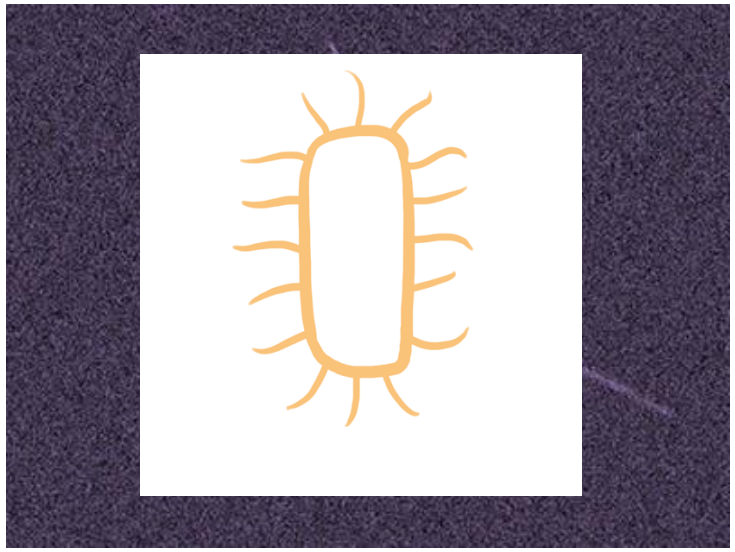
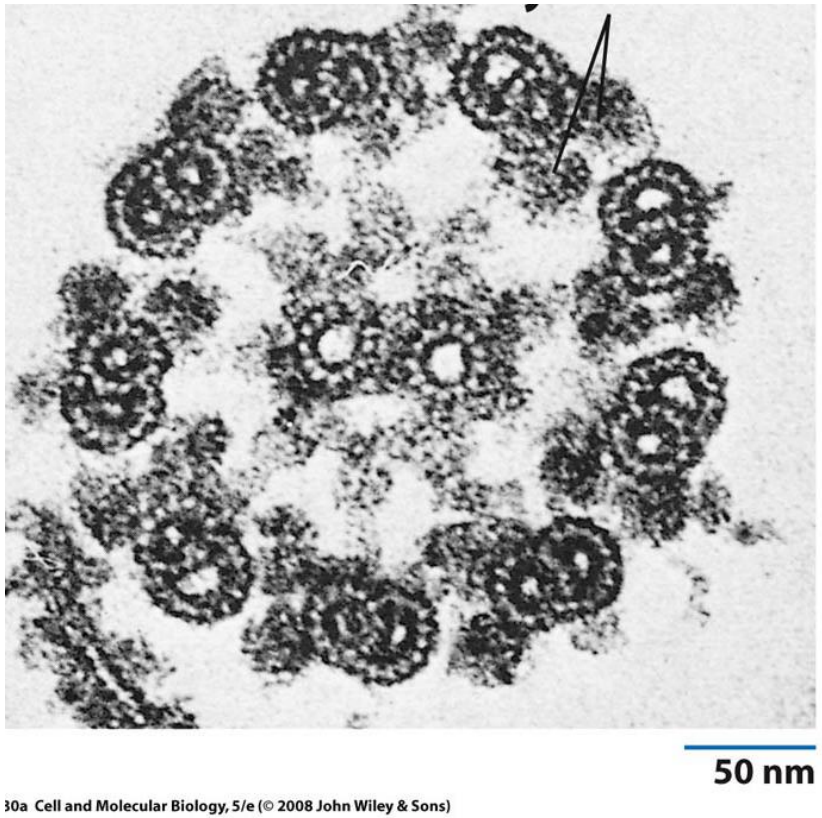
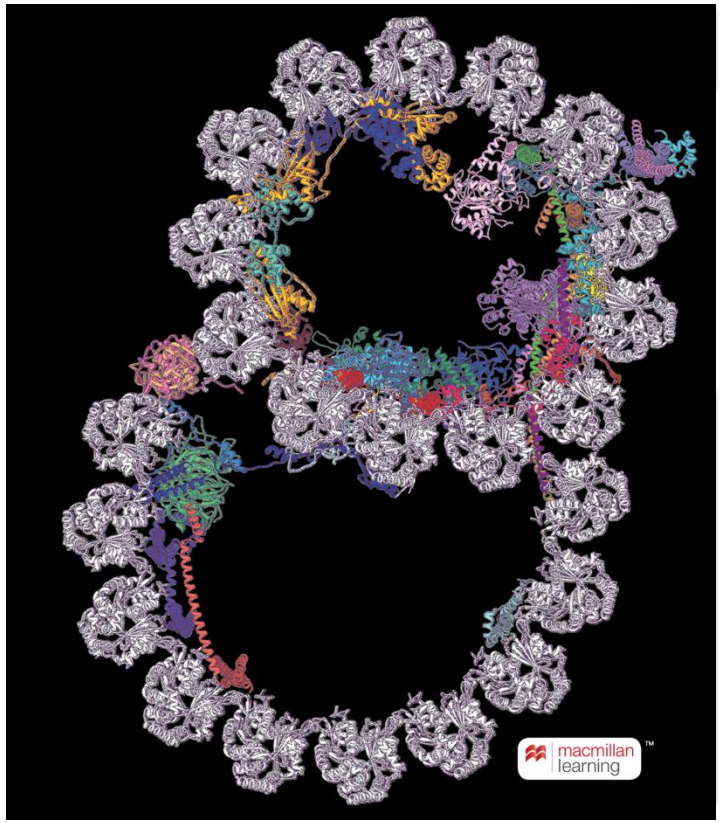
biol303- cytoskeleton 2
microtubules, cilia and flagella

now playing: whip it devo





30a Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)



Lodish Ch 18

overview

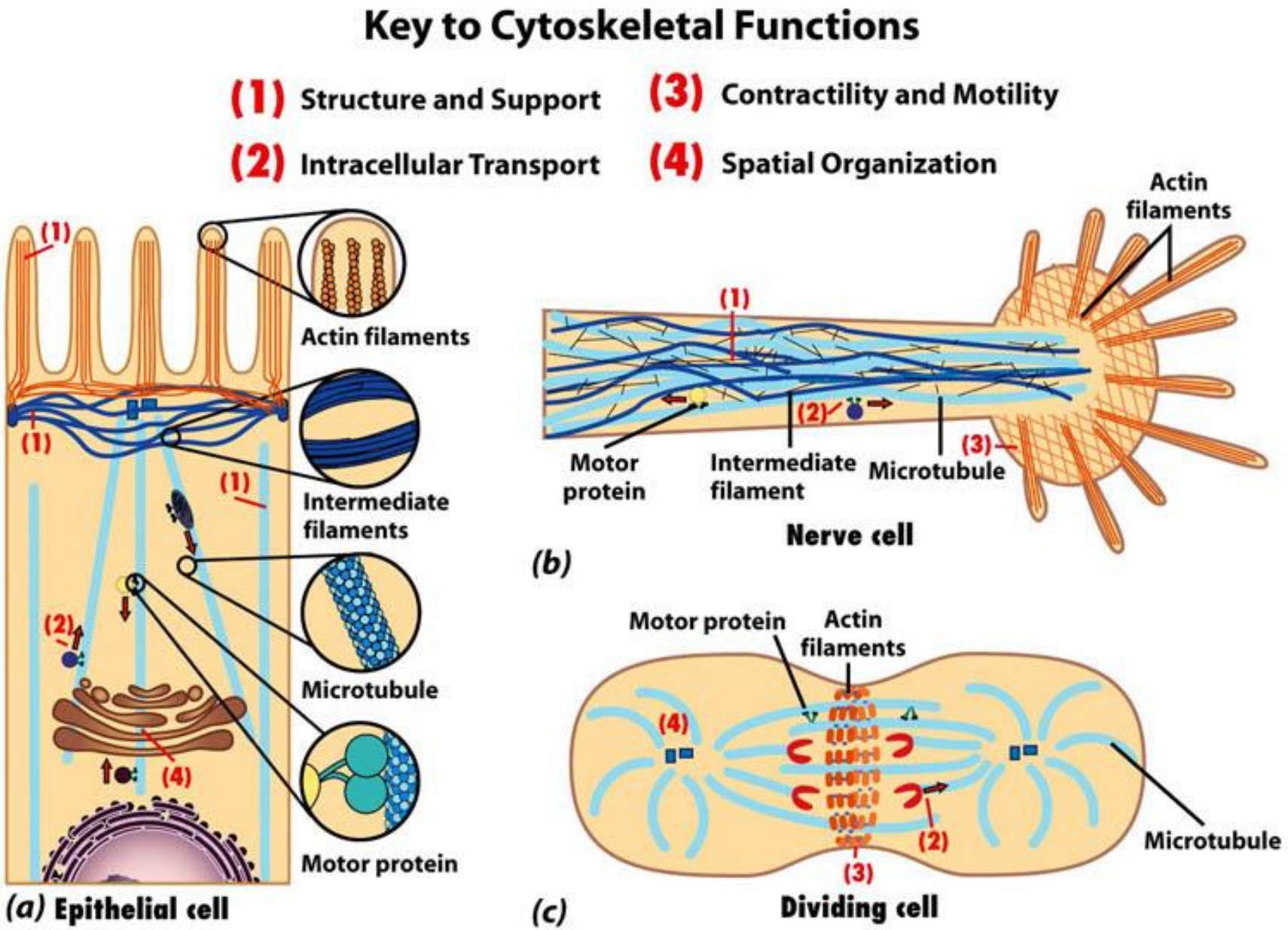


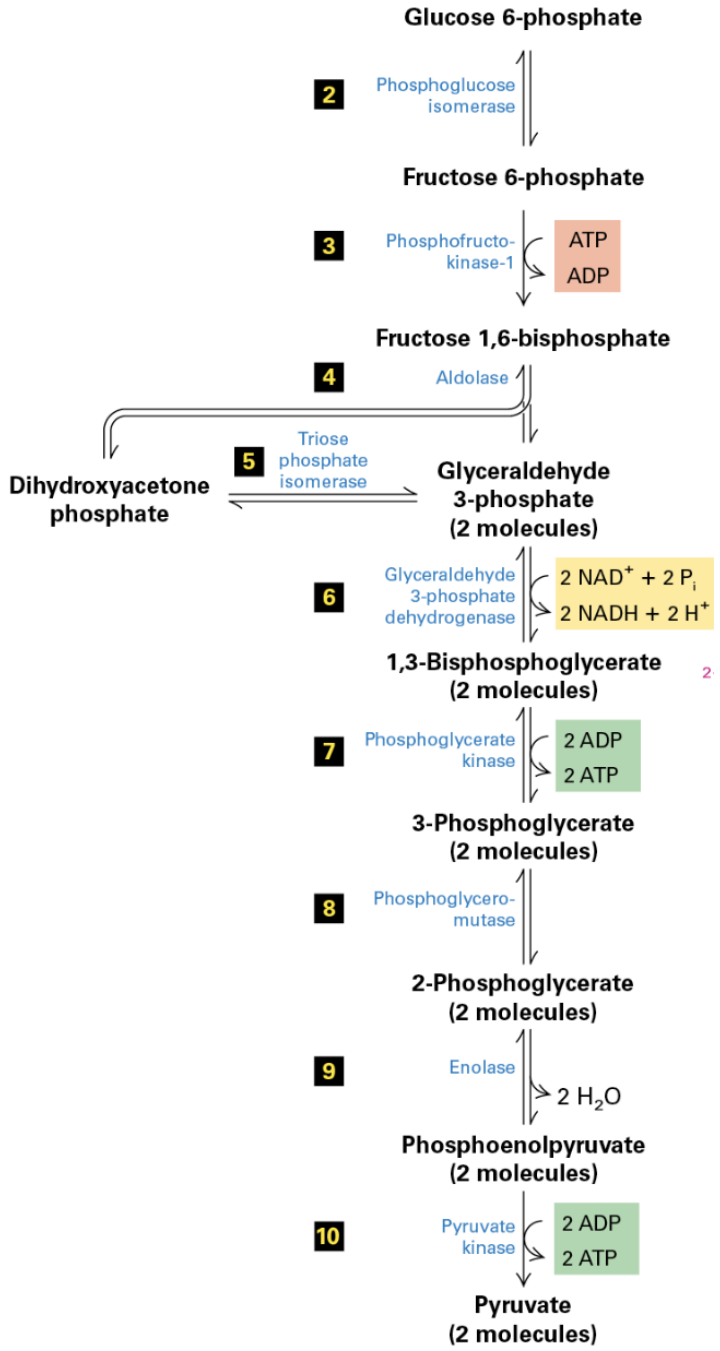
Figure 9-1 Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

