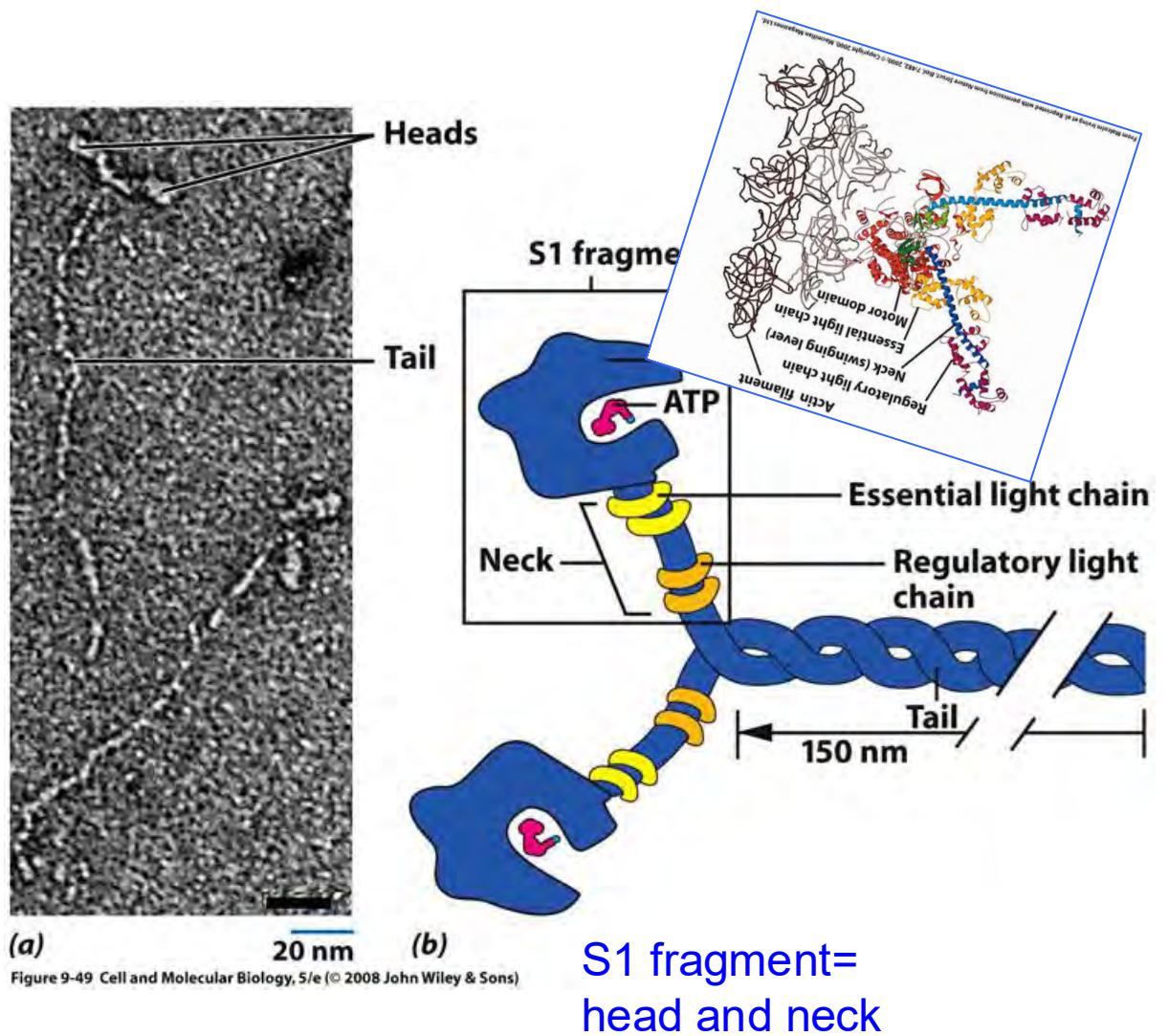


Figure 17-37 Essential Cell Biology, 4th ed. (© Garland Science 2014)

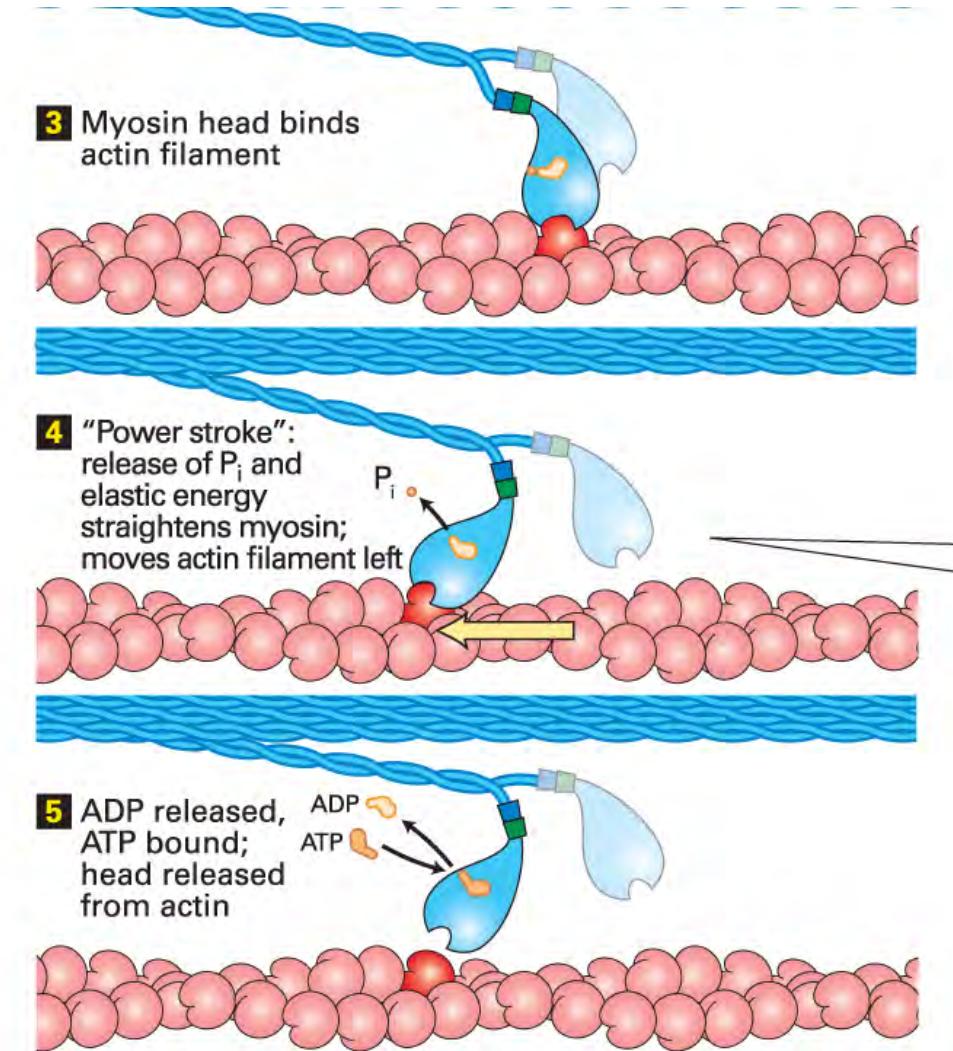
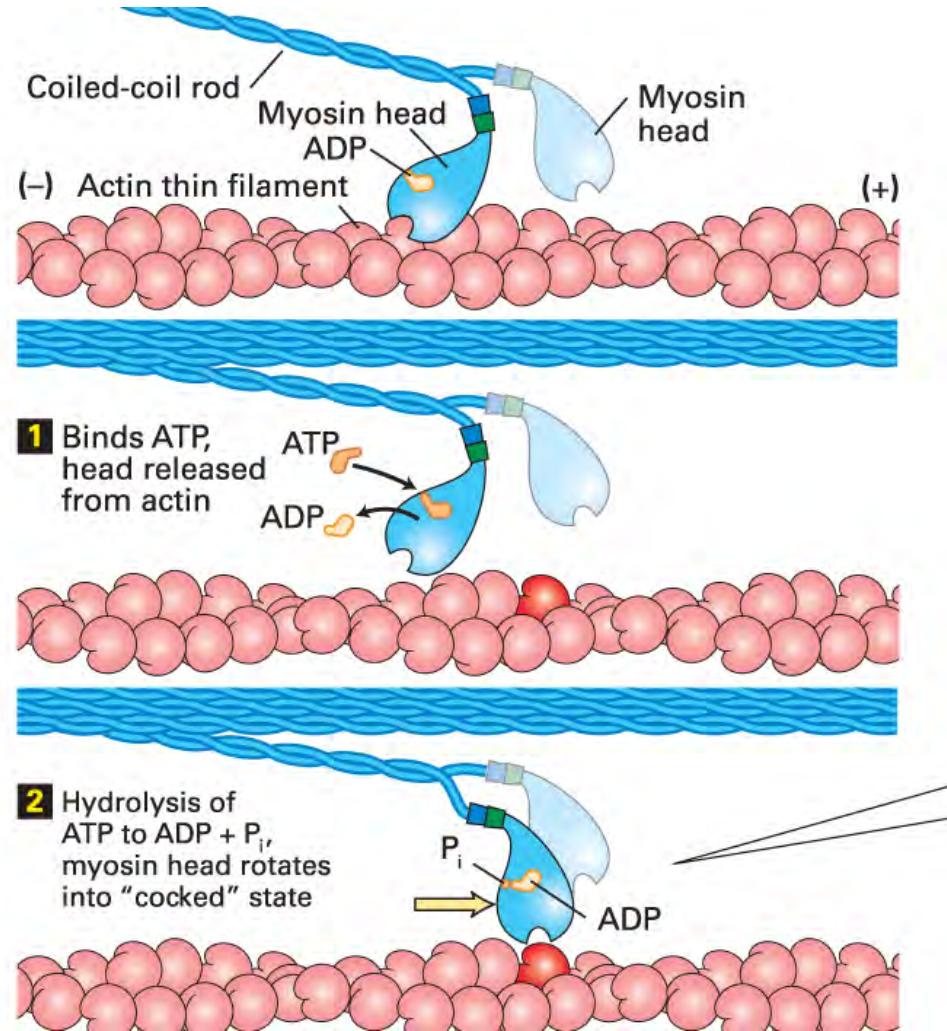
20 μm

Myosin family motors associate with **Actin** filaments



- two major classes: conventional (**type II**) primarily in **muscle**, and unconventional myo I-XIX
- head domain binds **actin** and hydrolyzes **ATP** to move in power stroke
- Tail domain: 6 polypeptides 2 heavy 4 light chains
- 10nm step size
- **most myosins move to the PLUS end (Except Myo6)**

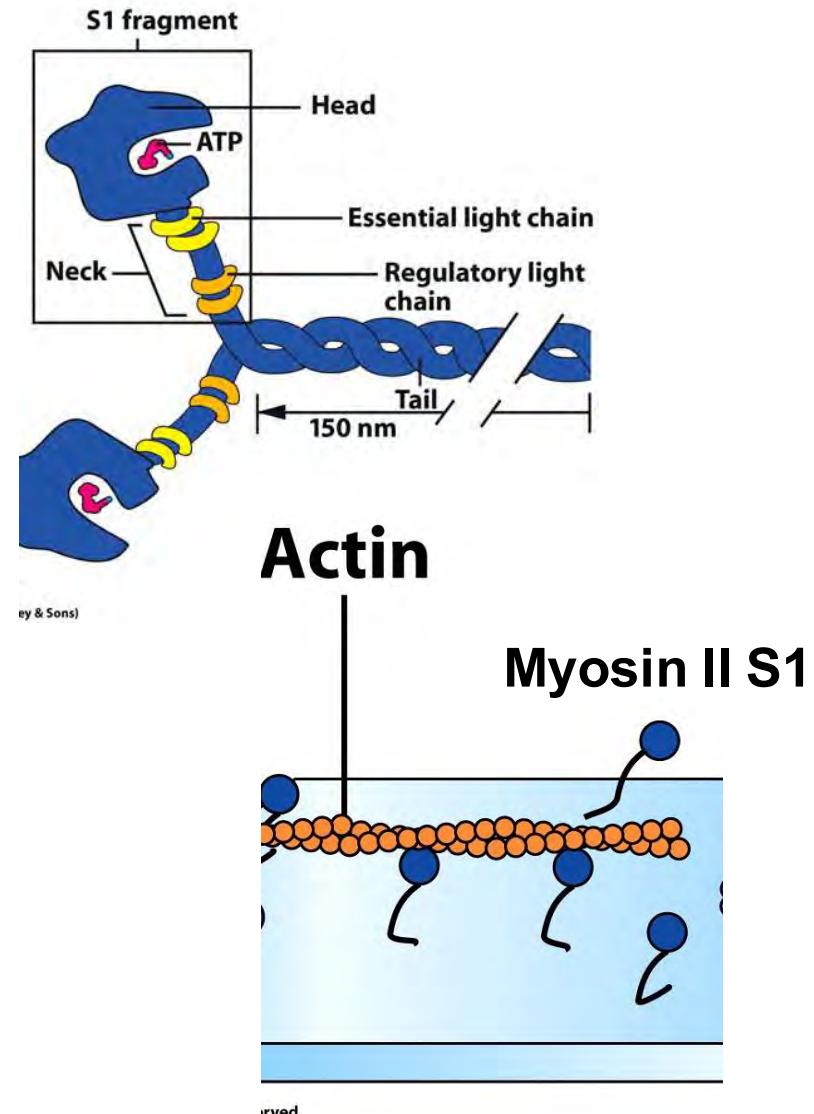
Myosin motors use ATP to walk along Actin filaments



poll 2: Myosin motors associate with Actin filaments

in an experiment, you bind myosin S1 fragments to a coverslip and add stabilized, labeled Actin filaments and ATP. what happens?

