# (3) Types of cytoskeletal fibers

Microtubule


25 nm

Actin filament

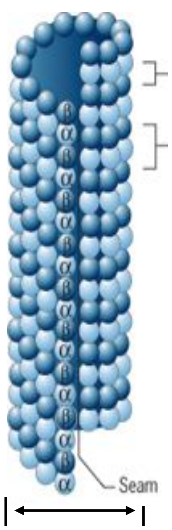
8 nm

Intermediate filament

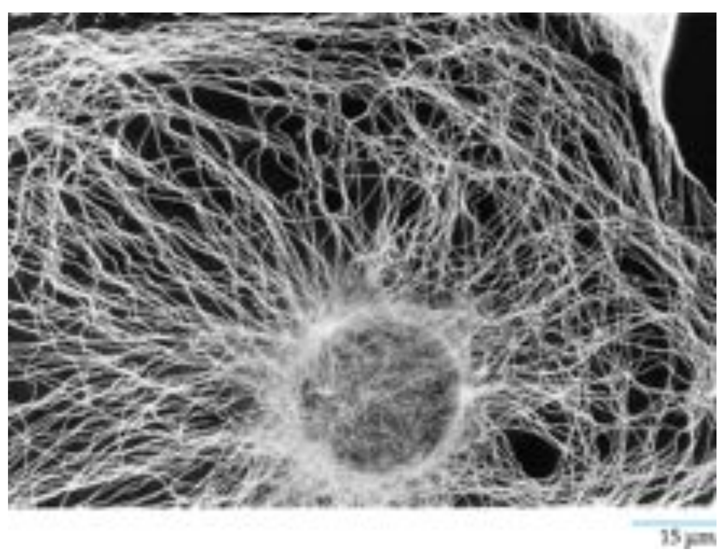
10 nm

Fibers made of polymerized protein

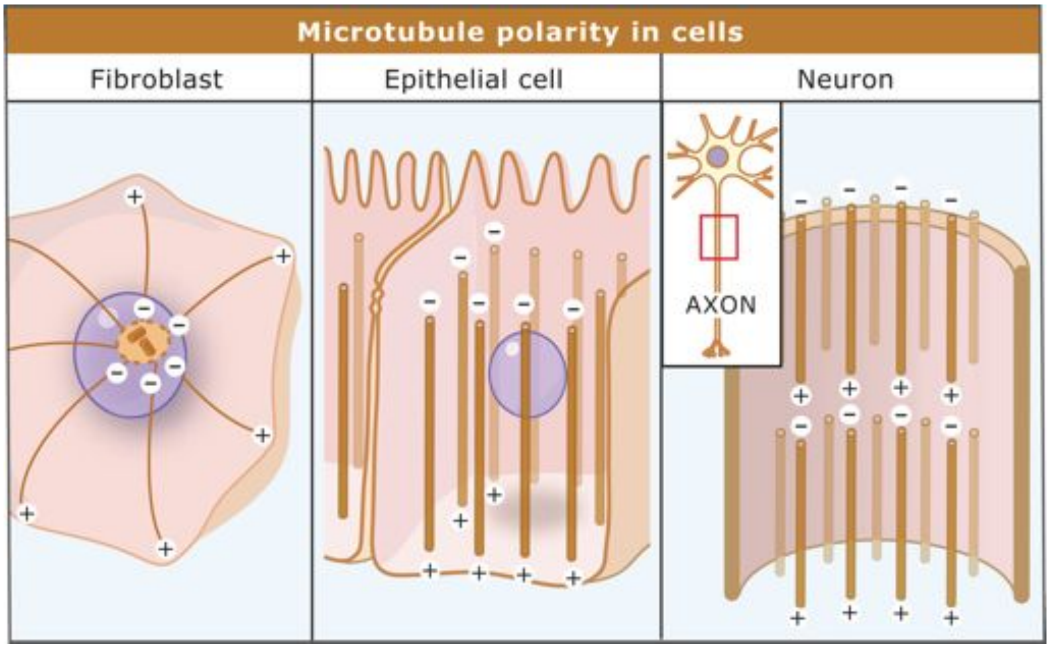
Microtubule




25 nm

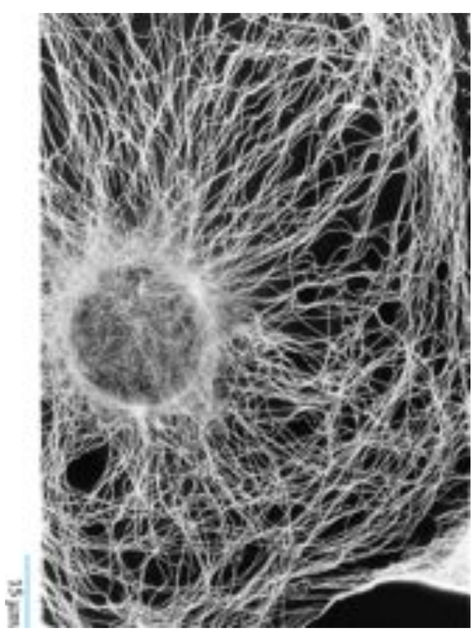


## Cell Shape and Transportation:

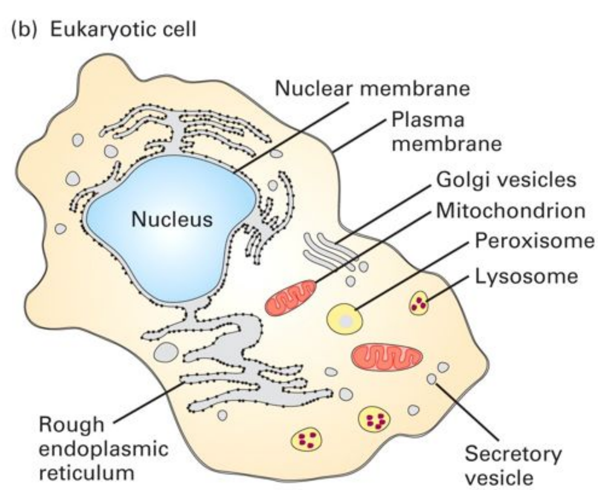



(Cells, Figure 7.14)

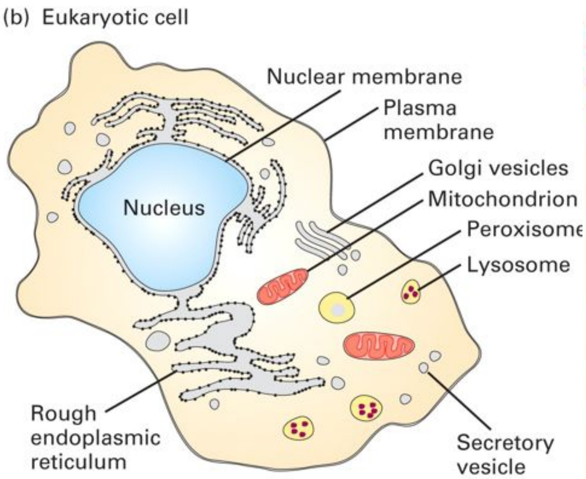
# Organization of the Cell

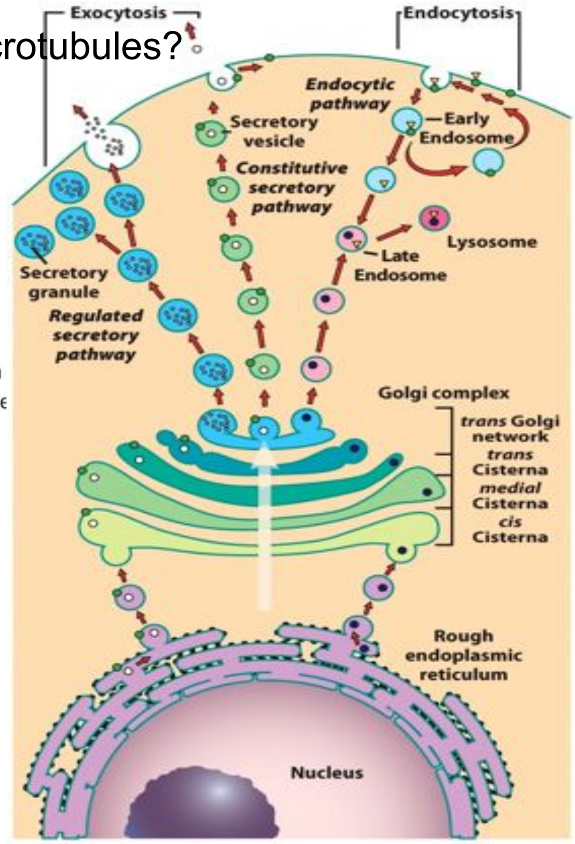


Organelles are not passive blobs?



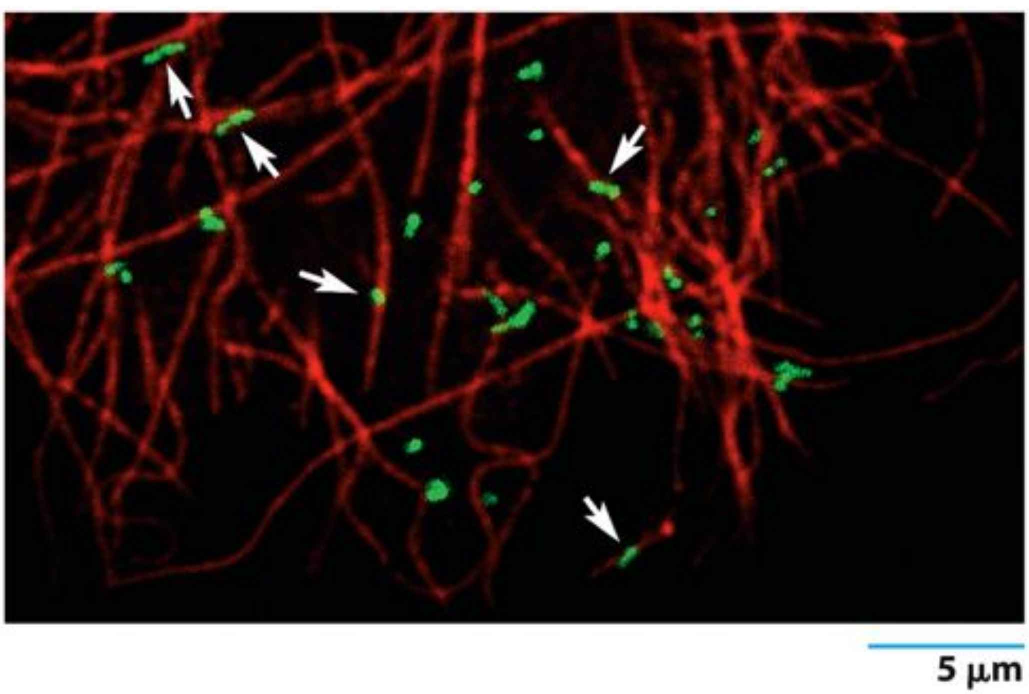
Do these organelles move on microtubules?






## A snapshot

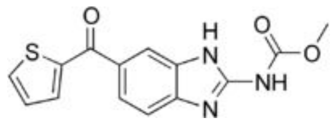




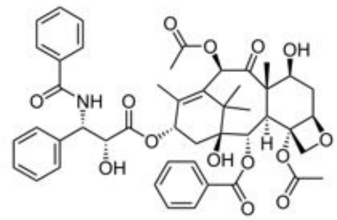

(Figure 9.2)

# Can we stop it?

Nocodazole

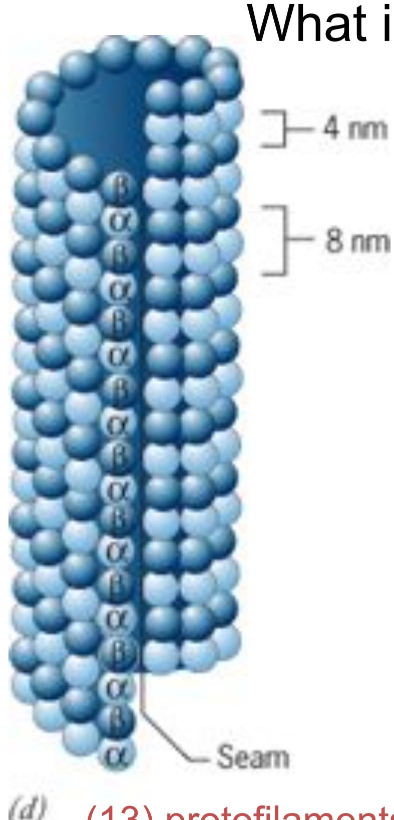


Taxol



Drugs can be used to study the functions of Microtubules

What is the structure?




GTP/GDP

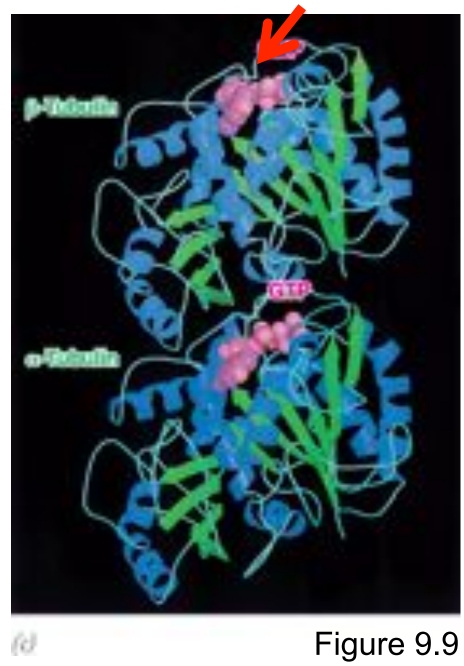
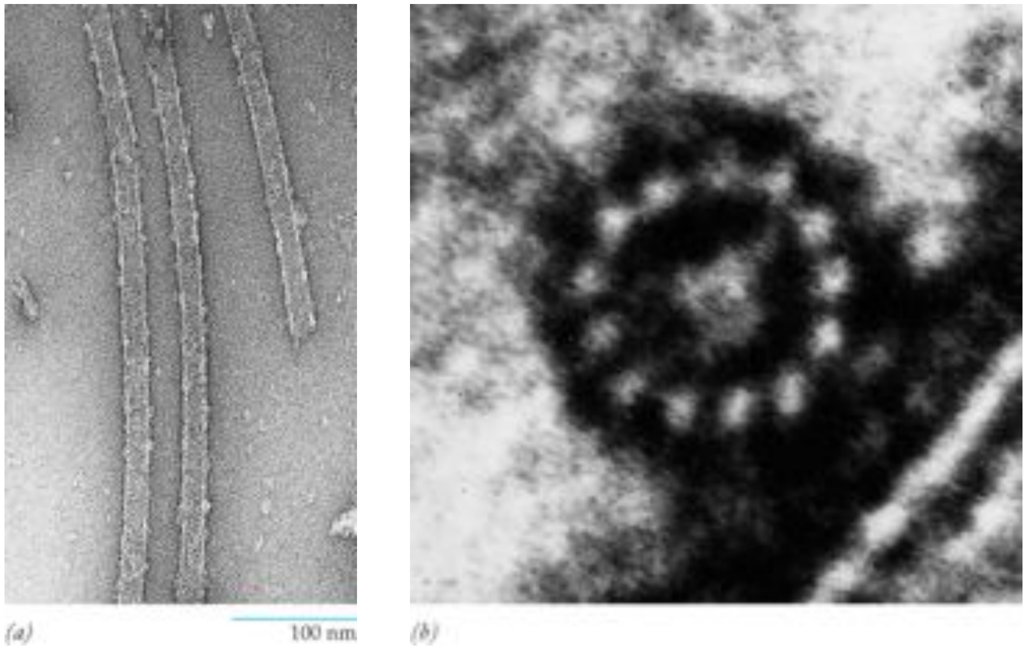


Figure 9.9

(13) protofilaments align to form a hollow tube

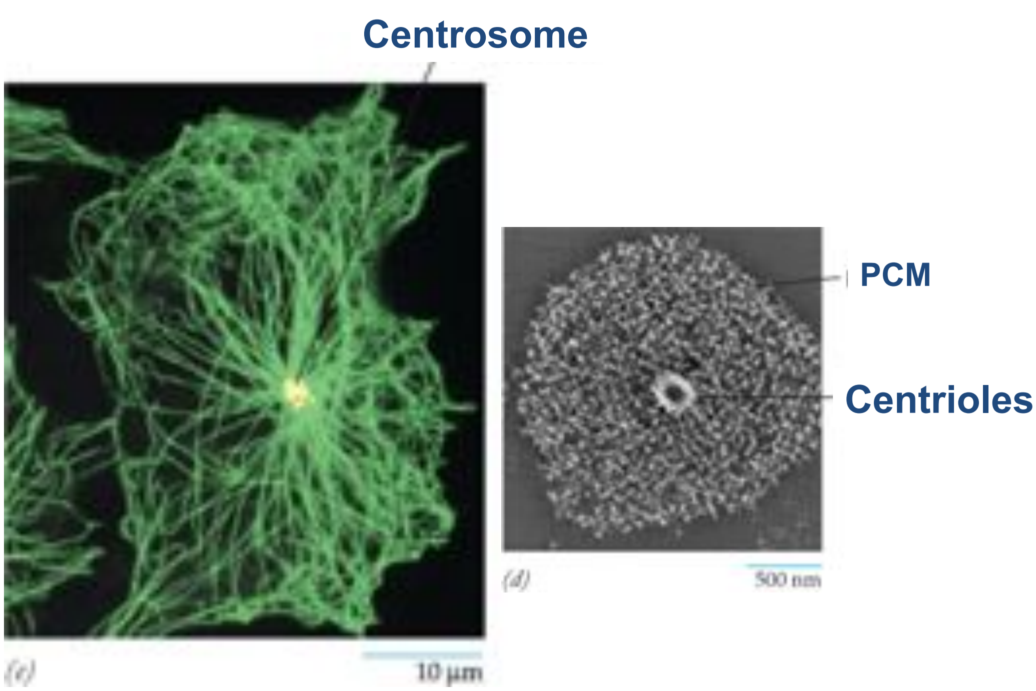
What is the structure? Microtubules are polymers

## How can we see this?

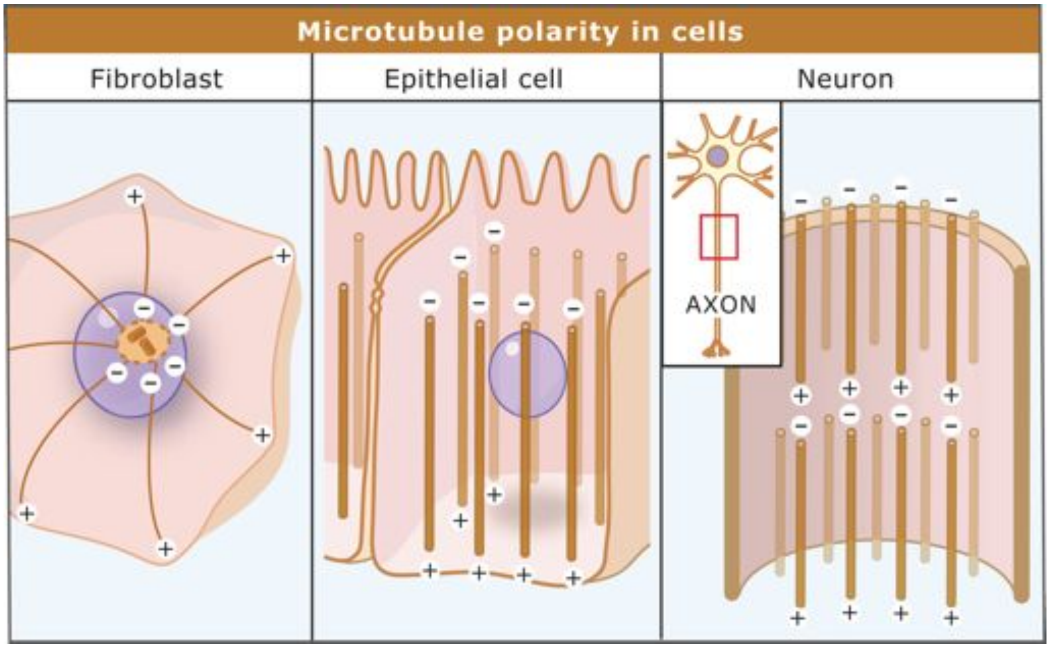



Self-assembly into polymer/microtubule

Microtubule Organizing Center (MTOC)

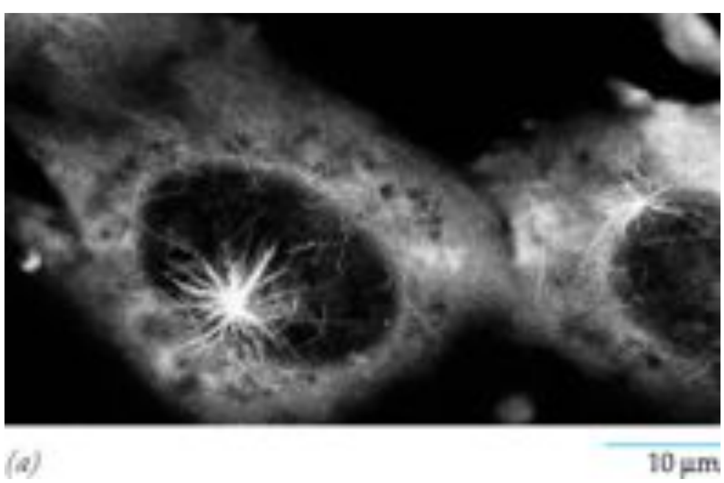


# Transportation and Polarity:




(Cells, Figure 7.14)

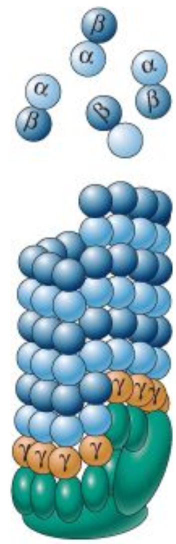
## Experiment to tell you where the MTOC is?




