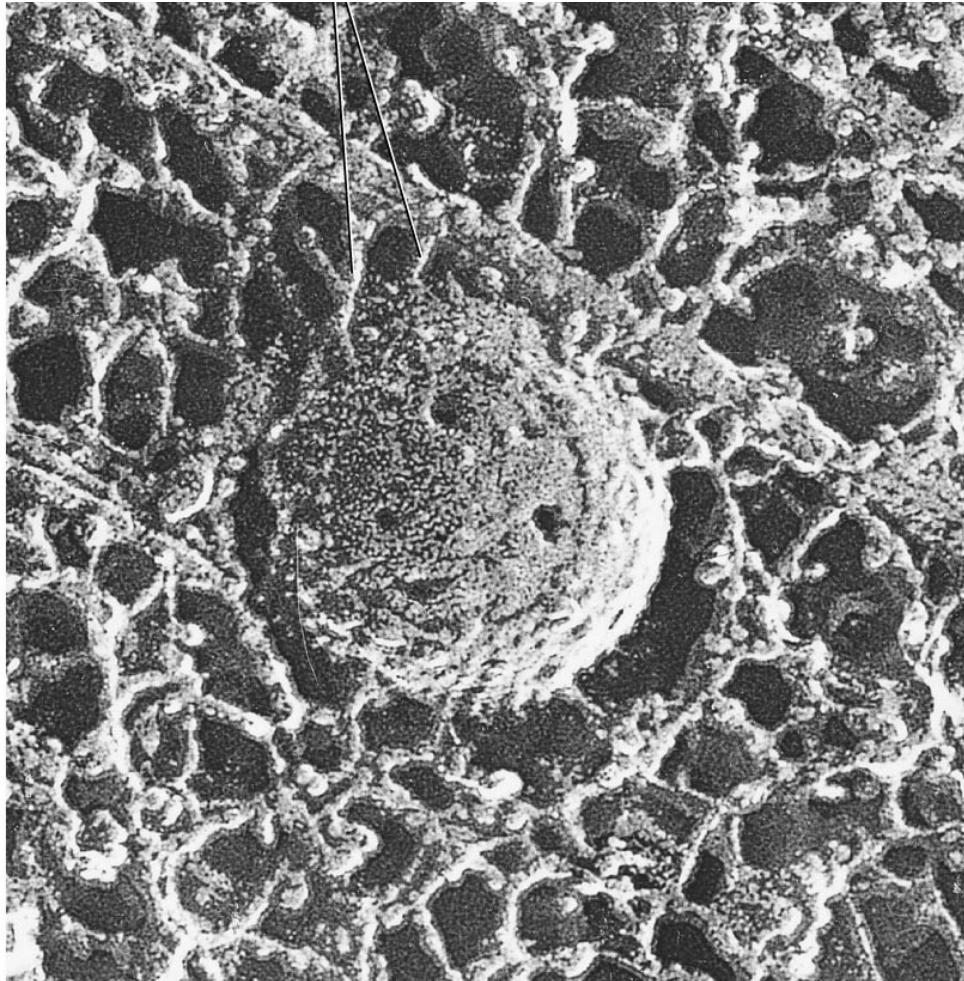


Cytoskeleton III: Molecular Motors



Motor Proteins

- Use energy derived from hydrolysis of ATP to produce mechanical force.
- Binds to cytoskeleton (e.g. AFs or MTs).
- Produces net movement of protein or “cargo”.
- Divided into 3 families:
 1. Myosins: move along AFs
 2. Kinesins: move along MTs (+)
 3. Dyneins: move along MTs (-)

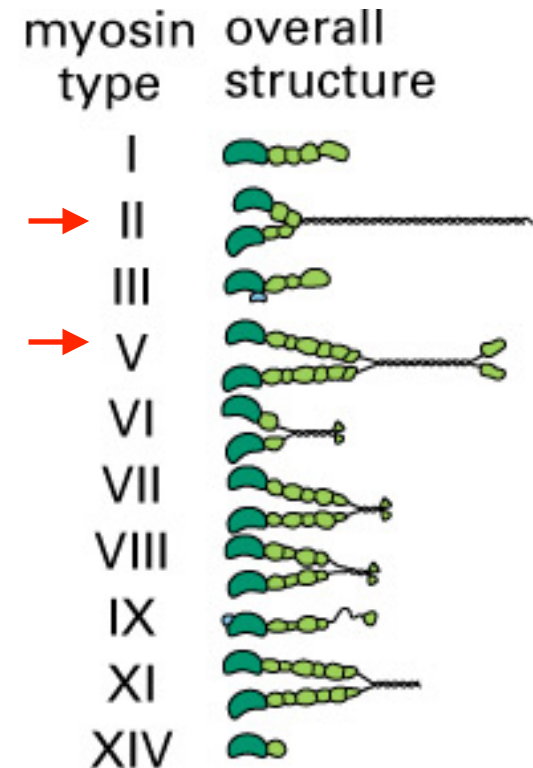
Myosins

Diverse family of motor proteins.

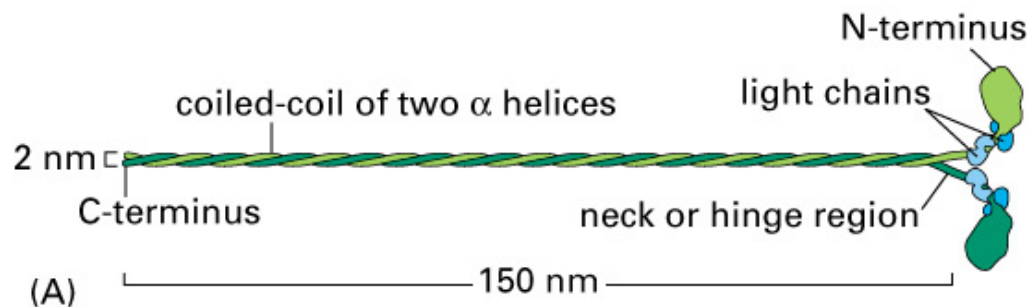
- Most eukaryotes (I, II, V).
- Plants (XI, XIII).
- Protozoa (XIV).
- Head region conserved.

Functions

- “Conventional” myosins
 - e.g. type II: muscle, cytokinesis.
- “Unconventional” myosins
 - e.g. type V: organelle transport.