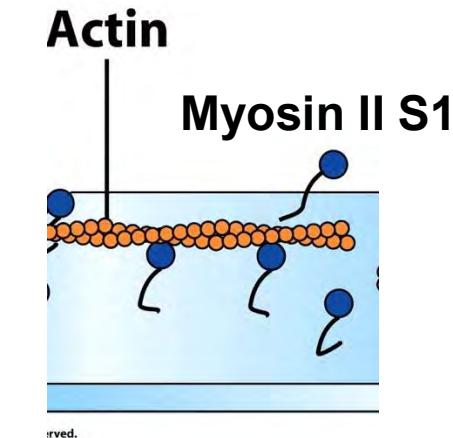
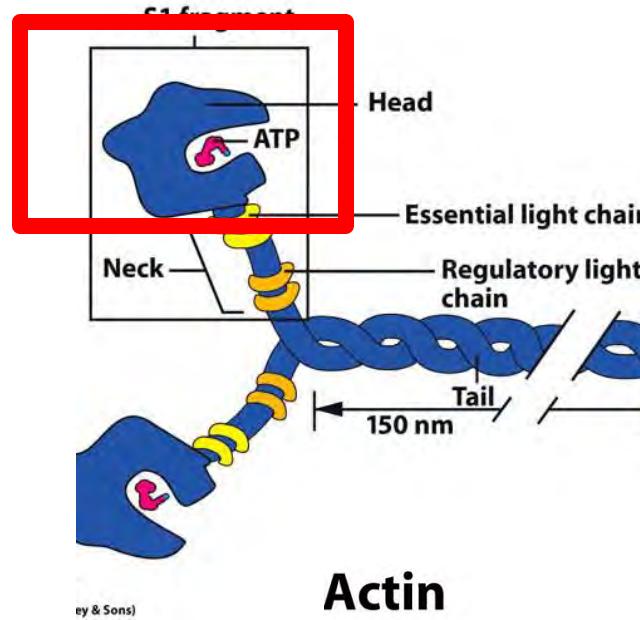
- a) the actin binds but doesn't move
- b) the actin binds and slides around
- c) the actin is hydrolyzed



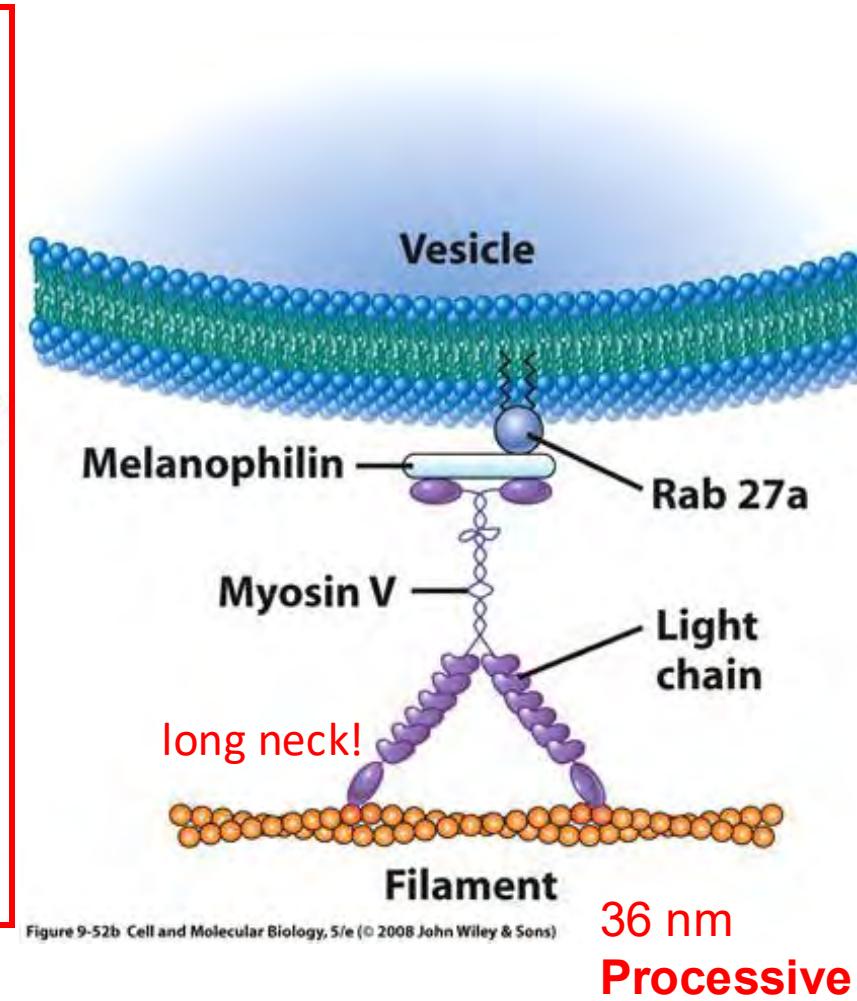
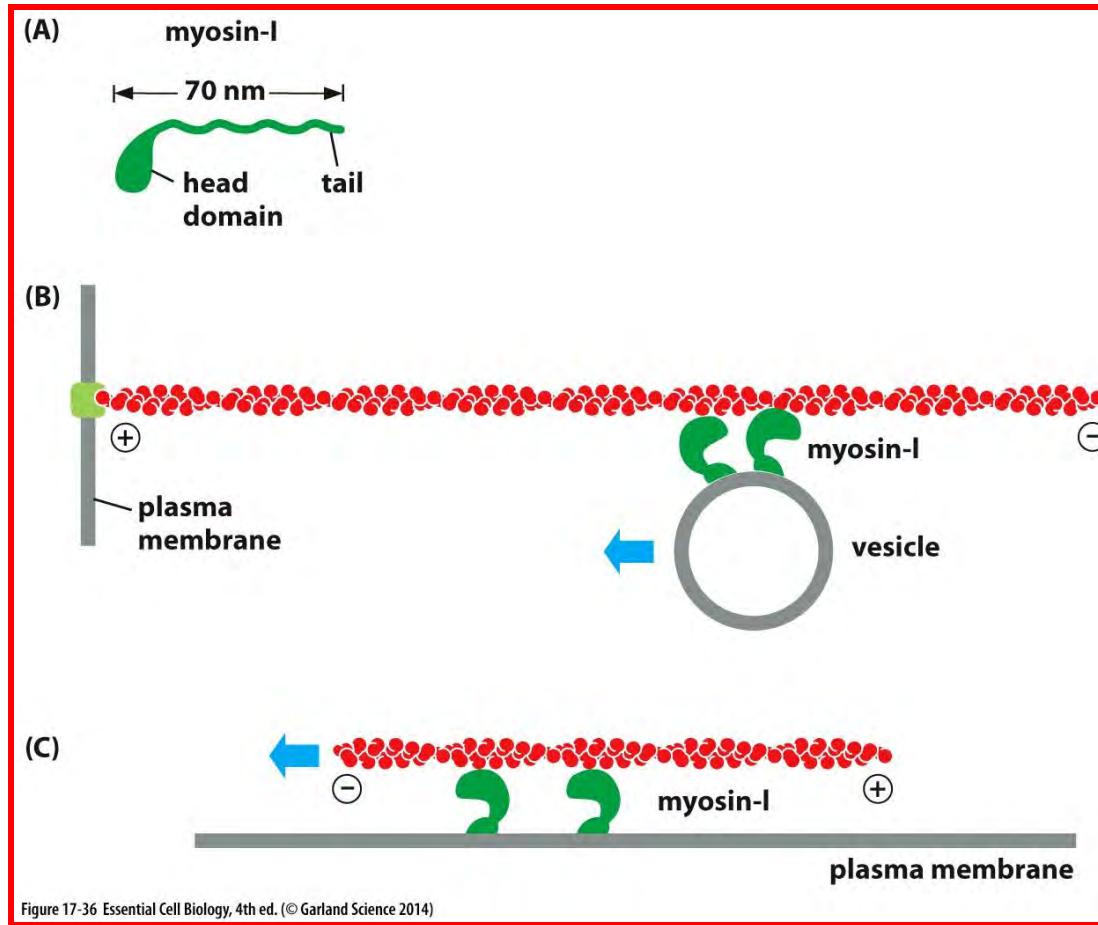
thought experiments: Myosin motors associate with Actin filaments

What if you bind myosin heads (not S1) to a coverslip and add stabilized, labeled Actin filaments and ATP. what happens?

What if you bind mutant myosin S1 fragments that cannot complete ATP hydrolysis to a coverslip and add stabilized, labeled Actin filaments and ATP. what happens?



unconventional myosins walk on actin filaments and can carry cargo



with the right motor, cargo can switch tracks

- myosins I, V, and VI are associated with cytoplasmic vesicles and organelles
- myoVI (reverse-moves to minus end) associated with clathrin-coated vesicles and early endosomes