Colcemid washout experiment

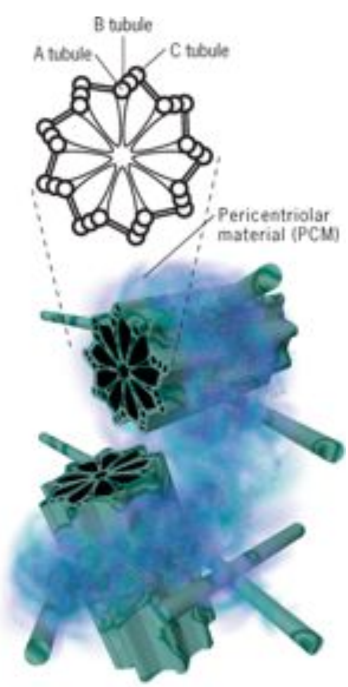
Figure 9.19

# Microtubule Assembly

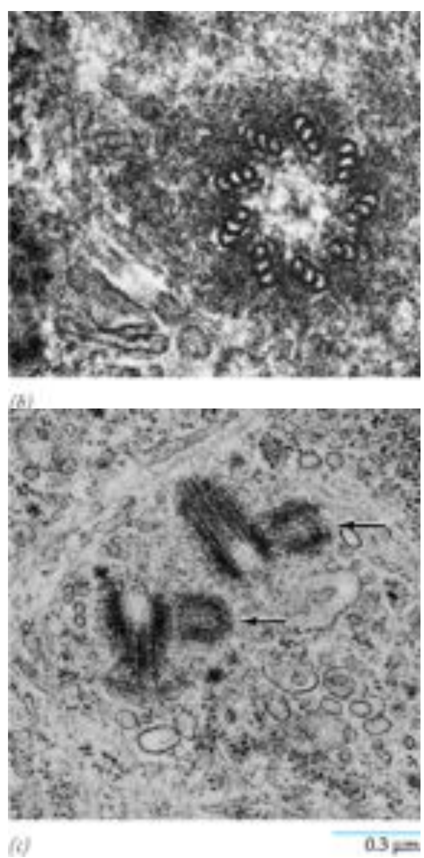



(c)

(13) protofilaments align to form a hollow tube = microtubule
lateral bonds give tubule strength

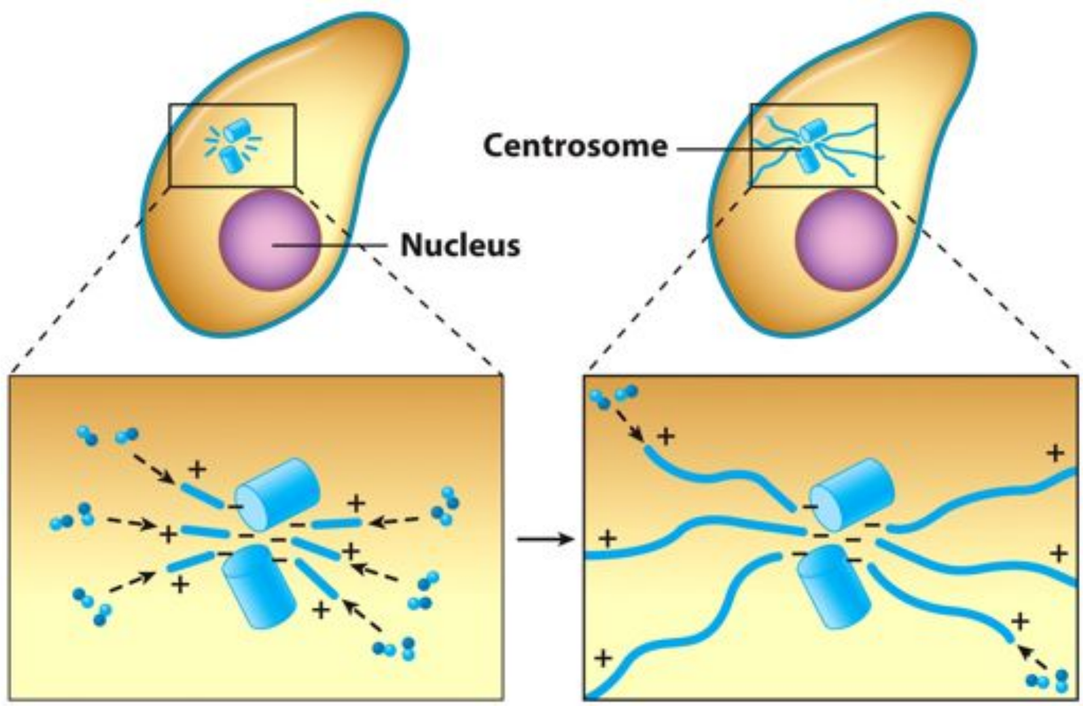



(a)



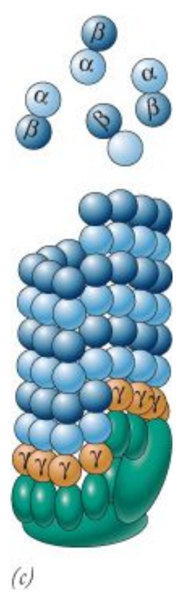

(b)

(c)





# Microtubule Assembly




(13) protofilaments align to form a hollow tube = microtubule

lateral bonds give tubule strength

## Where are subunits added?

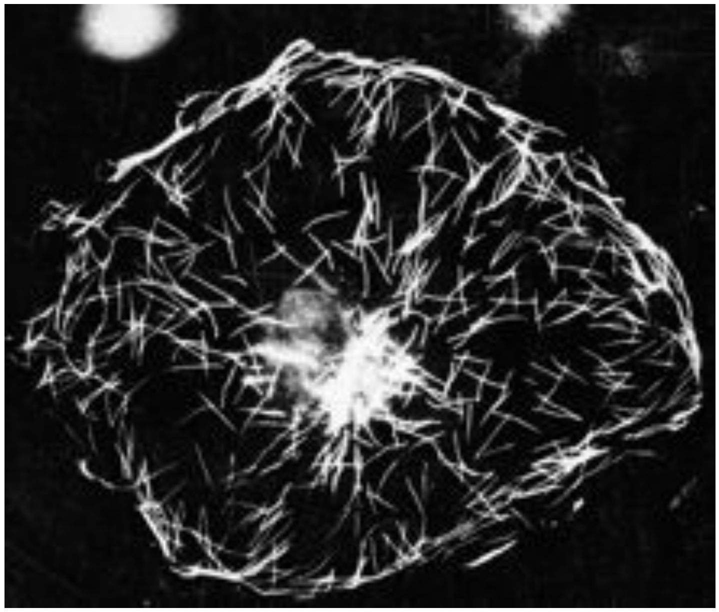


Figure 9.26

Injected with biotin-Tubulin (1 minute)

# FRAP

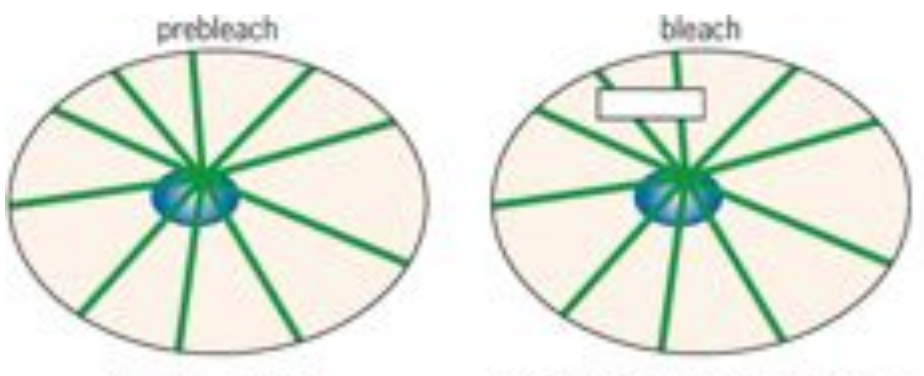



Figure 9.8

# FRAP:

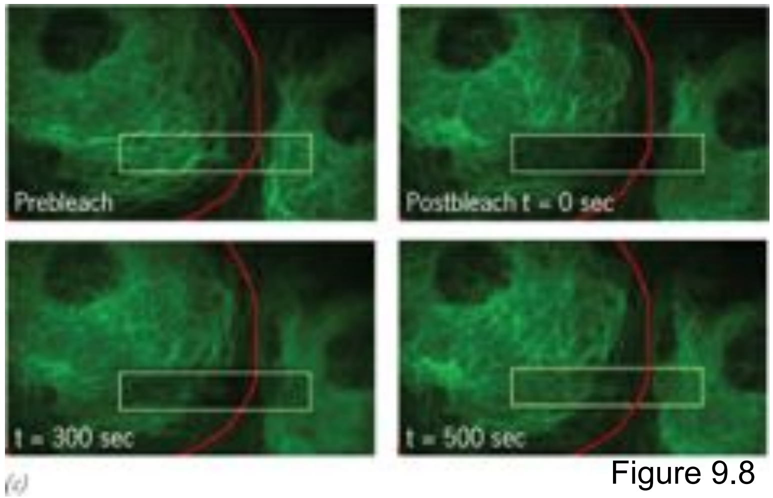
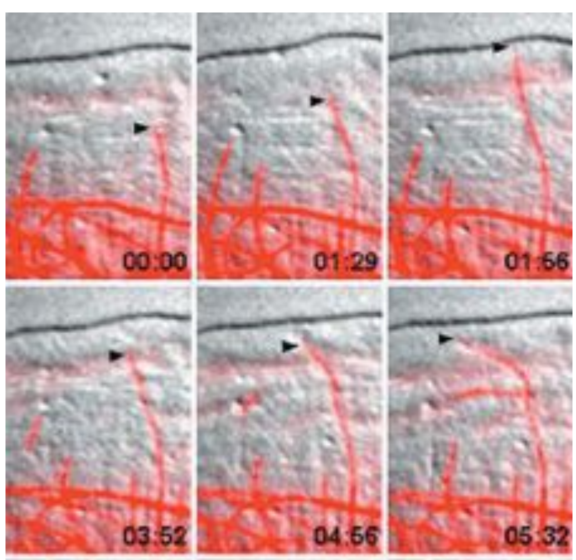



Figure 9.8

Can tell you about the dynamics of molecules in the polymer

# We can watch MT dynamics




Injected with rhod-Tubulin

Figure 9.4

# Dynamic Instability (Speckles)

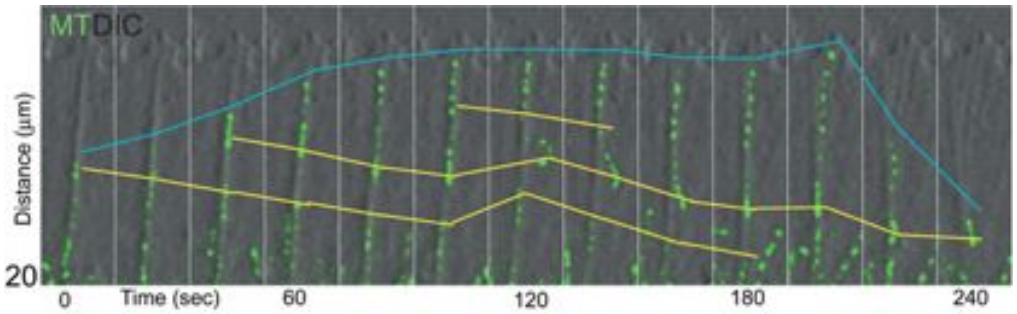
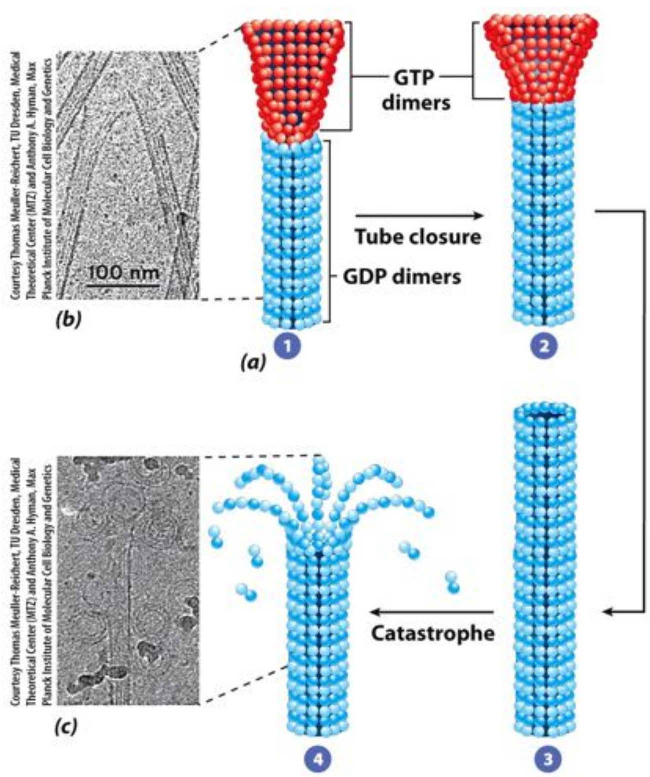



Figure 9.27

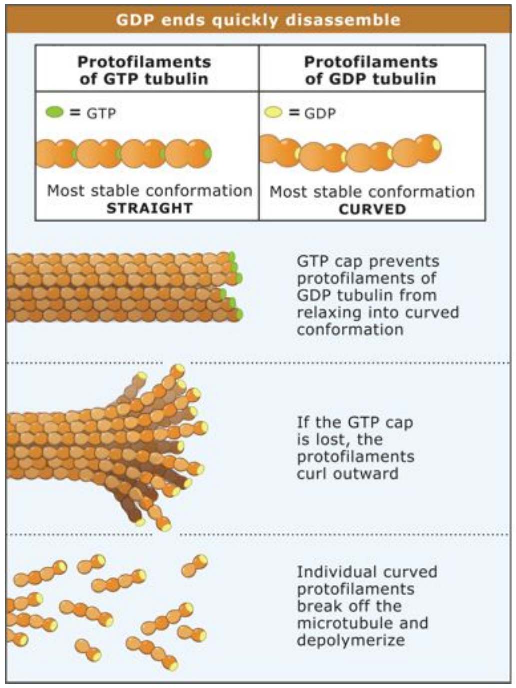
Inject with GFP-Tubulin

# GTP hydrolysis changes conformation and stability of MTs



# (+) end cap regulates stability of MT

GTP cap:

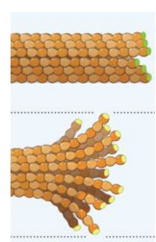



(+) end
β-tubulin

GTP-bound structure is different than GDP-bound

# Stability of microtubules

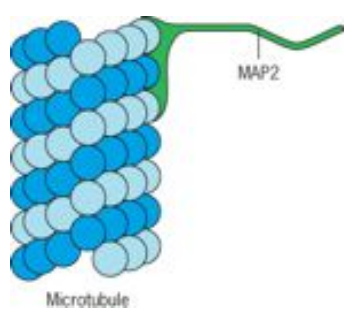
1) GTP vs GDP bound cap

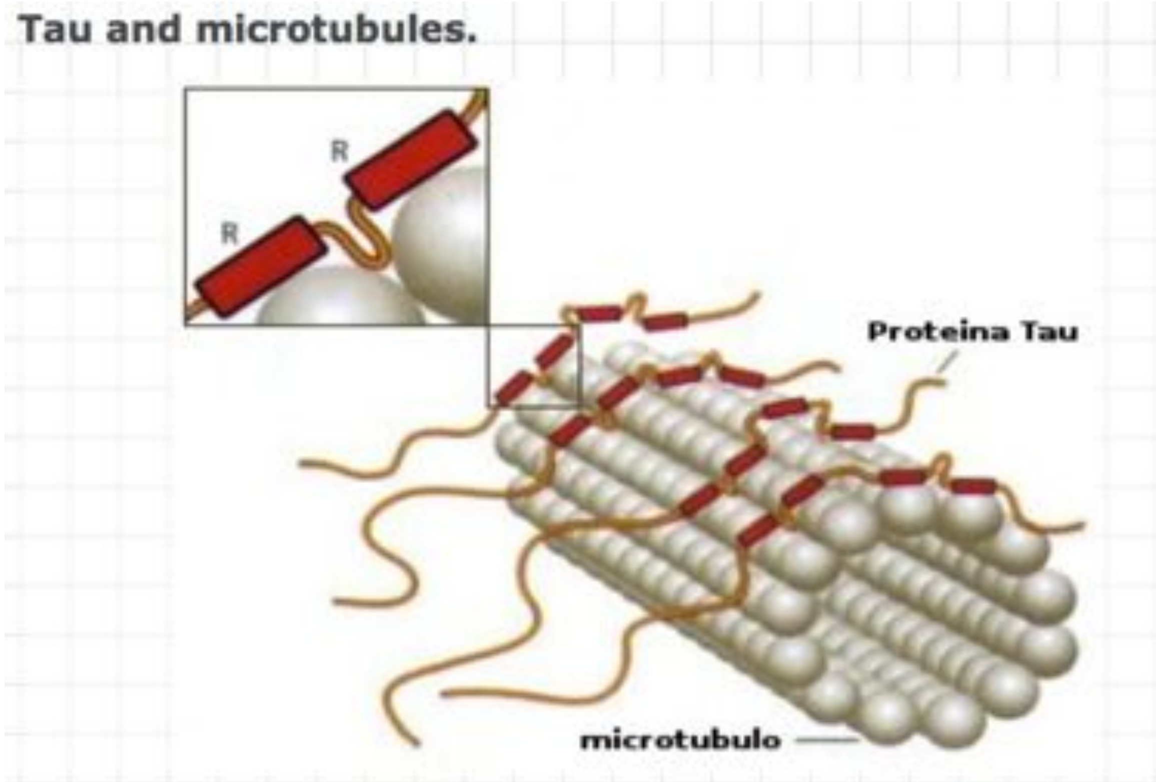


GTP

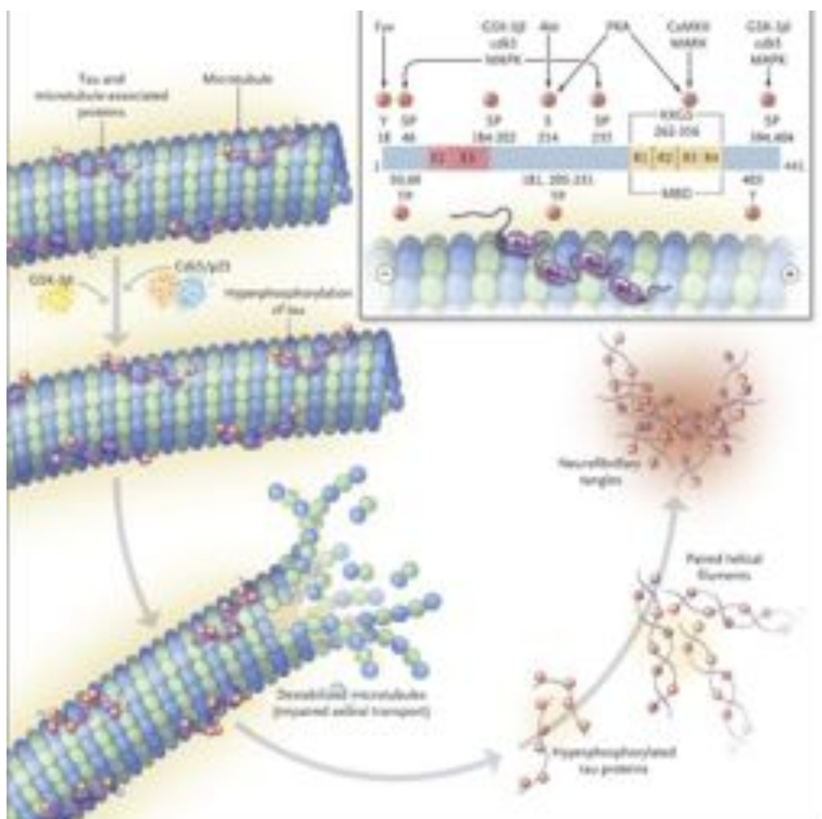
GDP

2) Microtubule associated proteins (MAPs)





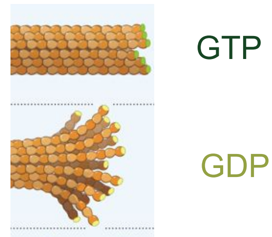

From website: flipper e nuvola



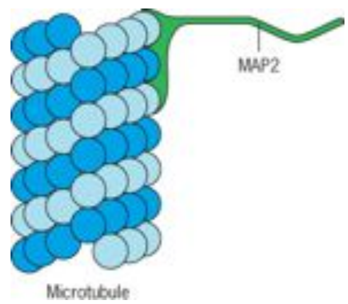
From website: flipper e nuvola

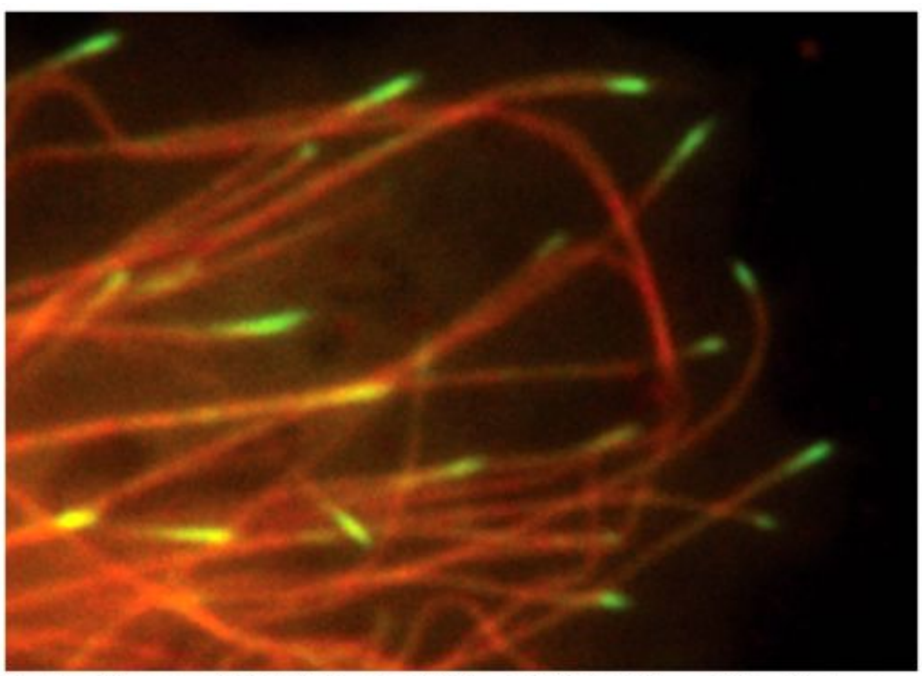
# Stability of microtubules

1) GTP vs GDP bound cap



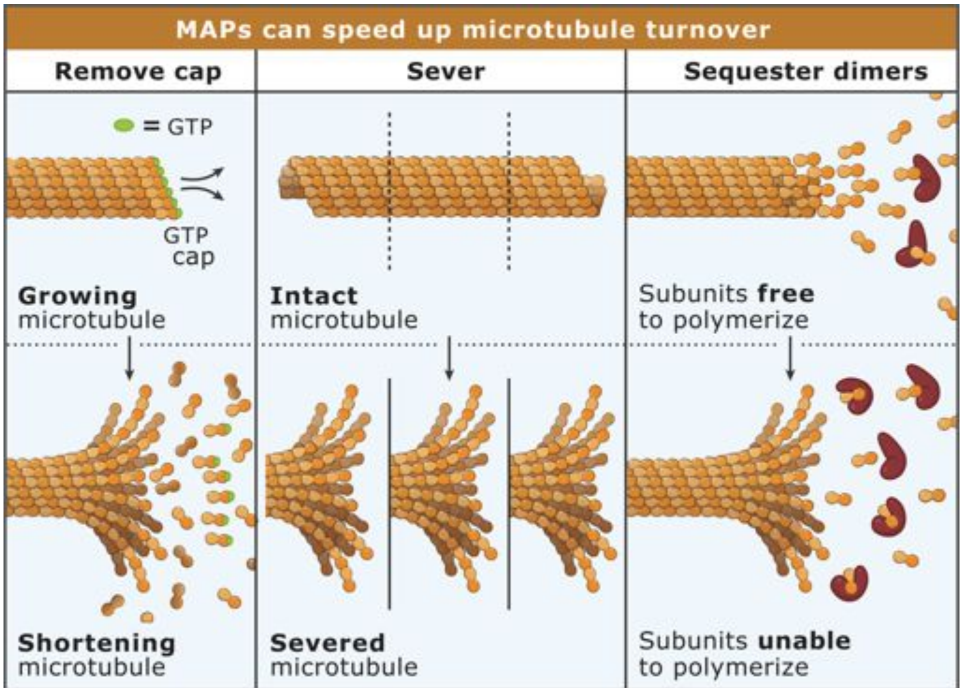

2) Microtubule associated proteins (MAPs)



From Anna Akhmanova and Michel O. Steinmetz, J. Cell Science 123, 3415, 2010; by permission of the Company of Biologists. Courtesy Anna Akhmanova and Ilya Grigoriev, Utrecht University, The Netherlands. http://jcs.biologists.org/content/123/20/3415.full?sid=f97c0f52-c919-4739-a062-0d83b8e8f68b