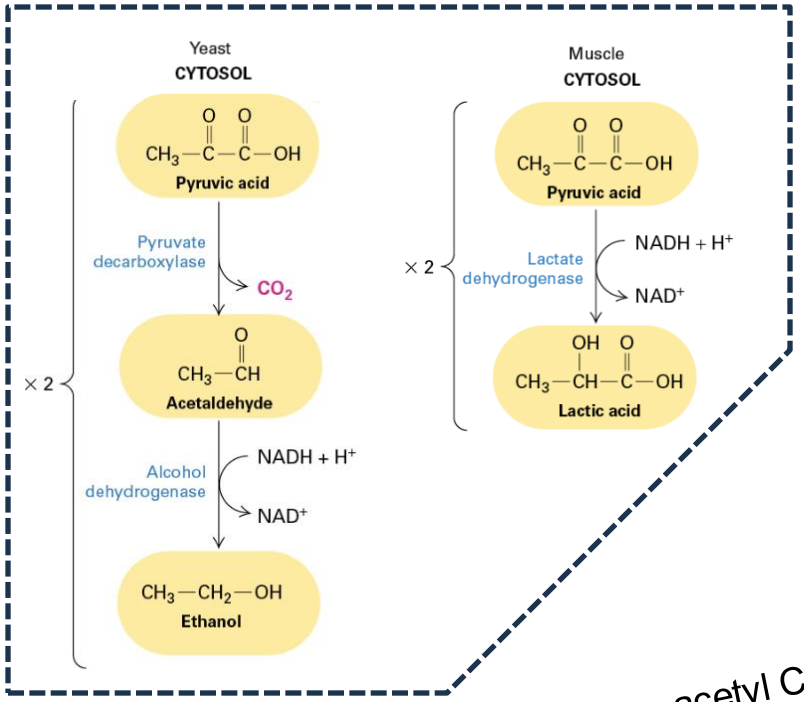
administrative

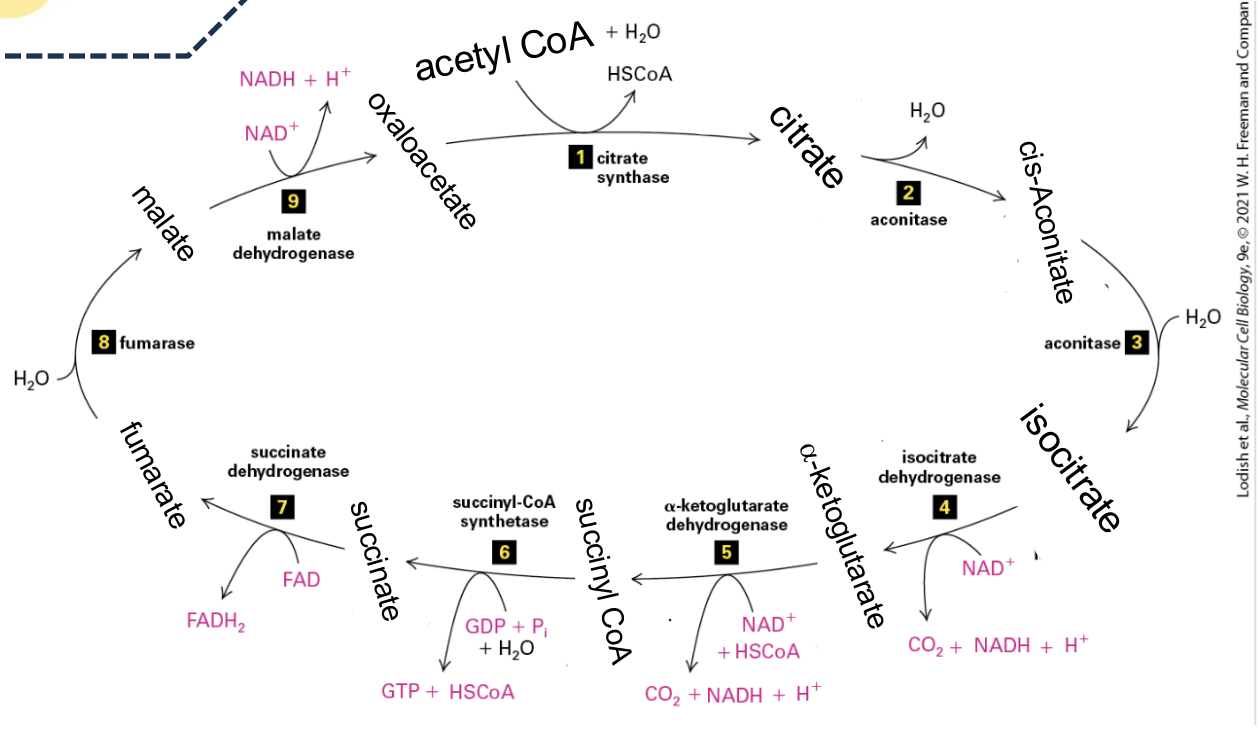
- error on Achieve assignment and related things…
- There is discussion homework this week (week 7) and Achieve homework due Friday
- project instructions and grading rubric is posted- more details to follow as you need them – next step is a figure summary
- No Friday office hours with msg- instead: Thurs Oct 16, 4:30-5:30pm this week only
- Exam 2 is in one week- some old exam questions are posted for practice; use your resources (discussion board, SI-PASS, study groups, tutoring, office hours) and know your vocabulary. There will be a reference sheet for substrates/products/enzymes (but not structures) for glycolysis, gluconeogenesis, fermentation, and the Krebs cycle

Metabolic reactions reference sheet

(make sure you know which pathway here is which!)







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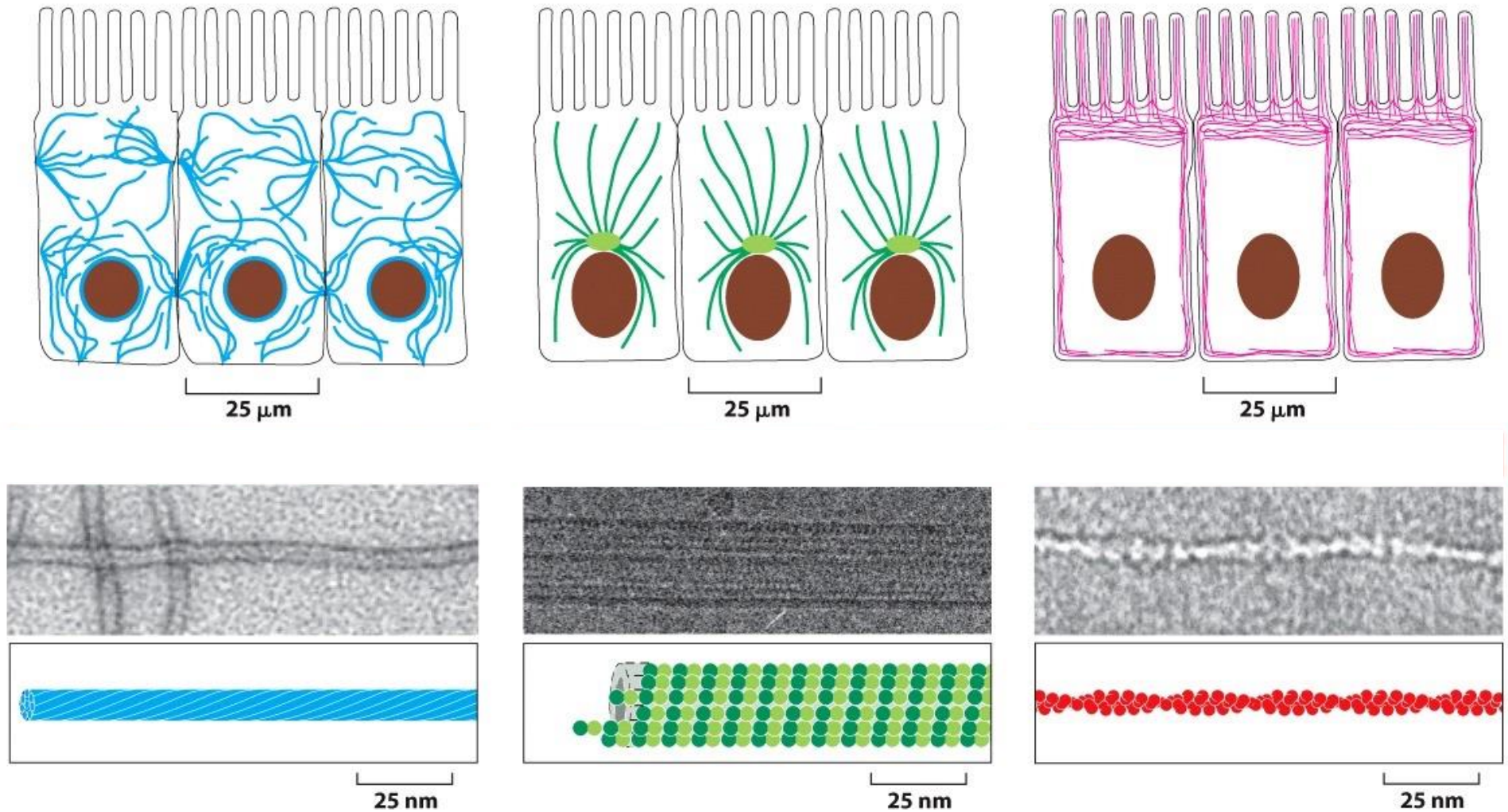
three types of cytoskeletal filaments…



three typ…oskeletal filam…d…

Intermediate Filaments

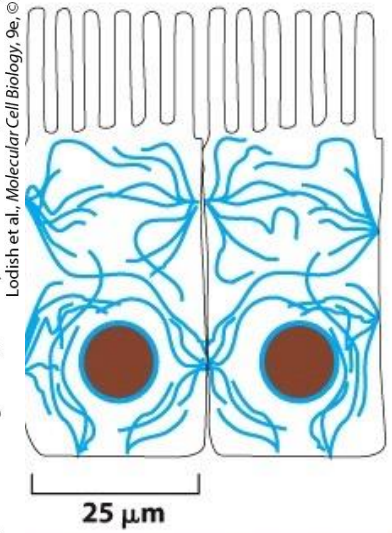
- IF subunits don't bind a nucleotide
- Great tensile strength
- Assembled onto preexisting filaments
- Less dynamic
- Unpolarized
- No motors
- Cell and tissue integrity

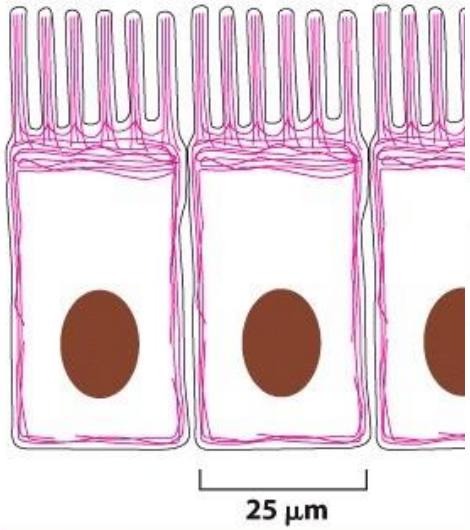
Microtubules

- αβ-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

(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





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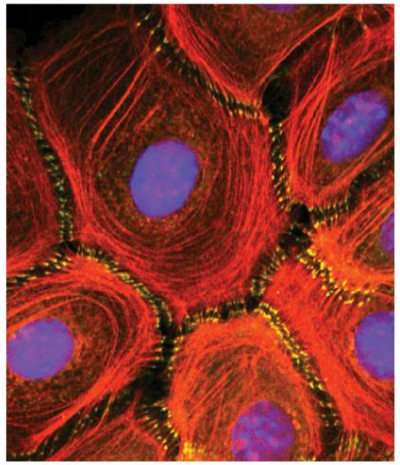

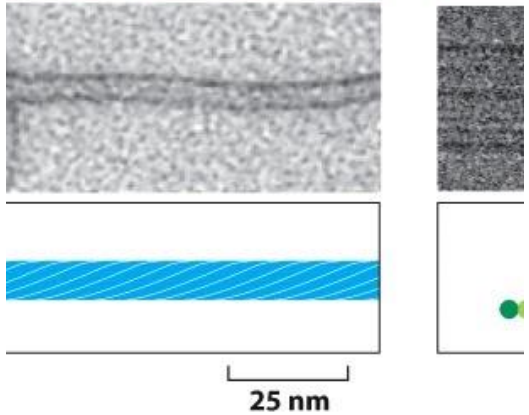

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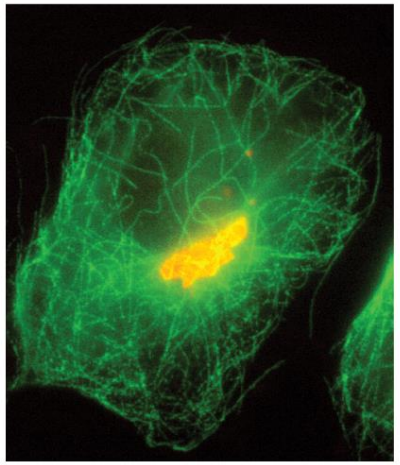