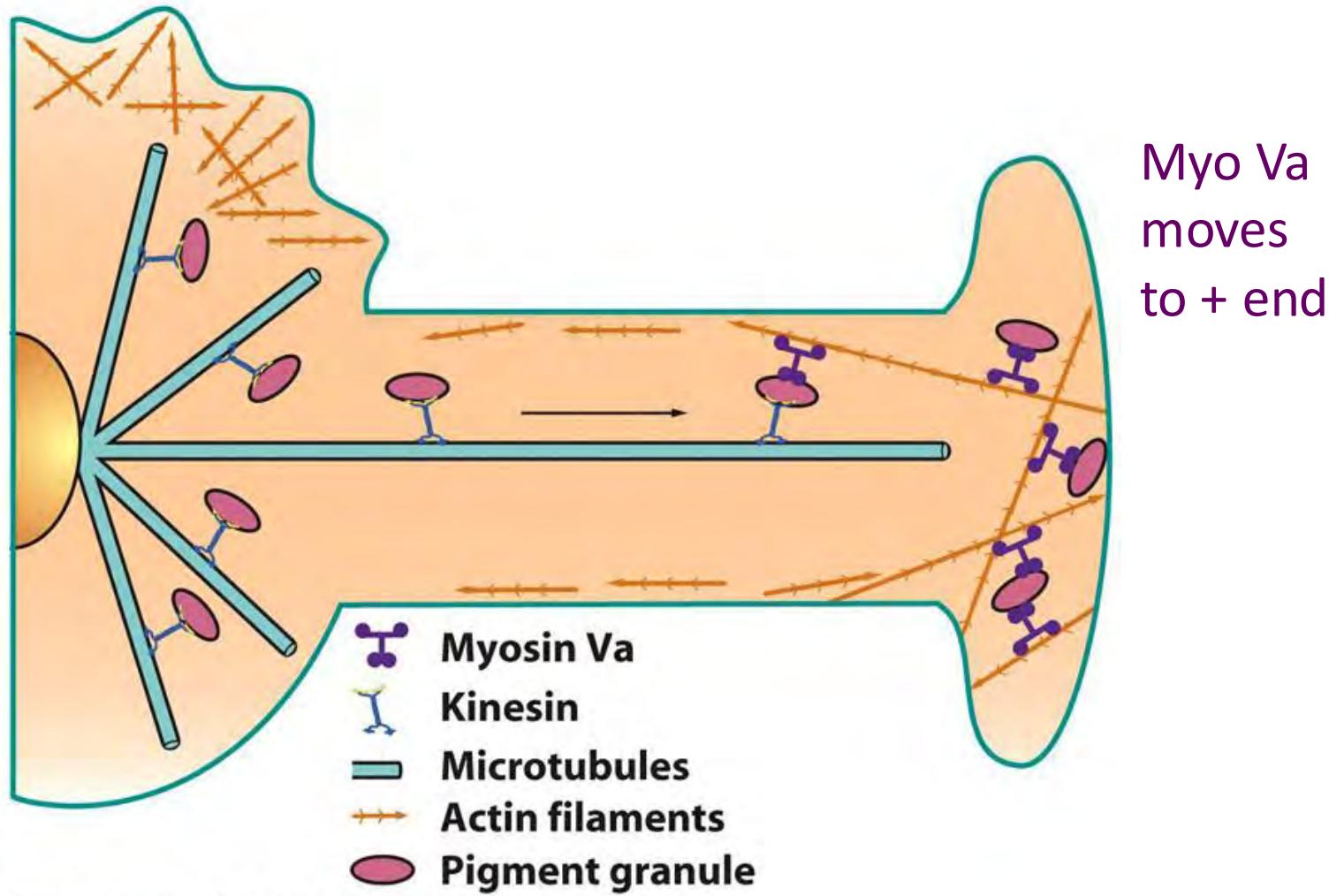
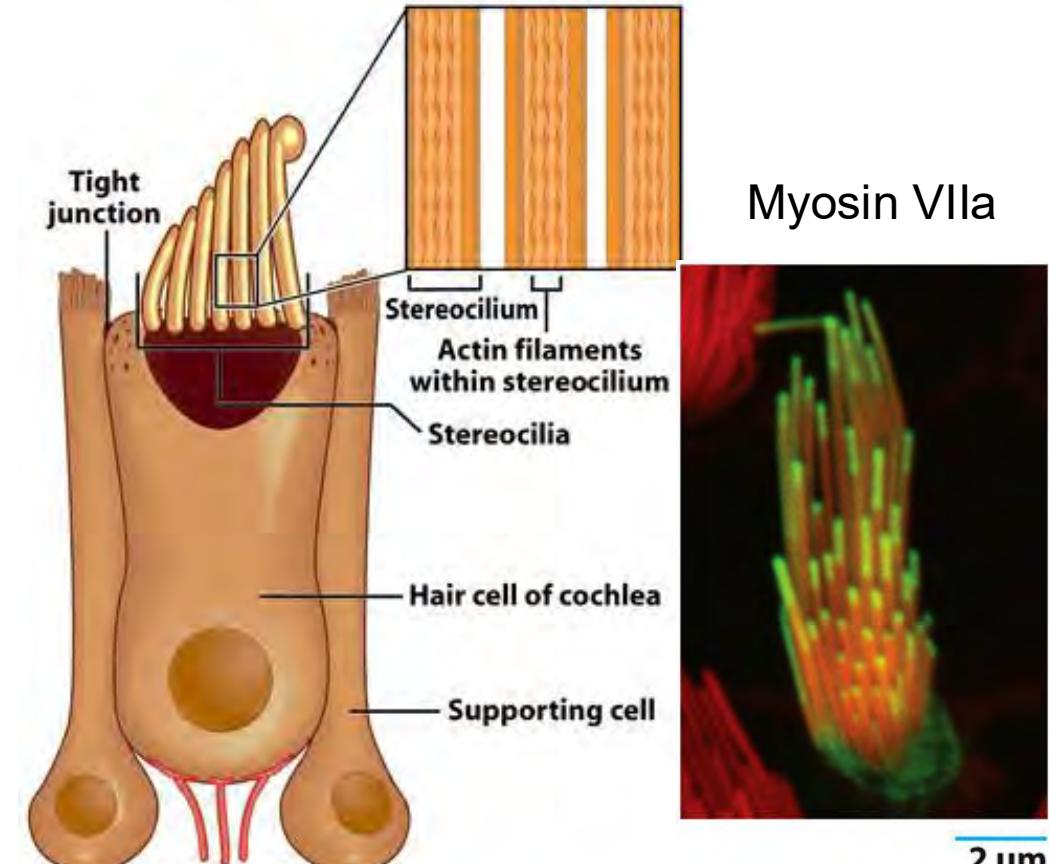
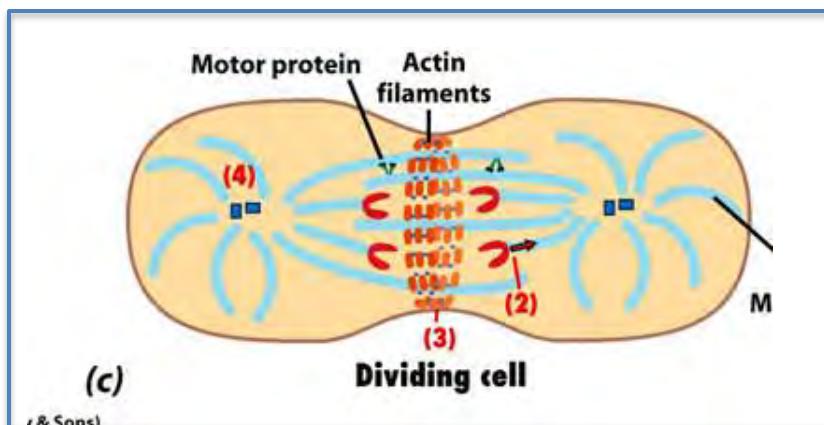
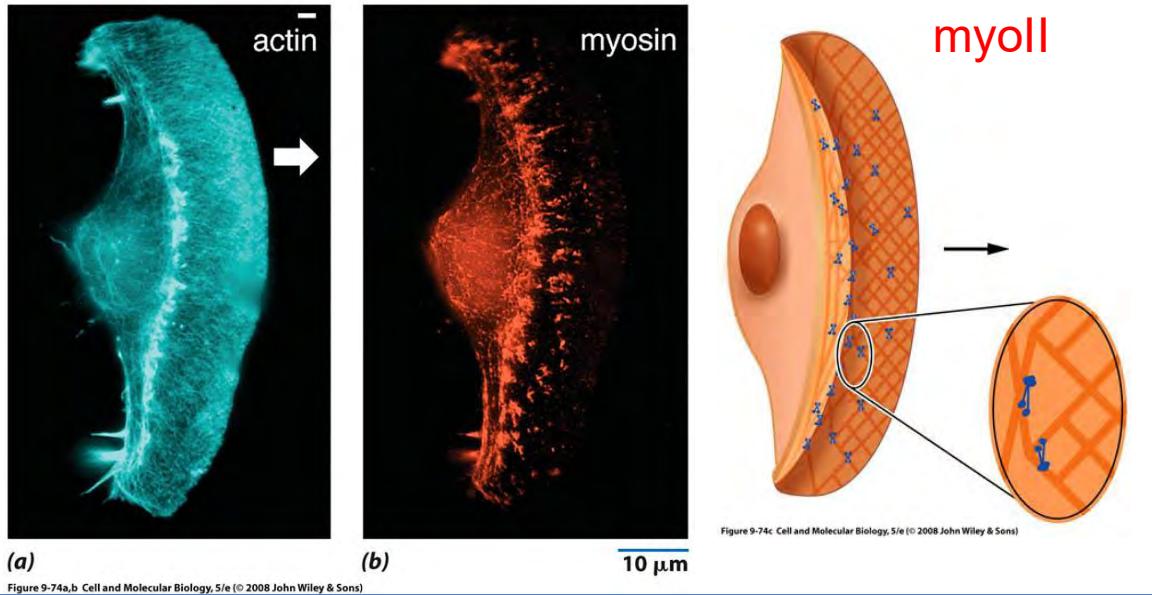


Figure 9-53 Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

many cell structures need both actin and myosin (actinomyosin)

migrating fish keratocyte



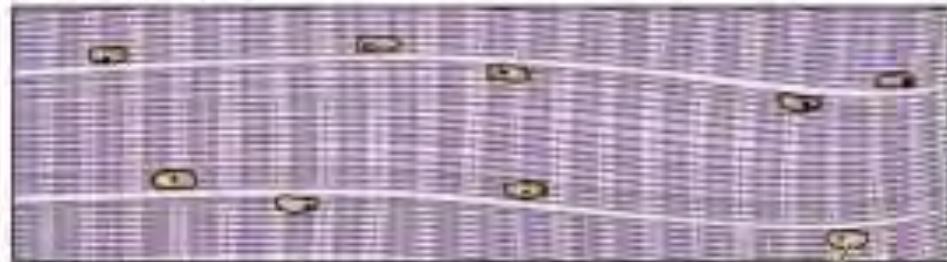
Hair cell of inner ear
stereocilia are
not true cilia

green- GFP G-actin (barbed ends)
red- phalloidin (binds F-actin)

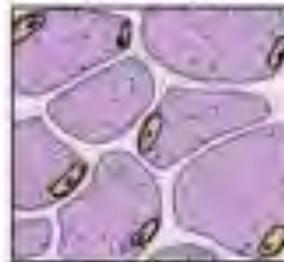
types of muscles

Muscle types

Skeletal muscle



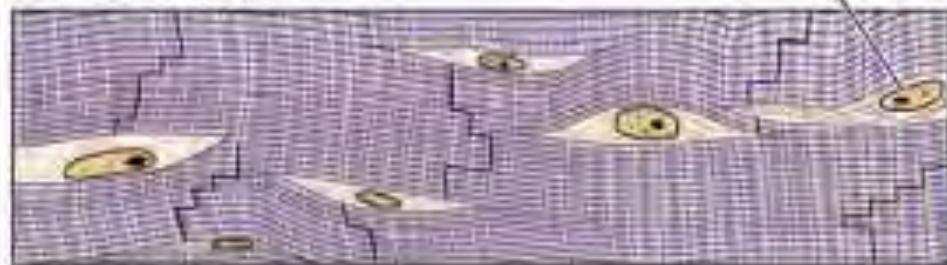
Cross sections



Activity

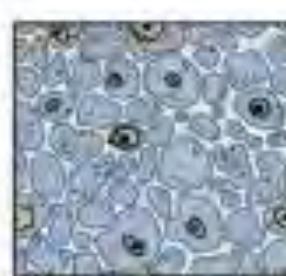
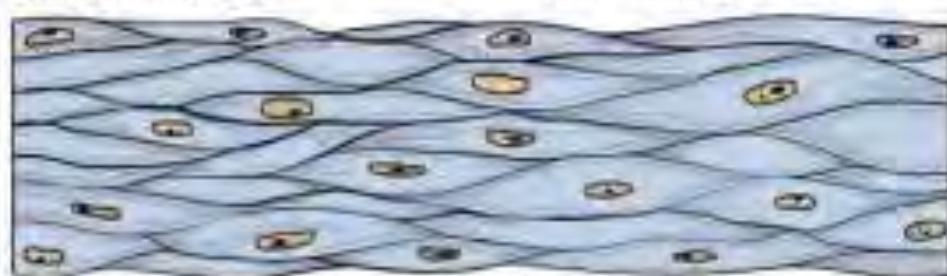
Strong, quick discontinuous voluntary contraction

Cardiac muscle



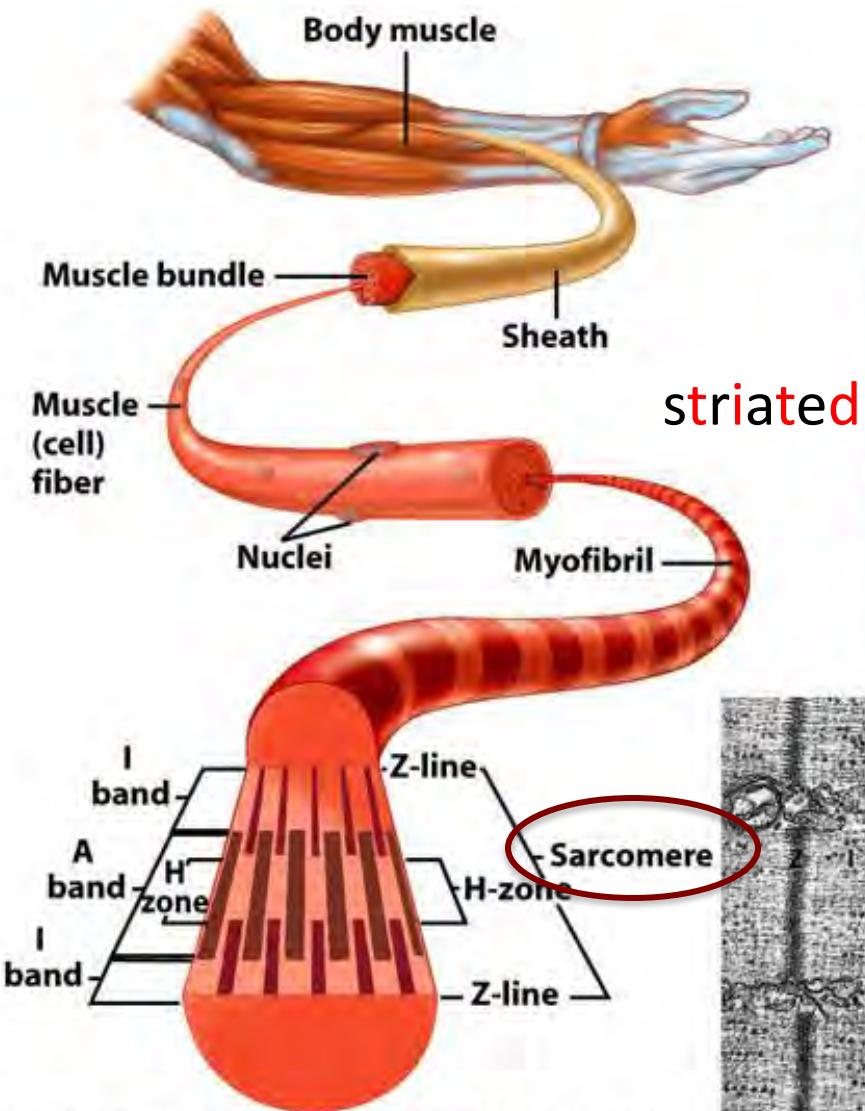
Strong, quick continuous involuntary contraction