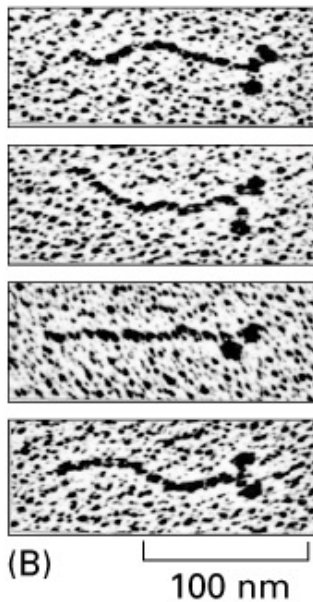


Myosin II



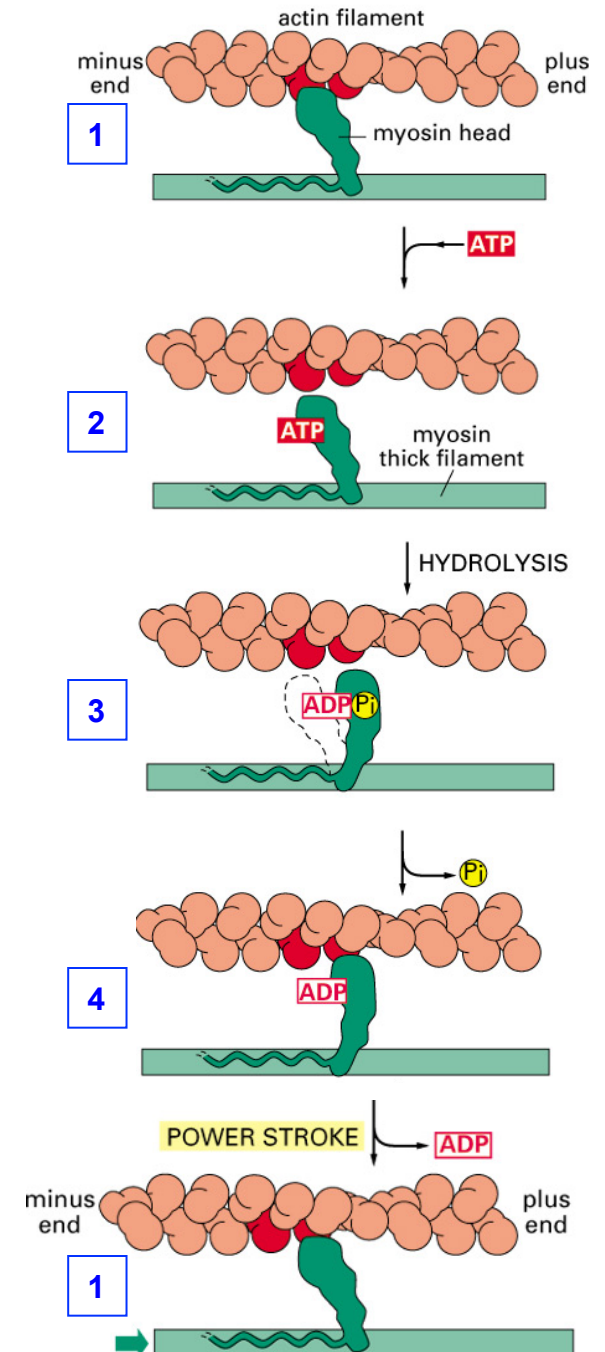
Structure

- Heavy chains (1 × ■, ■).
 - heads, tails.
- Light chains (2 × ■, ■).
 - “essential” and “regulatory” chains. ☰
- Heads are catalytic, provide force.
- Tails mediate dimerization with other myosins.
- Light chains amplify conformational change.



The Myosin Cycle

1. **Attached**: no ATP, locked.
2. **Release**: ATP bound, conformational change (away from AF).
3. **Cocked**: hydrolysis, conformational change toward (+) end of AF.
4. **Force-generating**: weak binding, P_i release, power stroke, ADP lost.



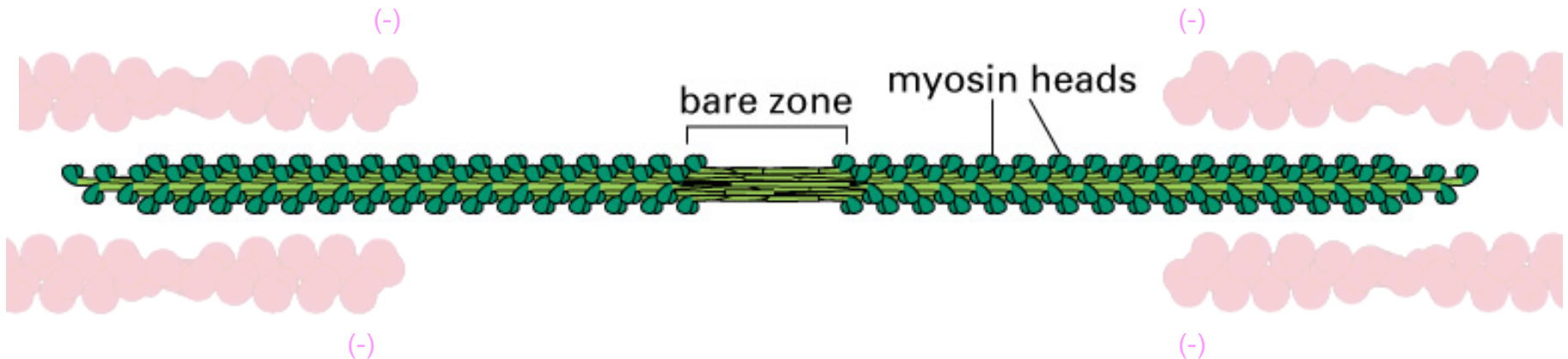
Myosin Cycle



Thick Filament Formation



- Tail-tail interactions.
- Heads oriented in opposite direction.
- Formation of bundles in sarcomere of muscle cells.



The Sarcomere

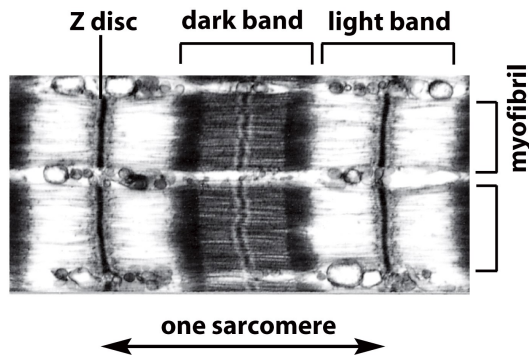


Figure 16-74b *Molecular Biology of the Cell* (2008)

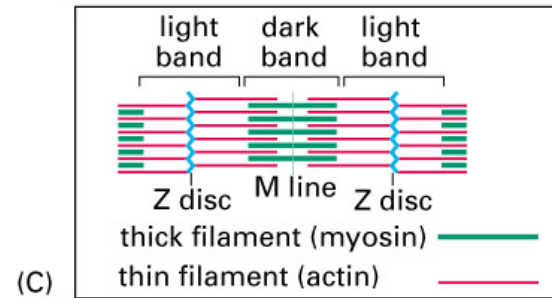
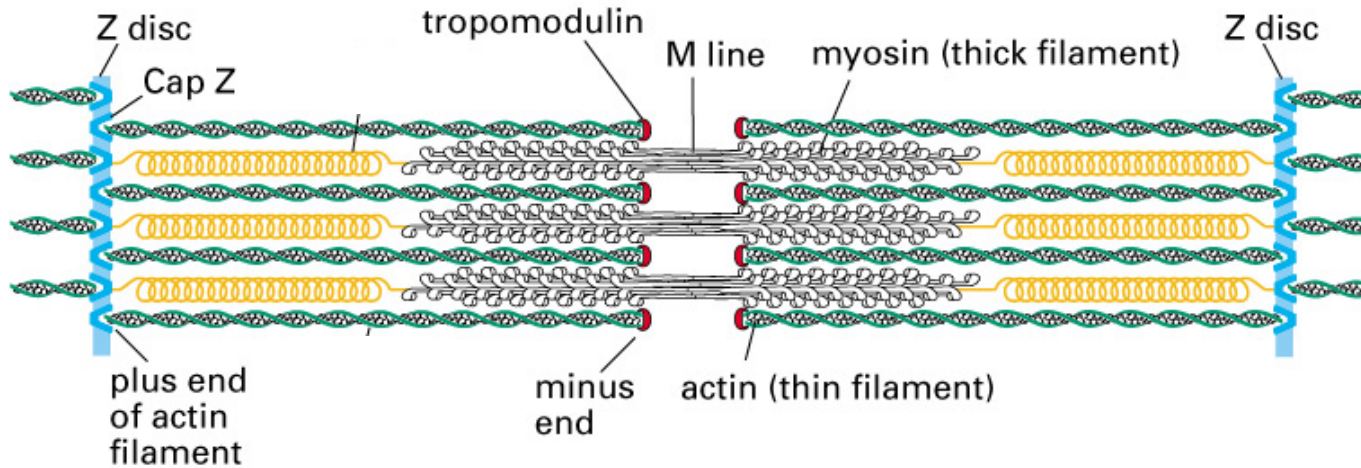