# MAPs can destabilize MTs

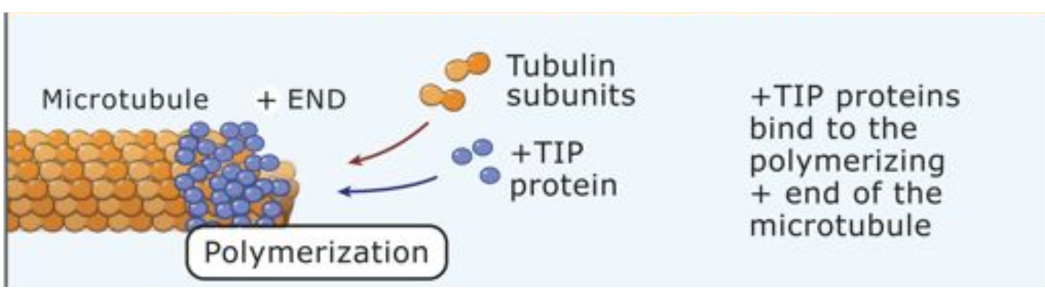



MCAK (GAP)

katanin

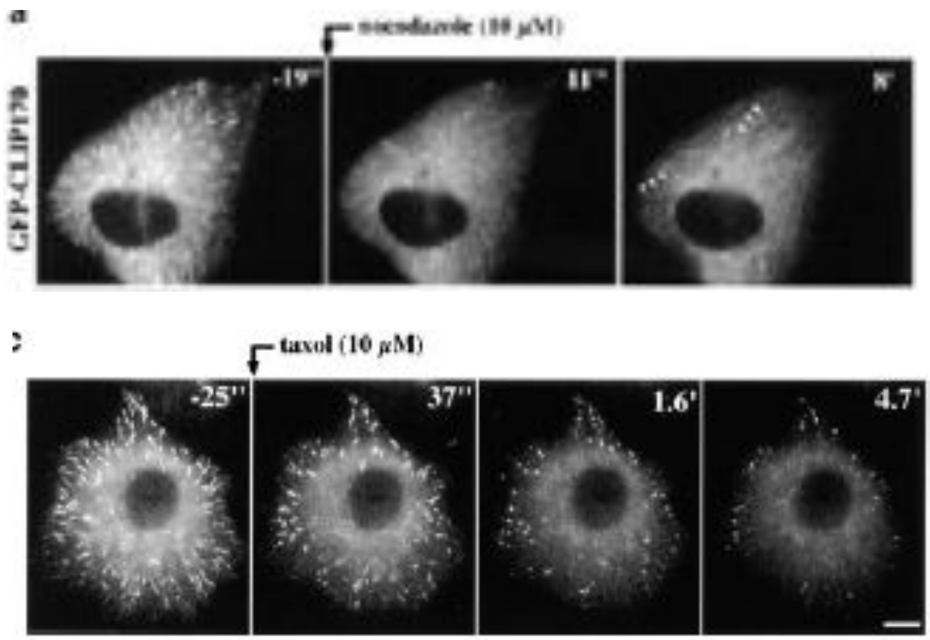
Colchicine (drug)

# How do (+) TIPs stabilize the (+) tip?

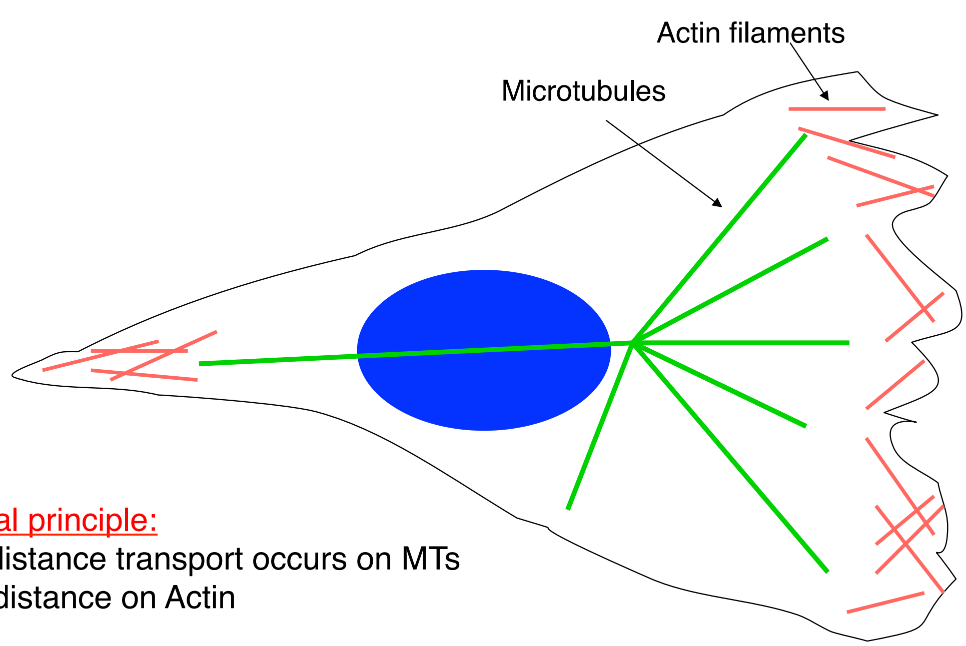



XMAP215
CLIP170 (function)

# Clip170

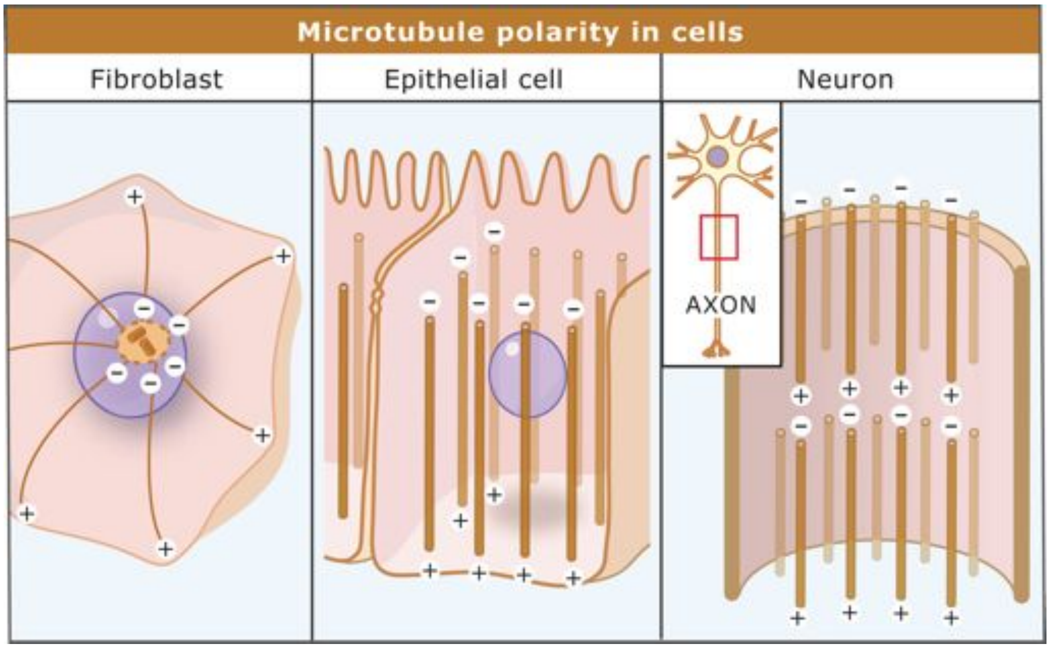



## Role of the Cytoskeleton and Motor Proteins in Membrane Trafficking

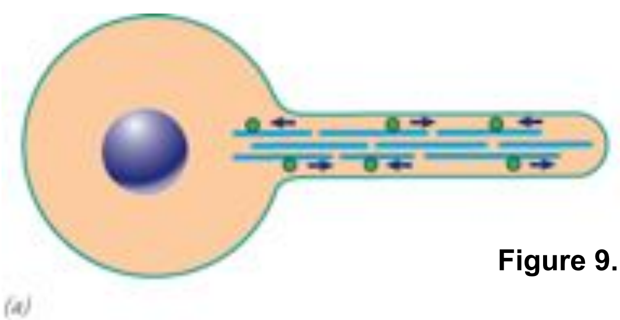



<u>General principle:</u>
Long-distance transport occurs on MTs
Short-distance on Actin

# Transportation and Polarity:




(Cells, Figure 7.14)



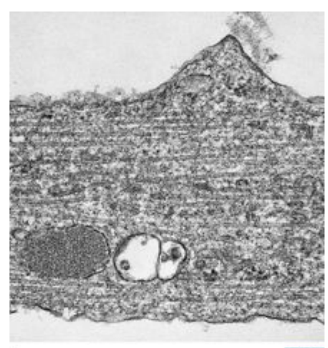
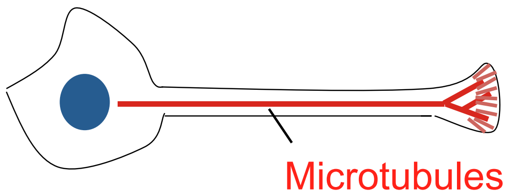


**Figure 9.13**

Which direction do mitochondria travel on microtubules?


Pilling and Saxton, MBOC 2006

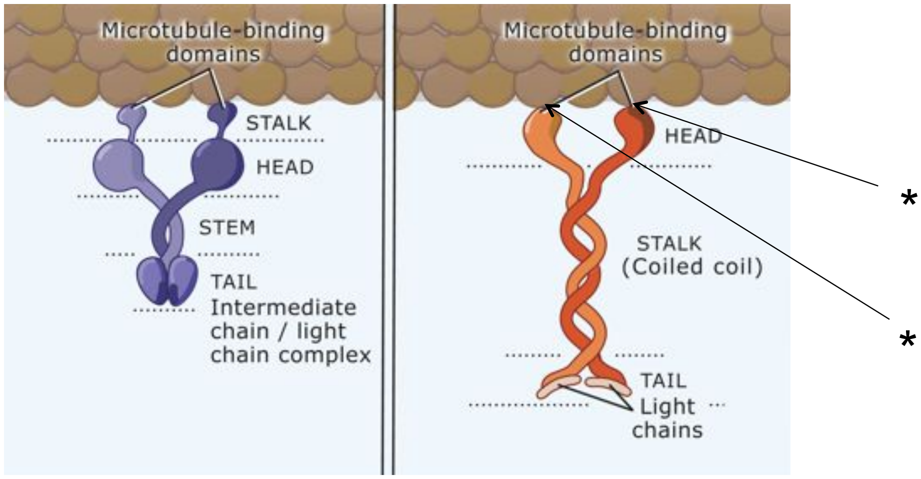



## Microtubule motor proteins

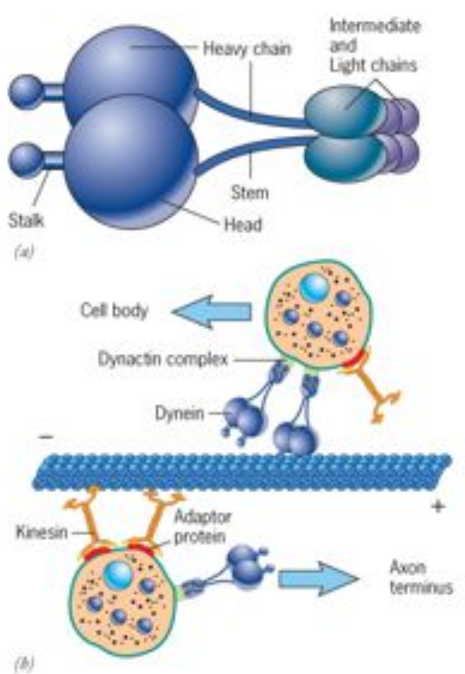
MOSTLY Plus-end directed motors----- kinesins

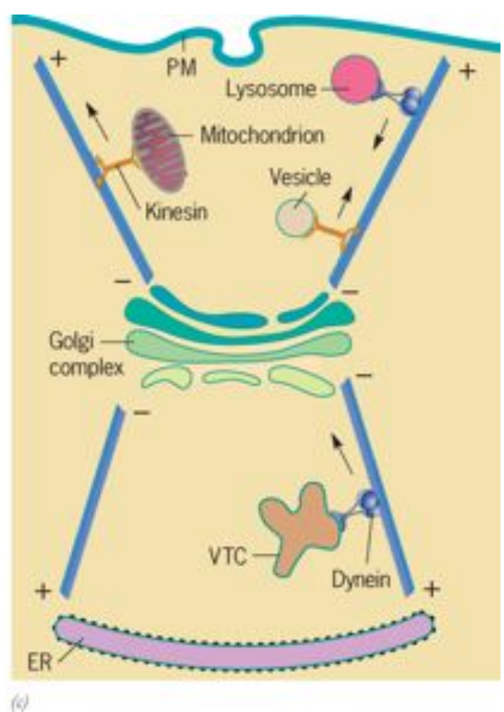
Minus-end directed motors----dyneins

# Motors “walk” along MTs towards the plus or minus end

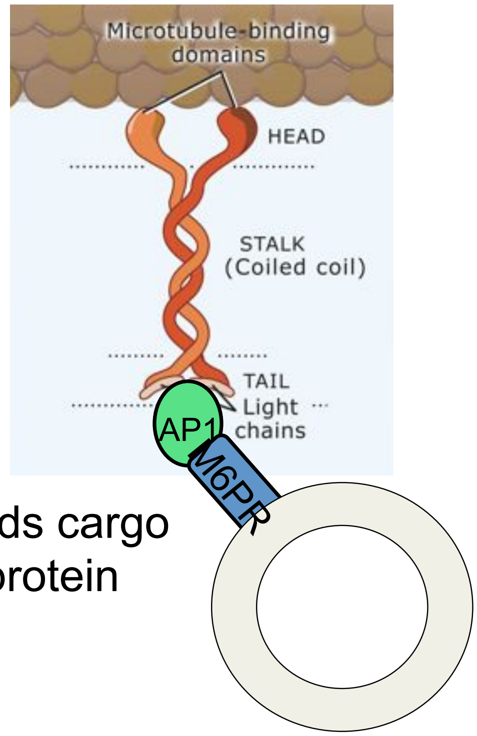



dyneins (-, only)    kinesins (+, mostly)



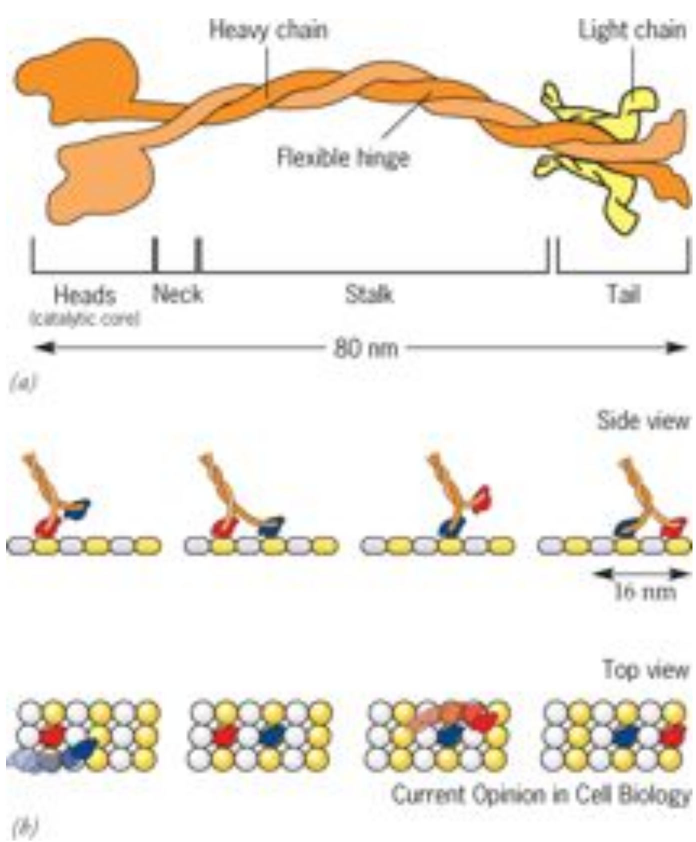
# How cargoes are loaded onto motors:



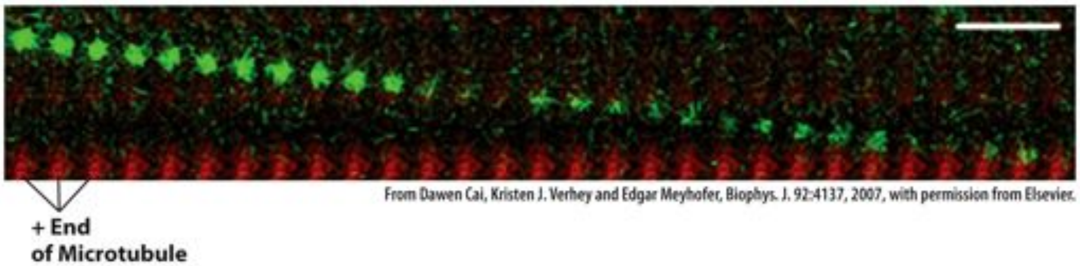

Tail domain binds cargo via adaptor protein

vesicle

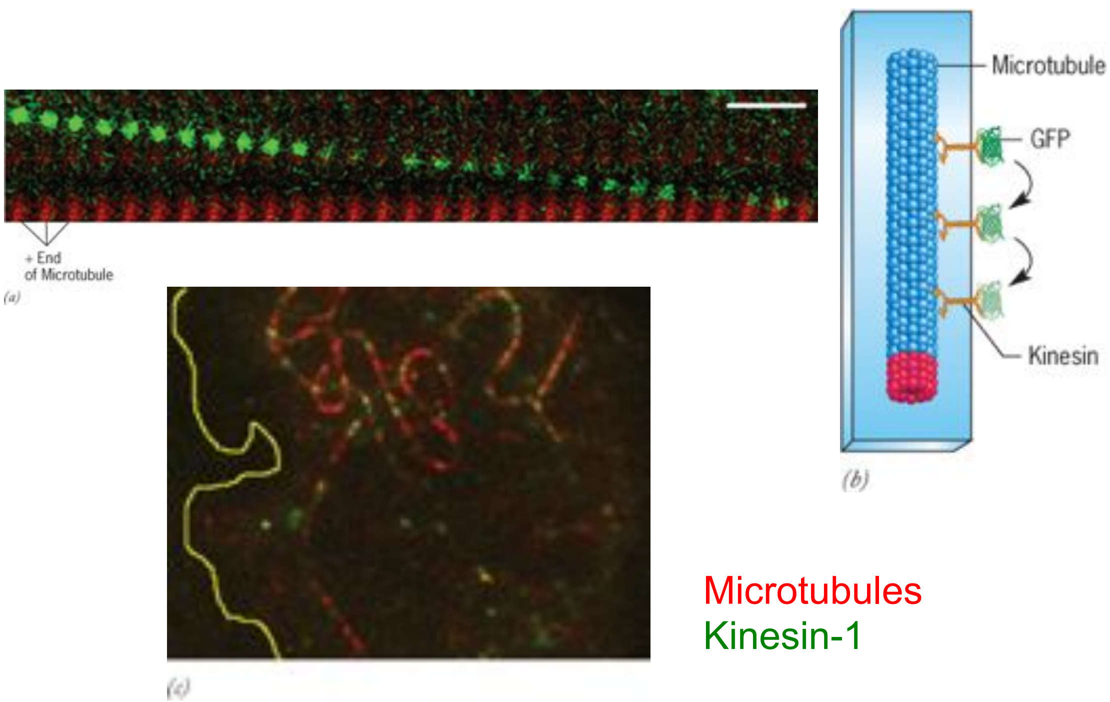
<u>Kinesin:</u>



# What can measure from this movie?



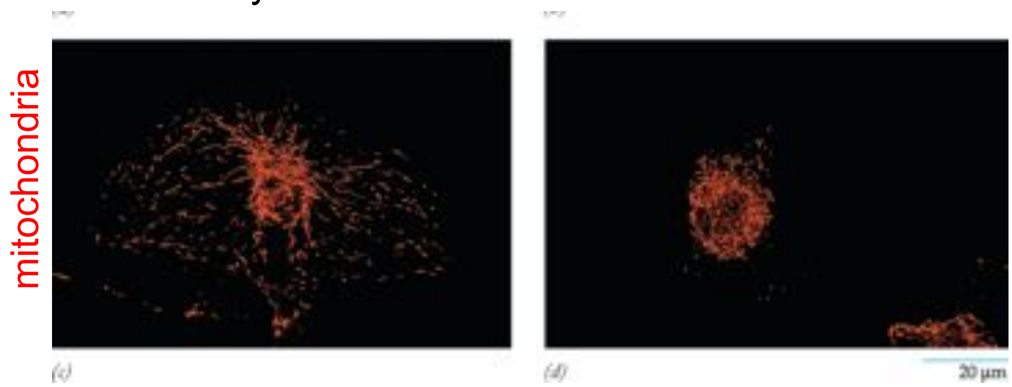

## Watching Kinesin-1 move –speed, processive



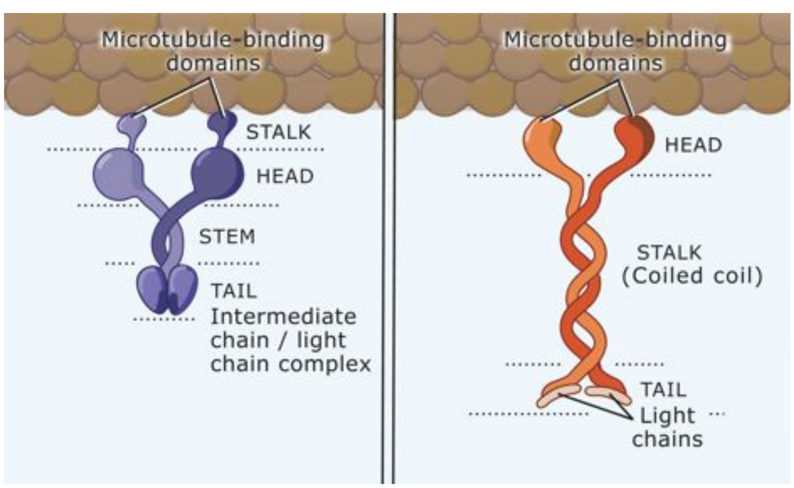

Microtubules
Kinesin-1

**Figure 9.6**

How do you determine which motor is involved?