Smooth muscle



Weak, slow involuntary contraction

skeletal muscle (and cardiac muscle) cells are comprised of myofibrils



within myofibril, **sarcomere**=
smallest contractile unit

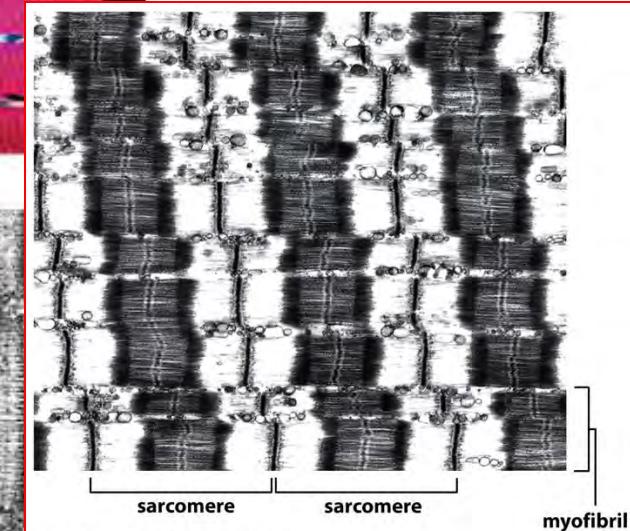
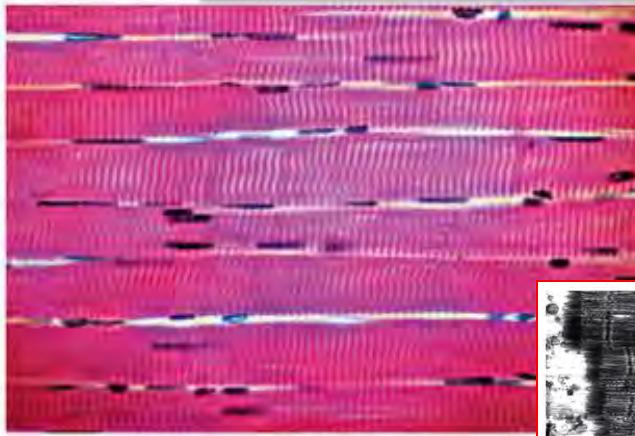


Figure 17-40b Essential Cell Biology, 4th ed. (© Garland Science 2014)

basic anatomy of a sarcomere

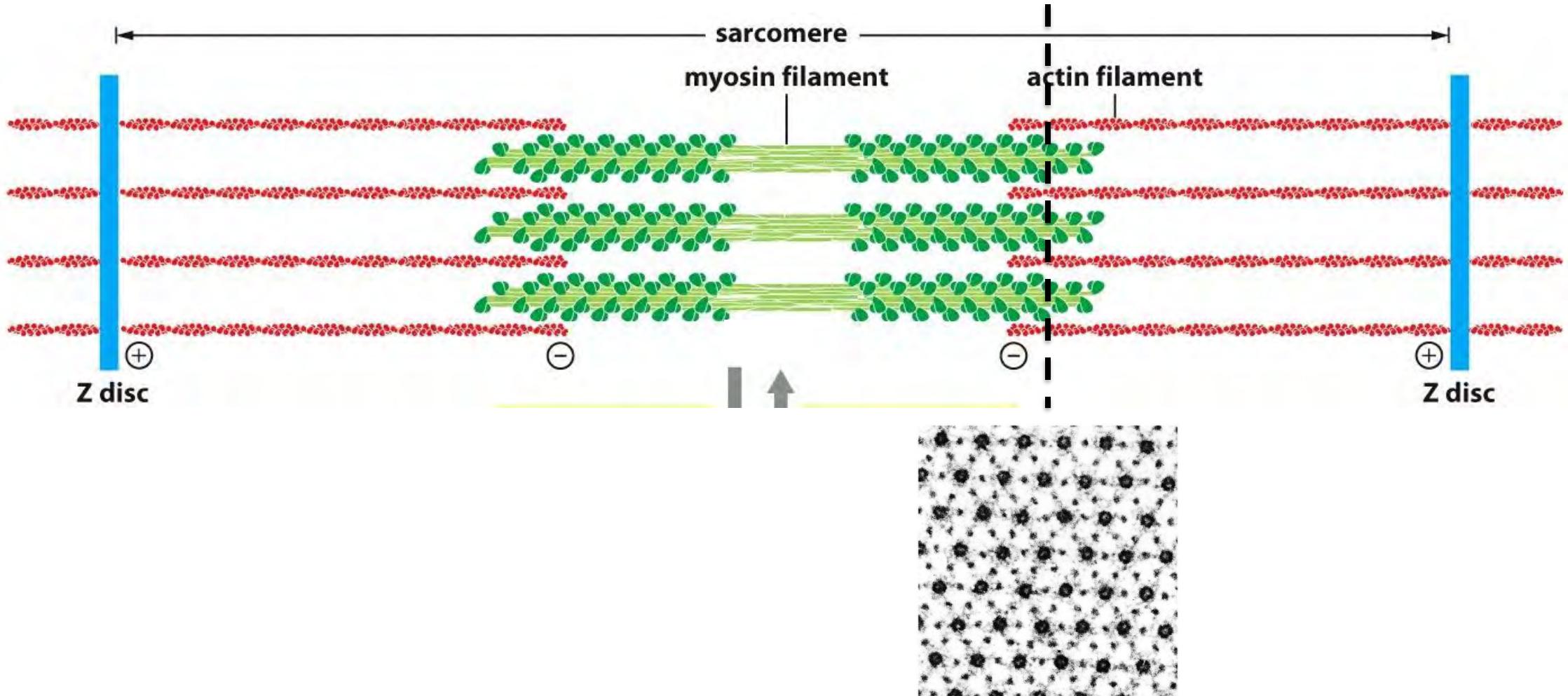
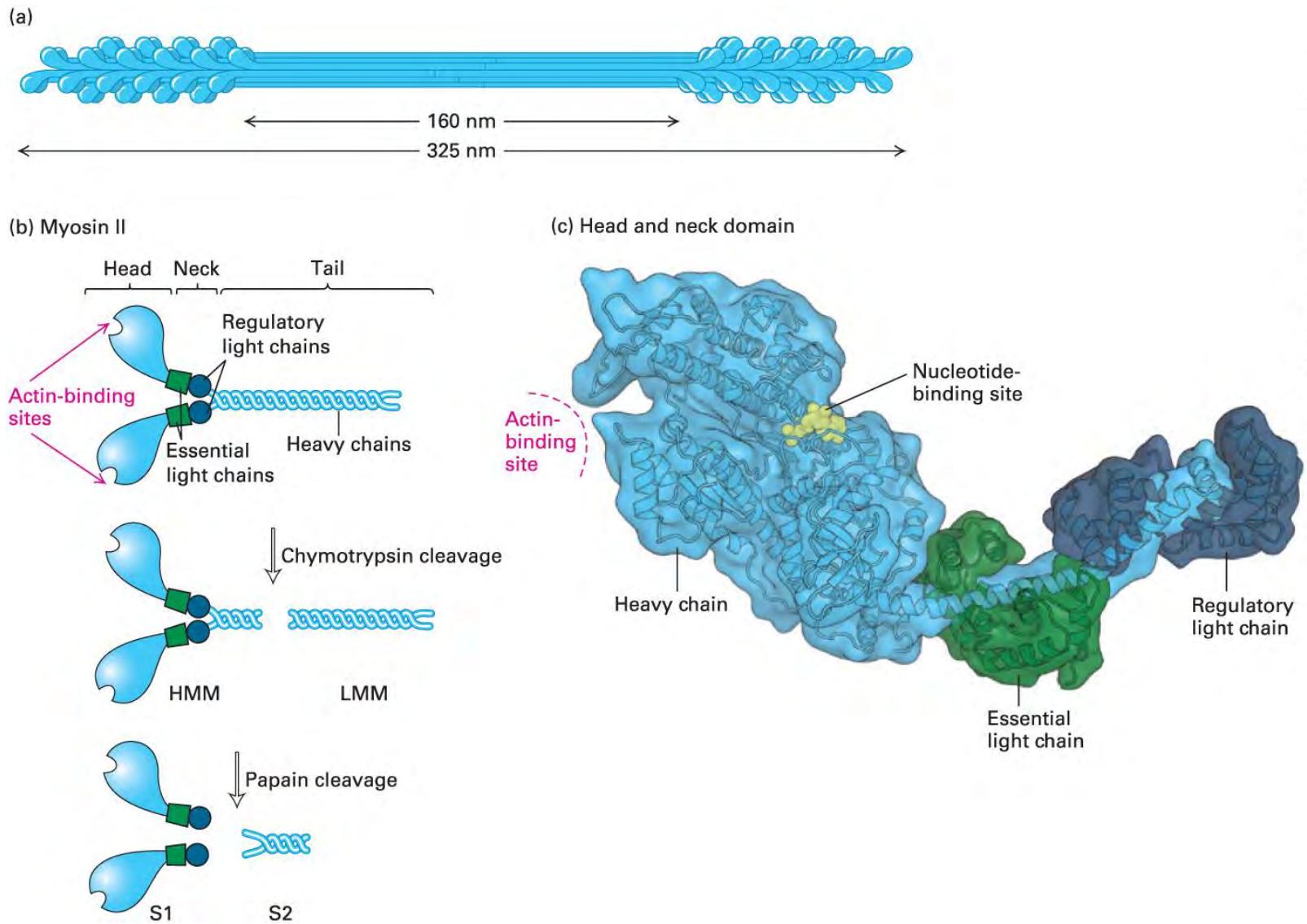