Figure 16-69 part 2 of 2. *Molecular Biology of the Cell*, 4th Edition.



Z disc = α actinin + CapZ

Figure 16-72. *Molecular Biology of the Cell*, 4th Edition.



Ca²⁺-dependence of Muscle Contraction



No Ca²⁺, tropomyosin blocks myosin-AF binding



Ca²⁺ released from SR and binds to troponin C



Conformational change in troponin I



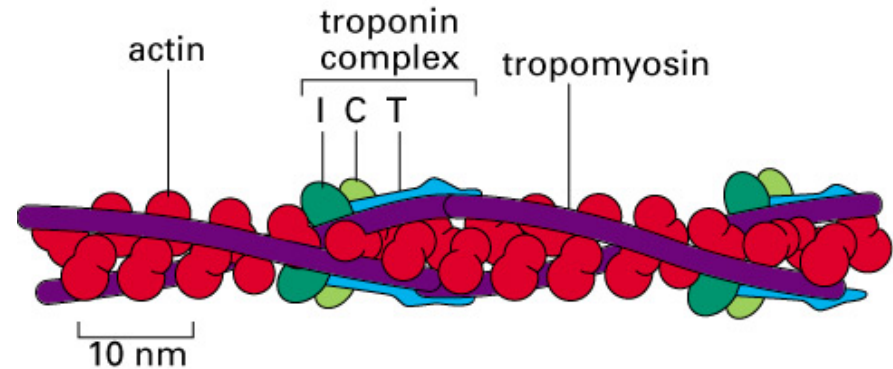
Tropomyosin (bound to Troponin T) moves and exposes binding sites



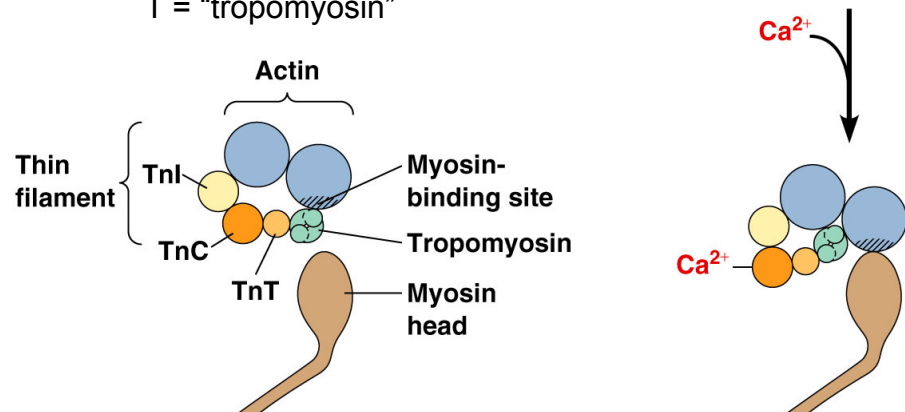
Myosin-AF binding



Muscle contraction



I = "inhibitory"
C = "calcium"
T = "tropomyosin"