Motors “walk” along MTs towards the plus or minus end



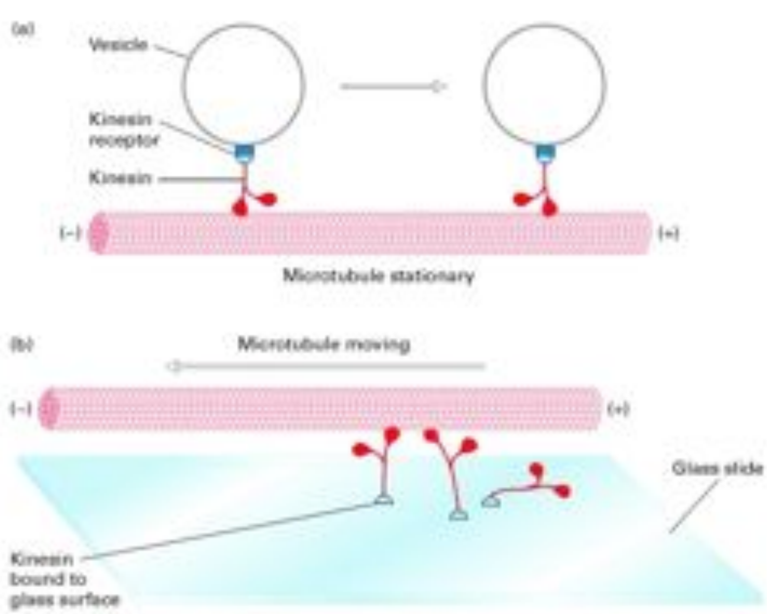

dyneins (-, only)    kinesins (+, mostly)

## kinesins:

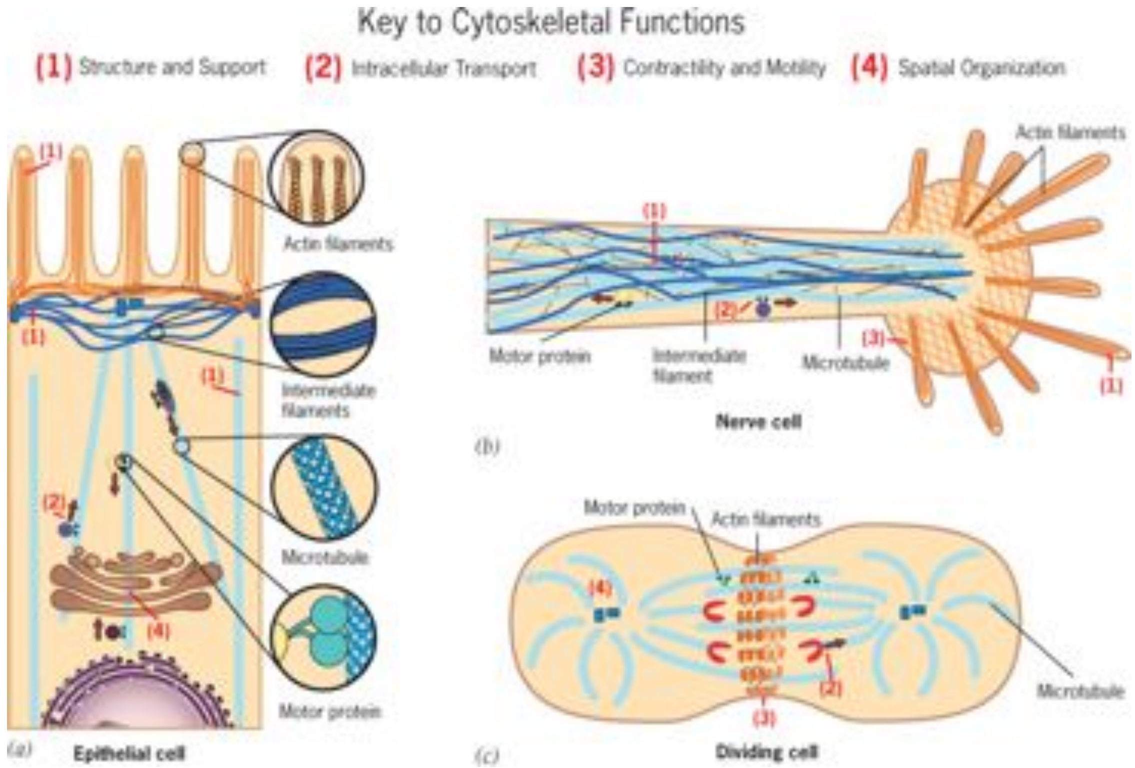
-MT (+) end
-motor domain at N-terminus
-ATP-dependent

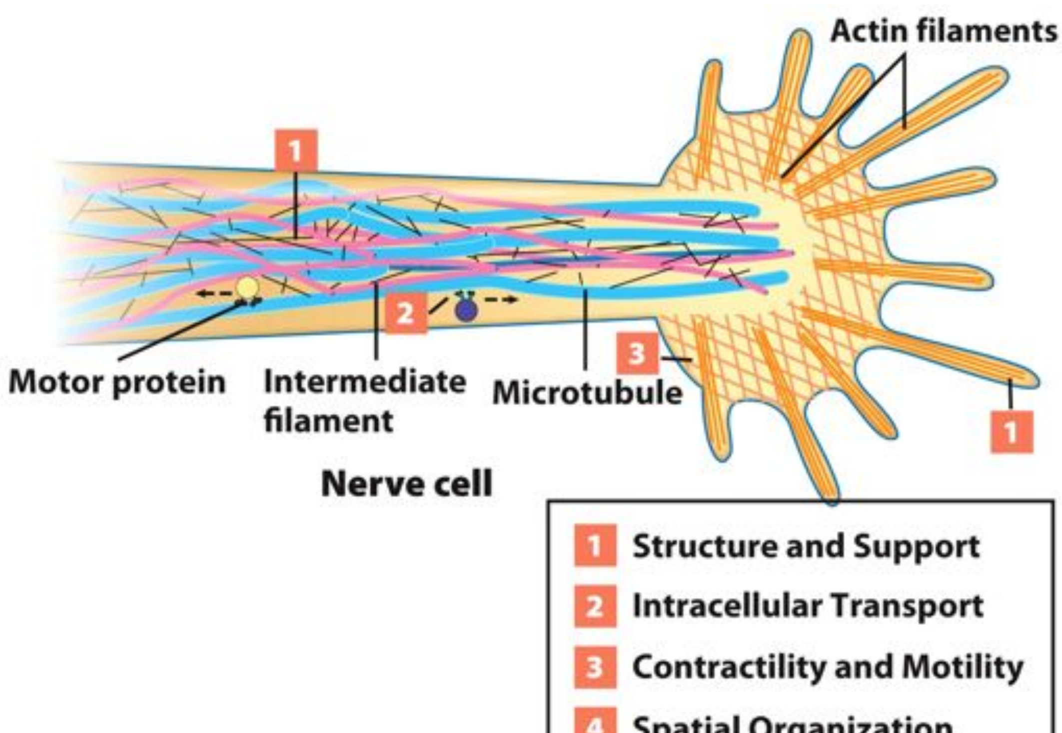
## dyneins:

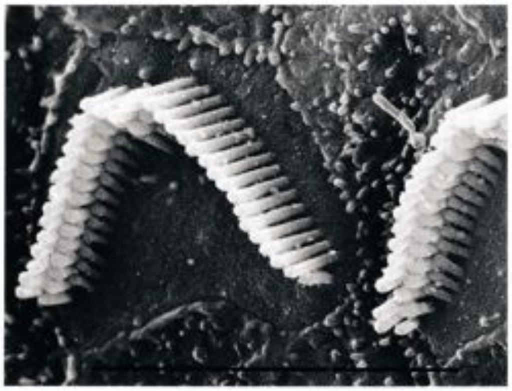
-MT (-) end
-motor domain at C-terminus
-cytoplasmic form (homodimer)
-ATP-dependent




Ron Vale and colleagues


Key to Cytoskeletal Functions
(1) Structure and Support
(2) Intracellular Transport
(3) Contractility and Motility
(4) Spatial Organization
Actin filaments
Intermediate filaments
Microtubule
Motor protein
(a)
Epithelial cell
Actin filaments
Motor protein
Intermediate filament
Microtubule
Nerve cell
(b)
Motor protein
Actin filaments
Microtubule
(c)
Dividing cell


Actin filaments
Motor protein
Intermediate filament
Microtubule
Nerve cell
1 Structure and Support
2 Intracellular Transport
3 Contractility and Motility
4 Spatial Organization





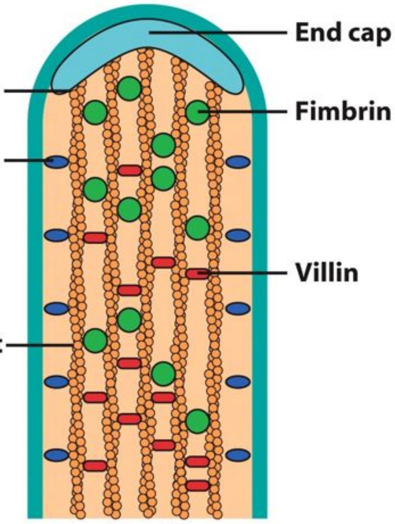
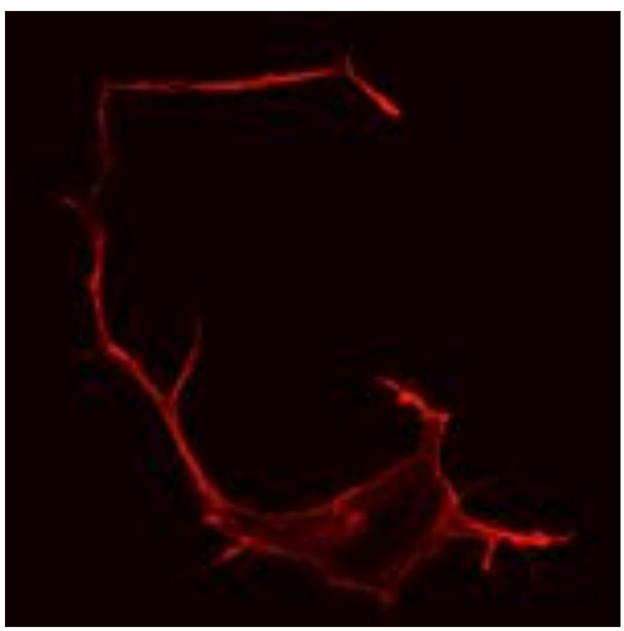




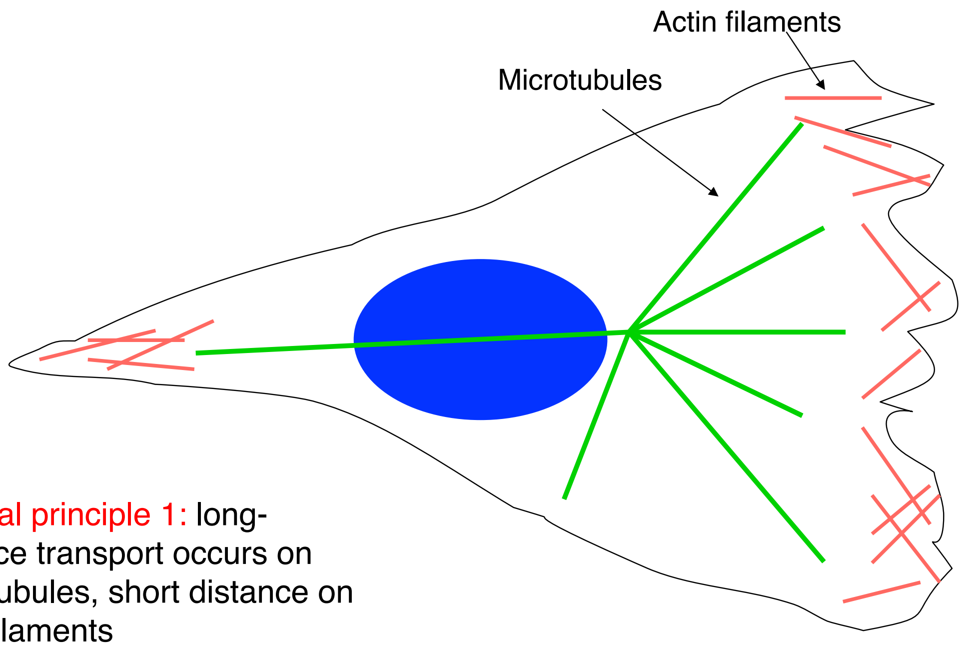

Figure 9.66

Picture of Neuron labelled for actin and its shape



General principle 2: Large structural changes occur with microtubules, small changes are with actin filaments

# Role of the Cytoskeleton and Motor Proteins in Membrane Trafficking

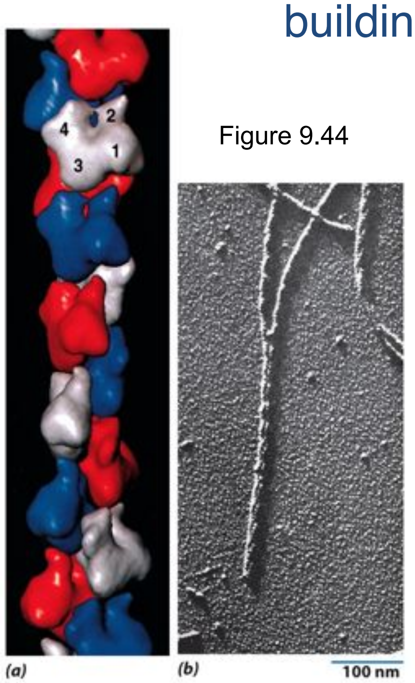



General principle 1: long-distance transport occurs on microtubules, short distance on actin filaments

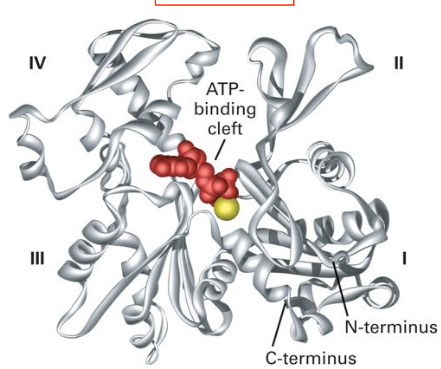
# Actin filaments

## building block = actin

Figure 9.44




(-) end




Lodish Fig. 19-3

Functions: structural support, contraction, migration

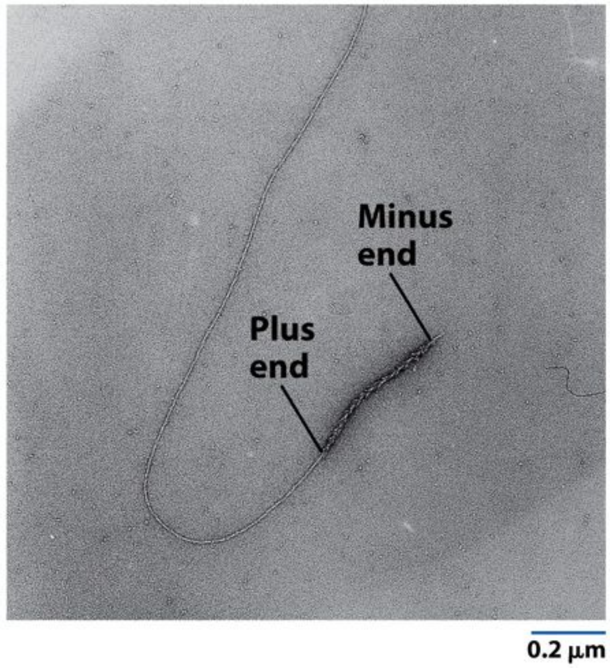




Figure 9.46

Rate and direction of growth depends on free actin concentration

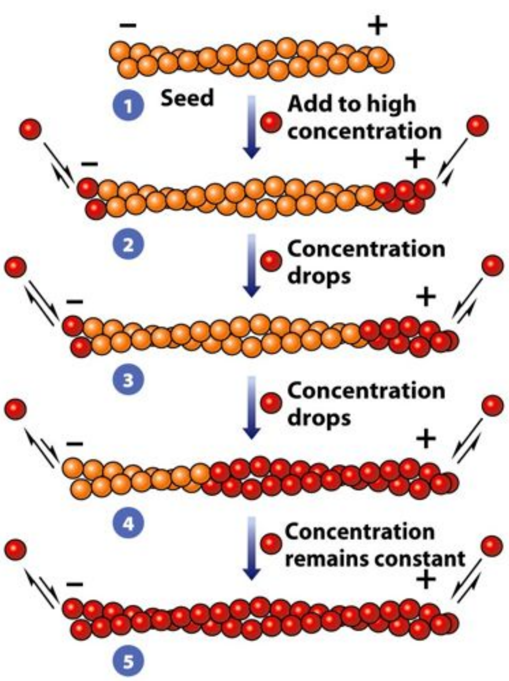


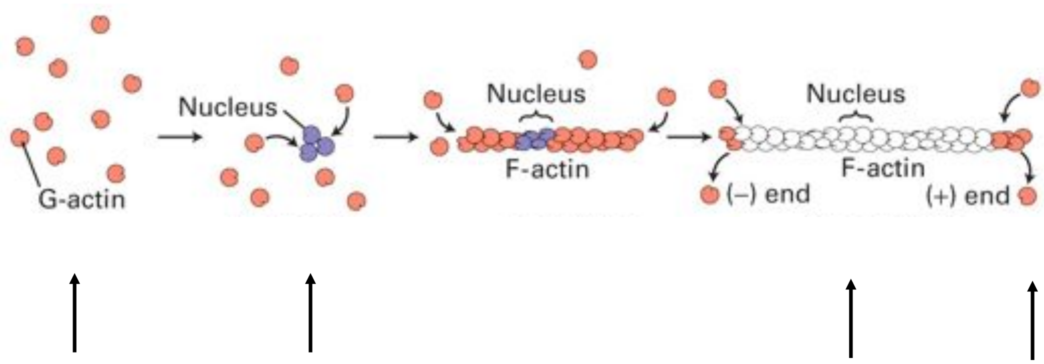


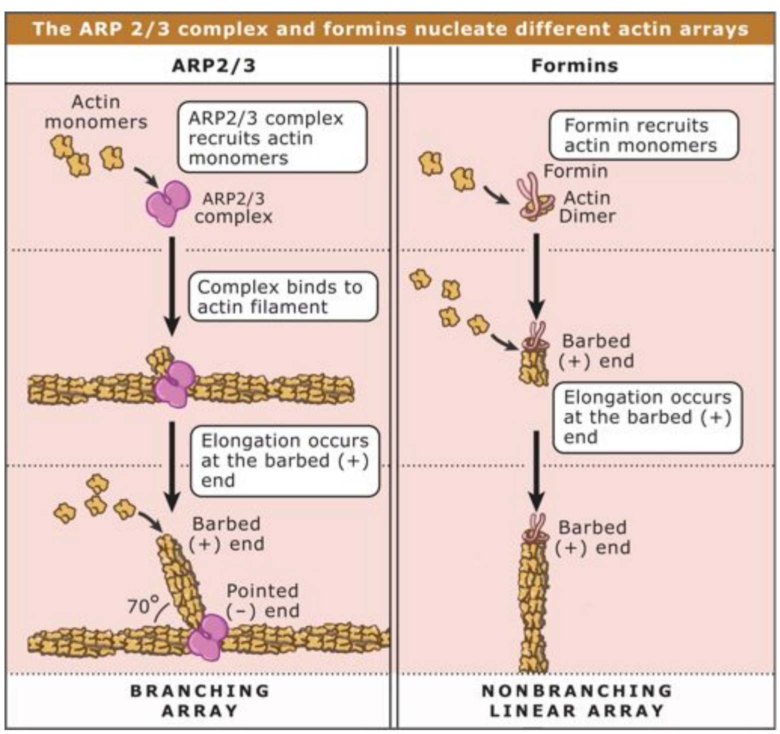
Figure 9.46

Critical concentration

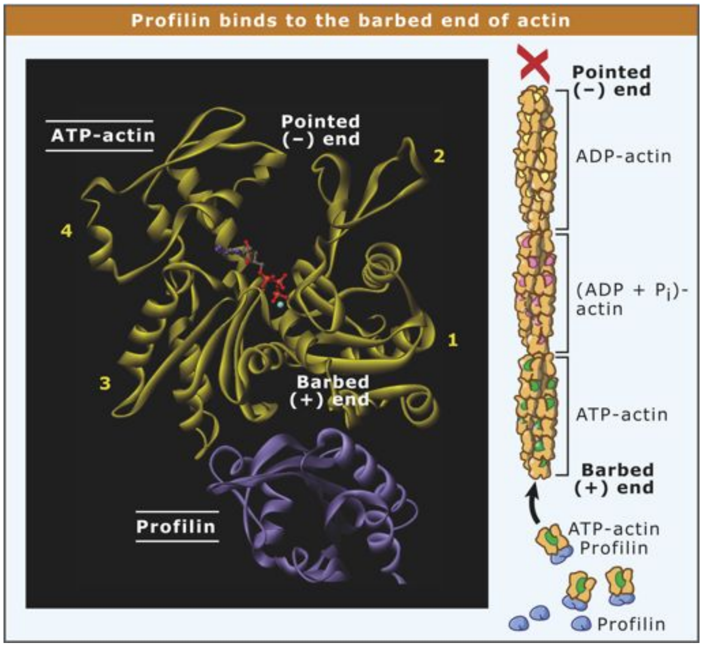
# Regulation by actin-binding proteins

## Actin polymerization



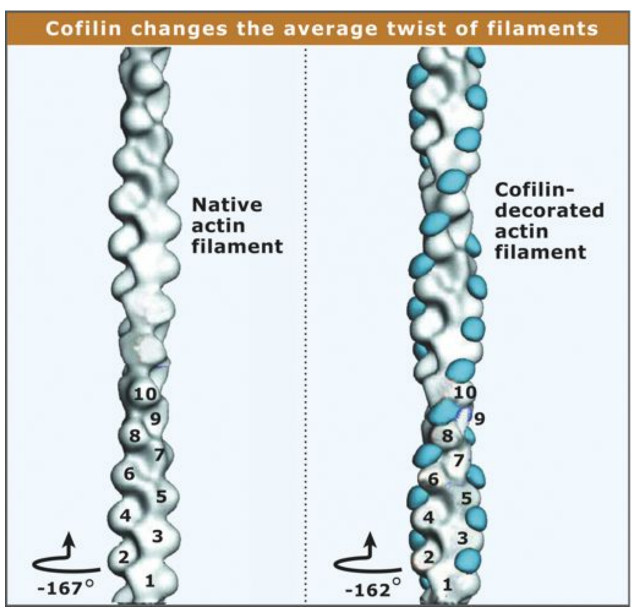



Proteins that regulate actin polymerization

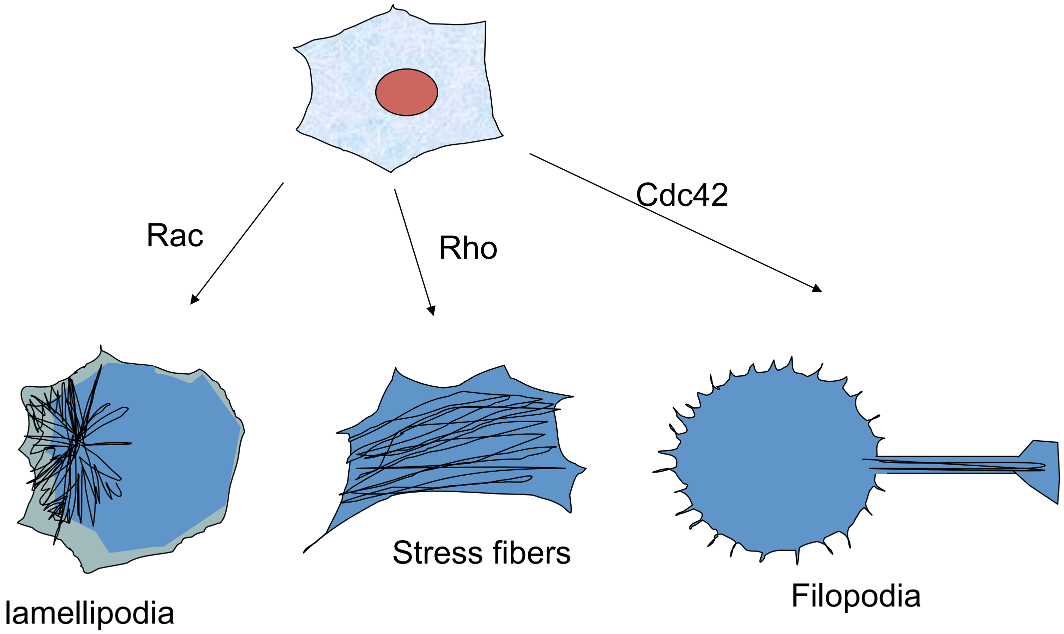



Therefore, the direction of growth is regulated

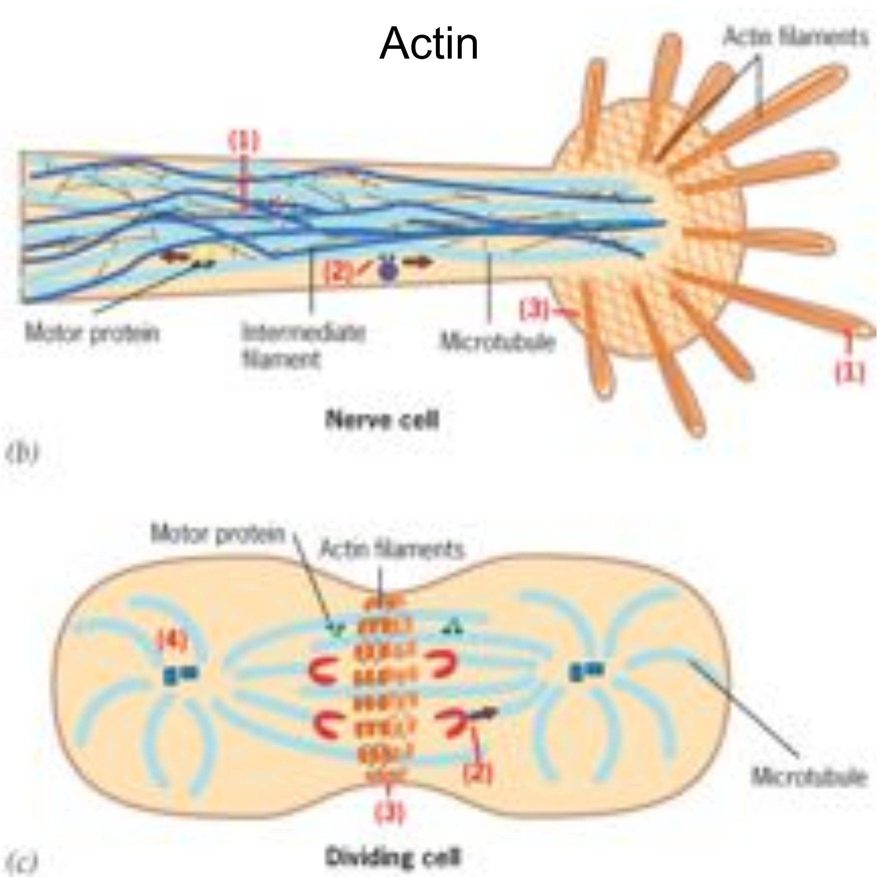
## Proteins that depolymerize actin filaments