Figure 9-5b Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

myosin II forms filaments



Myosin II forms *filaments* that pull on actin filaments

essential in muscle contraction

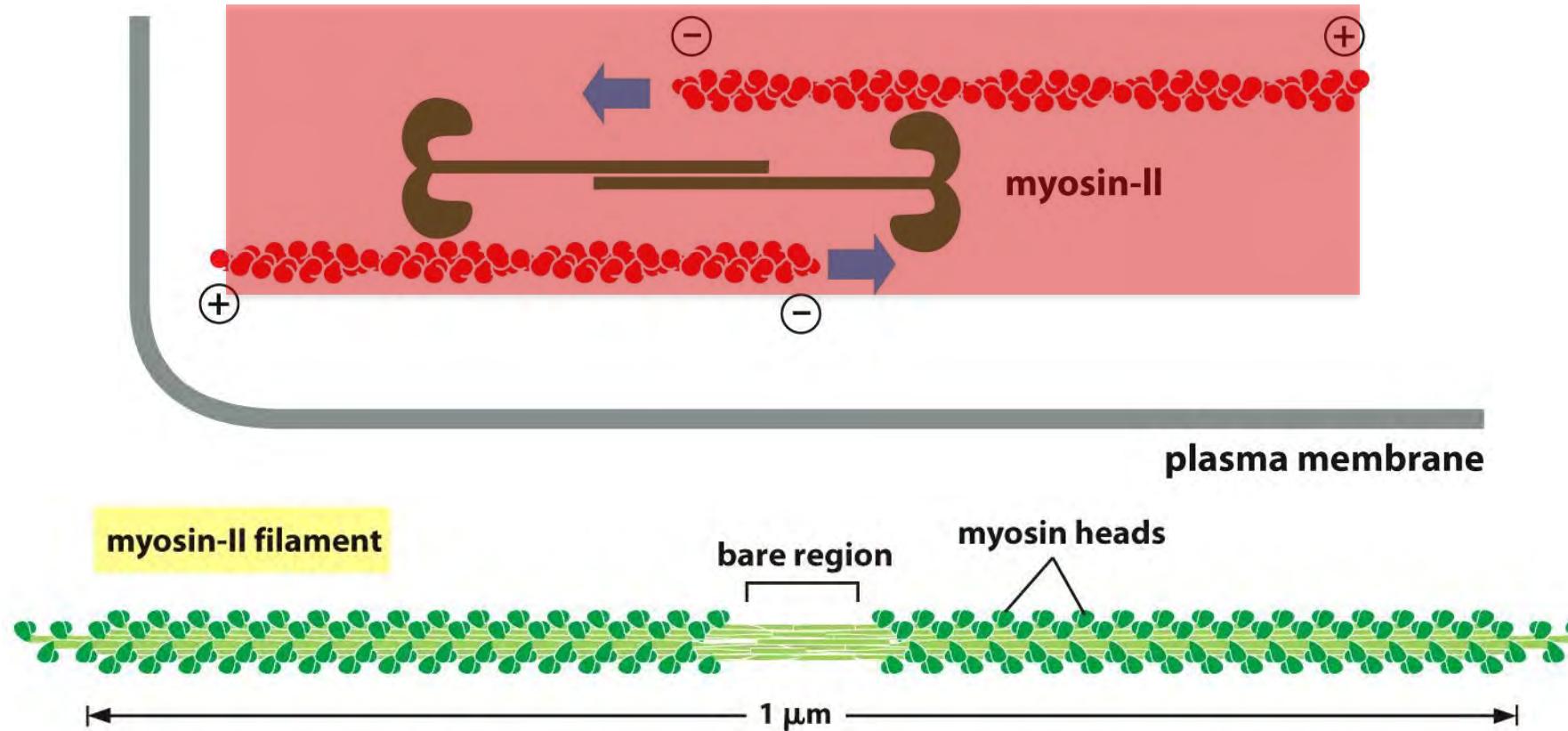
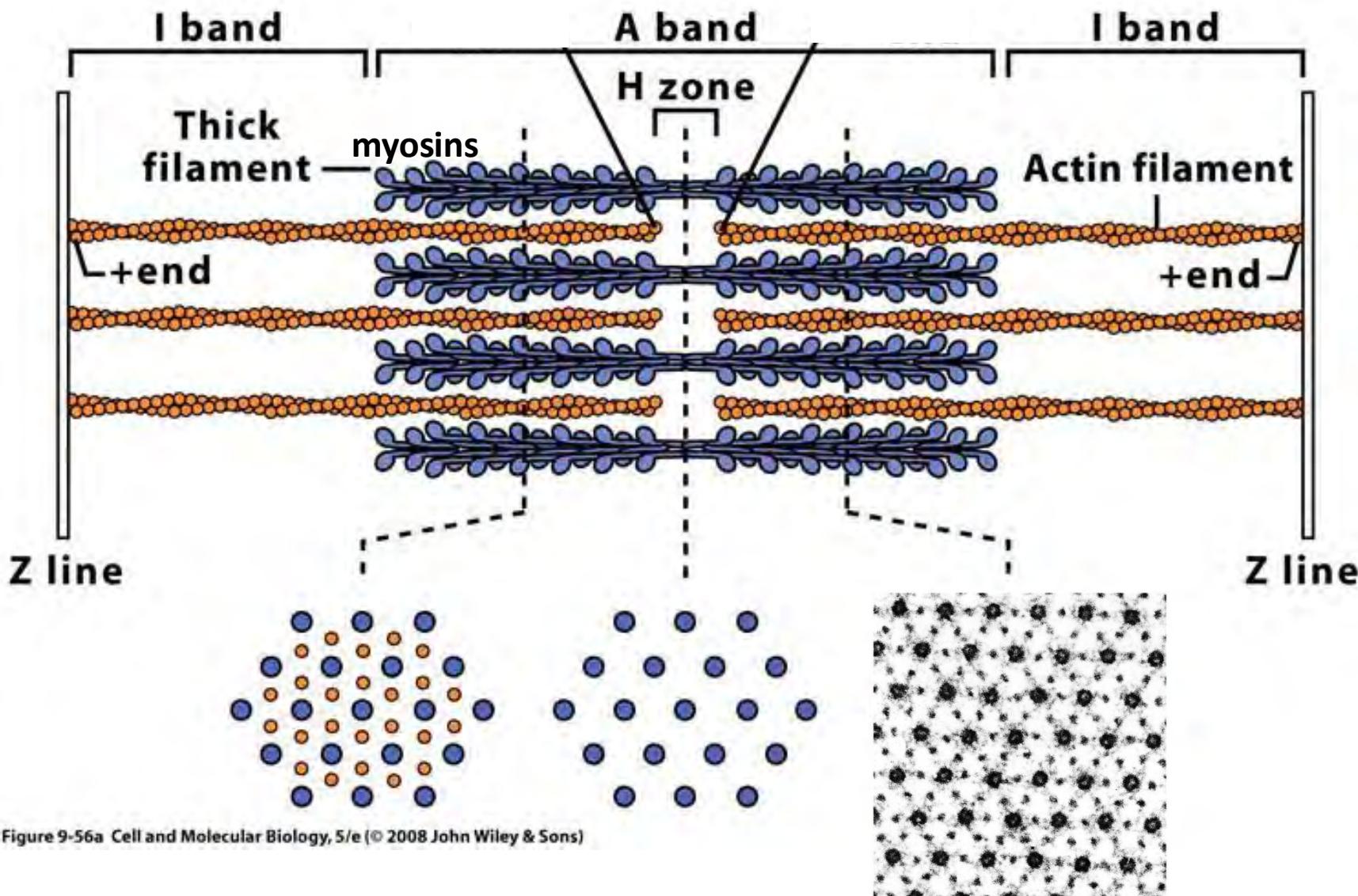


Figure 17-38b Essential Cell Biology, 4th ed. (© Garland Science 2014)

basic anatomy of a sarcomere



the sliding filament model

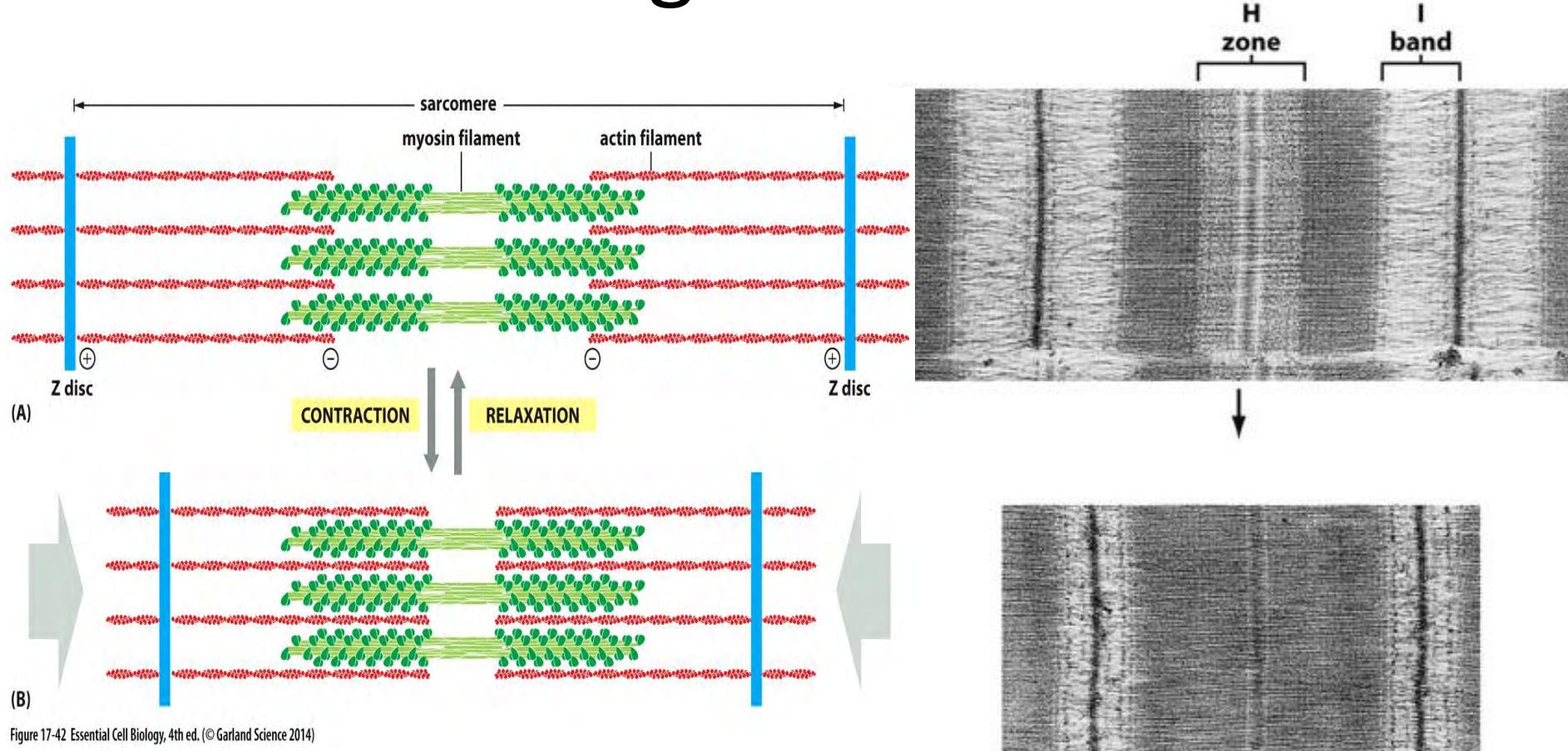


Figure 17-42 Essential Cell Biology, 4th ed. (© Garland Science 2014)

Thick (myosin) and Thin (Actin) Filaments
Do NOT change their length- only the extent of overlap
changes as sarcomere contracts/extends

many Myosins contact thin filaments at once

Huxley & Niedergerke,
Huxley & Hanson, 1954

major proteins of the sarcomere (1)

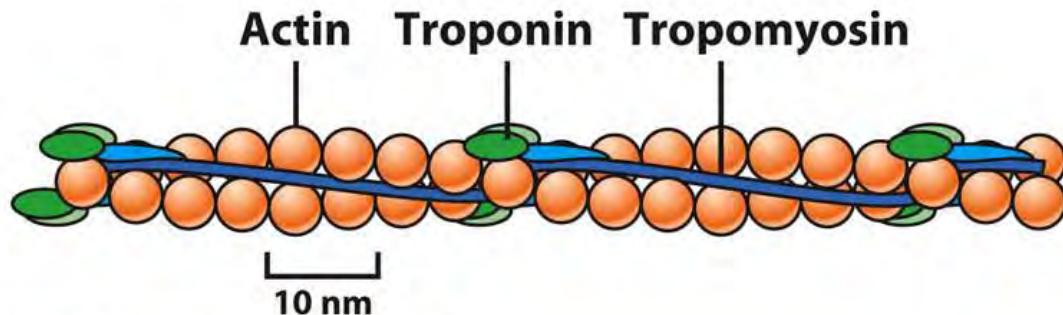


Figure 9-58 Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

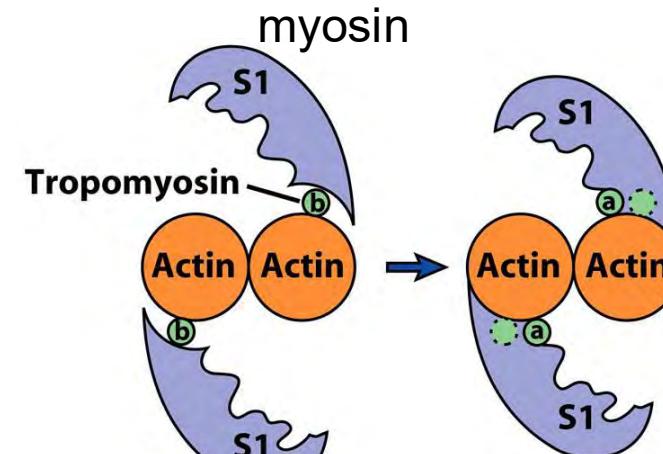


Figure 9-63 Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

1 **Tropomyosin** molecule associates with 7 **Actin** subunits
- its location along the Actin filament is Ca⁺⁺ dependent (via Troponin)

Troponin stabilizes filament by association with Actin and Tropomyosin

major proteins of the sarcomere (2)

Titin is the largest protein known – 38000 amino acids
(compare to: actin ~ 376aa, myosin ~ 1950aa, beta-tubulin ~ 440aa)
may prevent overstretching; positions myosins