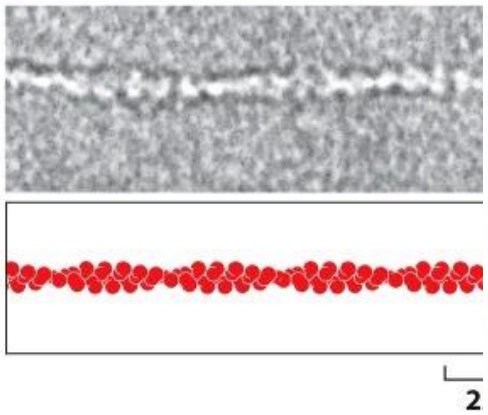

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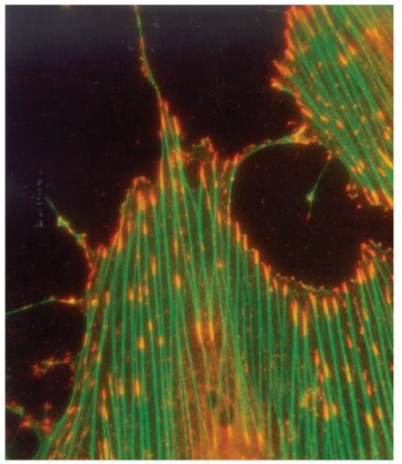
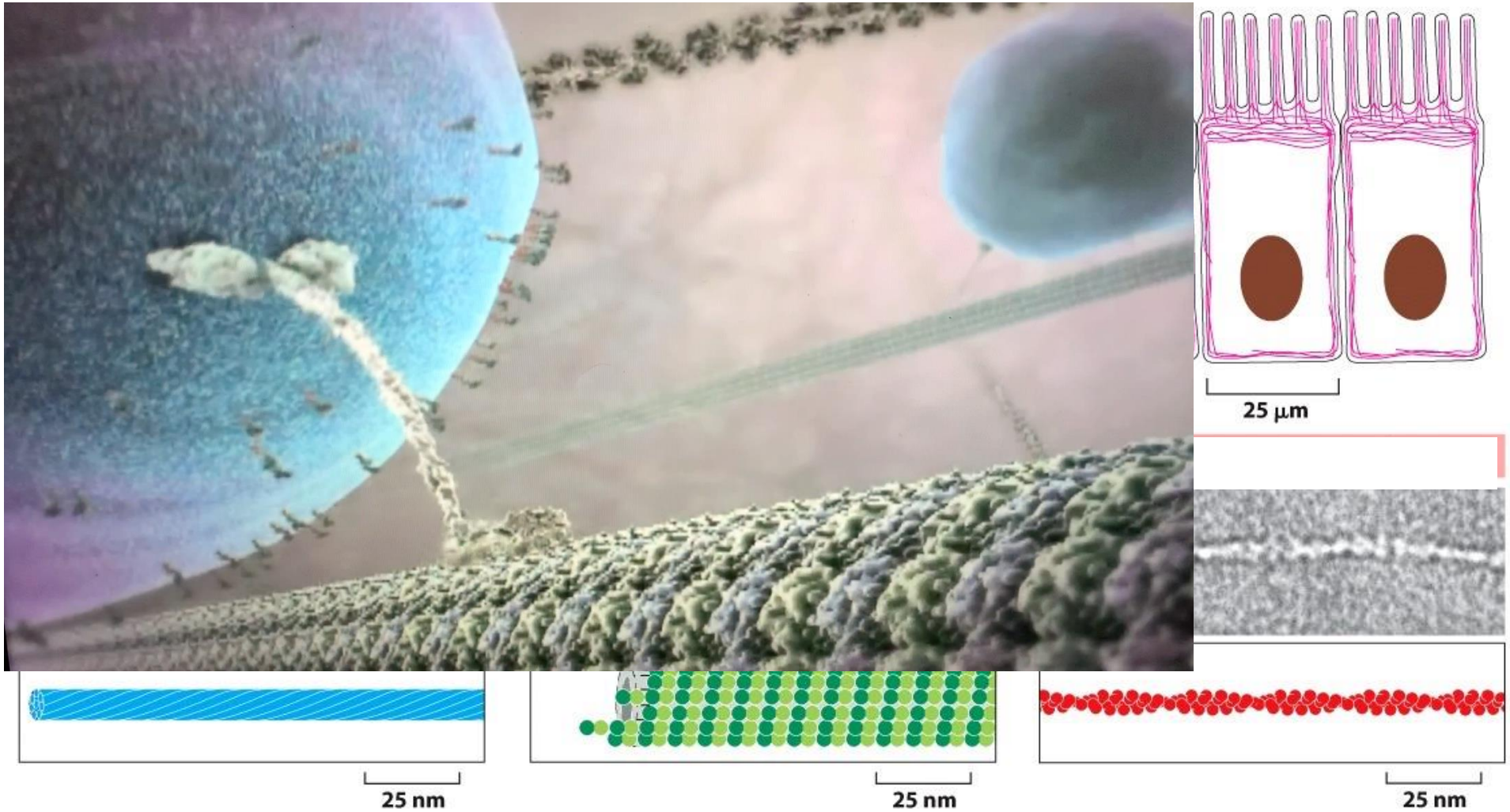
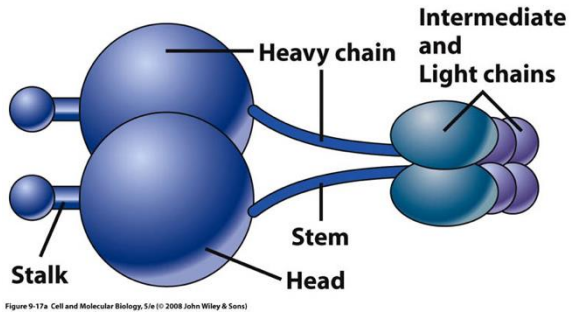


three types of cytoskeletal filaments… and…

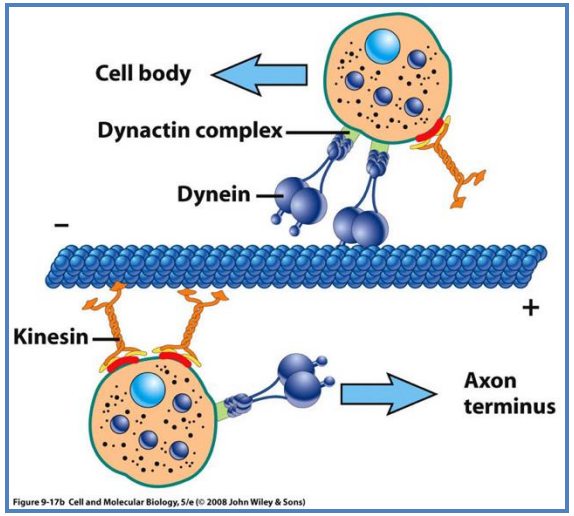


three major motor proteins

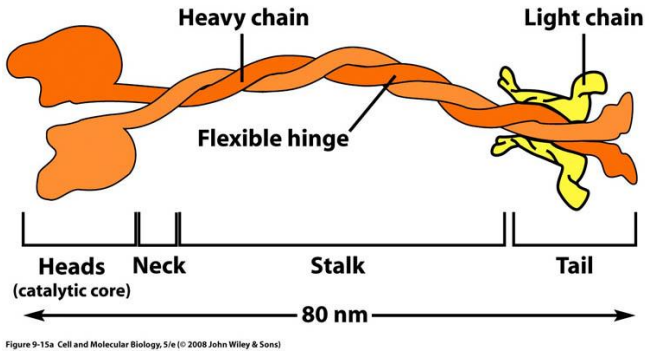
motor proteins use energy to move directionally along cytoskeletal polymers



Dynein(moves along Tub to - end)

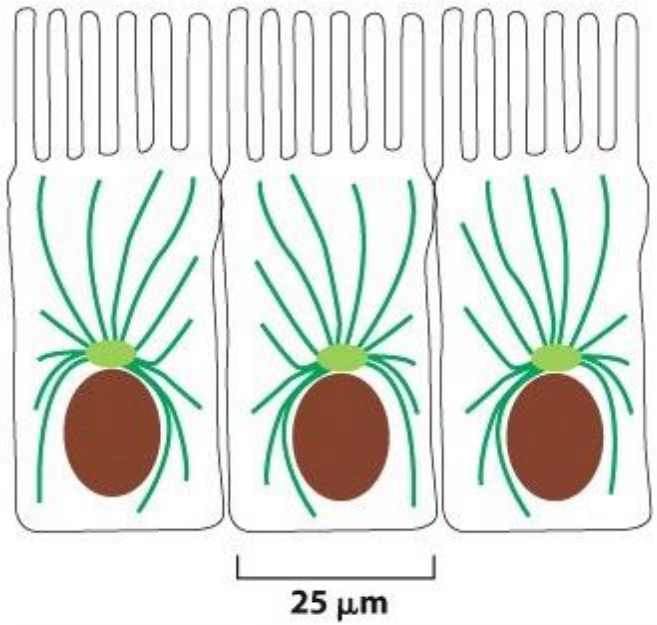


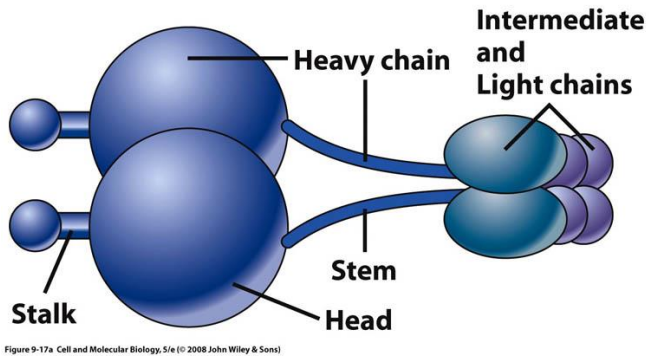
Myosin
(moves along Actin)



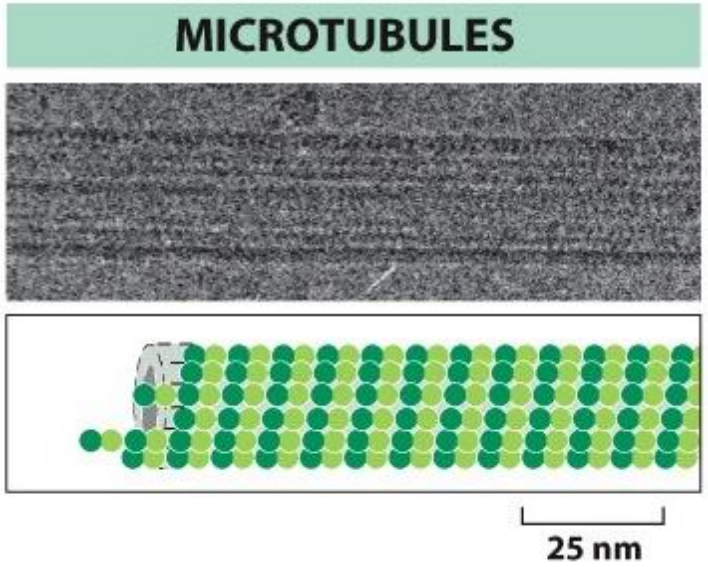
Kinesin
(moves along Tub to + end)

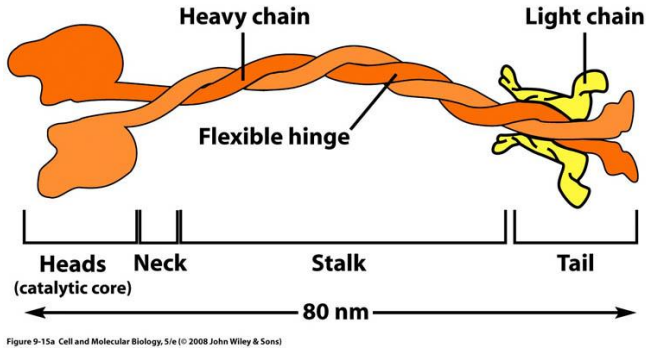
today: one filament, two motors





Dynein(moves along Tub to - end)

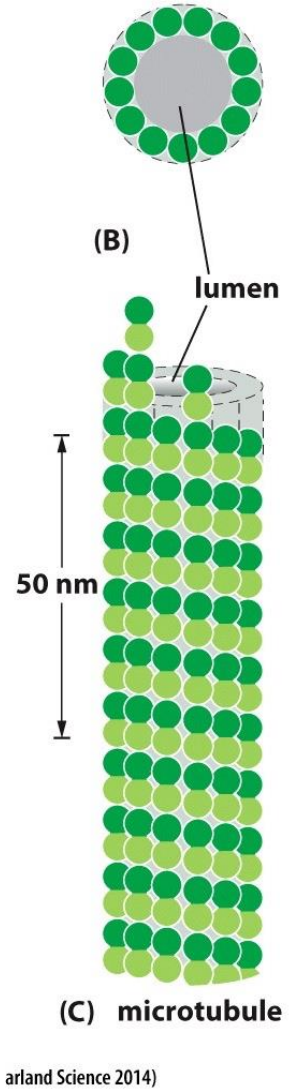




Kinesin
(moves along Tub to + end)

microtubule learning objectives

by the end of this lecture you should be able to:



arland Science 2014)

- describe the key functions of microtubules
- describe how microtubules assemble and breakdown
- know which molecules are required for microtubule assembly and stability and what drugs disrupt these
- explain “dynamic instability” and explain advantages of polymer structure
- compare and contrast the motor proteins associated with microtubules and their functions
- describe the organization of the axoneme structure and explain how microtubules and dynein contribute to flagellar and ciliary movement
- explain the importance of cilia in human biology

overview: **microtubules** contribute to structural support, organization, motility within the cell