Becker et al. World of the Cell

Myosin II S1 Fragment

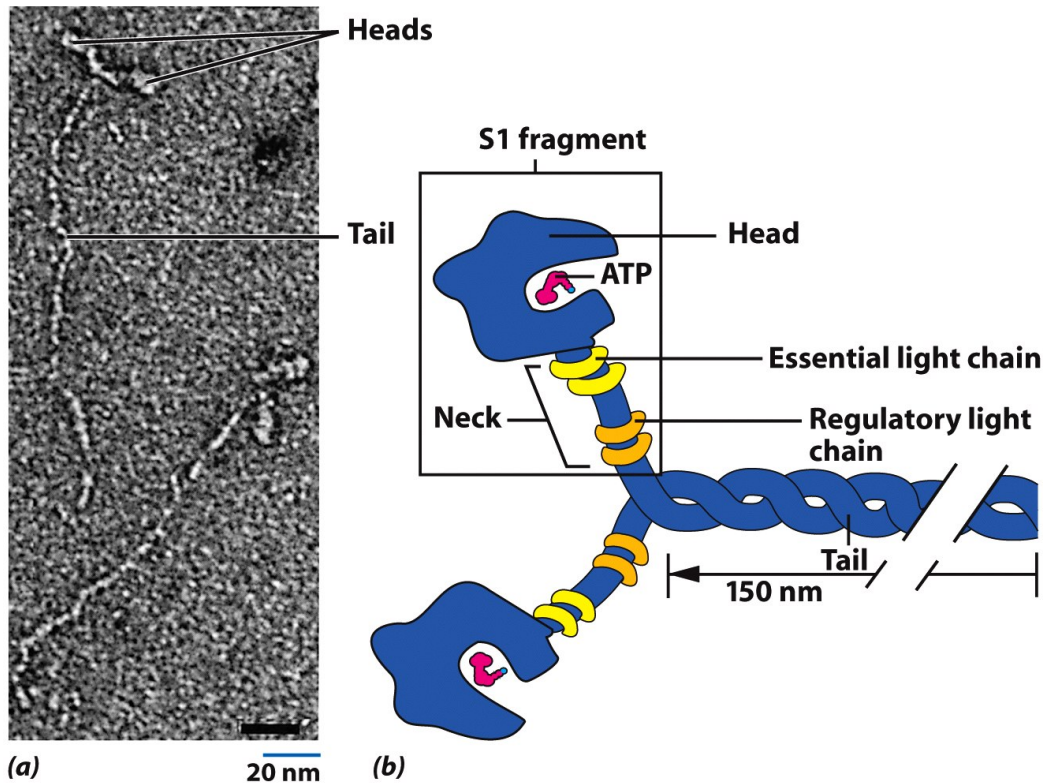


Figure 9-49 Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

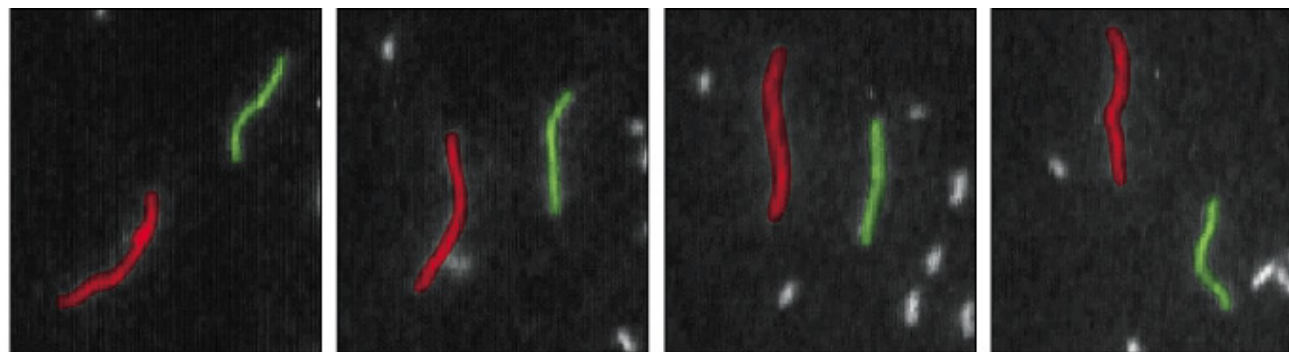
- Addition of protease enzyme.
- Myosin cleaved between neck and tail.
- S1 contains catalytic site.
- Can be immobilized on glass surface.
- Slide AFs *in vitro*.

Experimental Evidence



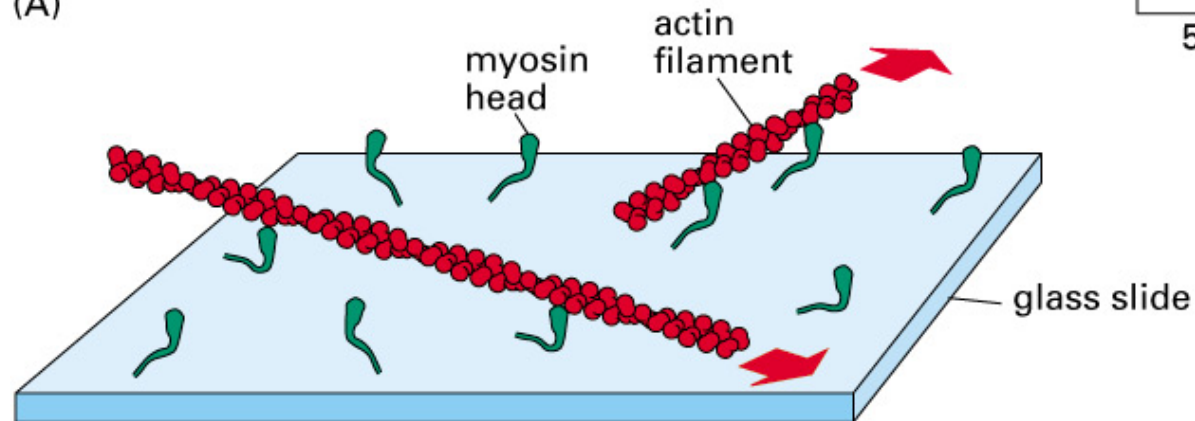
- S1 fragments propel AFs on glass slides

Myosin labelled
with phalloidan



(A)

5 μm



(B)

Spudich

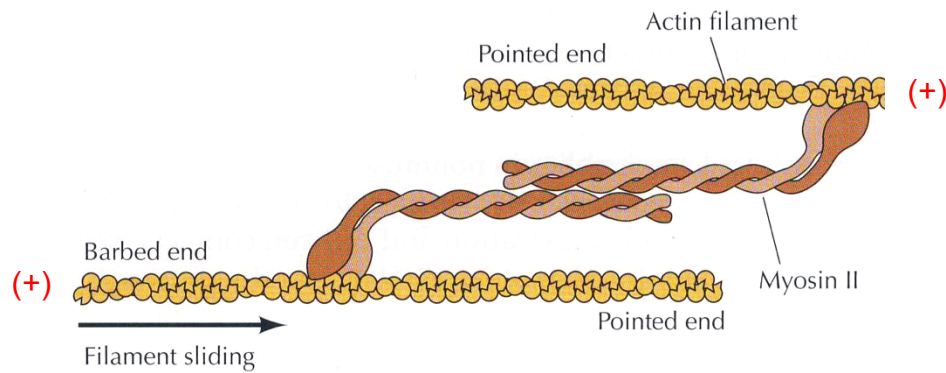
Figure 16-53. Molecular Biology of the Cell, 4th Edition.



“Contractile Ring”



- Important during cytokinesis.
- Myosin II and actin filaments.
- Redistribution of filaments.



Cytokinesis



Fig. 17-49B, Alberts

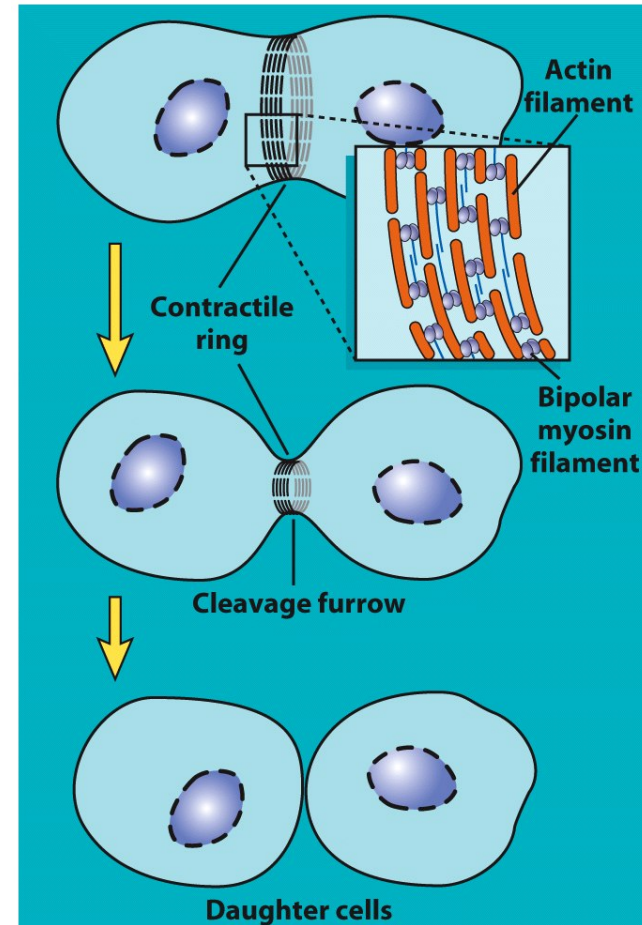


Figure 14-35a Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

Myosin V

- Long neck region.
- “Hand-over-hand” steps.
- *Processivity*.
- Carries cargo (organelles).