Nebulin may determine the size of the sarcomere

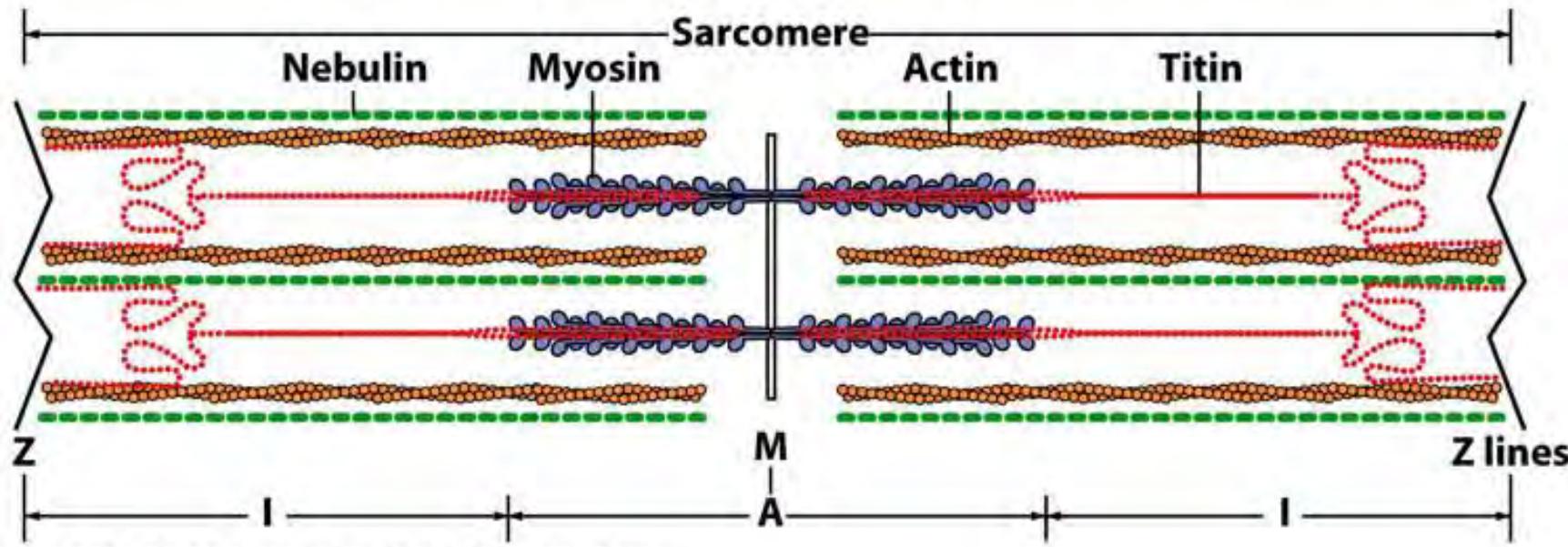
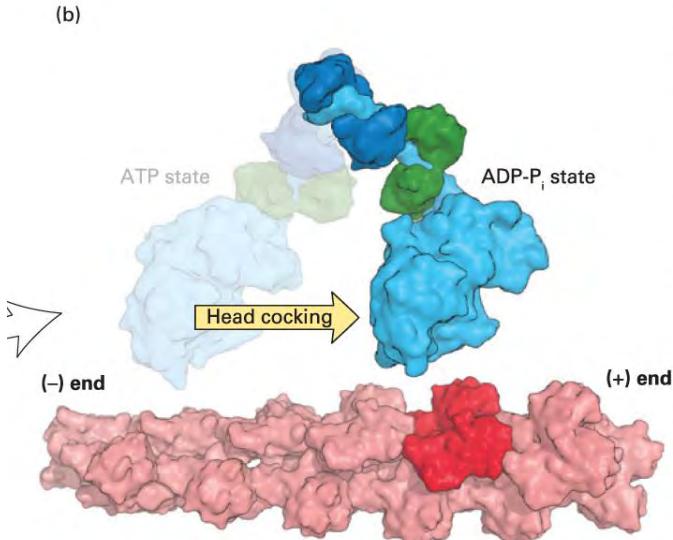


Figure 9-59 Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

Actin/Myosin interaction

(b)



Lodish et al., Molecular Cell Biology, 9/e, © 2002

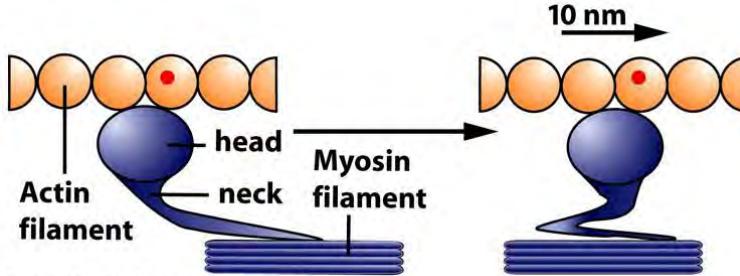


Figure 9-60a Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

myosin ratchets along the actin filament

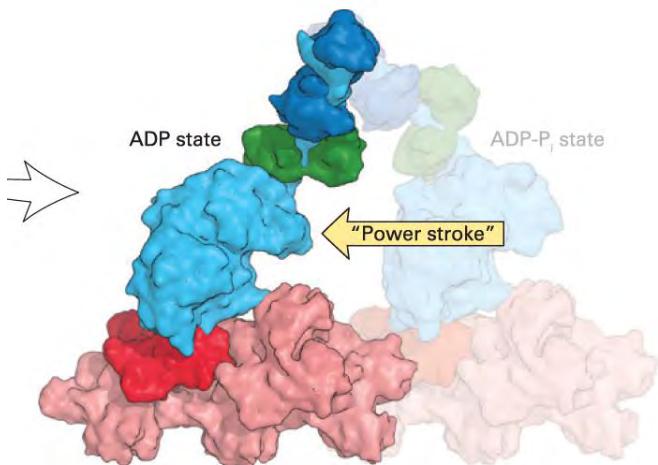
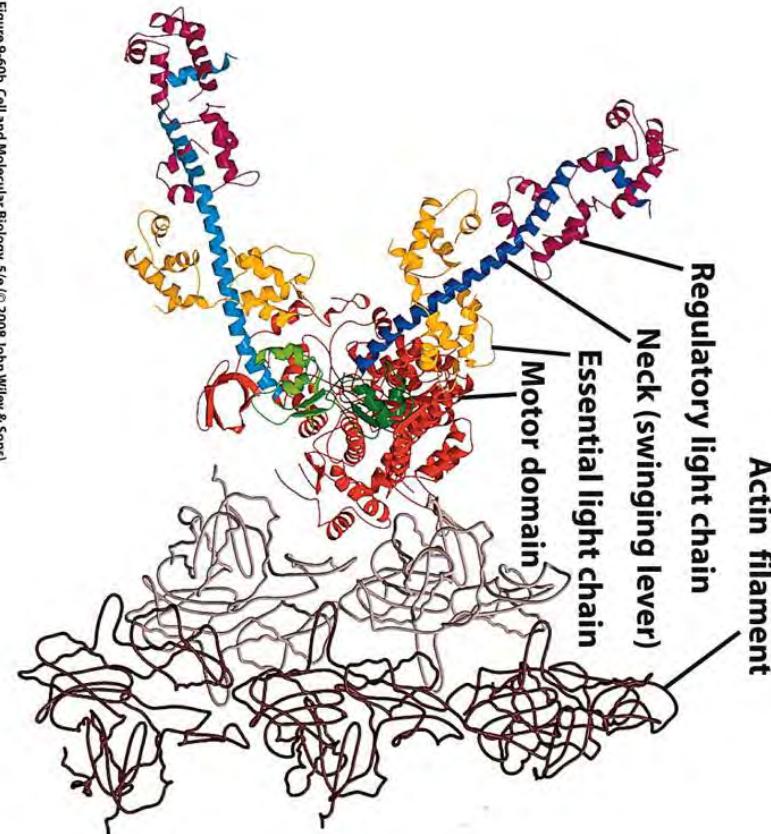


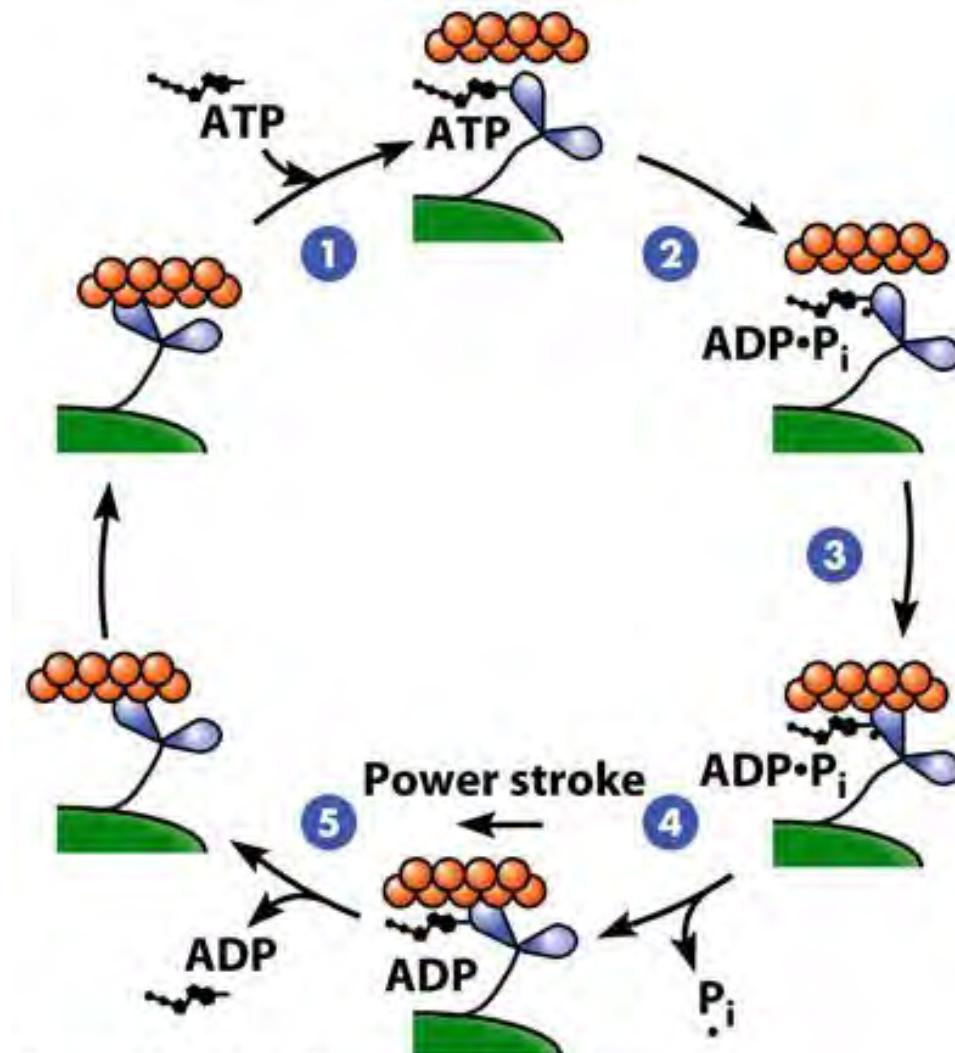
Figure 9-60b Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

interaction can be blocked by position of tropomyosin

non-processive (unlike what other motor?) – *lets go* – compensated by other myosin/actin interactions within the sarcomere (cross bridge)



“sliding” filaments require ATP



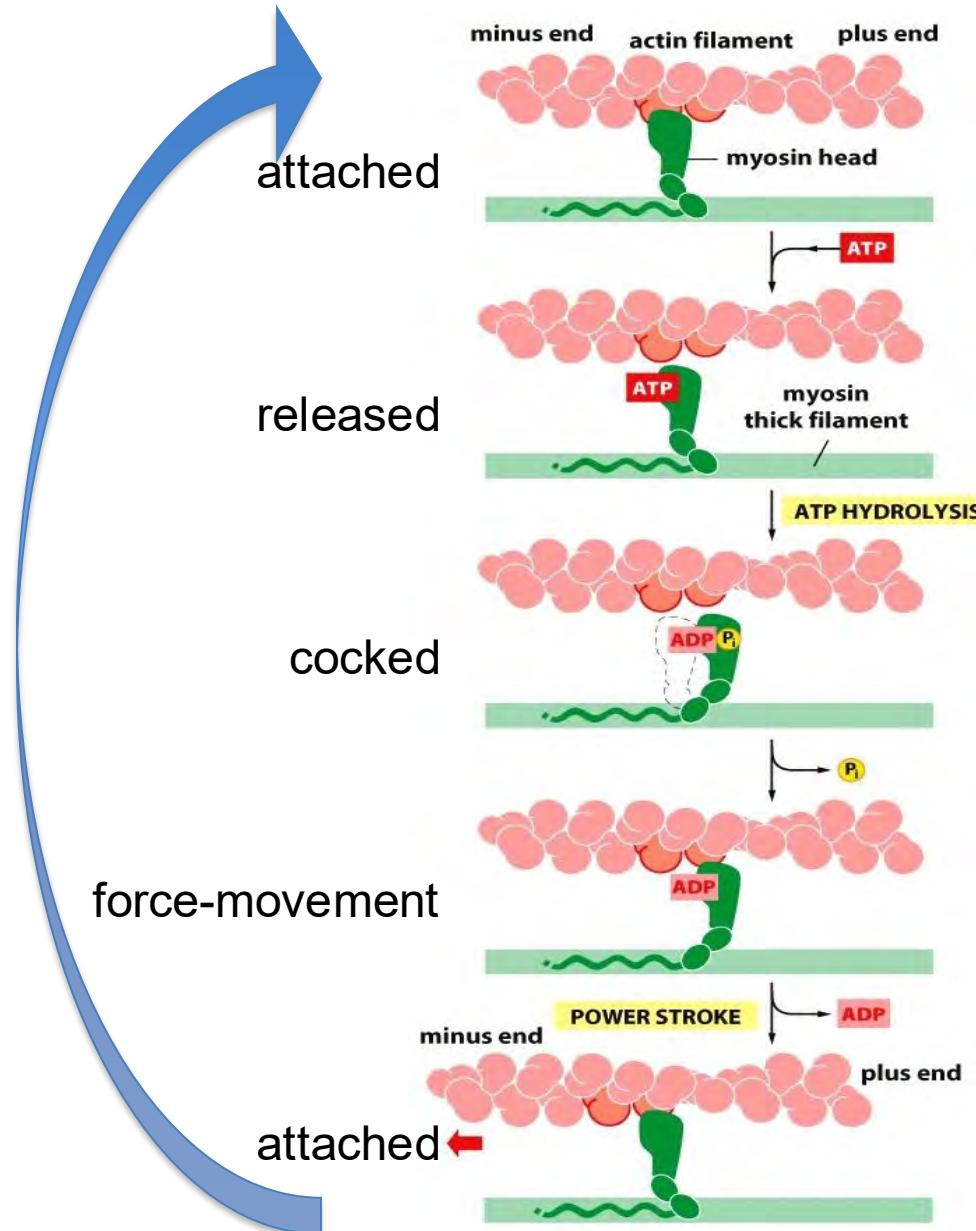
Myosin binds **ATP** and releases F-Actin