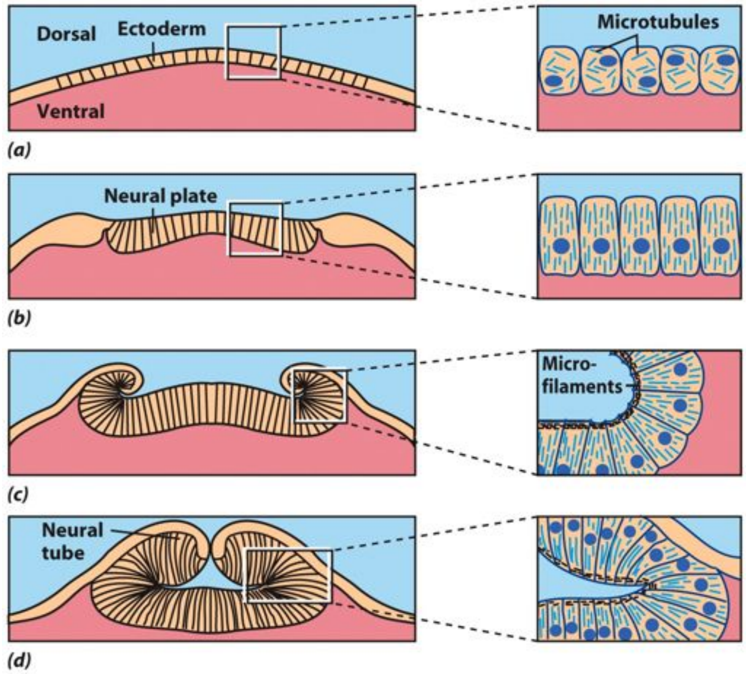
## Small G protein activation regulates actin organization




# Actin

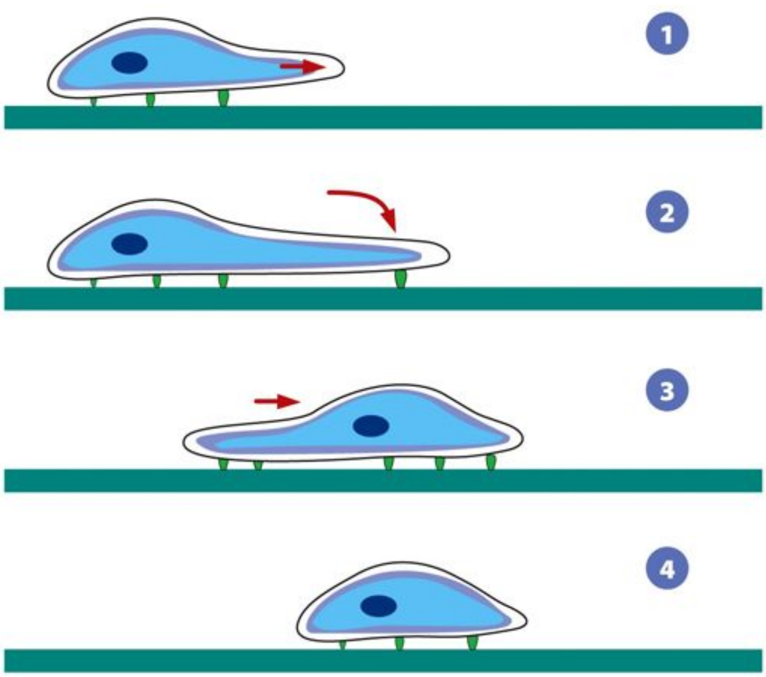


# Actin



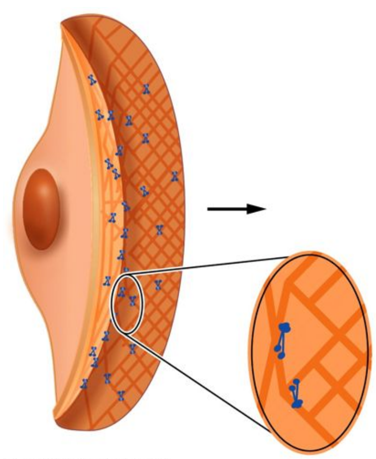
Dorsal
Ectoderm
Ventral
Microtubules
(a)
Neural plate
(b)
Micro-
filaments
(c)
Neural
tube
(d)



1
2
3
4



Directed cell motility

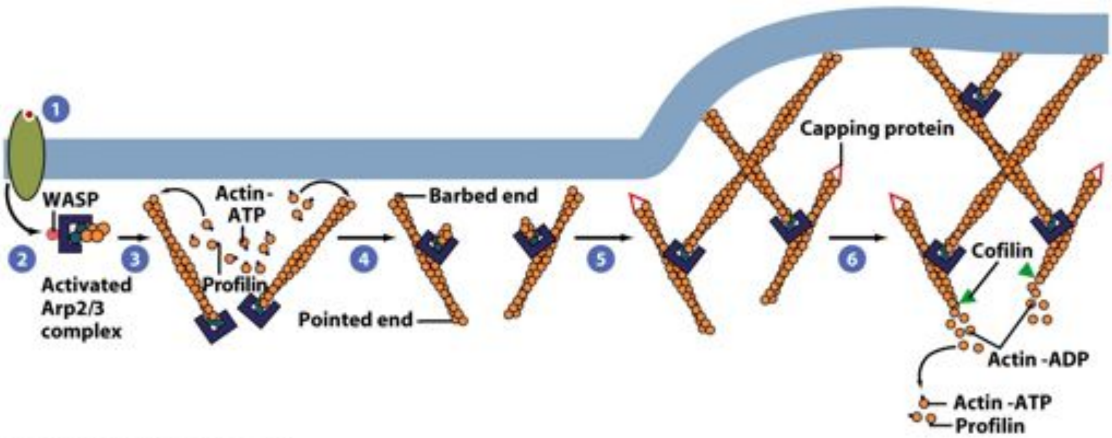
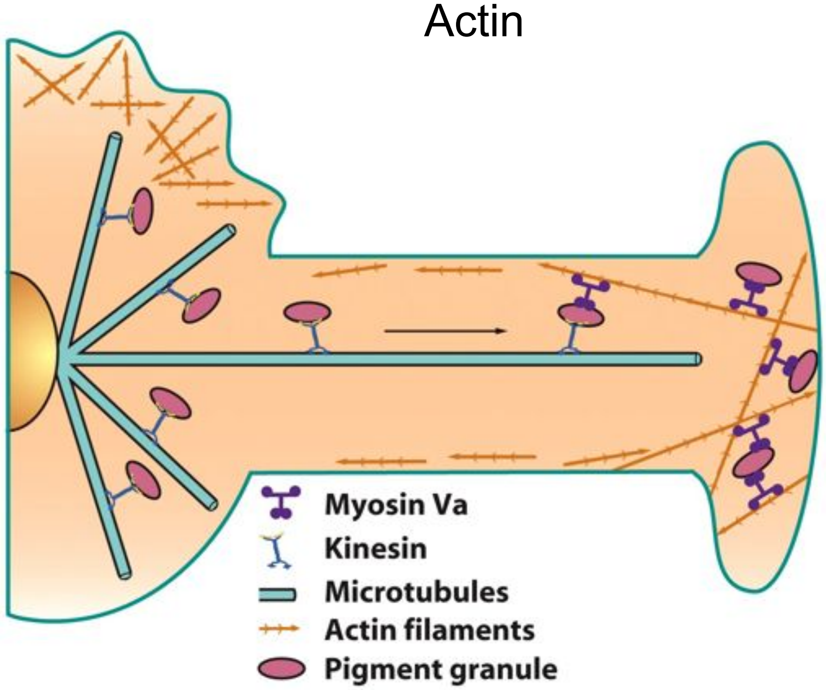


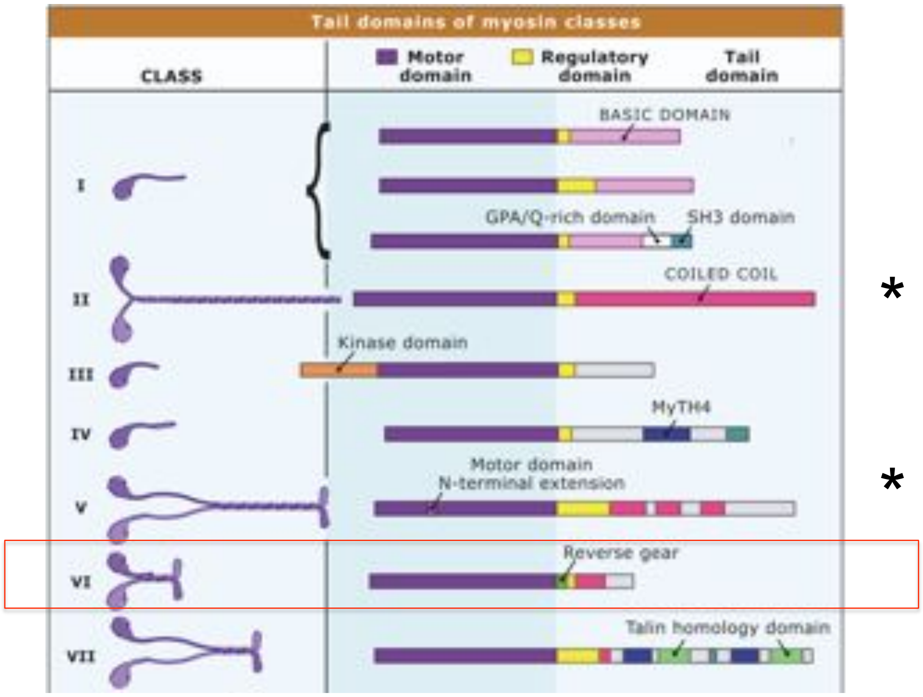



Figure 9.71

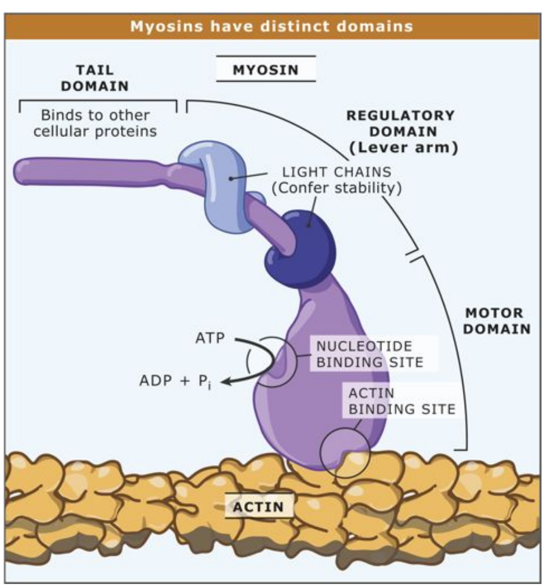
Actin

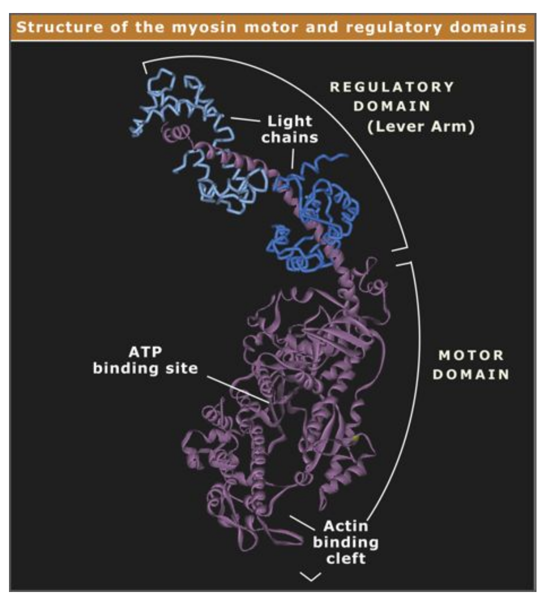


# Myosin: variety of tail domains



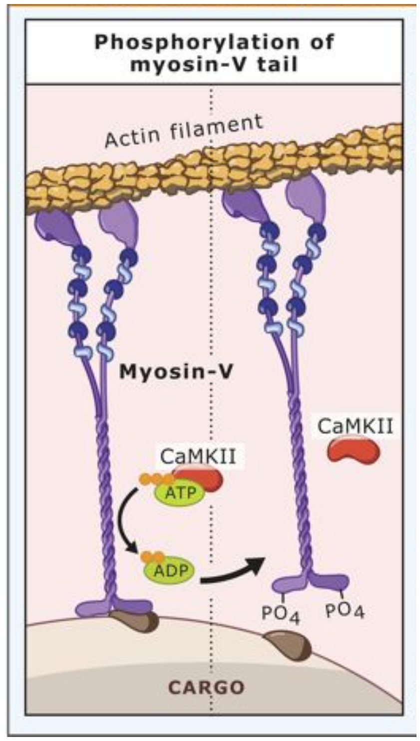

# Structure of myosin proteins




Binds tightly in the absence of ATP
ATP hydrolysis - power stroke - lever arm

Regulation of myosin by phosphorylation




Rab proteins on vesicles are linked to cytoskeleton

Also regulated by GTP-Rab state

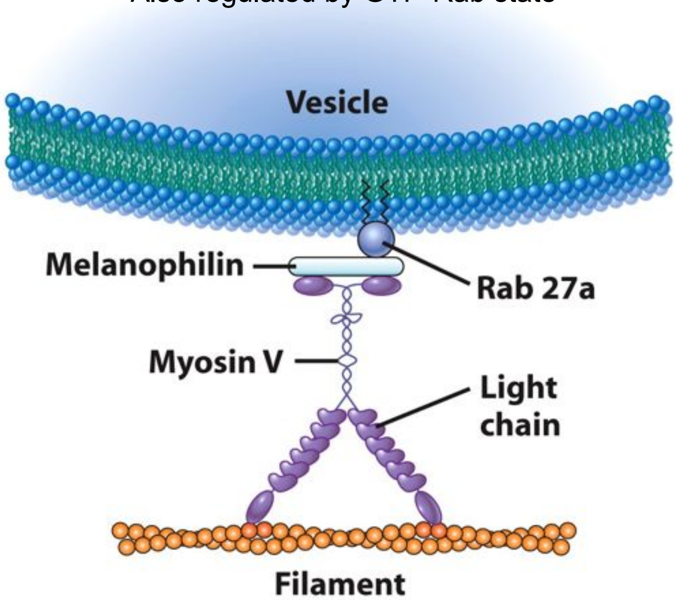
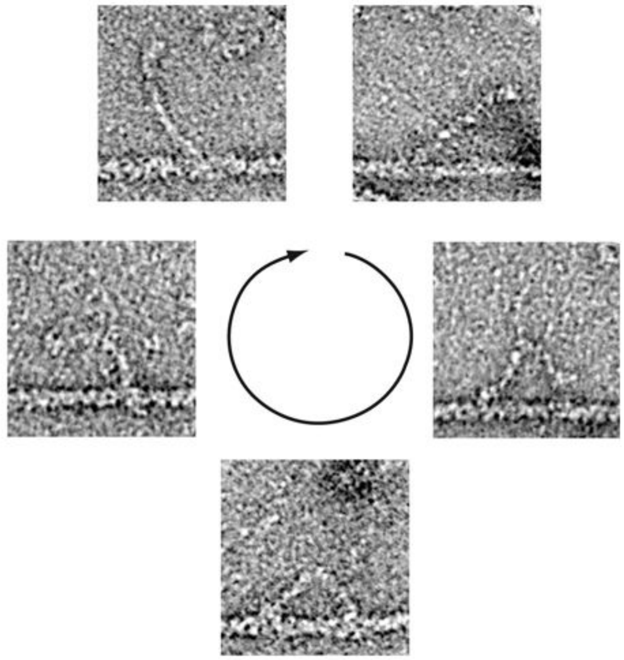
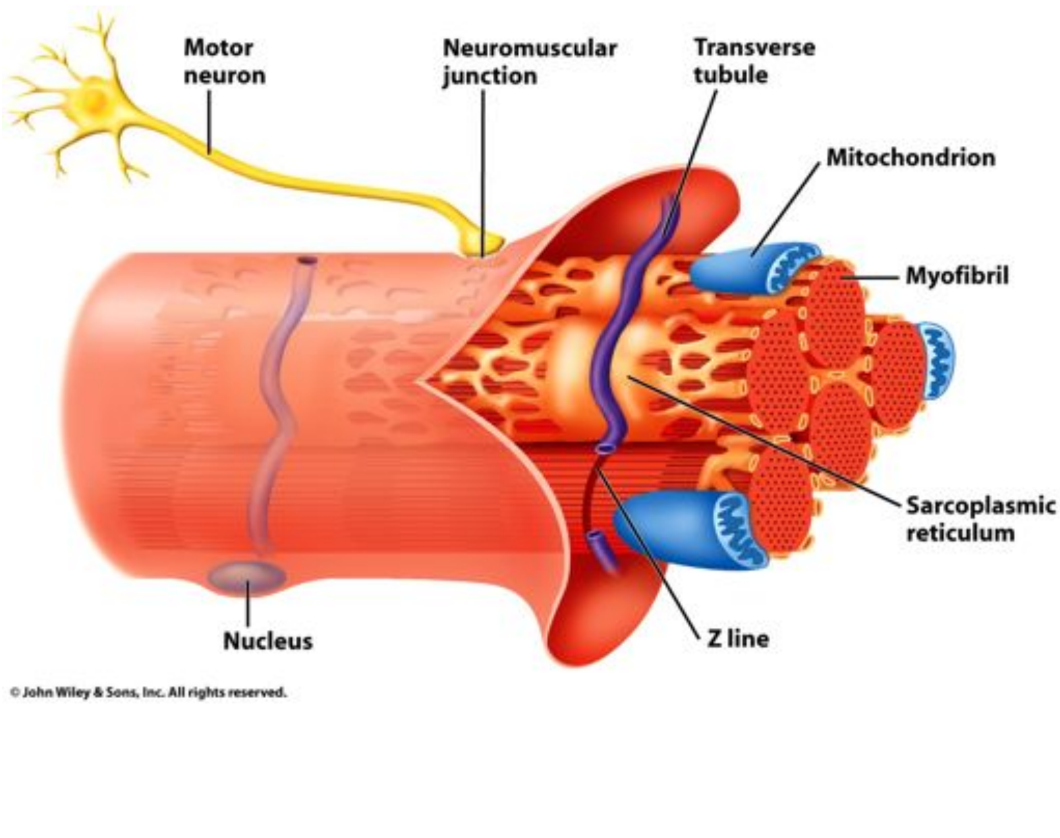


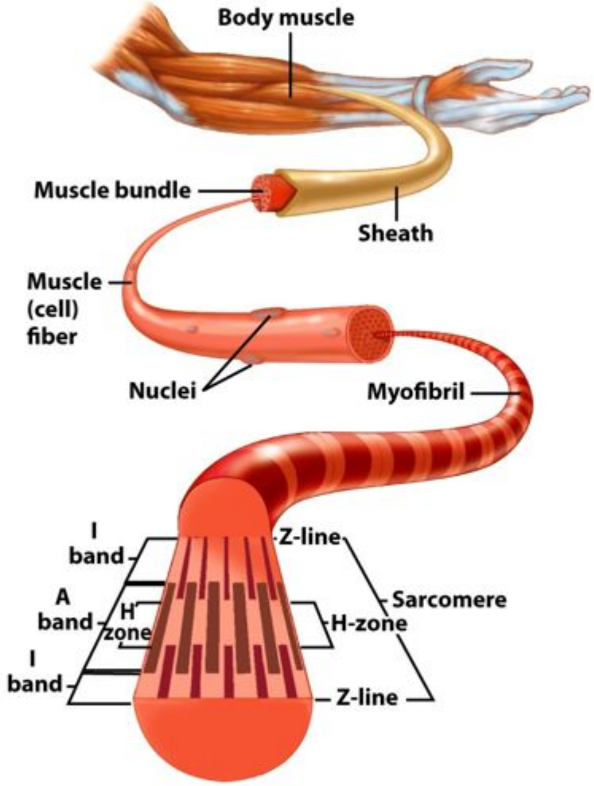



Figure 9.52



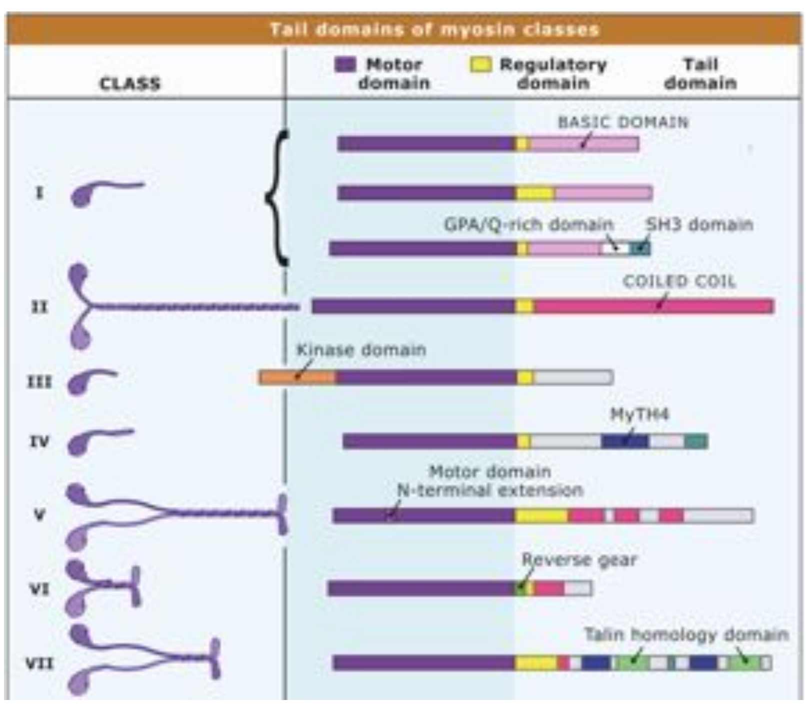
From Matthew L. Walker et al., courtesy of Peter J. Knight. Reprinted with permission from *Nature* 405:804, 2000; © Copyright 2000; Macmillan Magazines Ltd.