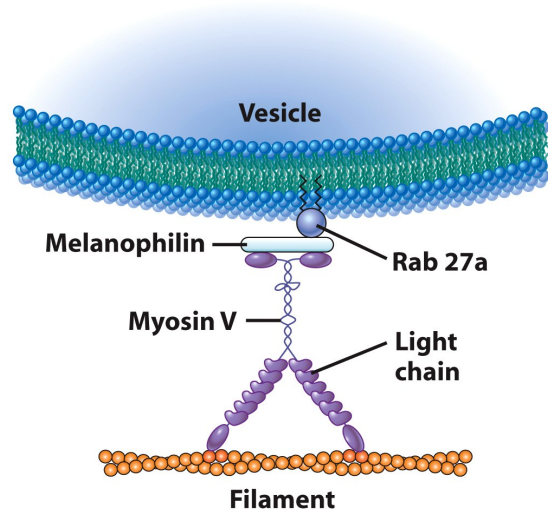
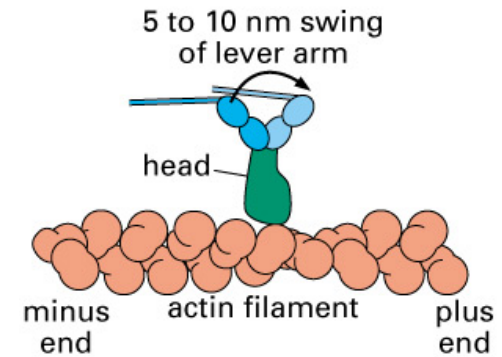


Figure 9-52b Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

Note: only 1 of 2 heads shown in figures

Myosin II



Myosin V

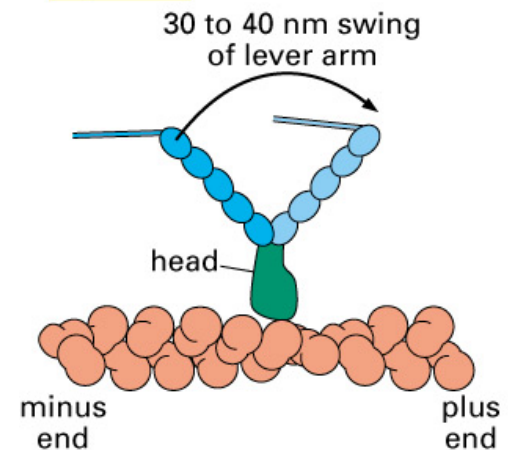
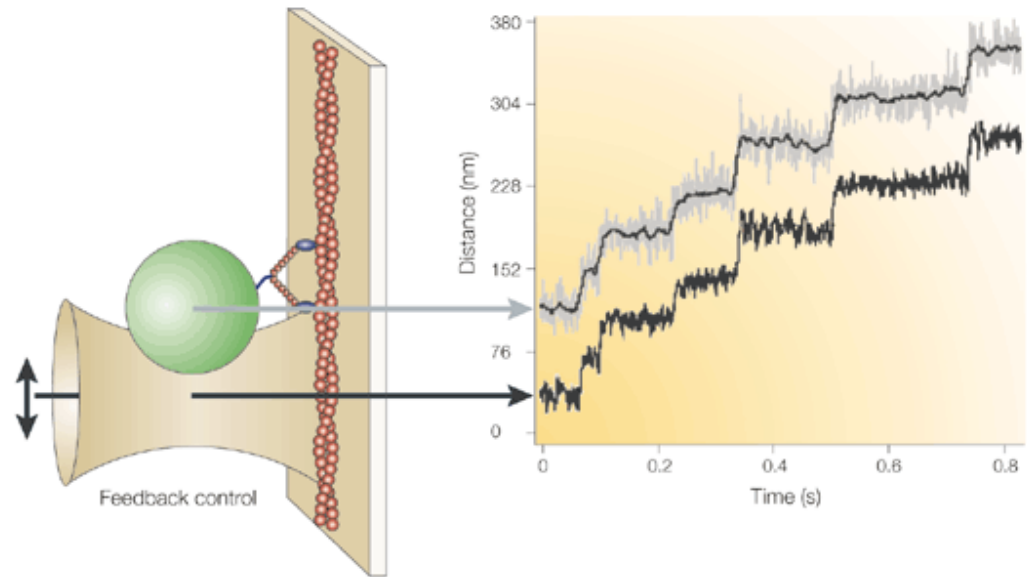


Figure 16-61. Molecular Biology of the Cell

Experimental Evidence



- Optical trap with feedback control.
- Polystyrene bead bound to myosin V.
- Steps measured as 30 – 40 nm.

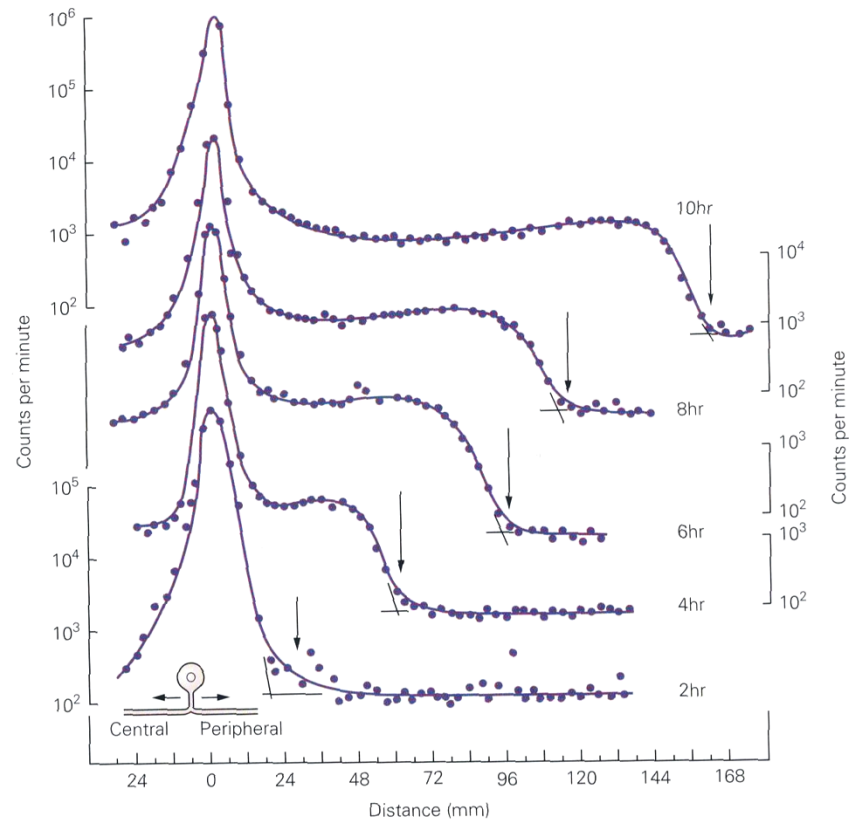


Nature Reviews | Molecular Cell Biology

Kinesins



- 1960s: Proteins involved in axonal transport were unidentified.
- Radioactive proteins containing ^3H -leucine moved along sciatic nerve in cat.
- 410 mm/day.