Myosin hydrolyses ATP and stores the energy, binds Actin

release of P results in tighter binding and movement of Myosin neck – Actin moves 10nm

(different than cycle for kinesin)

“sliding” filaments require ATP

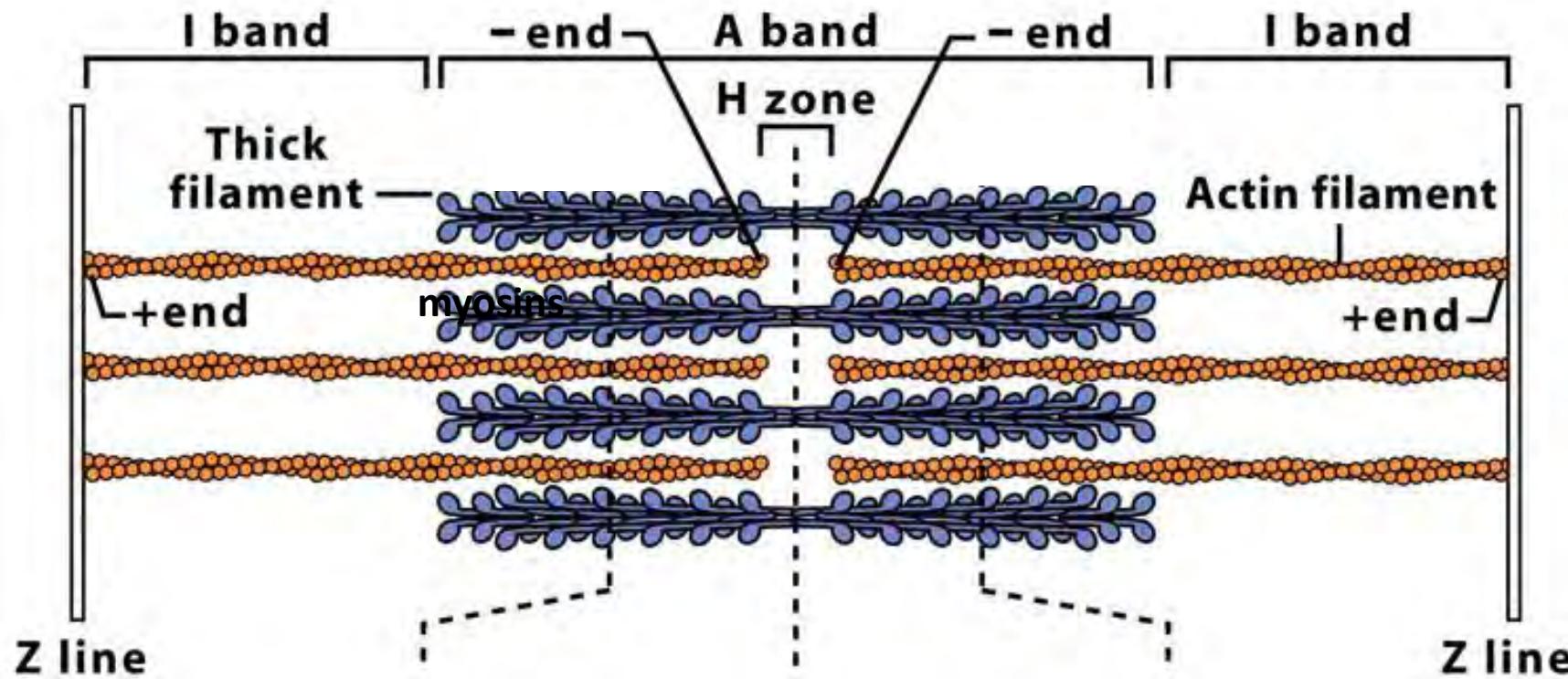


Myosin binds ATP and releases F-Actin

Myosin hydrolyses ATP and stores the energy, binds Actin

release of Pi results in tighter binding and movement of Myosin neck
– Actin moves 10nm

Sarcomere model



myosin

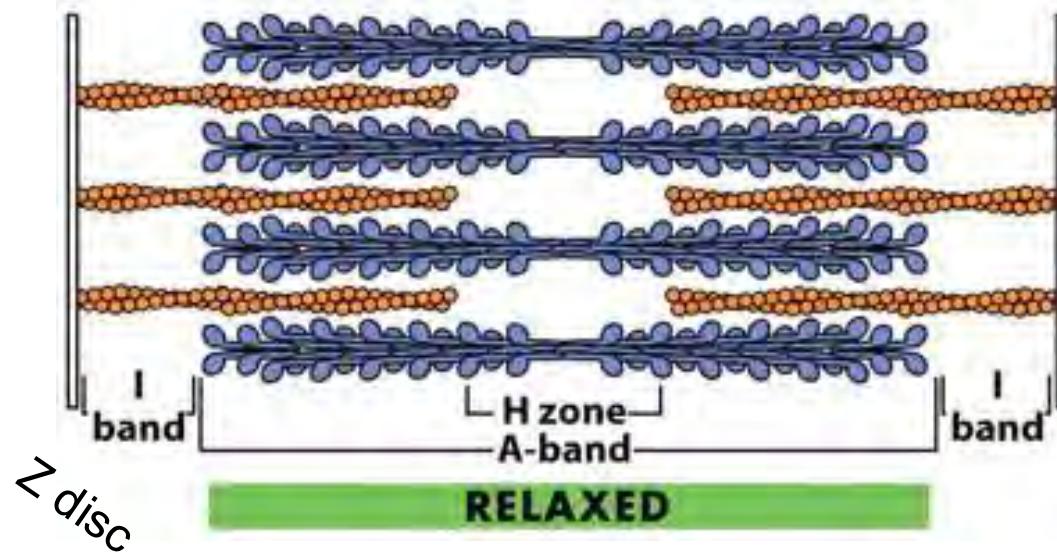


actin +Z



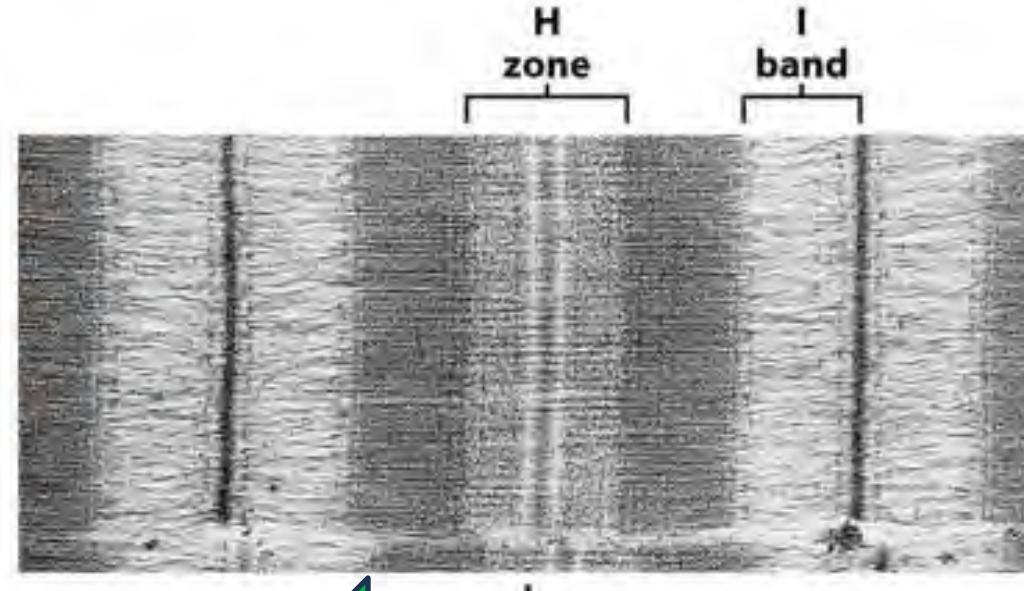
ATP

poll (3) predictions: if you stretched a relaxed myofibril, which bands/zones would NOT change in size?



The I band is where actin exists
doesn't overlap with myosin

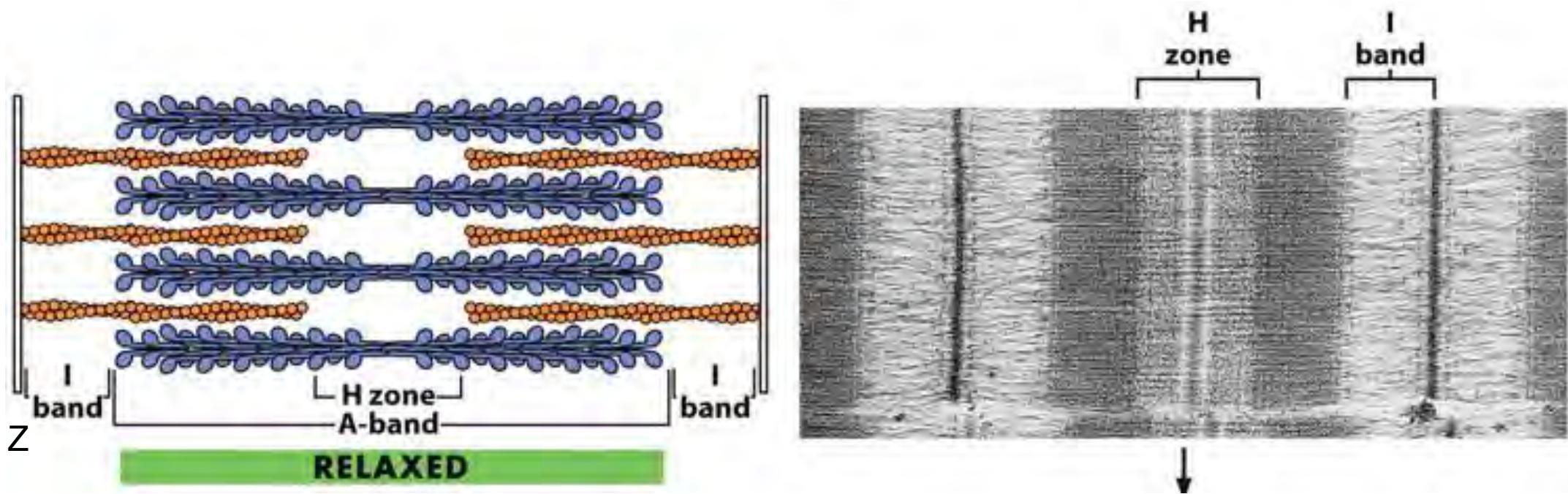
- a) A band
- b) I band
- c) H zone
- d) distance between Z lines/discs



Best answer – this reflects the thick filament, which doesn't change

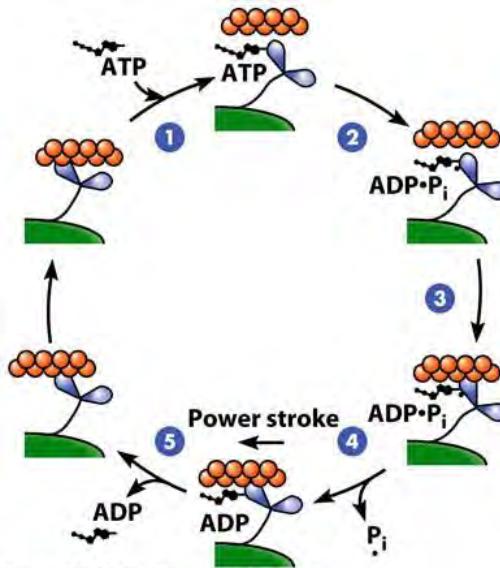
The H zone is where myosin filaments
don't overlap with actin

poll (4) predictions : in a stretched myofibril where sarcomeres are 50% longer, do you expect the contractile strength to be...