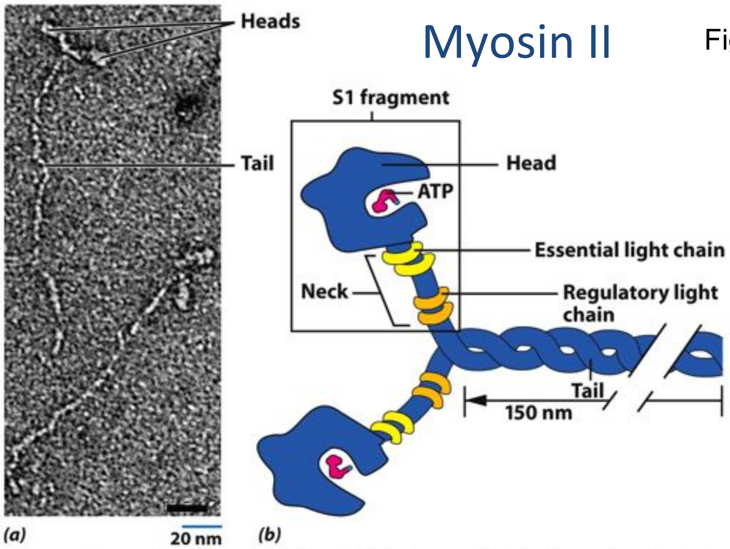



# Myosin: variety of tail domains



# Myosin II

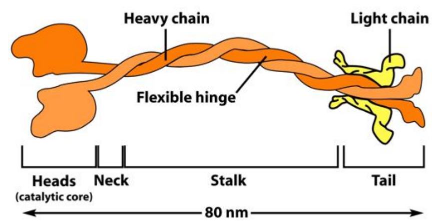
Figure 9.49



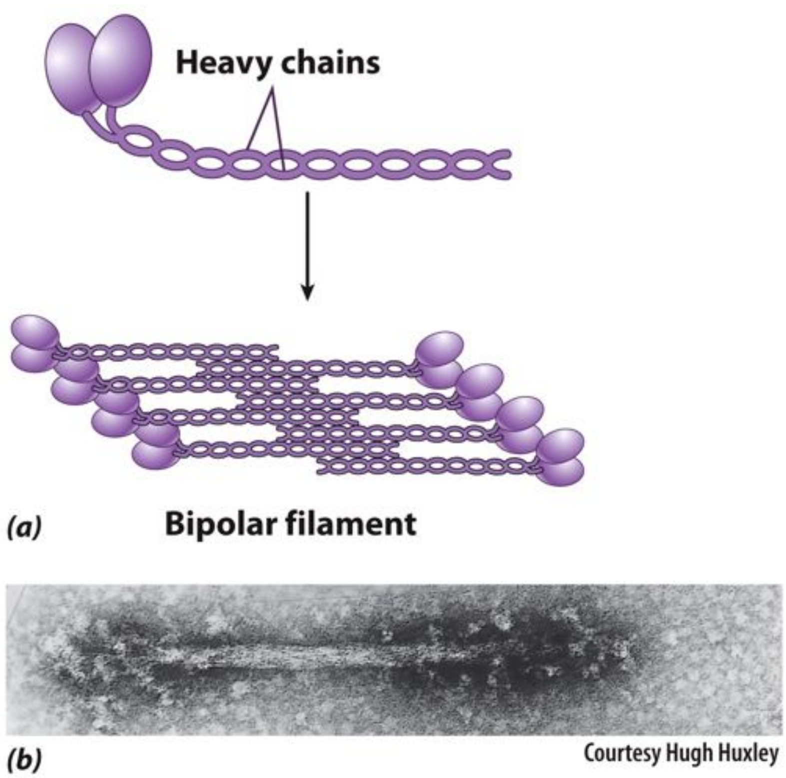



Myosin: mostly (+) end directed

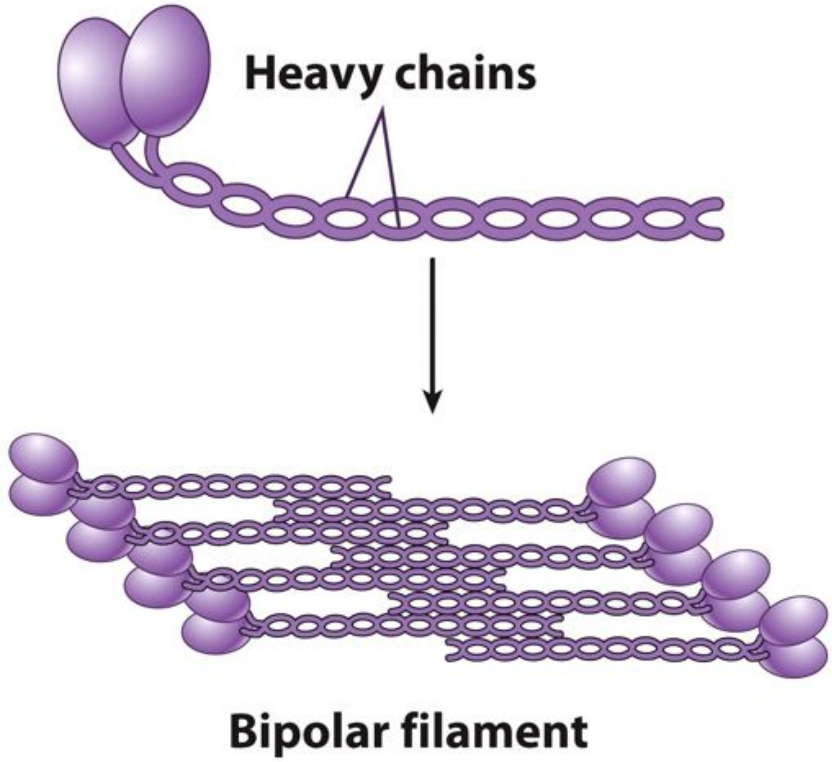
## Kinesin



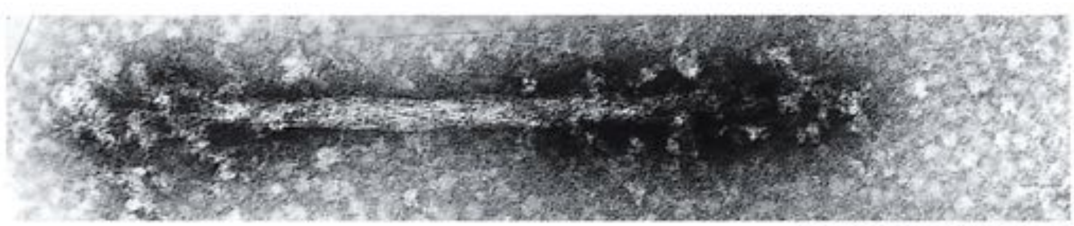


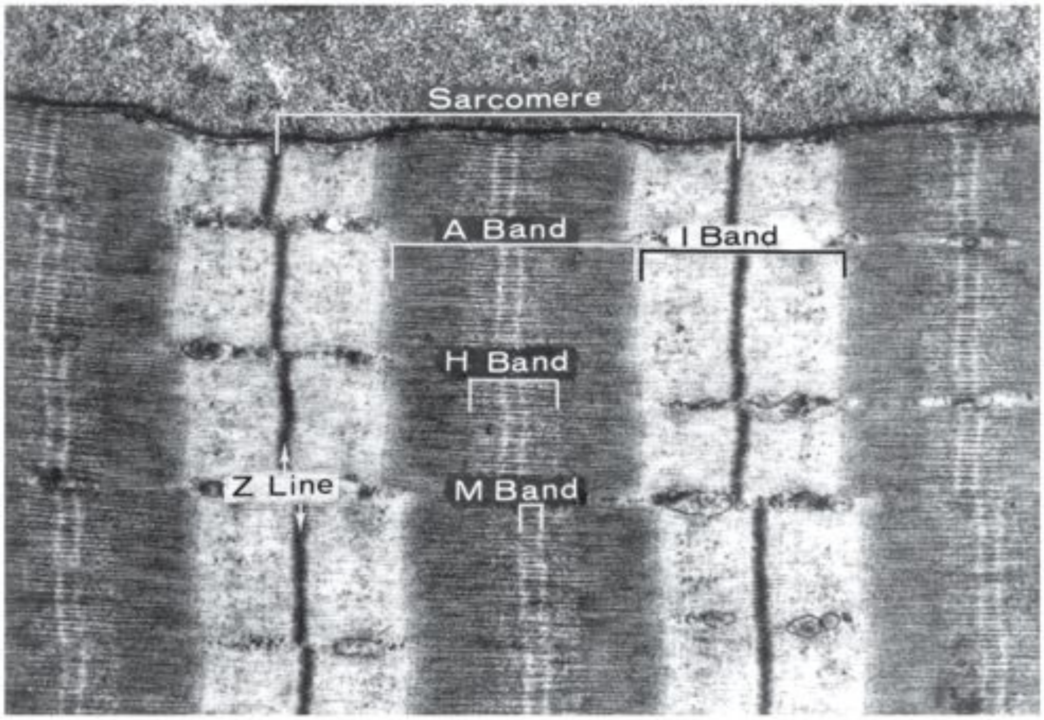

Courtesy Hugh Huxley



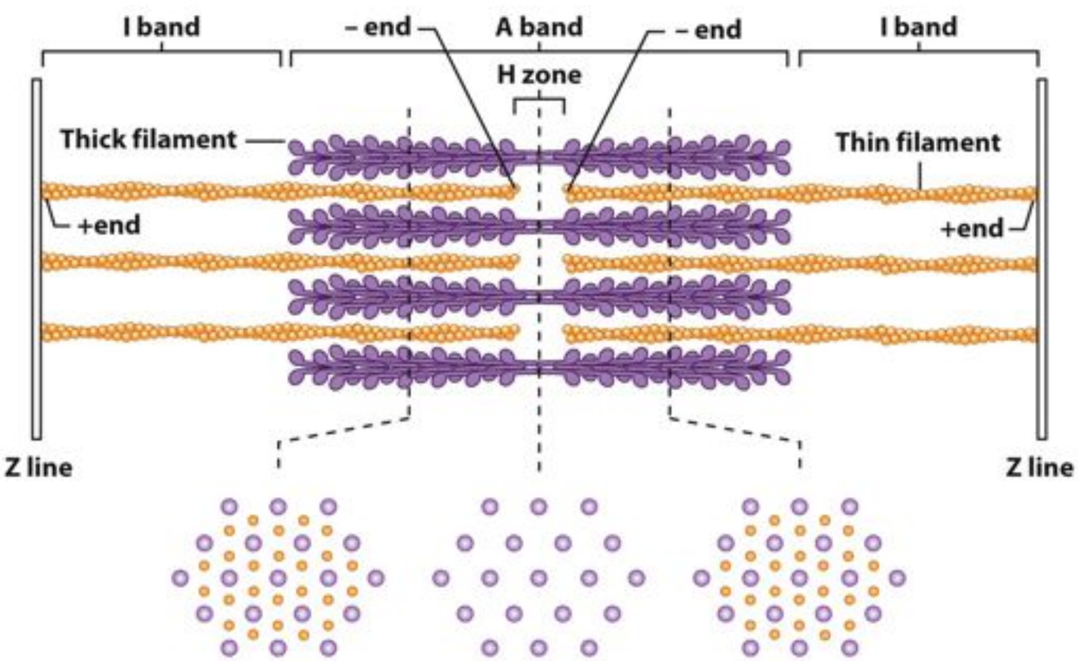


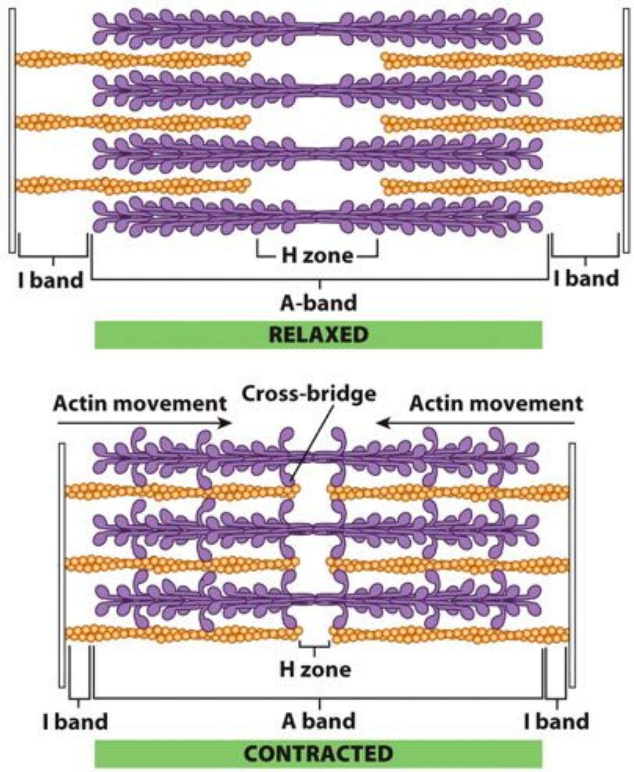



Courtesy Hugh Huxley

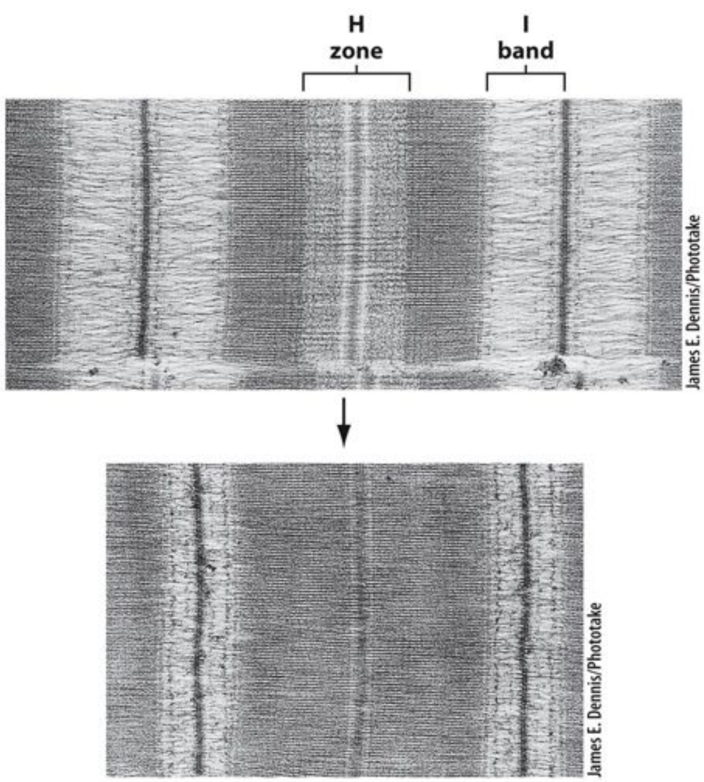


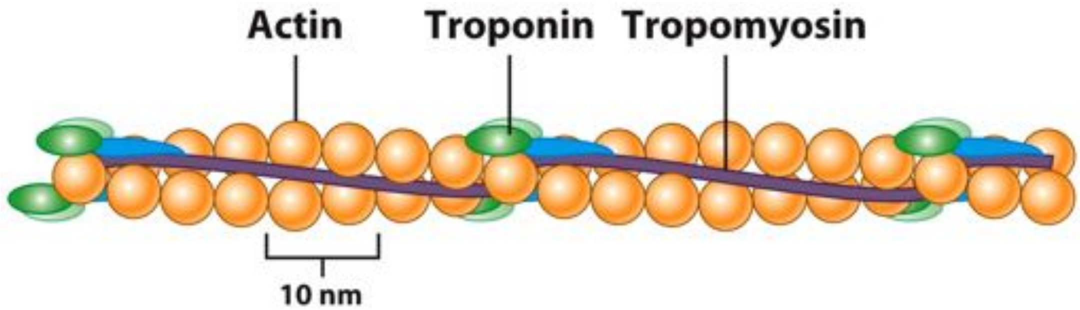

Don W. Fawcett/Photo Researchers, Inc.



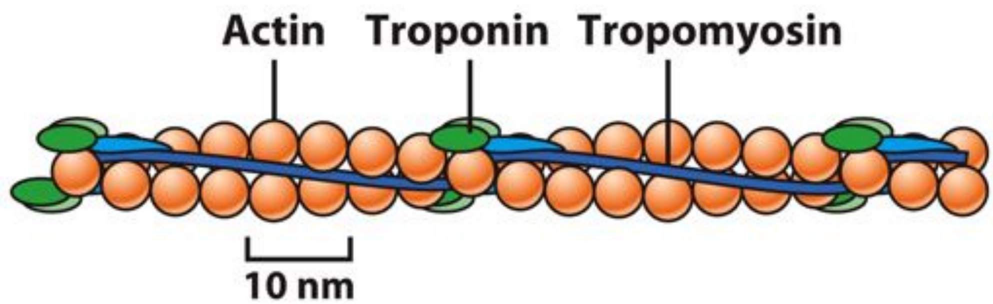

H zone
I band
A-band
I band
RELAXED
Actin movement
Cross-bridge
Actin movement
H zone
I band
A band
I band
CONTRACTED