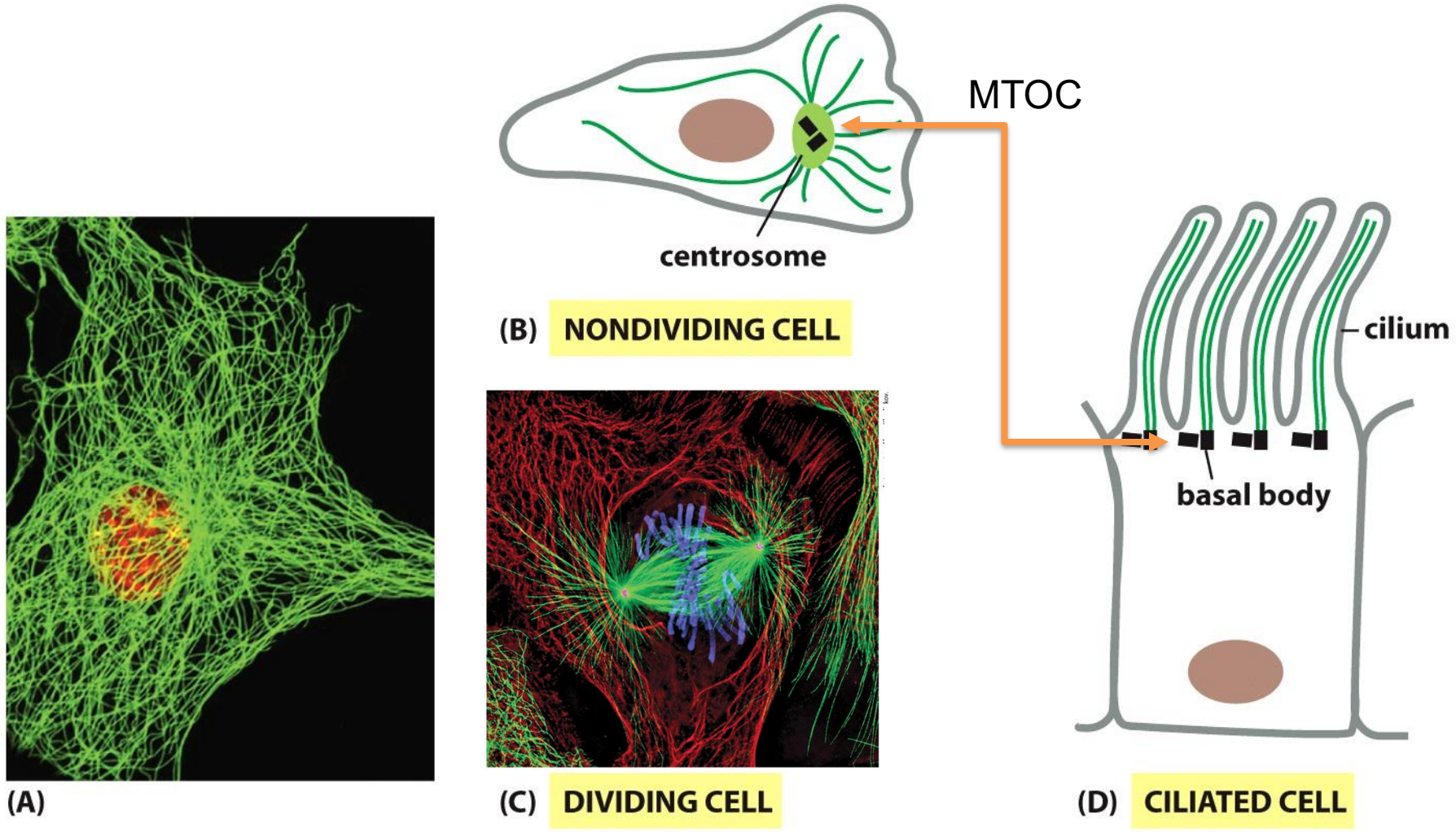


Figure 17-10 Essential Cell Biology, 4th ed. (© Garland Science 2014)

microtubules are polymers

+

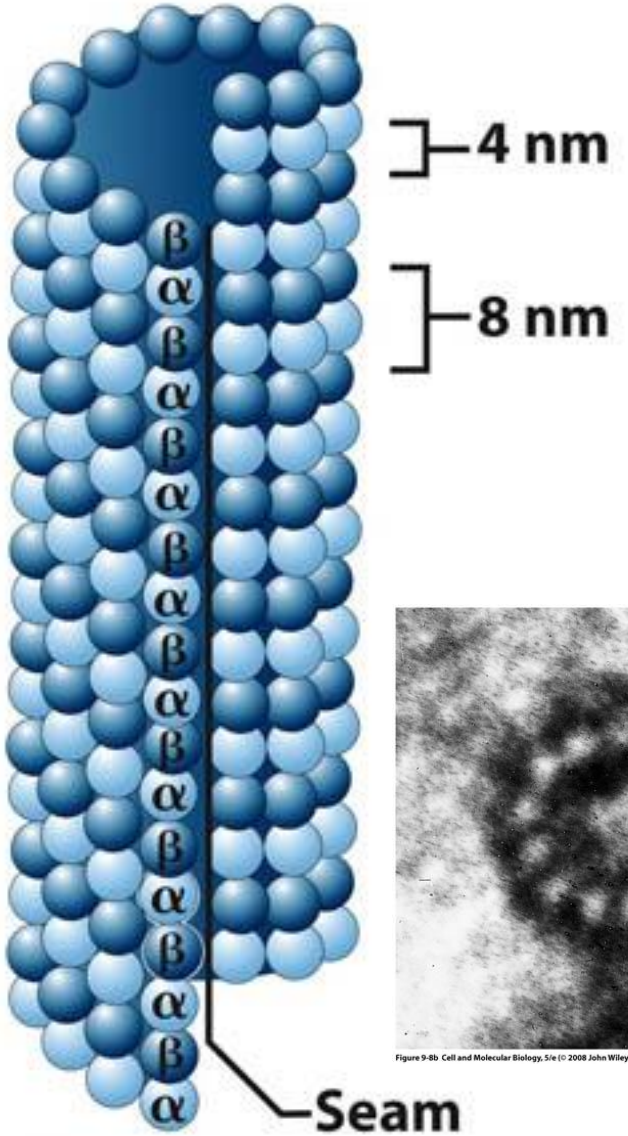
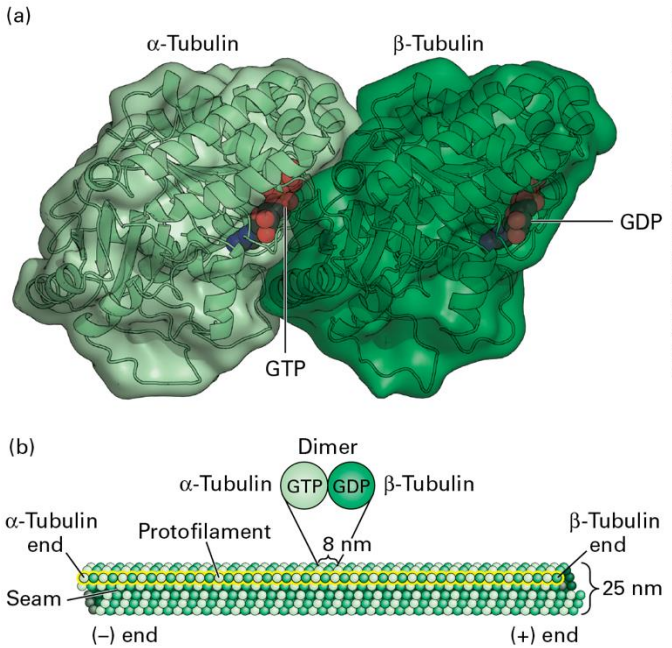


Figure 9-8d Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

Figure 9-8b Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

- *polarized* +/-, hollow tube ___**nm diameter**
- made up of α and β subunits (heterodimers) that can be added and released (13 protofilaments)
- stabilized by associated proteins



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

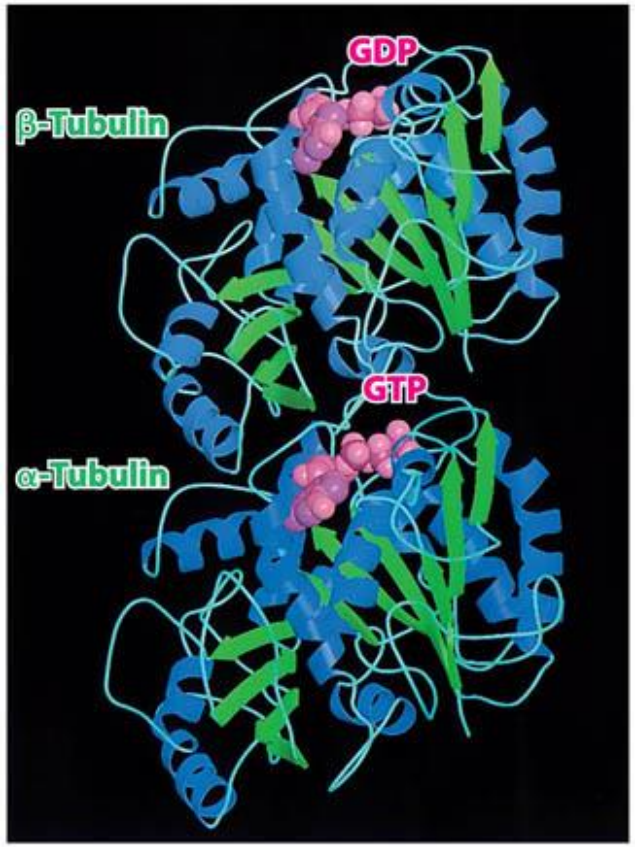
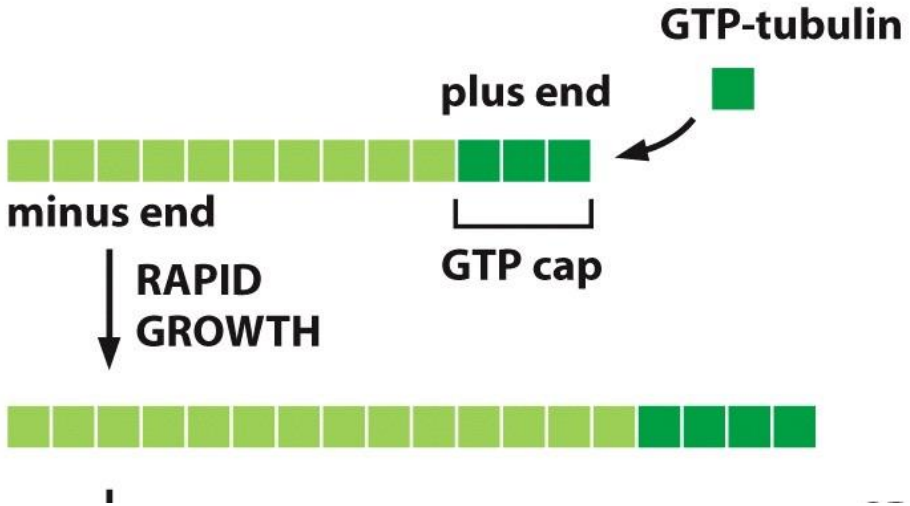
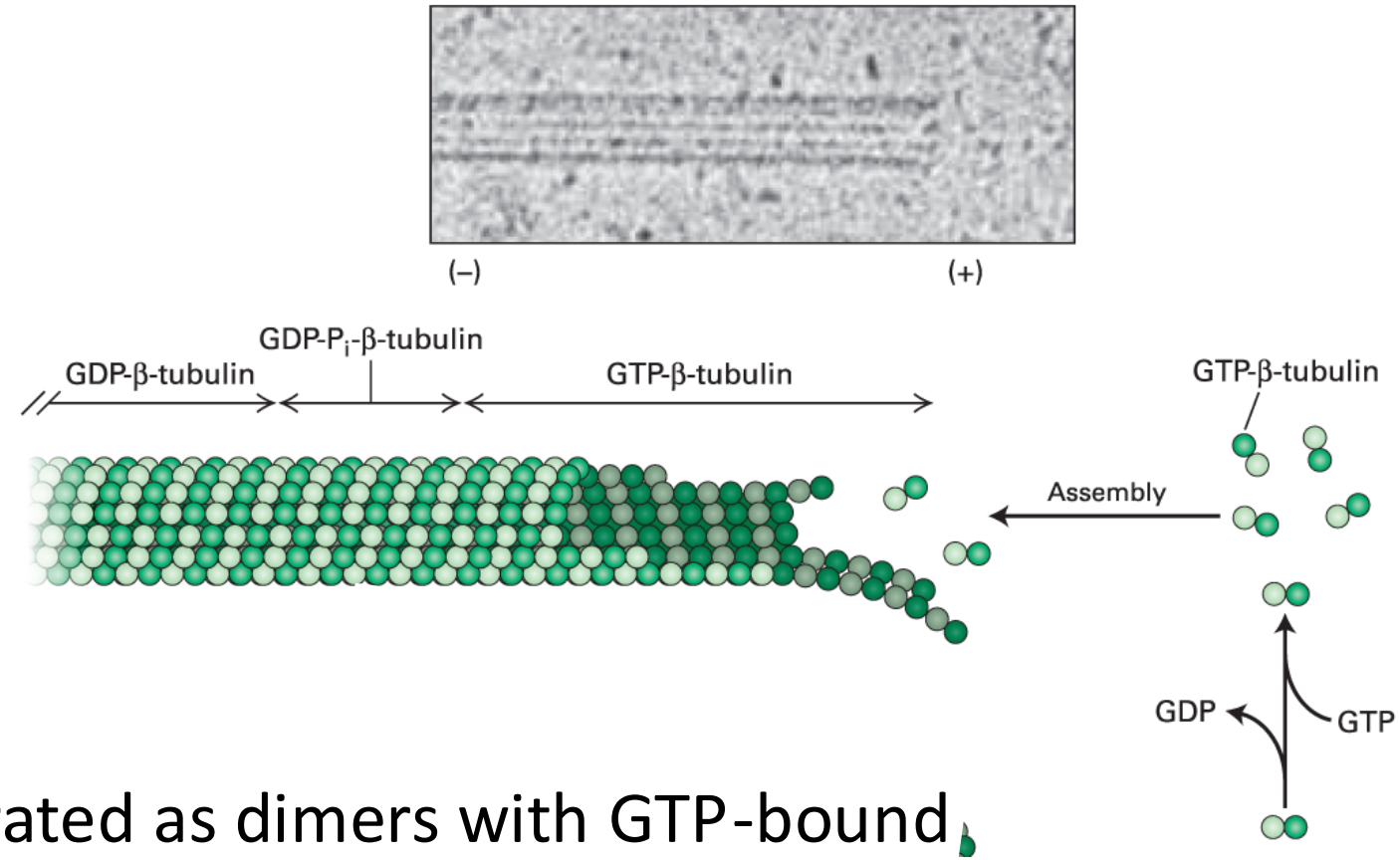


Figure 9-8c Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

microtubules can lengthen…





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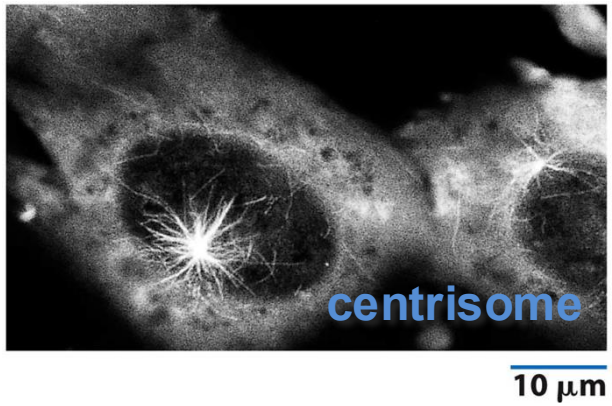
- subunits are rapidly incorporated as dimers with GTP-bound to the β-subunit, then GTP is hydrolyzed (expensive!)