Kinesins

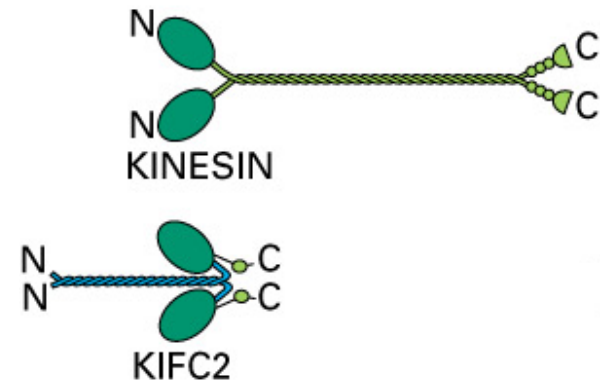


Discovery

- MTs adhered to glass coverslips.
- Axoplasmic extract from squid giant axon + ATP induced organelle movement.
- ATP but no axoplasm, no movement.
- With non-hydrolyzable form of ATP (AMP-PNP), vesicles bound to MTs but no movement.
- *Kinesin* isolated and identified as motor protein.

Kinesin Structure

- Similar basic structure as myosins.
- Head region conserved.
- Tail regions diverse.
- C-terminal domain attaches to cargo.
- Most travel on MTs toward (+) end.
- KIFC2 travels toward (-) end.



See also Fig. 16-63 in Alberts

Kinesin Structure



- ATP-binding sites (yellow) similar.
- MT vs. AF binding sites differ.
- Linker region interacts with catalytic core, thus swinging arm around.

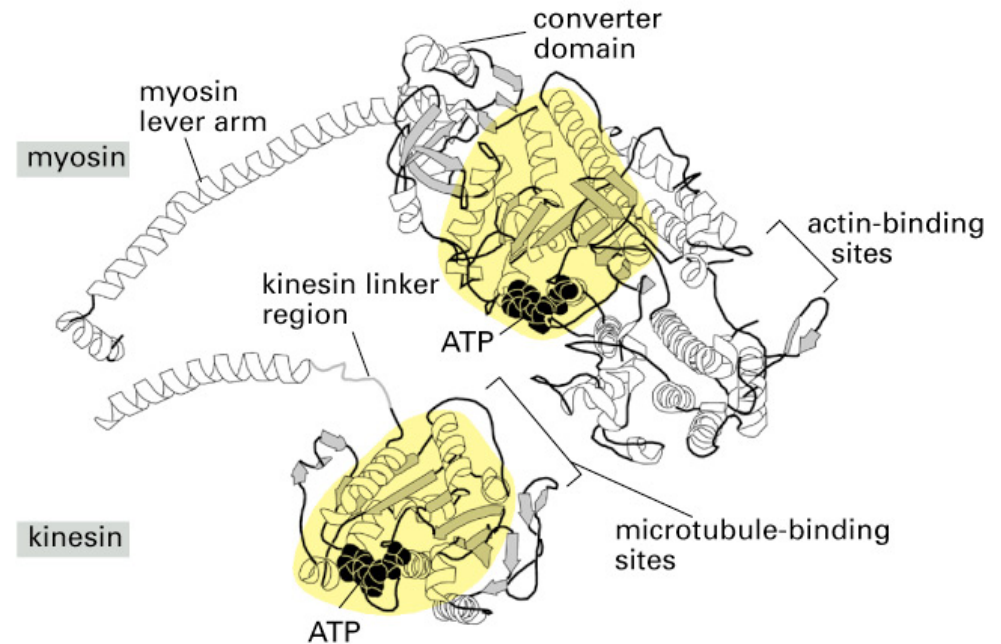


Figure 16-57. Molecular Biology of the Cell, 4th Edition.

Kinesin Cycle

- *Processive* steps along MTs.
- 1. ATP binding of leading head induces conformational change in its linker region; trailing head advances.
- 2. ADP induces weak binding of leading head.
- 3. Hydrolysis of trailing head induces detachment; ADP dissociates from leading head. (*Repeat...*)

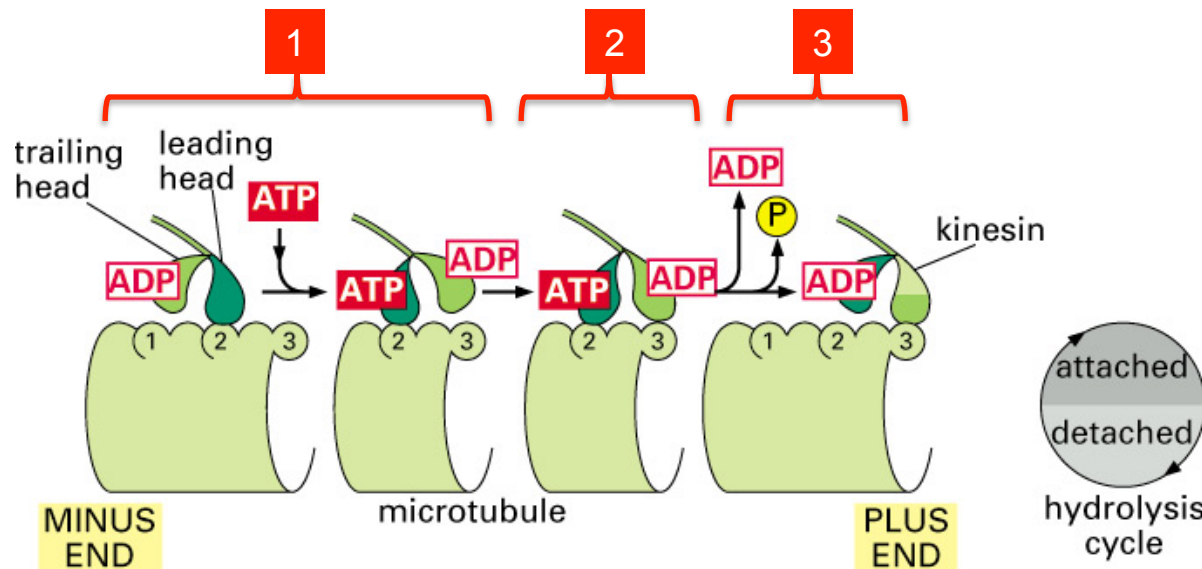


Fig. 16-62 starts at step 2

Longer hydrolysis cycle than with myosin II

Kinesin Cycle



Myosin and Kinesin Compared

Myosin II

Attach to AFs

Contract muscle

Rapid power stroke

No head coordination

5% of time attached

ATP binding → release

2 - 60 $\mu\text{m/s}$

< 8 pN theoretical force*

Kinesins

Attach to MTs

Transport organelles

Longer attachment

Processive steps

50% attached

ATP hydrolysis → release

< 2 $\mu\text{m/s}$

< 5 pN*

Note: binding will occur without ATP

Dyneins

- (-) end directed.
- MT motor proteins.
- 2 major divisions:
 - **Cytoplasmic**, e.g. retrograde vesicular transport.
 - Axonemal, e.g. beating of cilia and flagella.