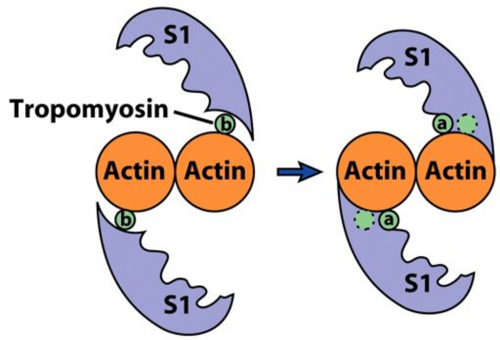

H
zone
I
band
James E. Dennis/Phototake
James E. Dennis/Phototake


Actin
Troponin
Tropomyosin
10 nm

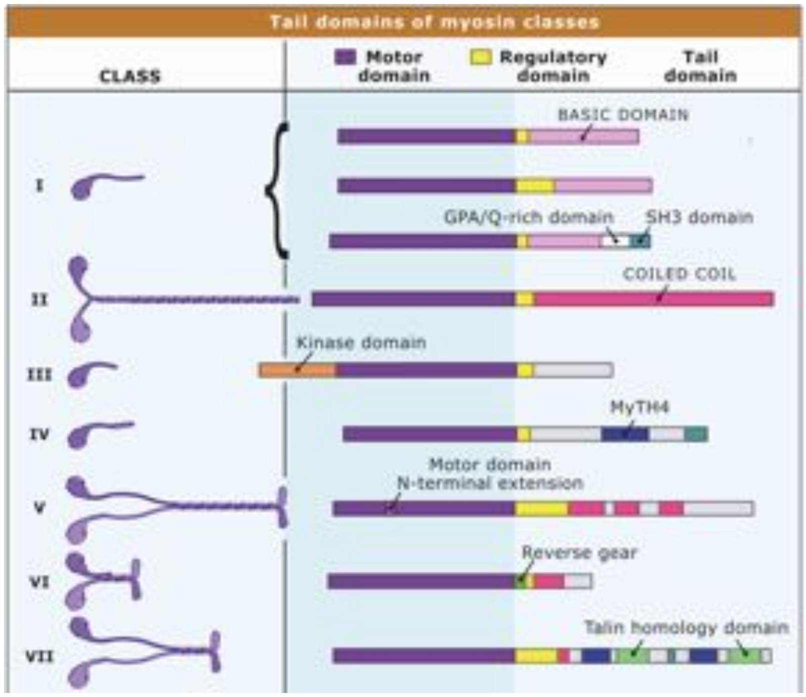



Actin
Troponin
Tropomyosin
10 nm



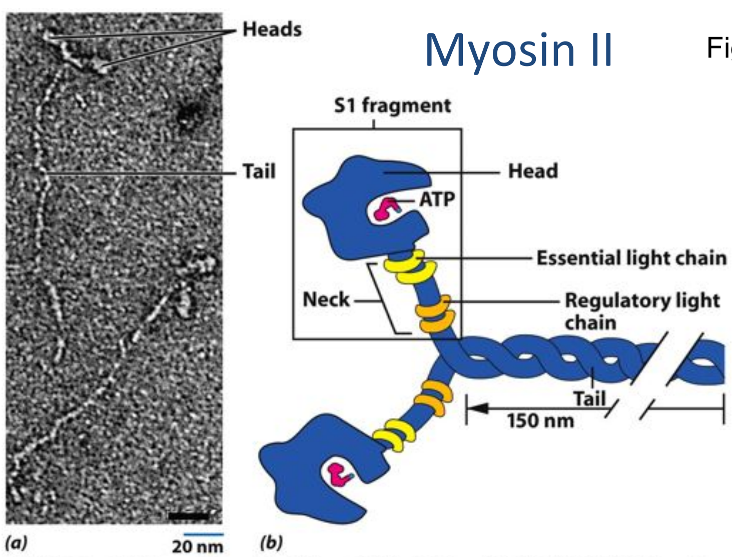

S1
Tropomyosin
b
Actin
Actin
b
S1
S1
a
Actin
Actin
a
S1

# Myosin: variety of tail domains




## Myosin II

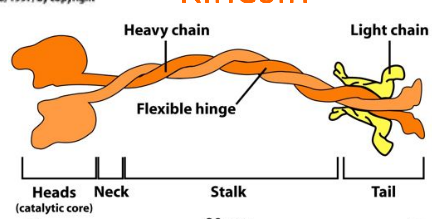
Figure 9.49

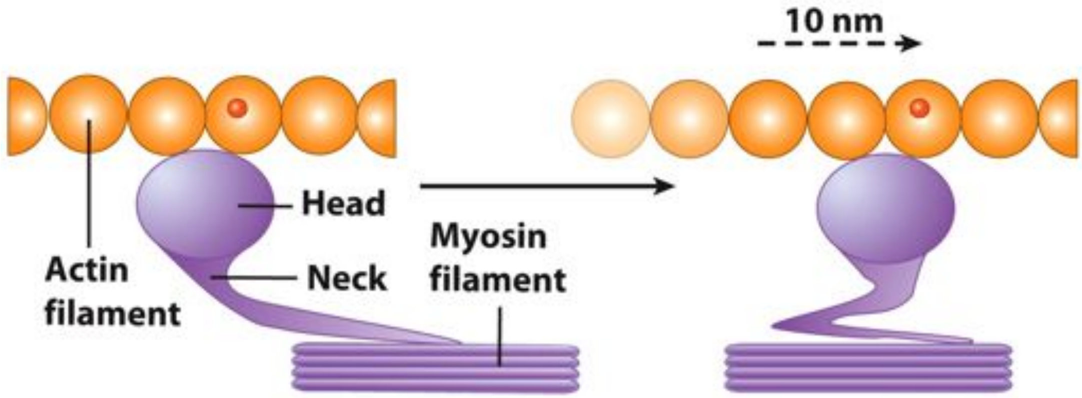




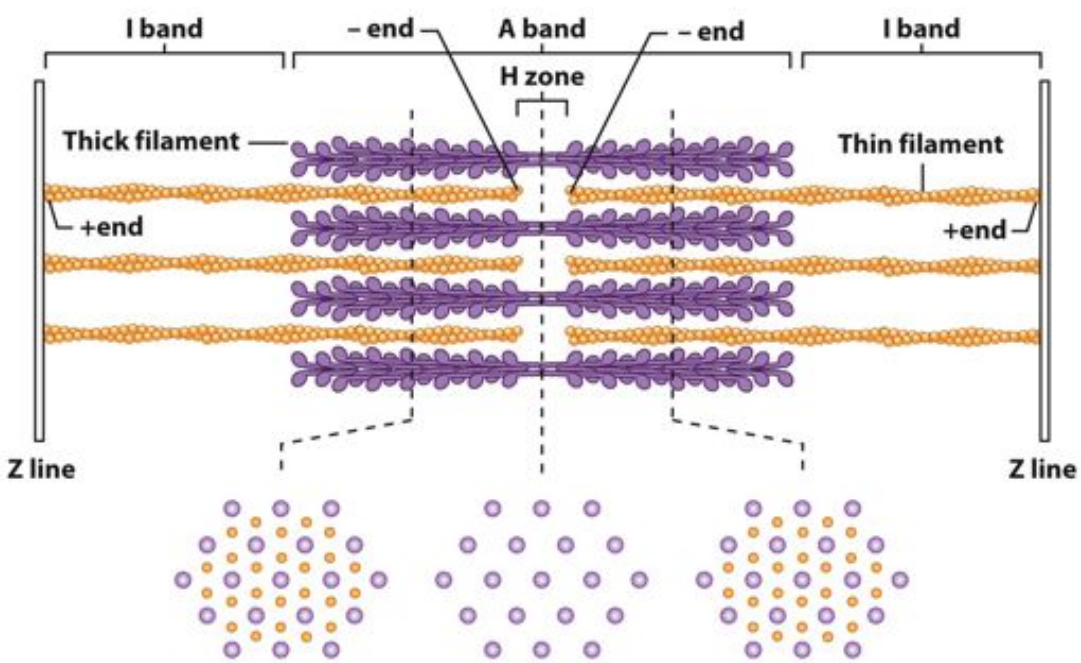

Myosin: mostly (+) end directed

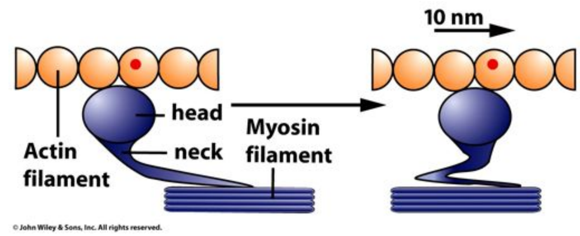
## Kinesin

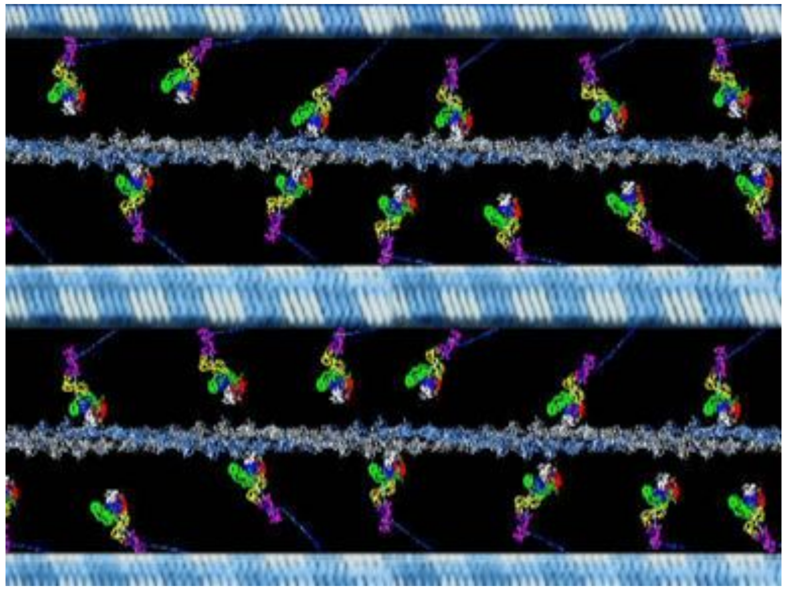


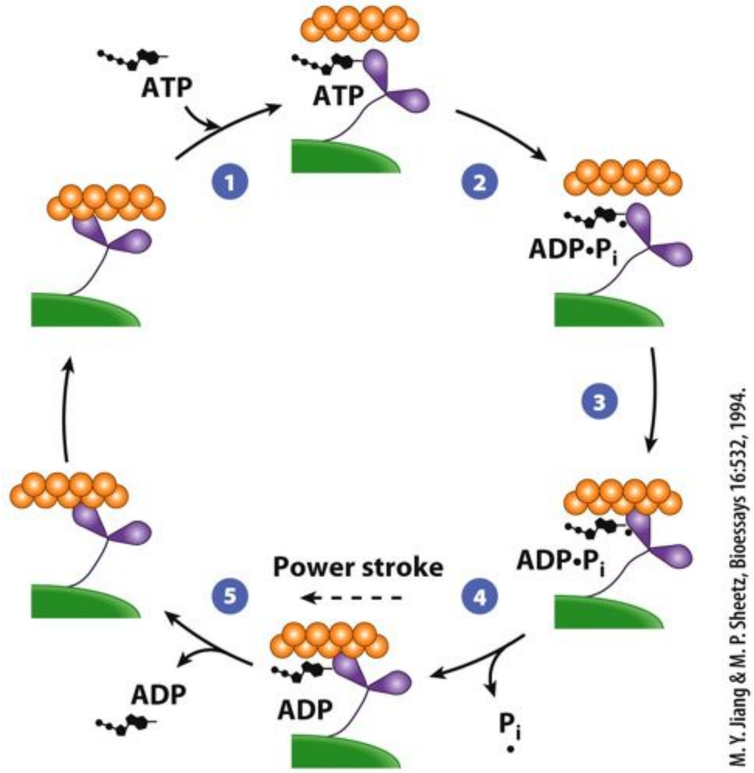

10 nm
Head
Myosin
filament
Actin
filament
Neck

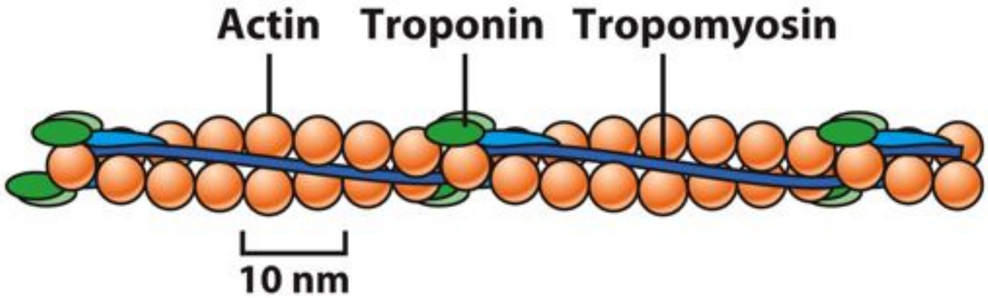



I band
– end
A band
– end
I band
H zone
Thick filament
Thin filament
+end
+end
Z line
Z line

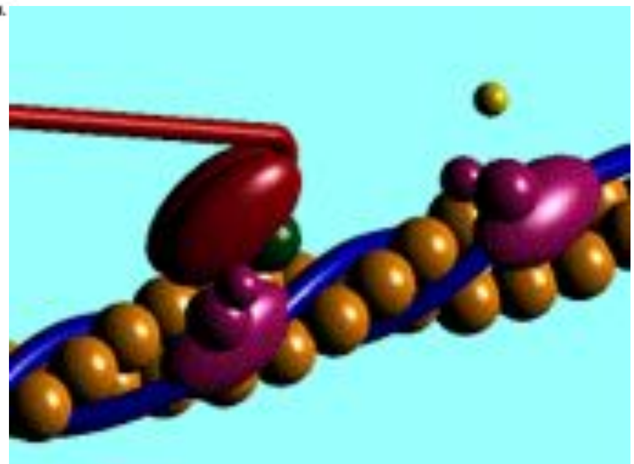

10 nm
head
neck
Actin filament
Myosin filament
© John Wiley & Sons, Inc. All rights reserved.

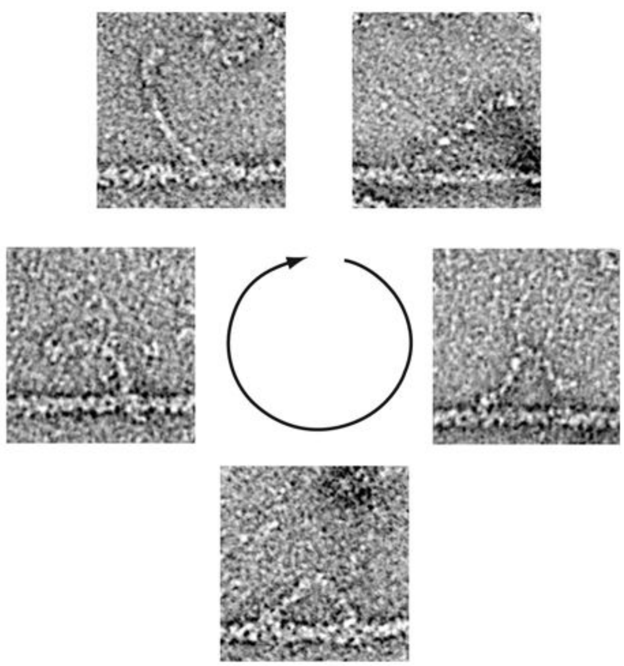



ATP
ATP
1
2
ADP•P$_i$
3
ADP•P$_i$
Power stroke
4
5
ADP
ADP
P$_i$
M. Y. Jiang & M. P. Sheetz, Bioessays 16:532, 1994.







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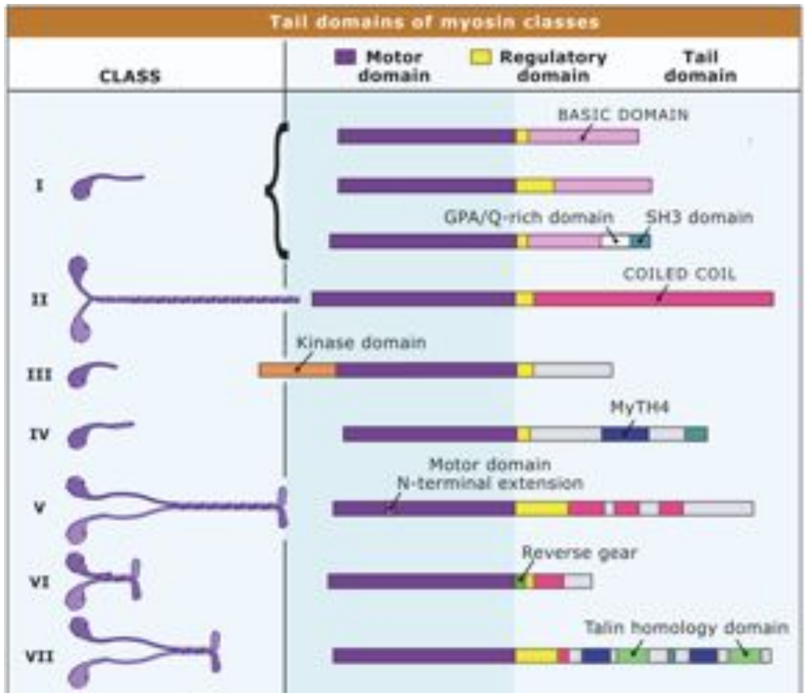
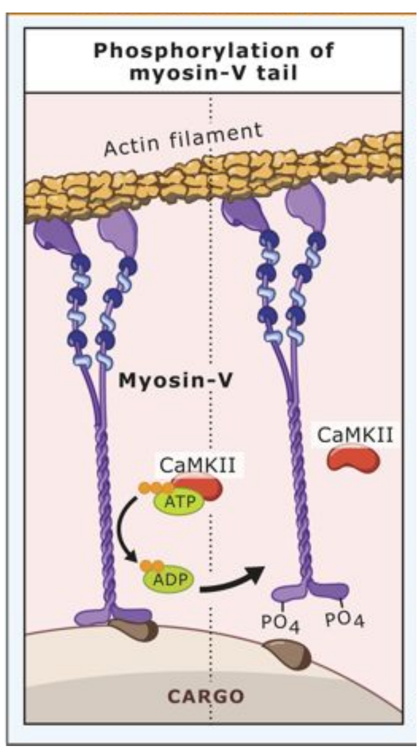
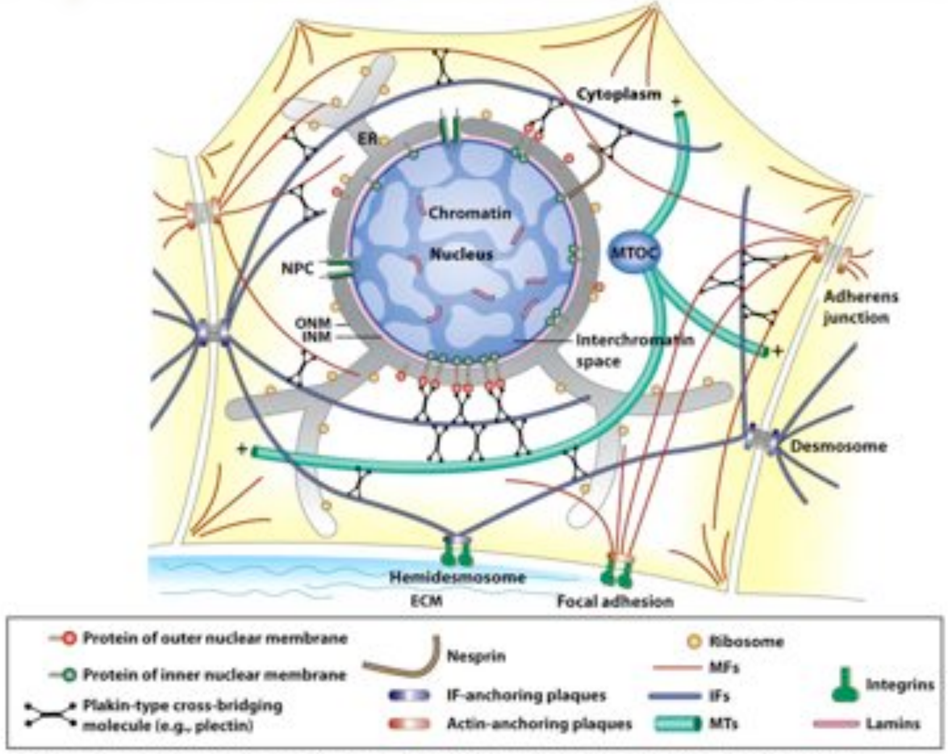
# Myosin: variety of tail domains




# Regulation of myosin by phosphorylation

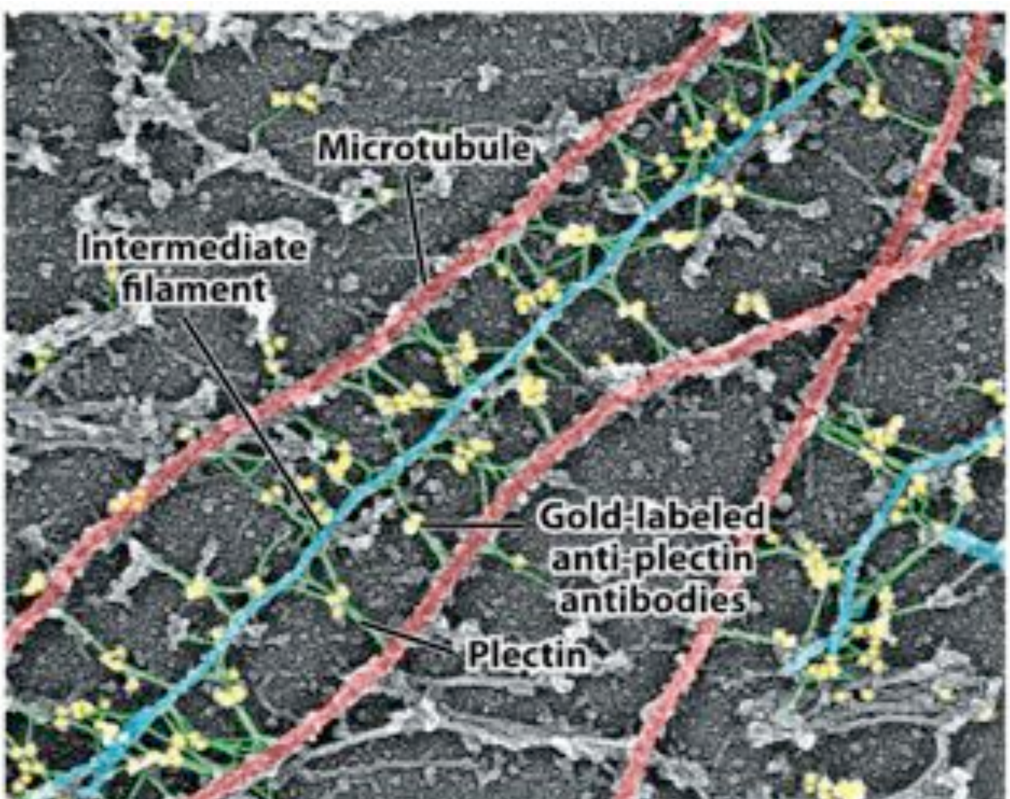


# The Cytoskeleton: Intermediate Filaments




Reprinted with permission from H. Herrmann et al., *Nature Revs. Mol. Cell Biol.* 8:564, 2007; © Copyright 2007, Macmillan Magazines Ltd.






Courtesy of Tatyana Svitkina and Gary Borisy

**TABLE 9.2 Properties and Distribution of the Major Mammalian Intermediate Filament Proteins**

| IF protein | Sequence type | Primary tissue distribution |
|---|---|---|
| Keratin (acidic) (28 different polypeptides) | I | Epithelia |
| Keratin (basic) (26 different polypeptides) | II | Epithelia |
| Vimentin | III | Mesenchymal cells |
| Desmin | III | Muscle |
| Glial fibrillary acidic protein (GFAP) | III | Astrocytes |
| Peripherin | III | Peripheral neurons |
| Neurofilament proteins | | Neurons of central and peripheral nerves |
| NF-L | IV | |
| NF-M | IV | |
| NF-H | IV | |
| Nestin | IV | Neuroepithelial |
| Lamin proteins | | All cell types (Nuclear envelopes) |
| Lamin A | V | |
| Lamin B | V | |
| Lamin C | V | |

More detailed tables can be found in *Trends Biochem Sci.* 31:384, 2006, *Genes and Development* 21:1582, 2007, and *Trends Cell Biol.* 18:29, 2008.

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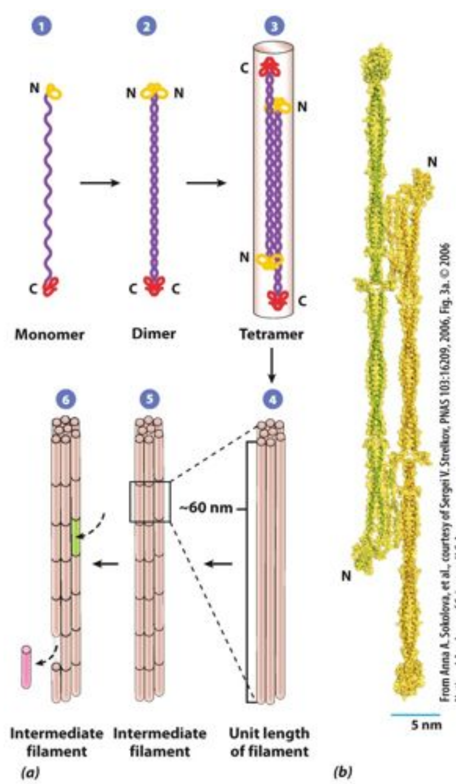
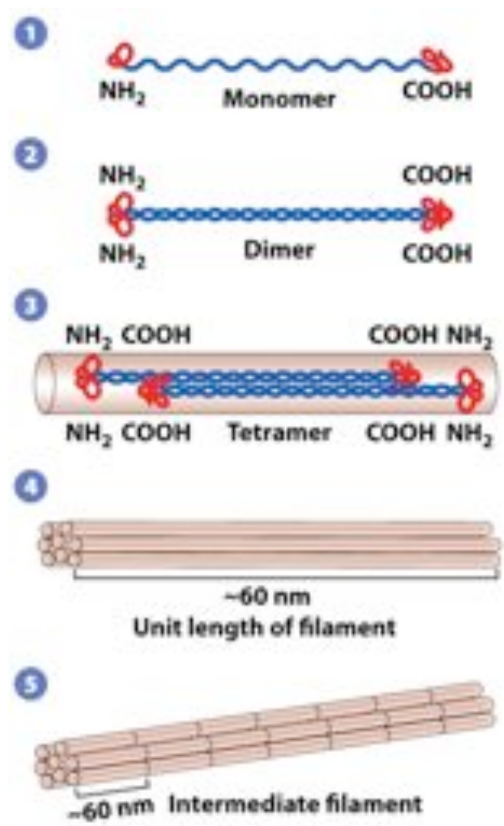
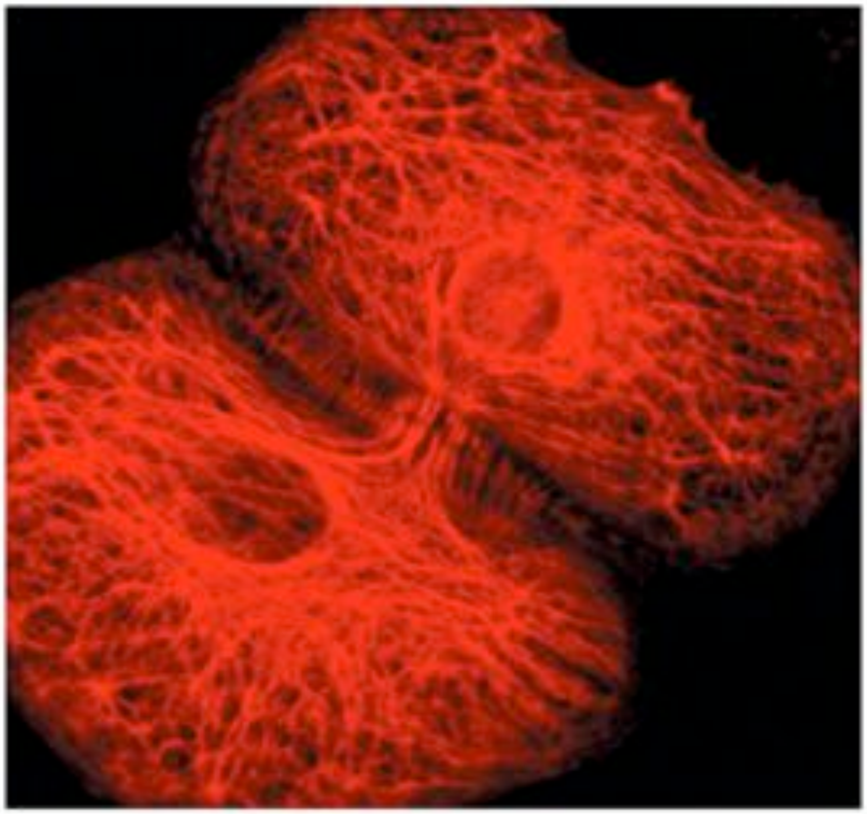


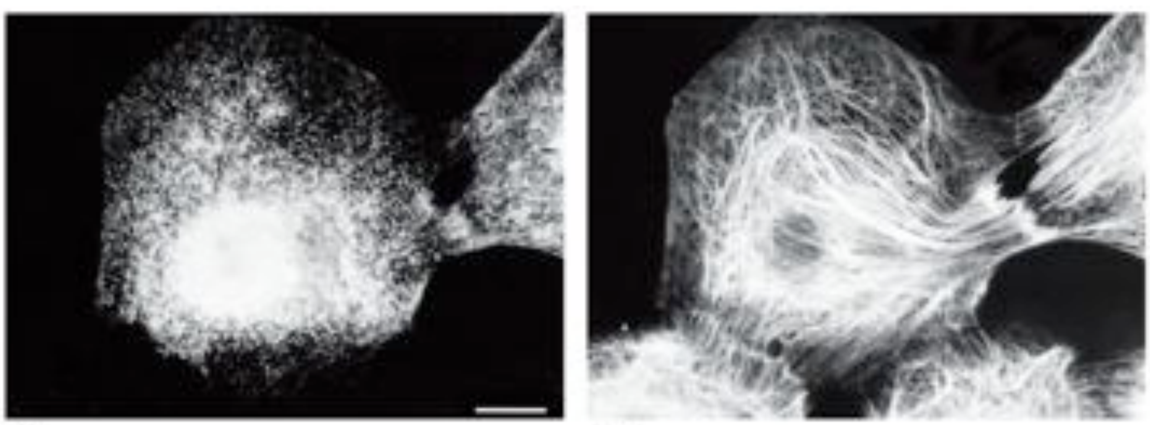

N
N
5 nm



1
NH2
Monomer
COOH
2
NH2
COOH
NH2
Dimer
COOH
3
NH2 COOH
COOH NH2
NH2 COOH
Tetramer
COOH NH2
4
~60 nm
Unit length of filament
5
~60 nm
Intermediate filament





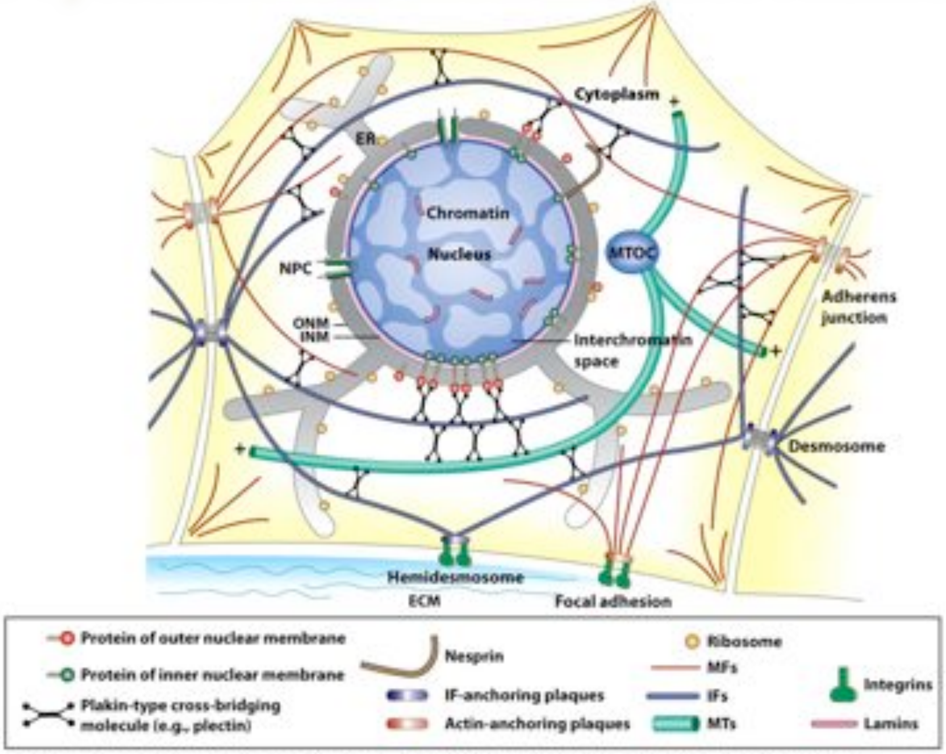
From Pierre A. Coulombe and M. Bishr Omary, Curr. Opin. *Cell Biol.* 14:110, 2002



(a) (b)

From Rita K. Miller, Karen Vikstrom, and Robert D. Goldman, *J. Cell Biol.* 113:848, 1991; by copyright permission of The Rockefeller University Press.