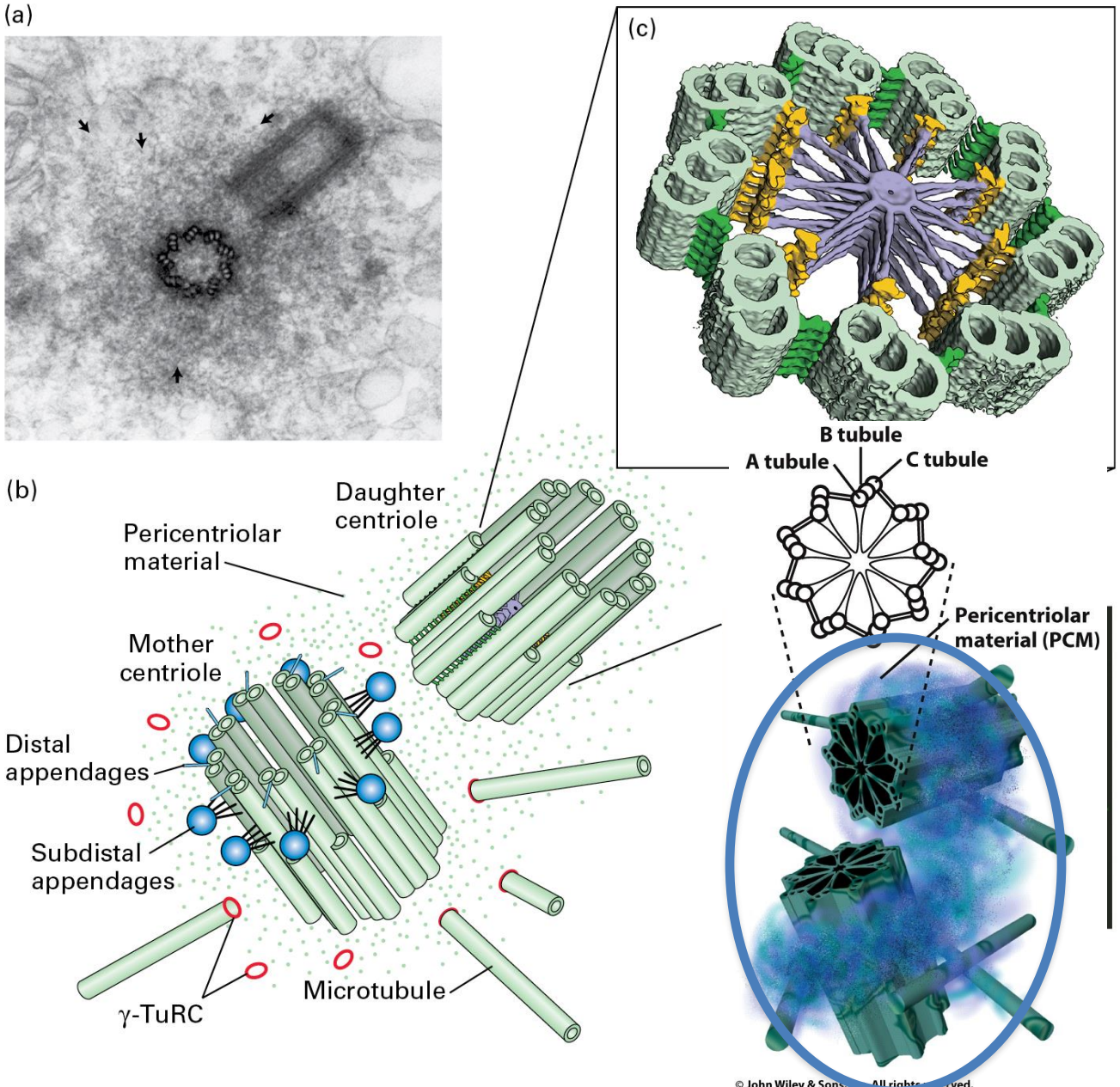
- **GDP on β is exchanged in the cytosol for GTP**

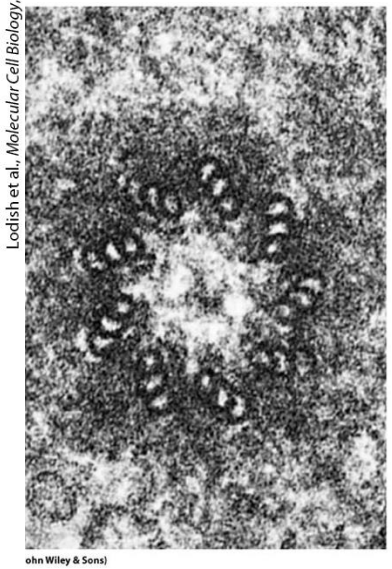
soluble pool of dimers in cytosol

MTOCs govern MT assembly



- **MTOC - microtubule-organizing centers**
  - control #, polarity, time, location, of nucleation
- centrosome **and basal body** in most animal cells (nucleus in plants)
- centrosome is made up of 2 centrioles and PCM (pericentriolar material)





Part (d) Courtesy of Ryoko Kuriyame.
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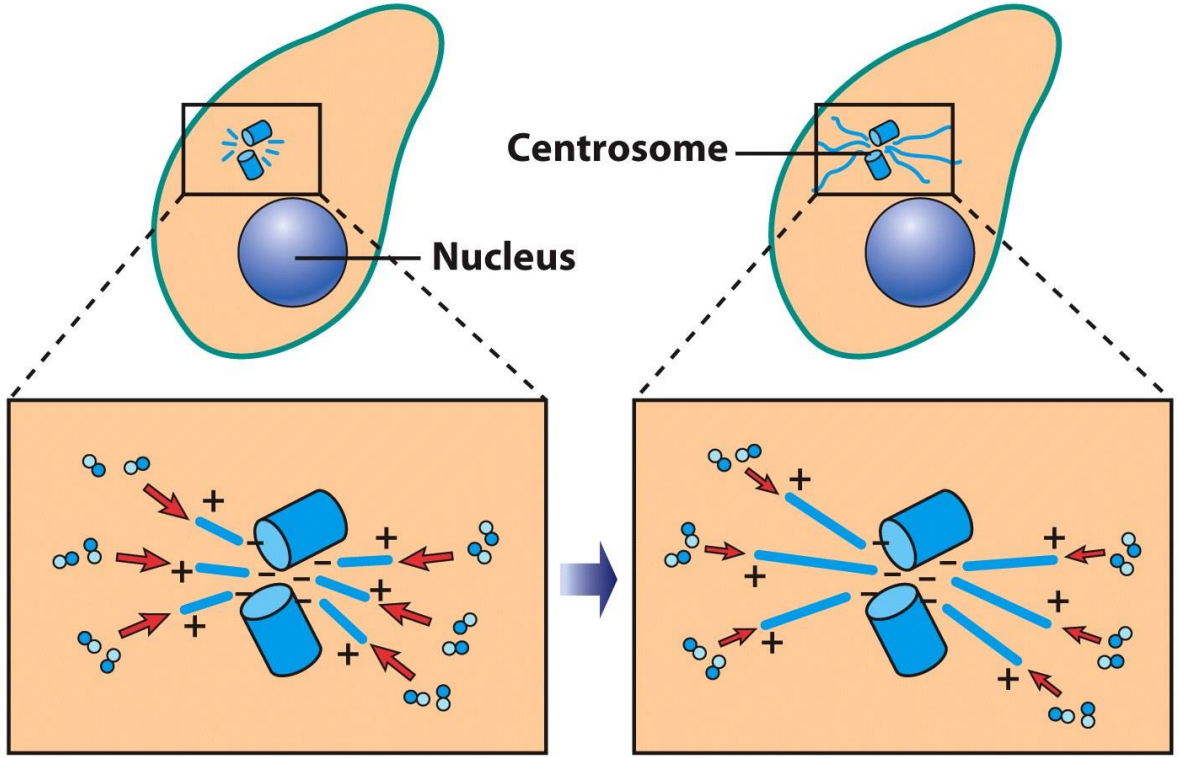
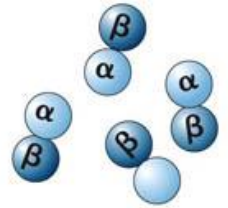
microtubule assembly



nucleation of MTs is slow
elongation is fast

minus end is associated with centrosome;

dimers are added to (and lost from) the PLUS end



subunit pool

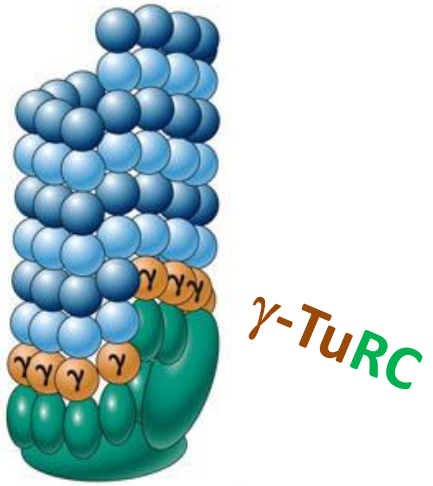


Figure 9-20c Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

γ-Tubulin sits in a ring in PCM and dimers bind to it to form microtubule

microtubules are very dynamic – grow & shrink

- subunits are rapidly incorporated as dimers with GTP-bound to the β-subunit, then GTP is hydrolyzed (expensive!)