- a) the same
- b) greater
- c) reduced

Best answer – fewer myosins would be able to catch on and pull



Biggestharrypotterfans | tumblr

Poll (5) postulate: for someone who was immobilized by the curse ***Petrificus Totalus***, in which all the skeletal muscles stiffen, in which state would you expect most of the victim's muscle myosin?

- a) bound to ATP
- b) bound to ADP and Pi
- c) not bound to either ADP or ATP

Best answer – see step 5 on cycle above

signaling into the muscle fiber

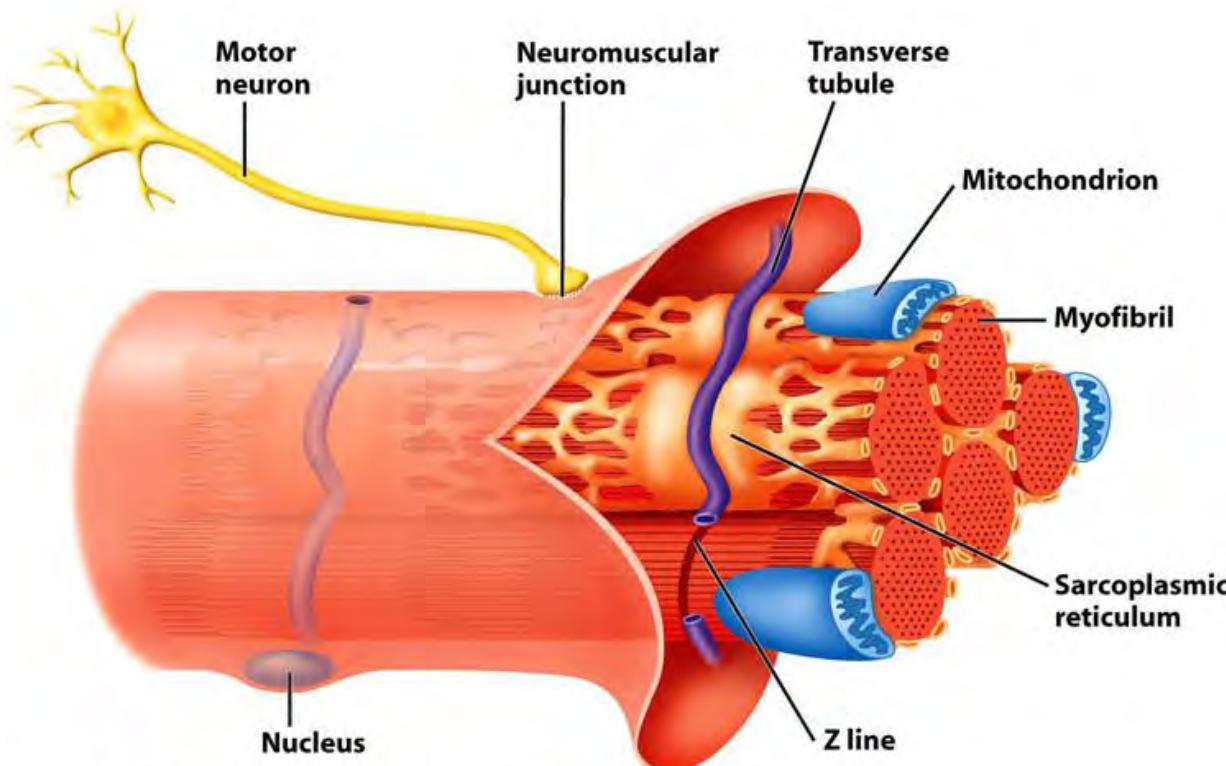


Figure 9-62 Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

motor neurons signal to a muscle at the neuromuscular junction

impulse causes the **sarcoplasmic reticulum** to release Ca^{2+} (stored there via Ca^{2+} ATPases) –

high Ca^{2+} relocates Tropomyosin and allows Myosin to bind Actin

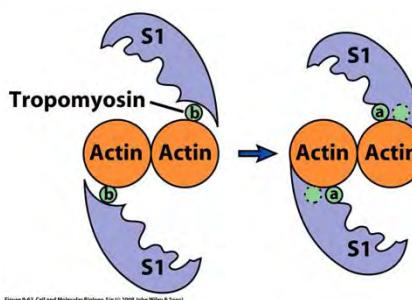


Figure 9-63 Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

muscle contraction triggered by Ca⁺⁺ release into cytosol

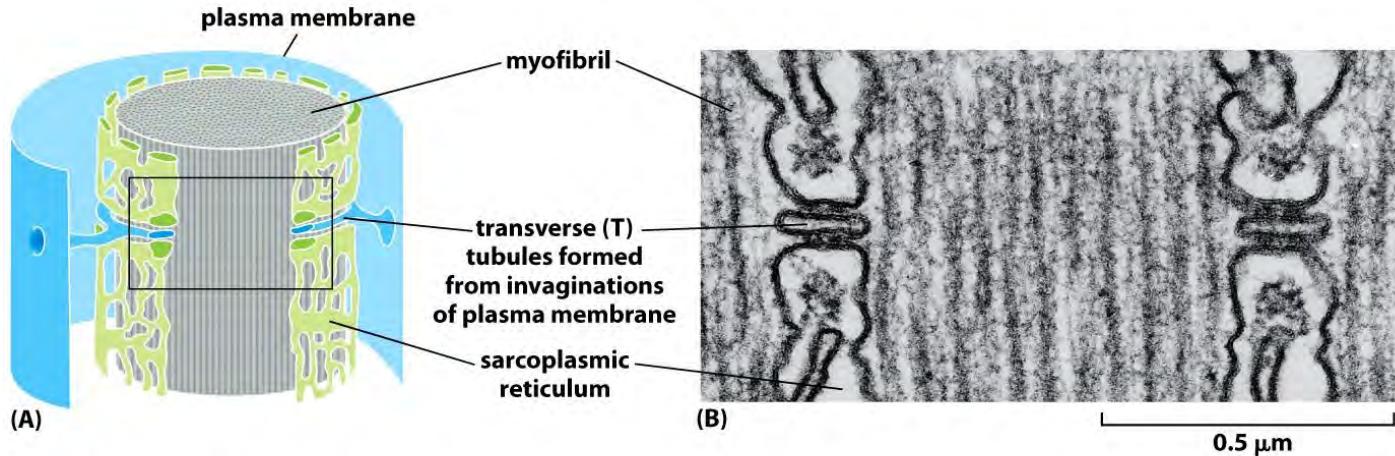


Figure 17-44 Essential Cell Biology, 4th ed. (© Garland Science 2014)

action potential opens Ca⁺⁺ channels;
cytosolic Ca⁺⁺ is required to allow sarcomere movements

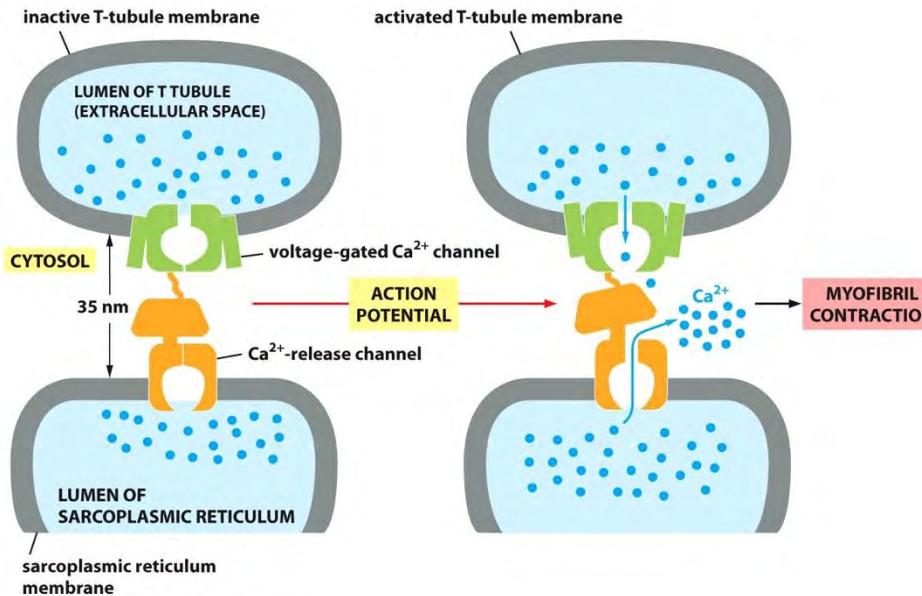
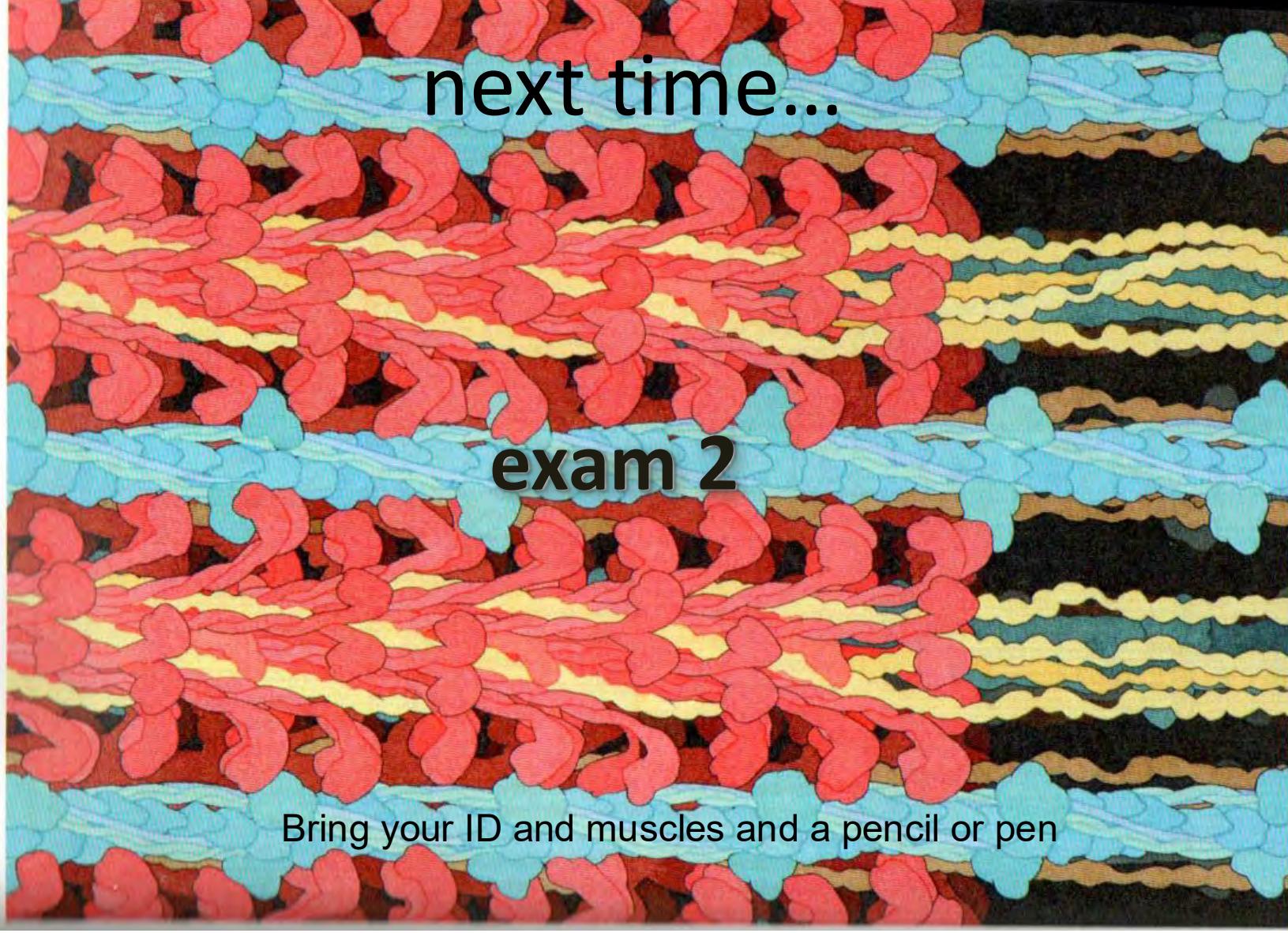


Figure 17-45 Essential Cell Biology, 4th ed. (© Garland Science 2014)



next time...

exam 2

Bring your ID and muscles and a pencil or pen