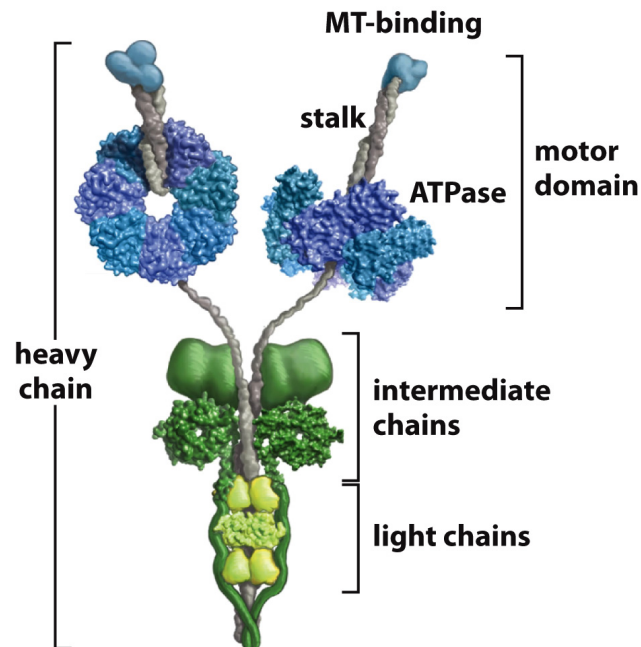
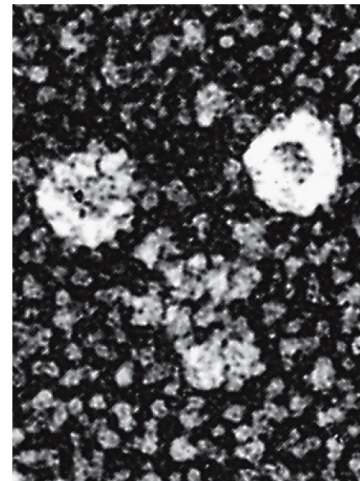
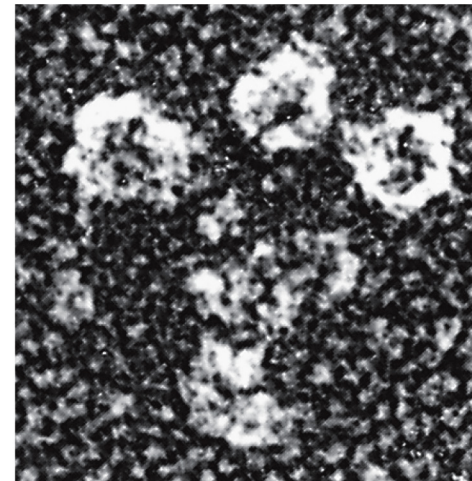


Figure 16-58b Molecular Biology of the Cell 6e (© Garland Science 2015)

cytoplasmic dynein



ciliary dynein



25 nm

Figure 16-58a Molecular Biology of the Cell 6e (© Garland Science 2015)

Cytoplasmic Dynein

- Dynein cannot bind directly to cargo.
- Attachment to MT mediated by *dynactin complex*
- So named because of inclusion of actin (red).
- Other accessory proteins.

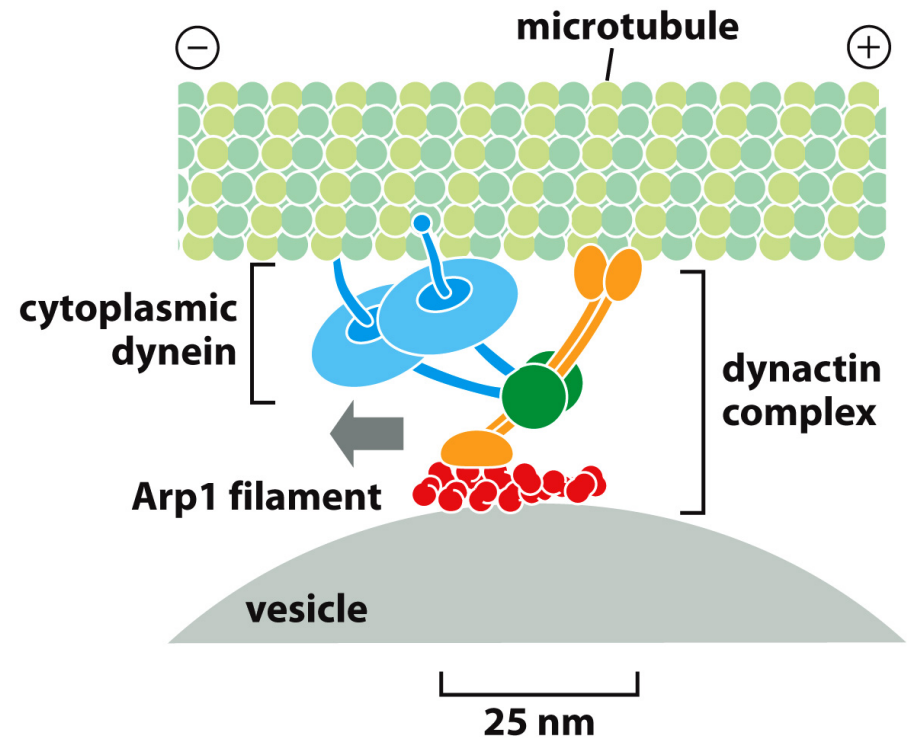


Figure 16-60 Molecular Biology of the Cell 6e (© Garland Science 2015)

Axonal Transport

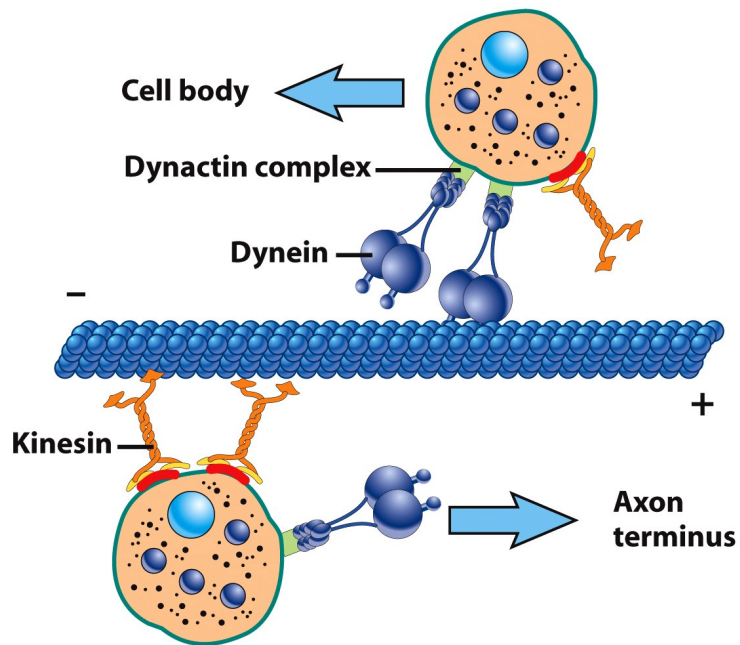
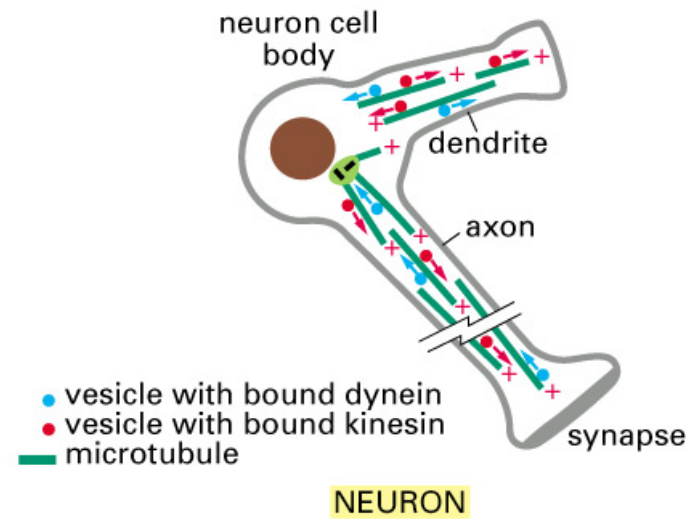