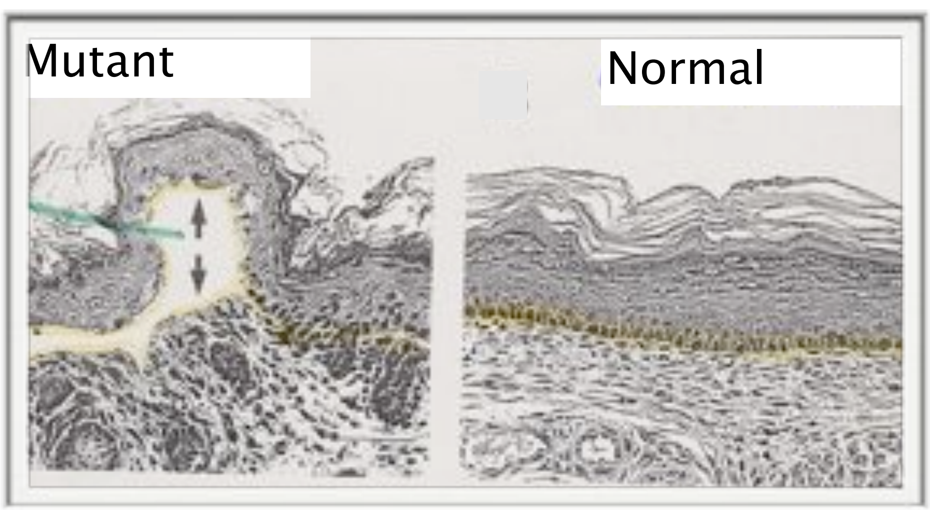
# The Cytoskeleton: Intermediate Filaments



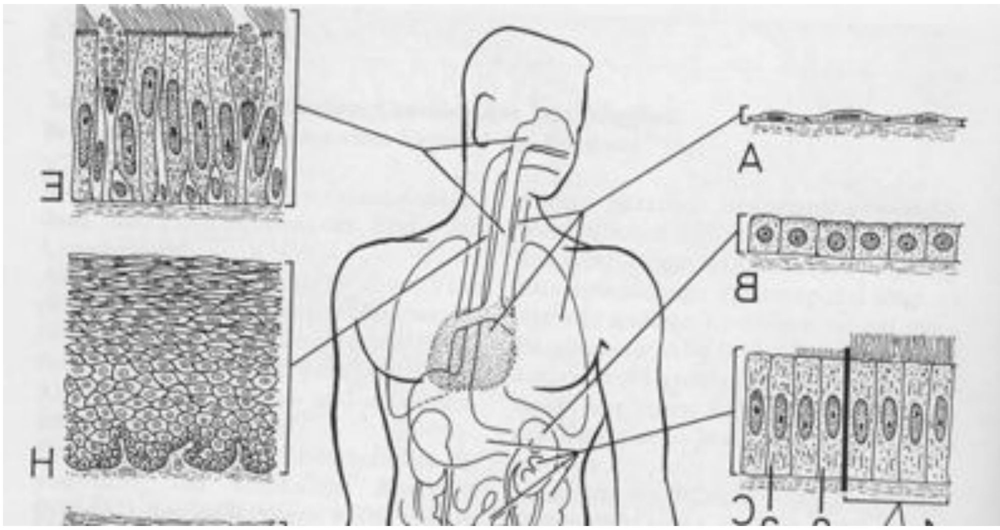





epidermolysis bullosa simplex



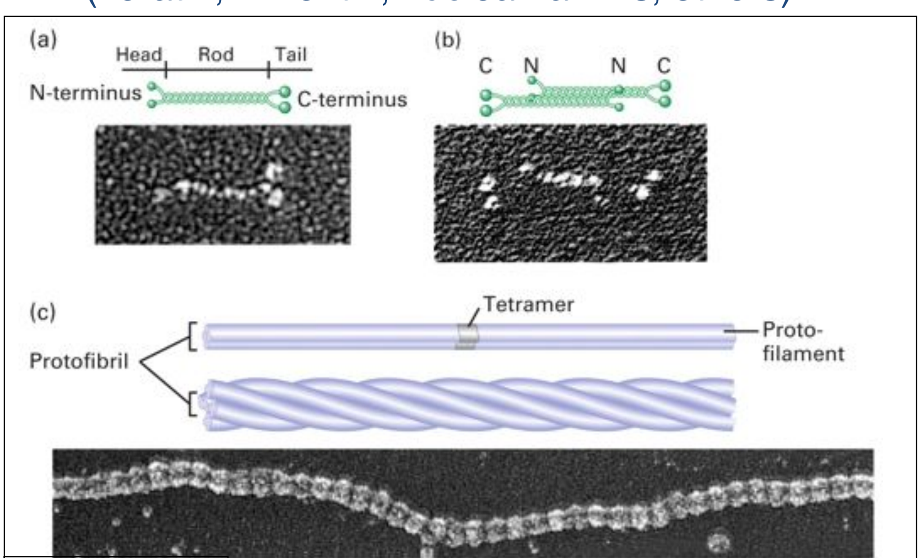
# Keratins form Intermediate Filaments that organize tissues




Places where you' ll find epithelial cells

# Intermediate filaments

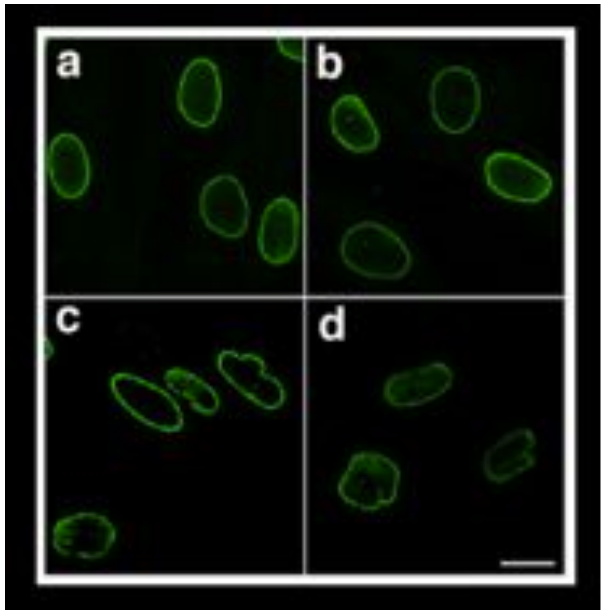
building block = variable
(keratin, vimentin, nuclear lamins, others)




Lodish Fig. 19-33

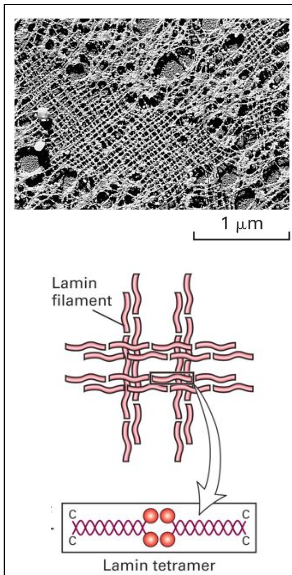
**Functions: mechanical integrity of tissue, cell, or subcellular organelle**

Laminopathies (HGPS, progeria)

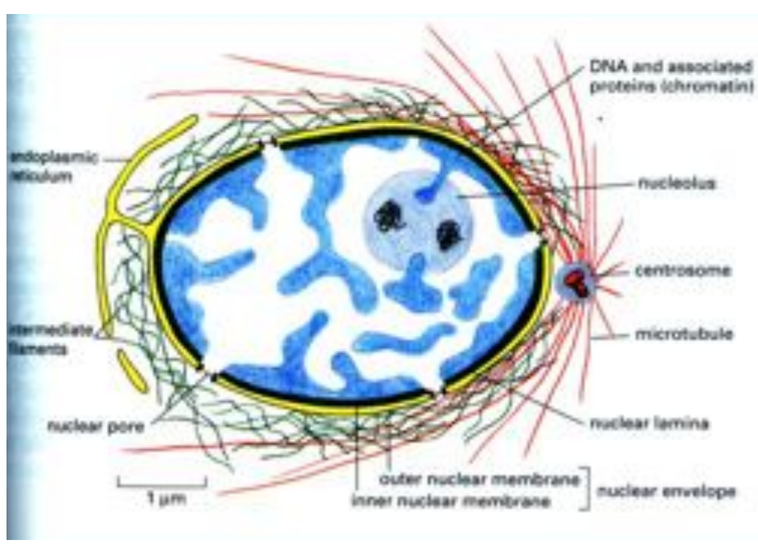



Some IFs are found in all cells.

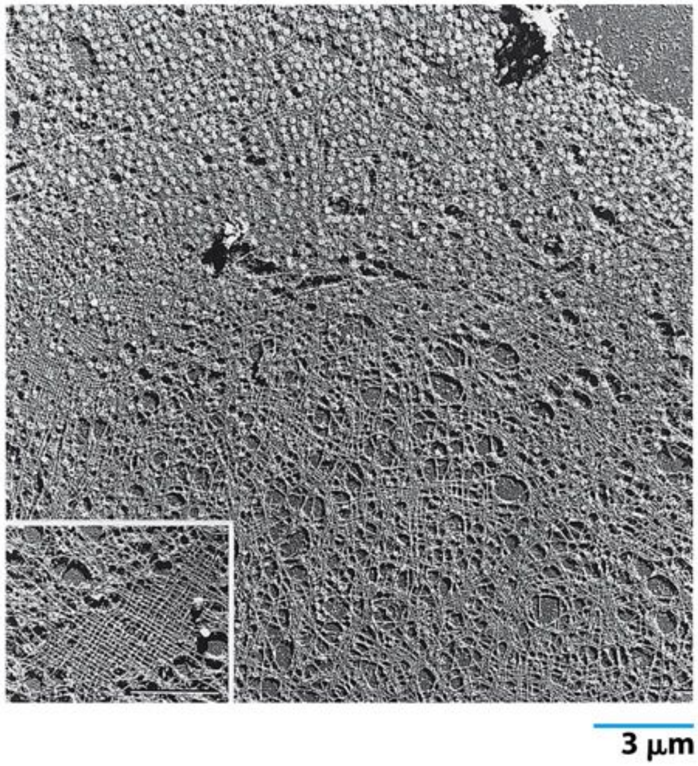
Nuclear lamins, a special type of IF, form a basket underlying the nuclear membrane, giving it strength and organization.



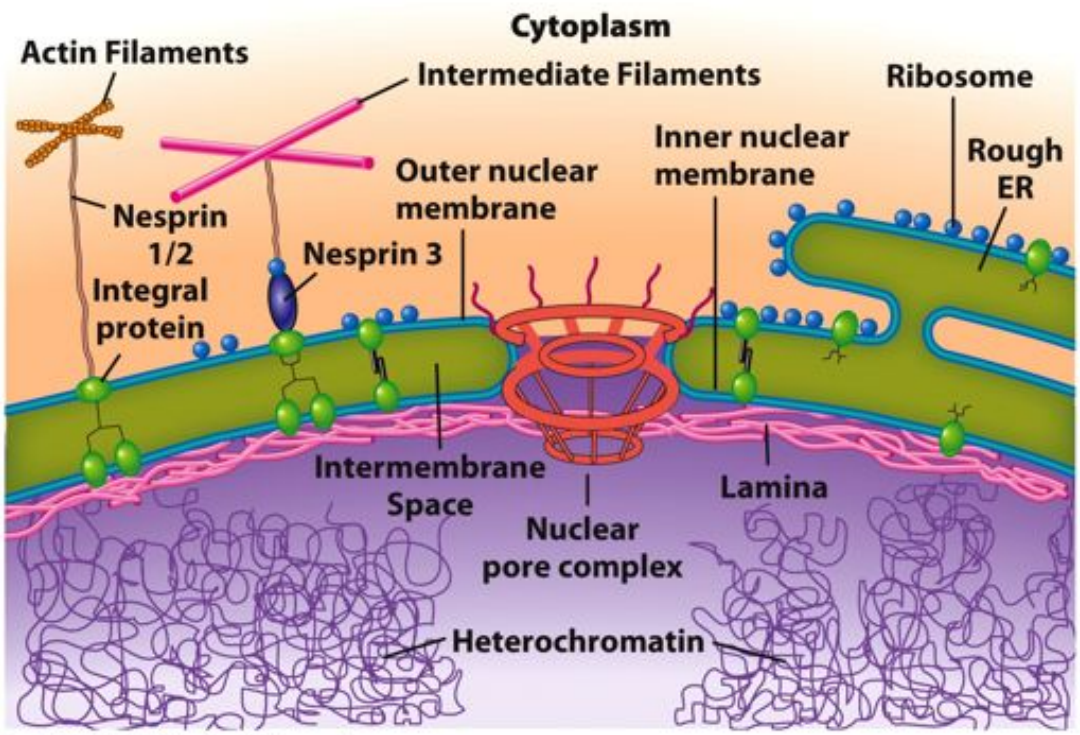

Lodish Fig. 19-33

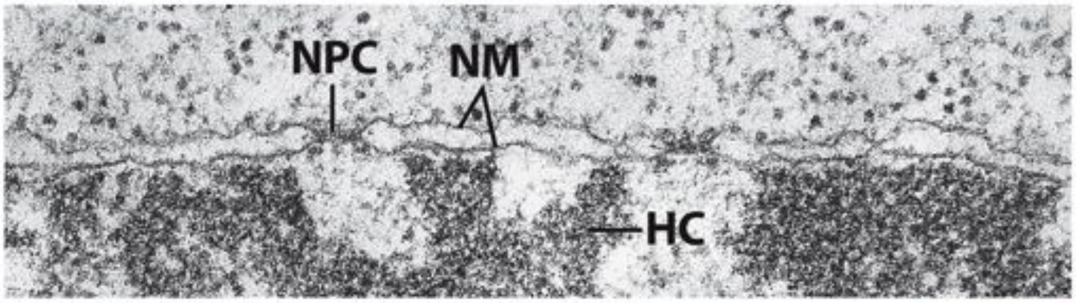



***Phosphorylated at head and tail during mitosis**


3 μm




Cytoplasm
Actin Filaments
Intermediate Filaments
Ribosome
Inner nuclear membrane
Rough ER
Outer nuclear membrane
Nesprin 1/2
Nesprin 3
Integral protein
Intermembrane Space
Lamina
Nuclear pore complex
Heterochromatin




From Werner W. Franke, et al., J. Cell Biol. 91:47s, Figure 8, 1981.
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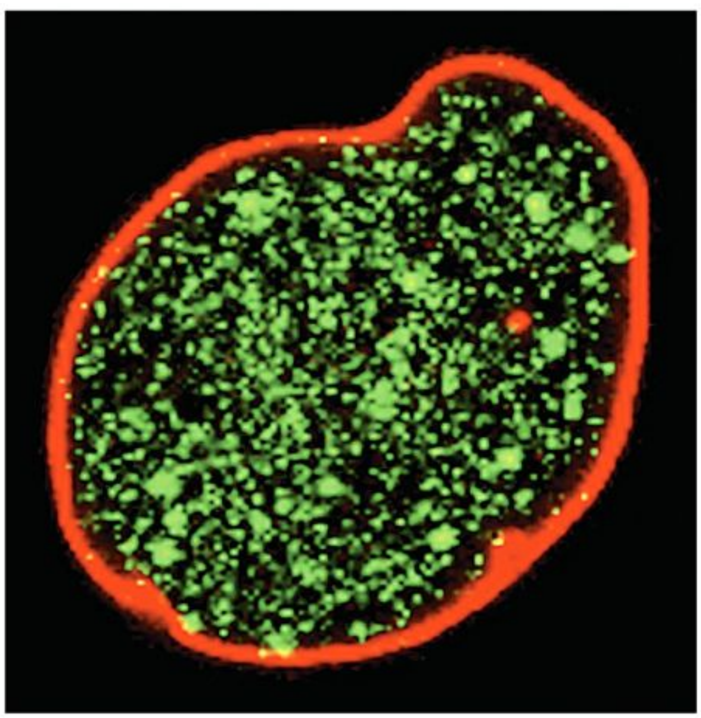
0.2 μm



From H. Ma, A.J. Siegel, and R. Berezney, J. Cell Biol. 146:535, Figure 2, 1999.
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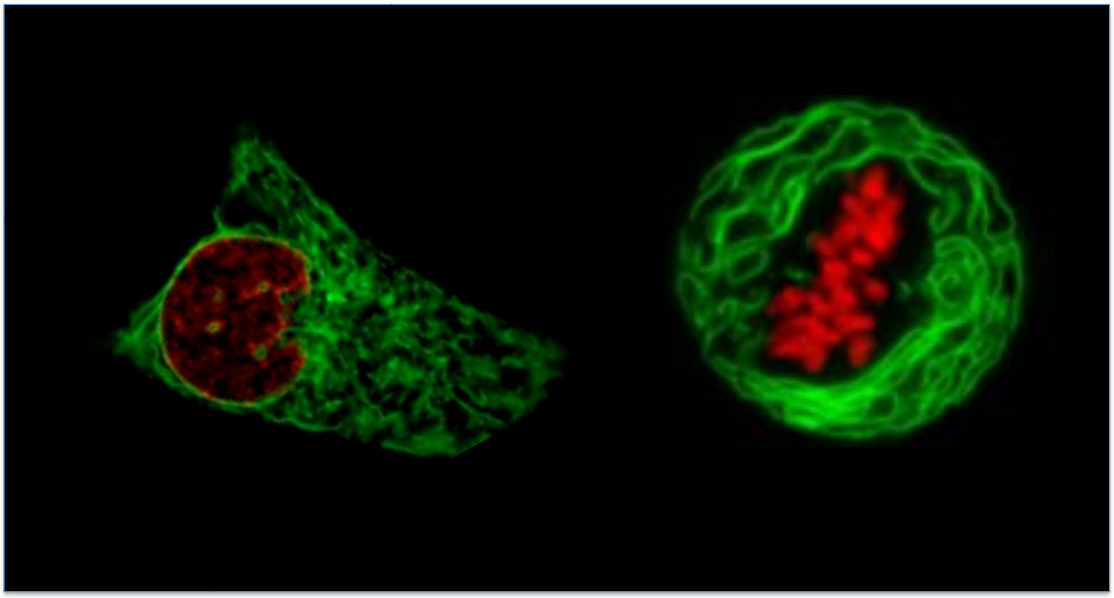
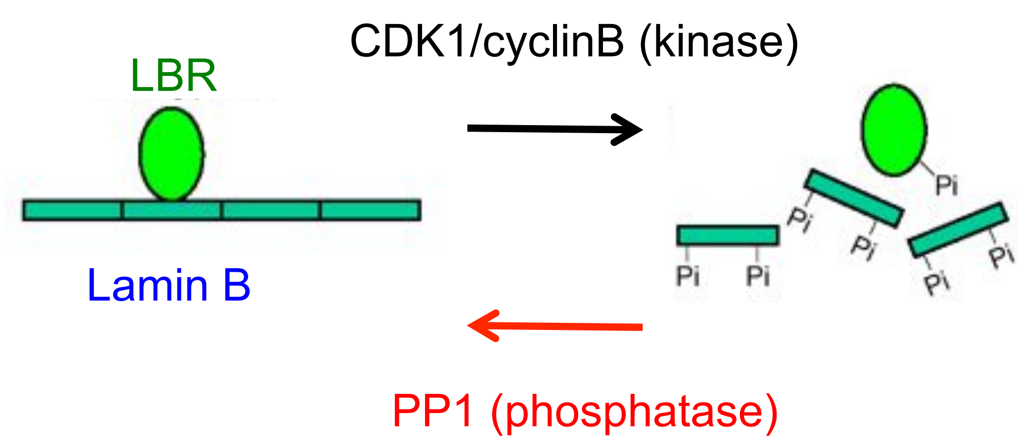
3 μm

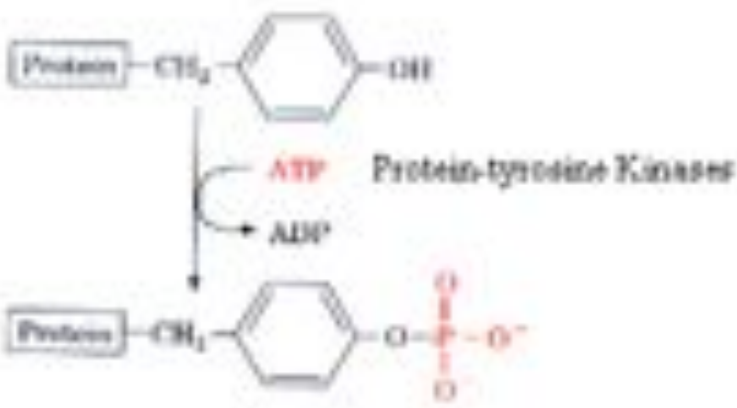
# Cell Cycle NE/Lamin Changes

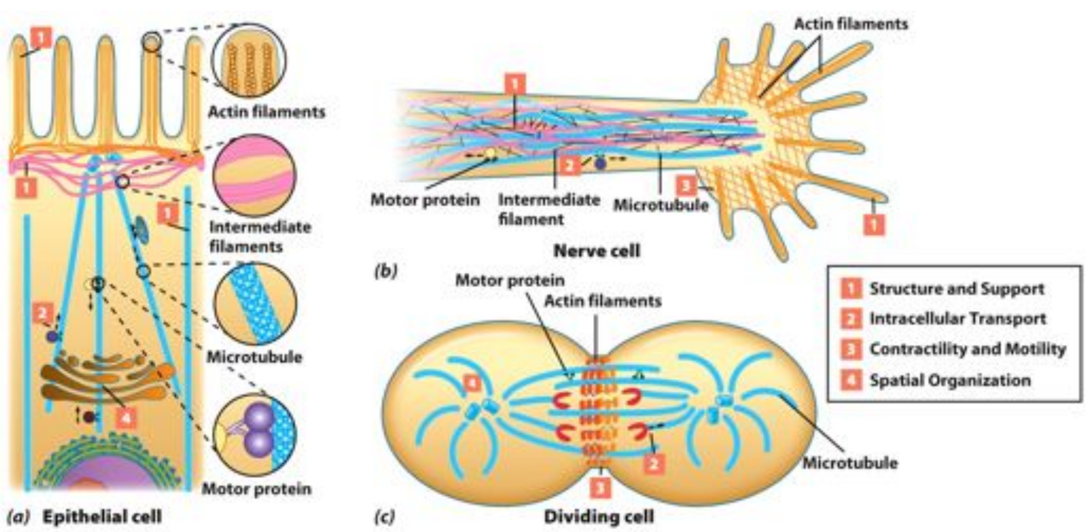


## Regulation of Nuclear Lamins



# Phosphorylation of Tyrosine Residues




(a) **Epithelial cell** (b) **Nerve cell** (c) **Dividing cell**