- if all GTP is hydrolyzed, the microtubule can rapidly **depolymerize**

- **GDP on β is exchanged in the cytosol for GTP**

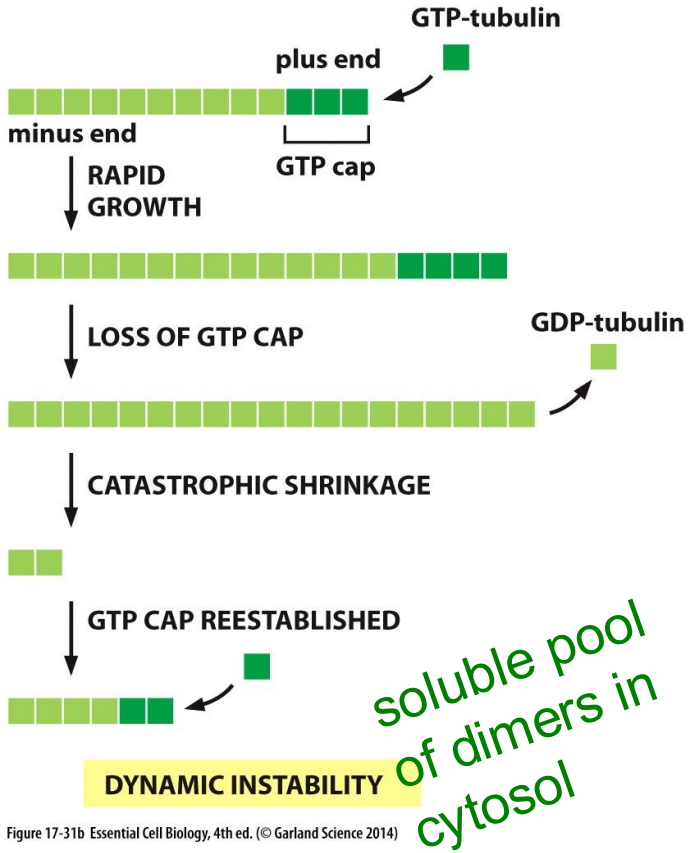
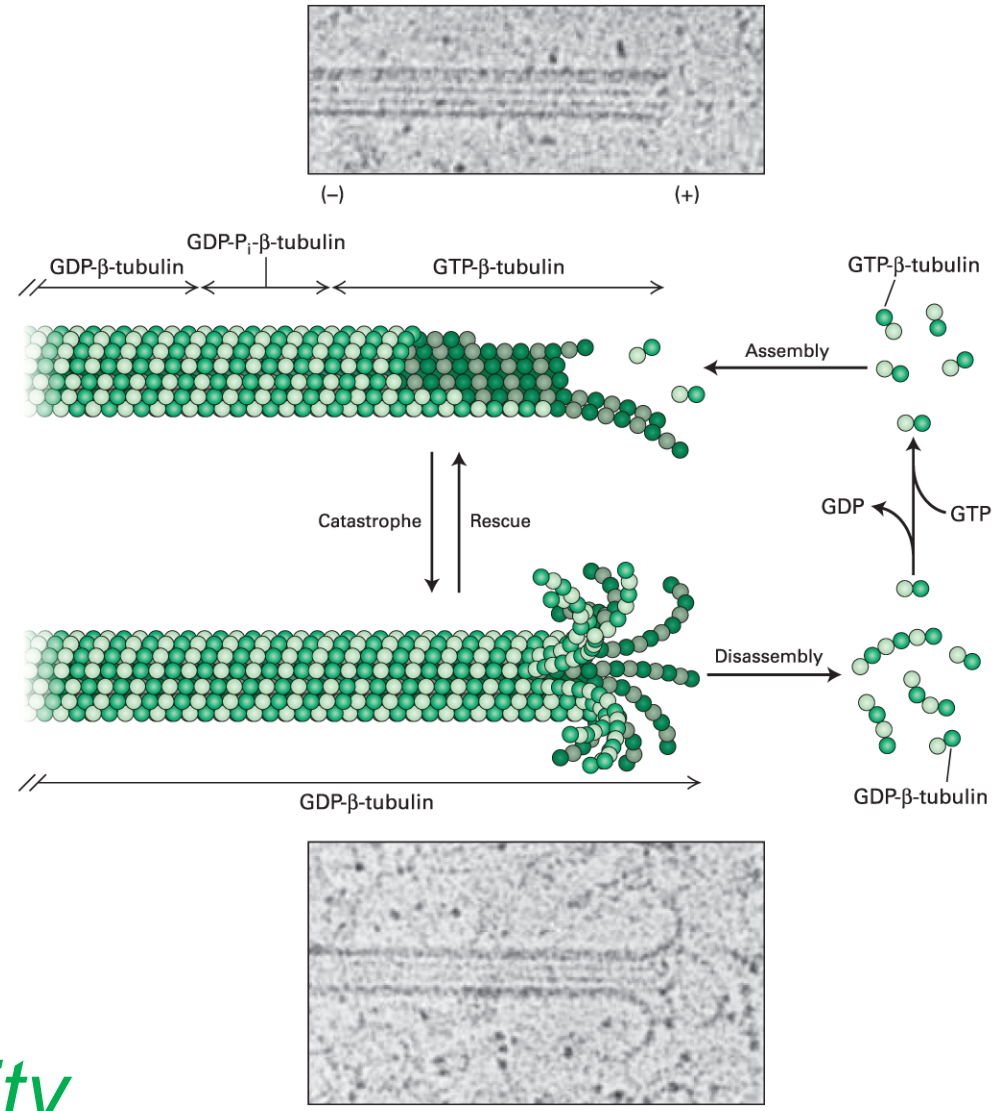


Figure 17-31b Essential Cell Biology, 4th ed. (© Garland Science 2014)

soluble pool of dimers in cytosol



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dynamic instability

microtubule assembly & disassembly

- assembly occurs at microtubule-organizing centers (MTOCs)

- requires energy from **GTP hydrolysis** (beta) to lengthen (expensive)

- *dynamic instability* at the plus end = polymerize and depolymerize

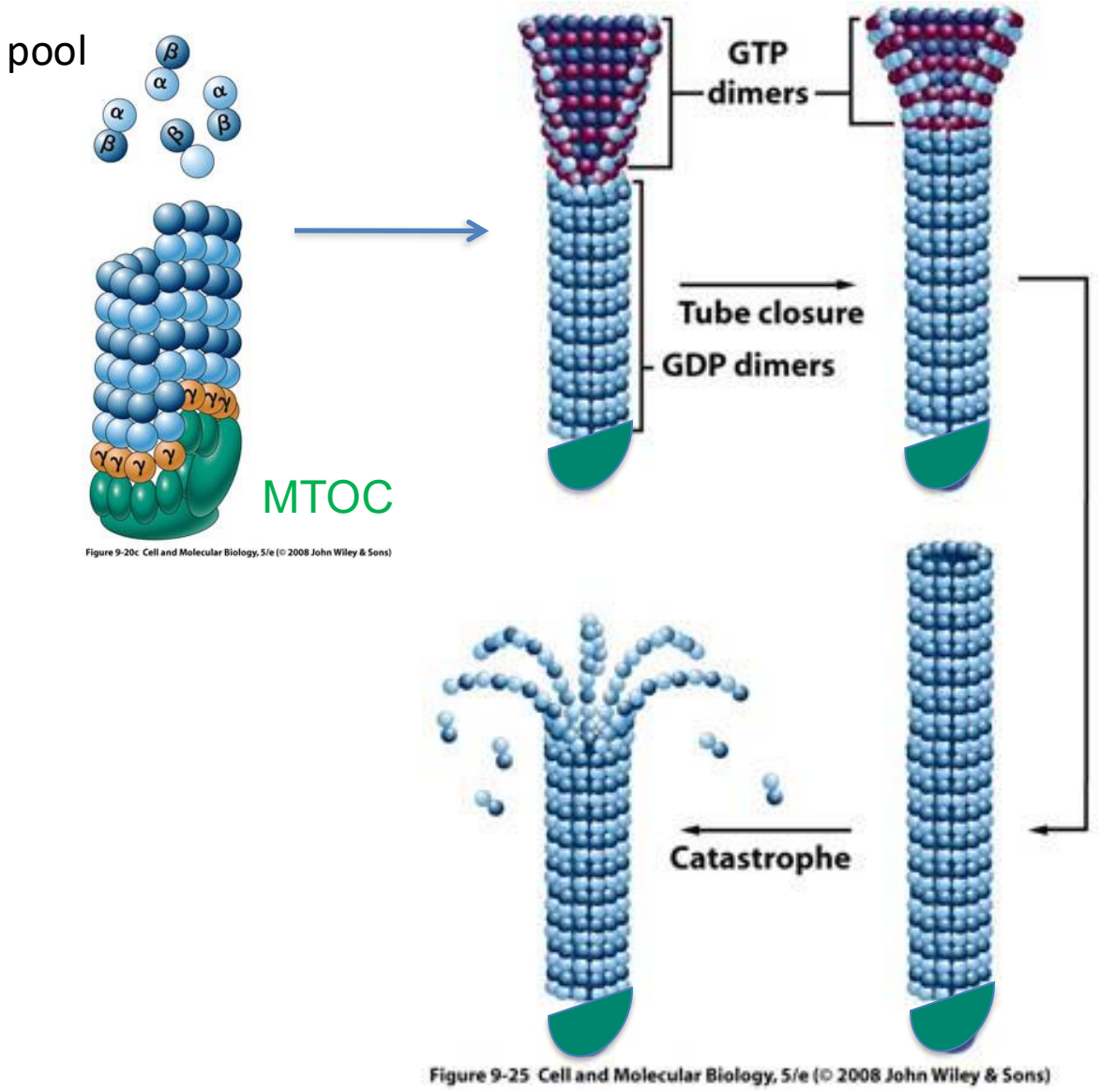
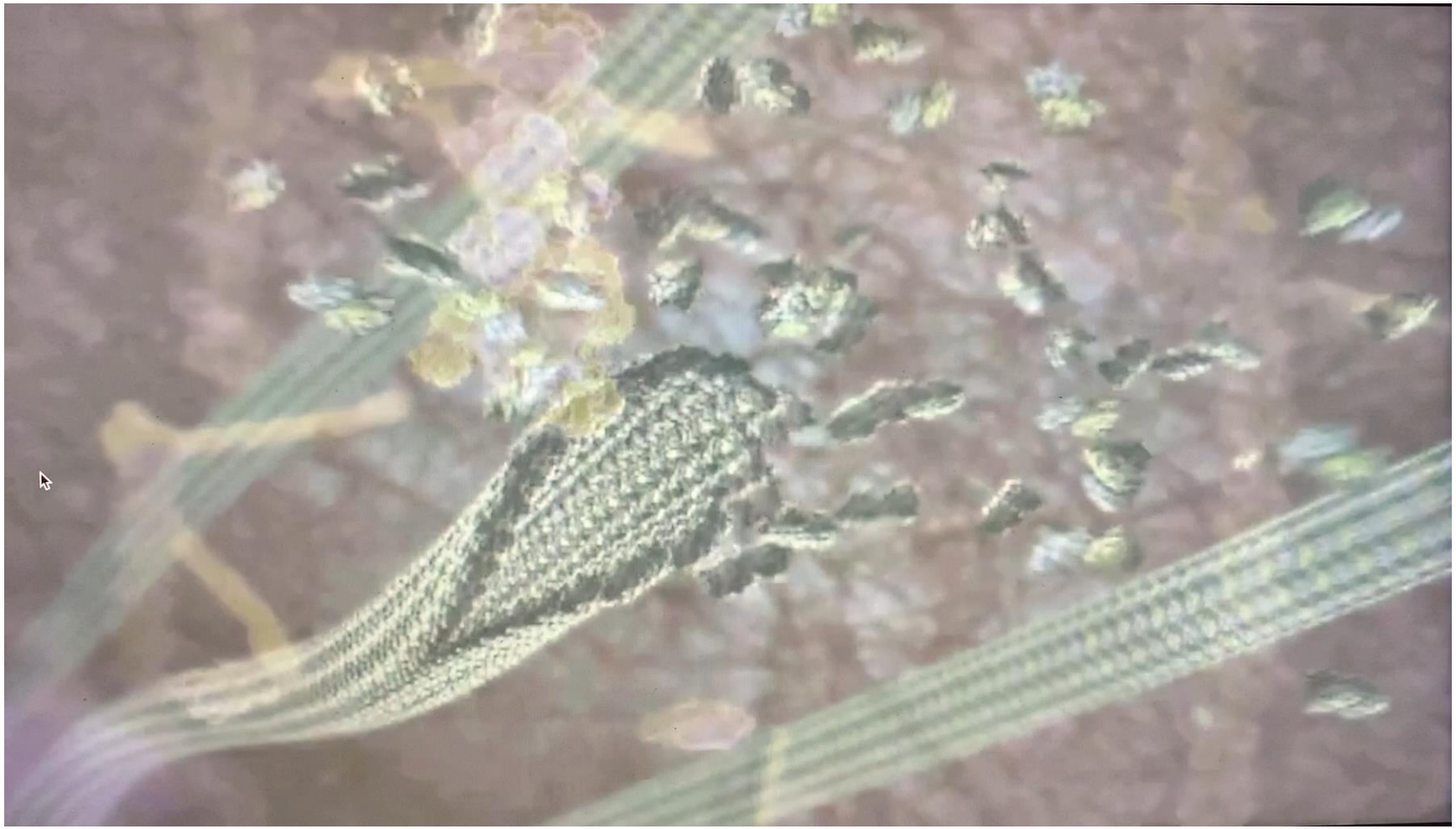


Figure 9-25 Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

microtubule assembly & disassembly



https://www.youtube.com/watch?v=wJyUtbn0O5Y

some treatments change microtubule dynamics

treatments that disassemble/destabilize microtubules (shift steady state):
cold, high Ca++, and

drugs:
colchicine – binds dimer, adds to tubule, blocks further addition
nocodazole,vinblastine - suppress addition to tubule, may detach from MTOC
taxol - inhibits disassembly and thereby assembly.
THESE are ANTI-CANCER agents because they interfere with cell division