Figure 9-17b Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

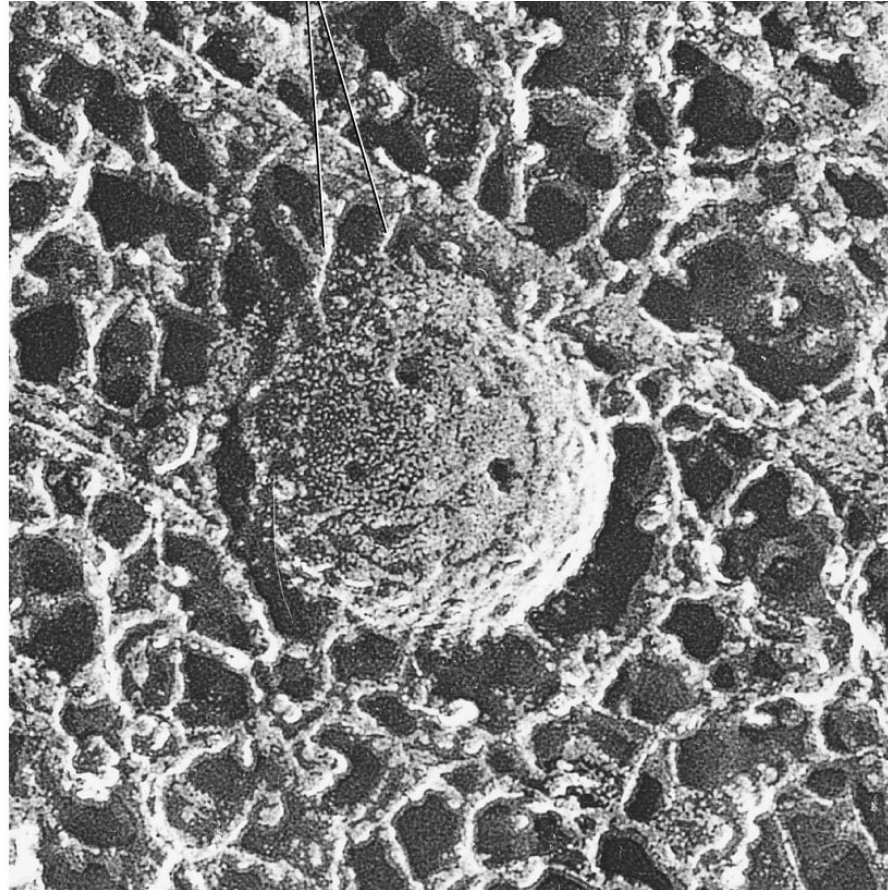


Organelles – Kin/Dyn



Axonal Transport

- SEM micrograph of vesicle being transported along axon MTs by kinesin or dynein.



Role of AF vs. MT-based Motors

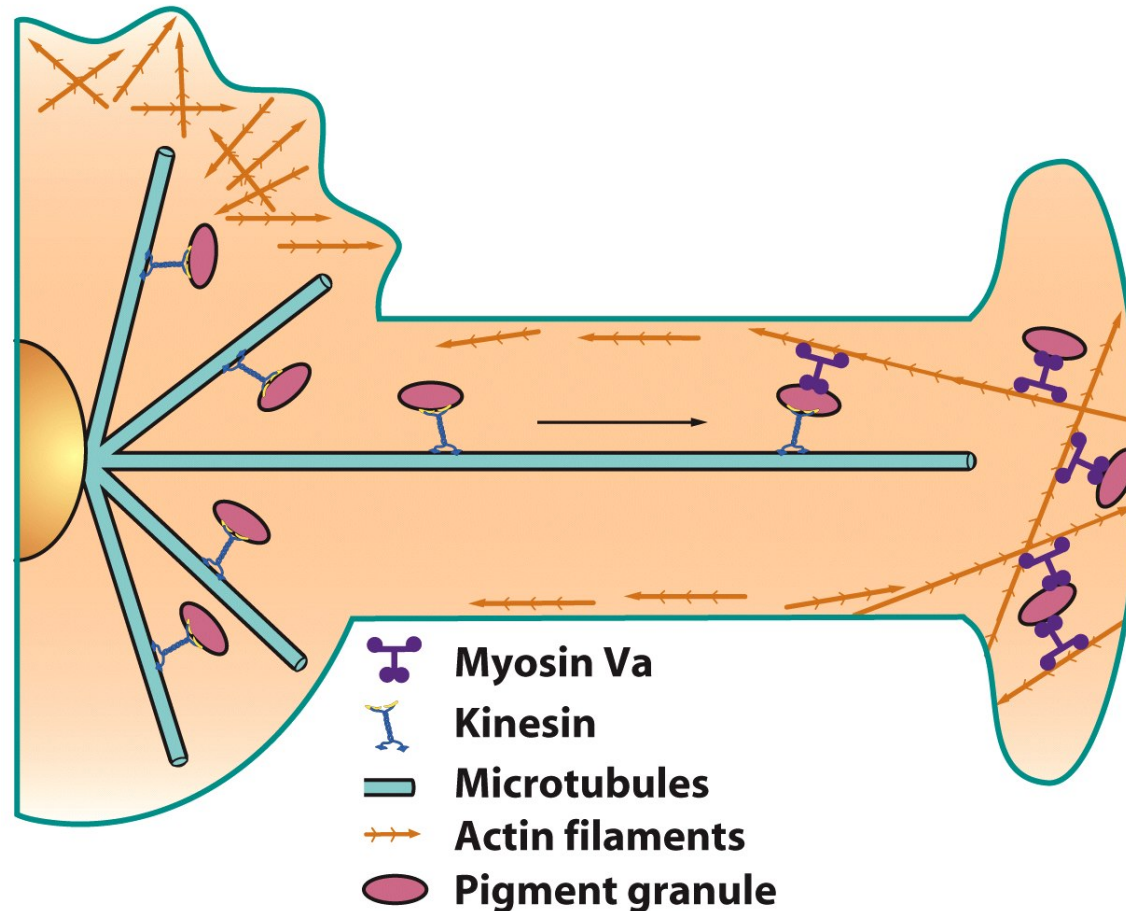


Figure 9-53 Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

Things to Consider...

1. Structure is related to function. Think about the differences between types of motors at the molecular level that lead to differences in function.
2. Why isn't there a single motor protein that performs all of the roles discussed?