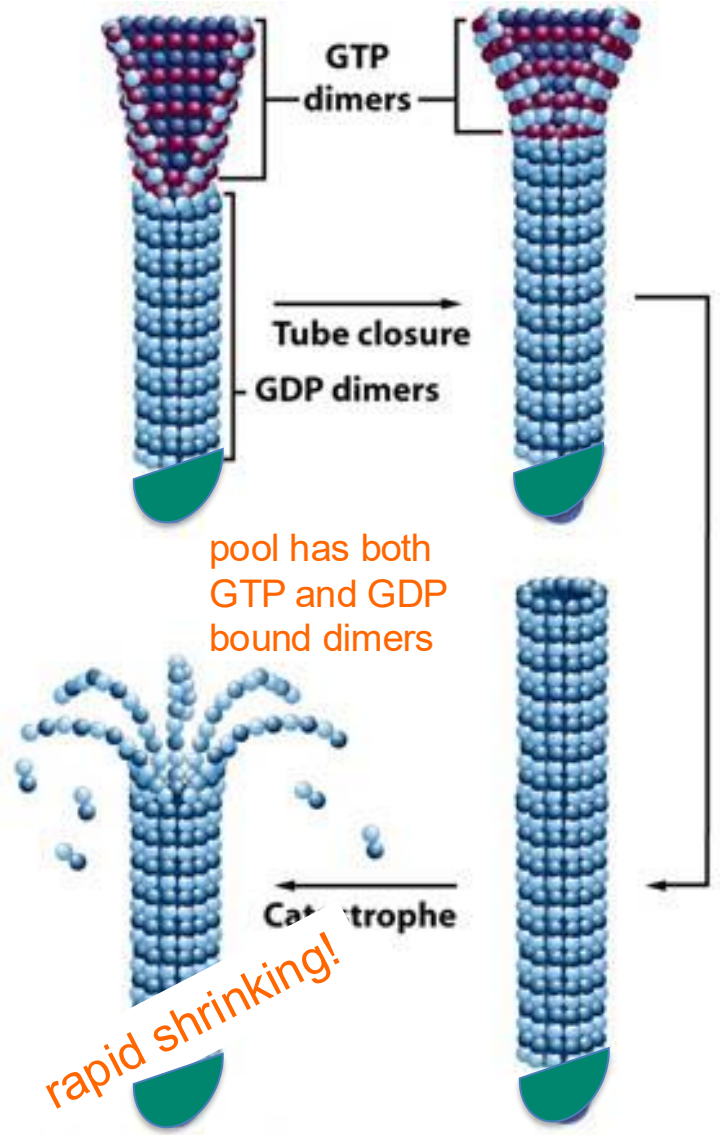
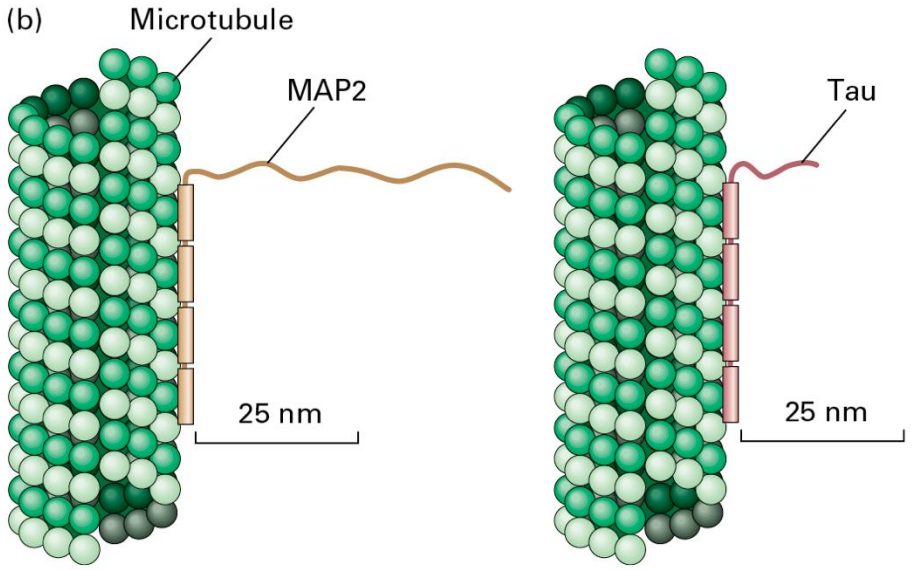
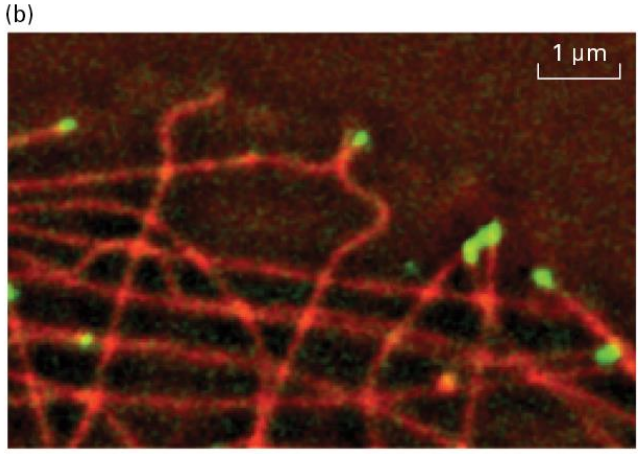
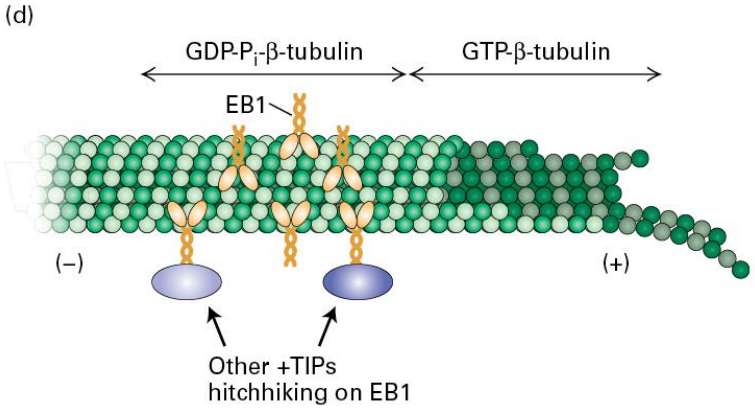


Figure 9-25 Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

dynamic microtubule structures are regulated in cells by Microtubule Associated Proteins (MAPs)







Parts (a–c) Courtesy of Dr. A. Akhmanova, Cell Biology, Utrecht University, The Netherlands, and Dr. M. Steinmetz, Biomolecular Research, Paul Scherrer Institut, Villigen PSI, Switzerland.
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- MAPs can **stabilize** and organize microtubules (Microtubule Associated Proteins , eg *tau, MAP2*)
- EB1 binds near the end of microtubules
- + end tracking proteins
- As can post-translational modifications (acetylation), dependent on the context

PollEv.com/msg303
Send **msg303** to **22333**

a microtubule experiment – poll 1

- you attempt to make tubulin polymers *in vitro*
- you add cell extracts to large amounts of α- and β-Tubulin, ATP, GTP, GDP, and adjust the temperature to 37.

Poll 1: after you get polymers, which of the following will be depleted from the test tube?

a) ATP

b) ADP

c) GTP

d) GDP

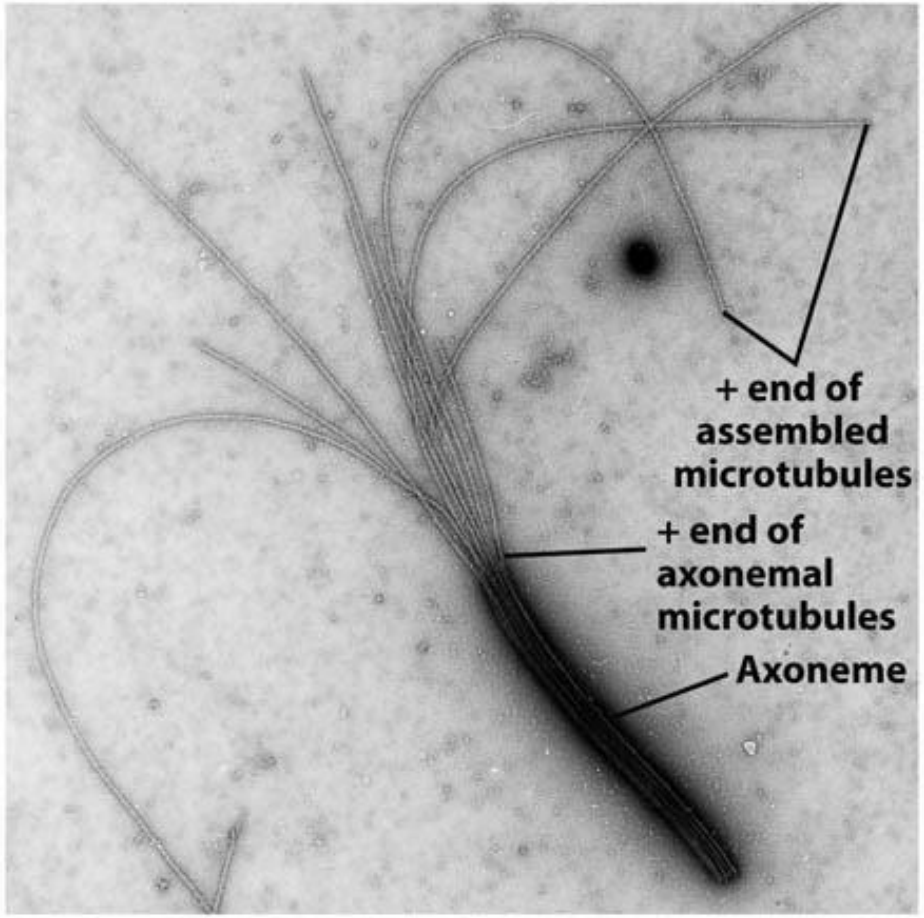


Figure 9-24 Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

an MT experiment – poll 2

- you attempt to make tubulin polymers *in vitro*
- you add cell extracts to large amounts of α- and β-Tubulin, ATP, GTP, GDP, and adjust the temperature to 37.

- you have made tubulin polymers *in vitro,* but they are *transient*, which is not great for taking pictures.

which drug might help you *maintain* the microtubules*?*

a) colchicine

b) phalloidin

c) nocodazole

d) taxol

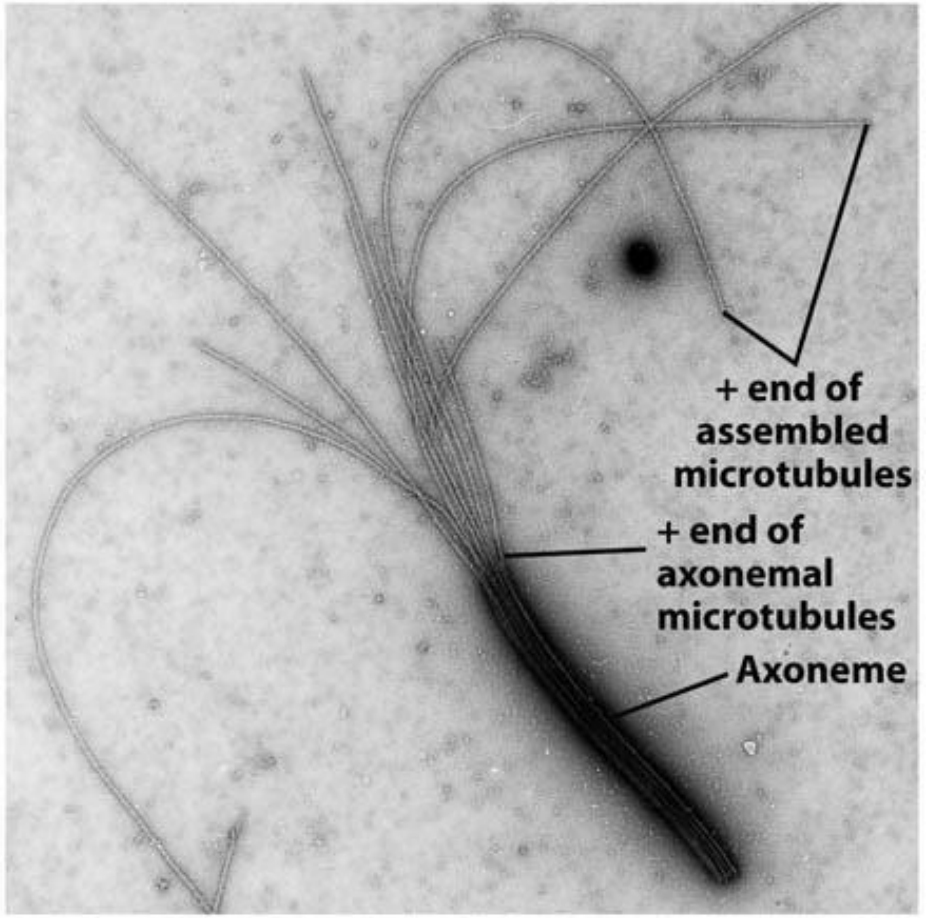


Figure 9-24 Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

About your in vitro microtubules…

after much work (EM), you realize your polymers are funny-looking because the tubes are too small in diameter ☹

Courtesy of R. H. Wade, Institut de Biologie Structurale, Grenoble, France.

Poll 3: which of these additions would help?

1) more GTP
2) more β-subunits
3) *in vitro*-made centrioles
4) centrosomes = γ-Tubulin ring complex

why all the dynamics? one example:

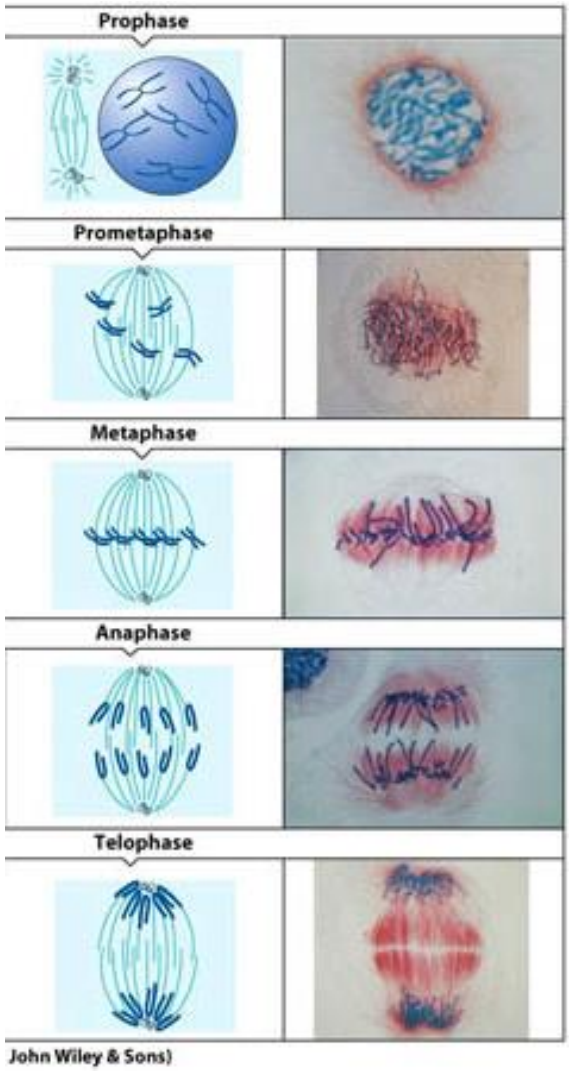
prophase

prometaphase

metaphase

anaphase

telophase



photos are of a plant cell

stages of mitosis (M phase)

mitosis example: prometaphase- movement of chromosomes to spindle equator requires:

tubulin dynamics and **motor proteins**

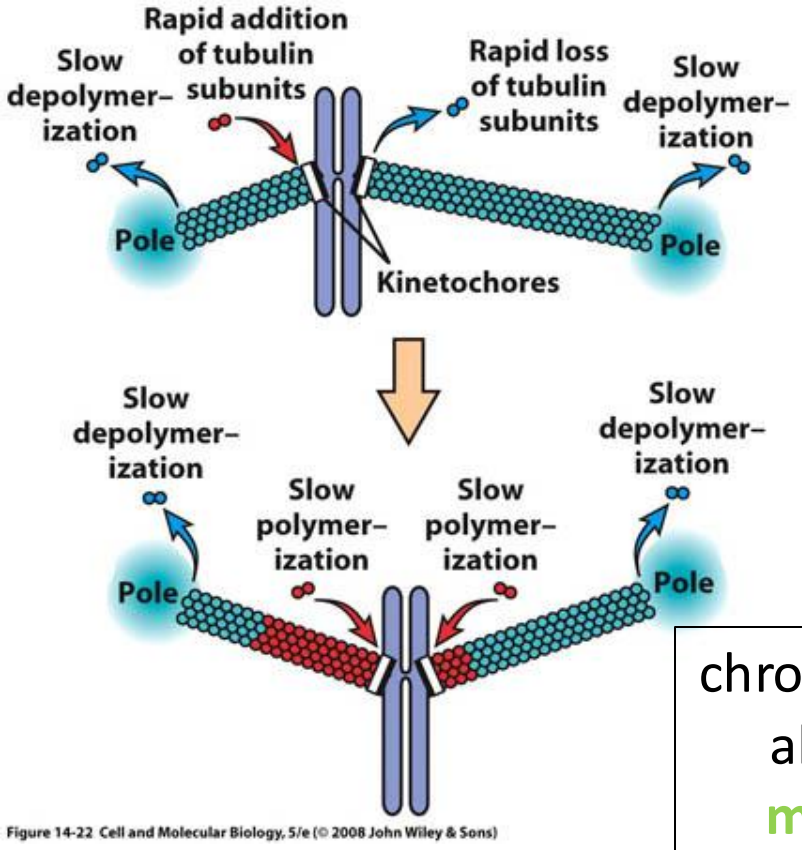
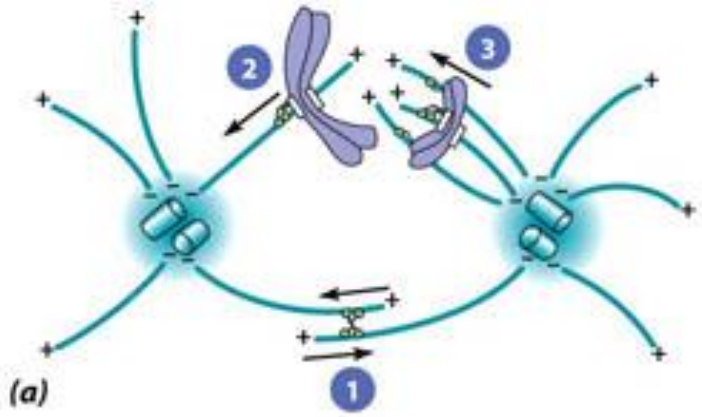


Figure 14-22 Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)



chromosomes align at the **metaphase plate**

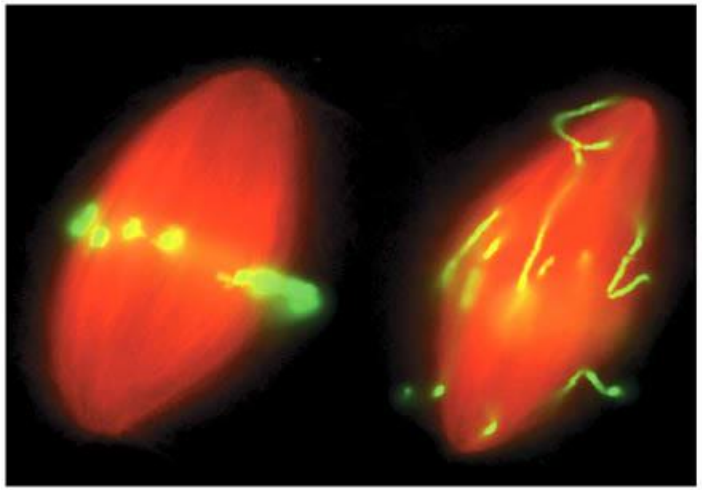
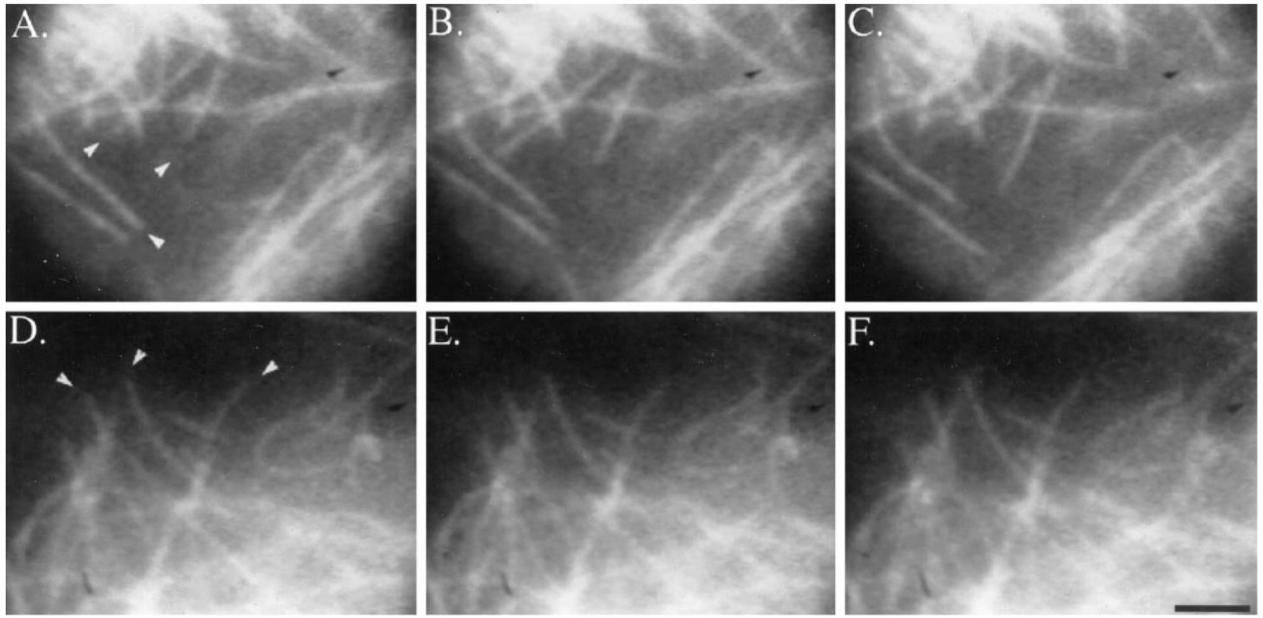
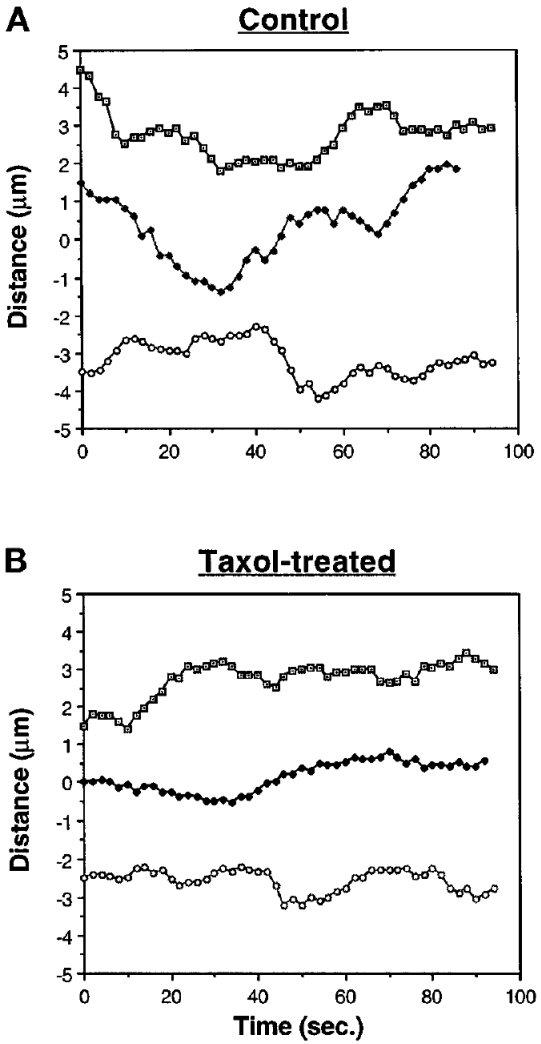


Figure 14-21 Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

Taxol-treated cells have less dynamic microtubules





- Caov-3 cells (ovarian adenocarcinoma)
  Control (top), or with 30nM taxol (bottom), scale bar 2um
- Data from primary paper: Yvon, Wadsworth, and Jordan (1999) “Taxol Suppresses Dynamics of Individual Microtubules in Living Human Tumor Cells.” *Molecular Biology of the Cell.* Vol 10, April, 947-959.

microtubule polarity is important and consistent within cell types

microtubules stabilize the location of organelles such as ER and Golgi.

ANIMAL CELLS

(pretend this is a cilium)
+plus

- minus (MTOC-basal body)

within the main part of a polarized cell
- minus (apical)

+plus (basal)

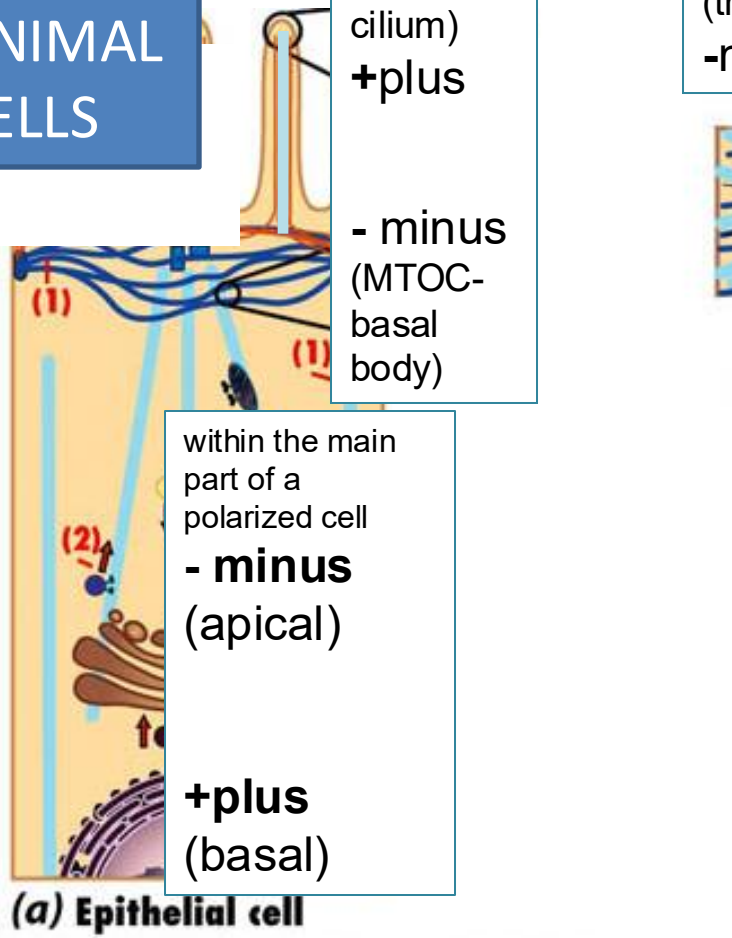
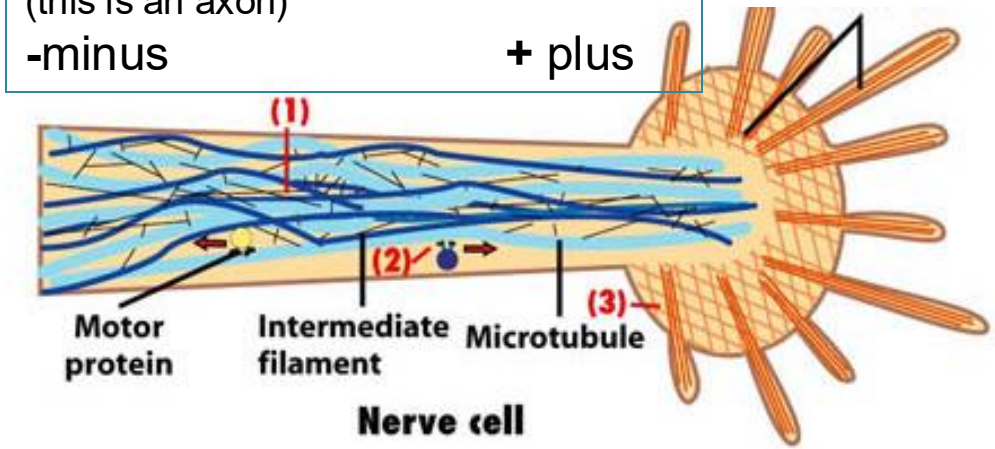


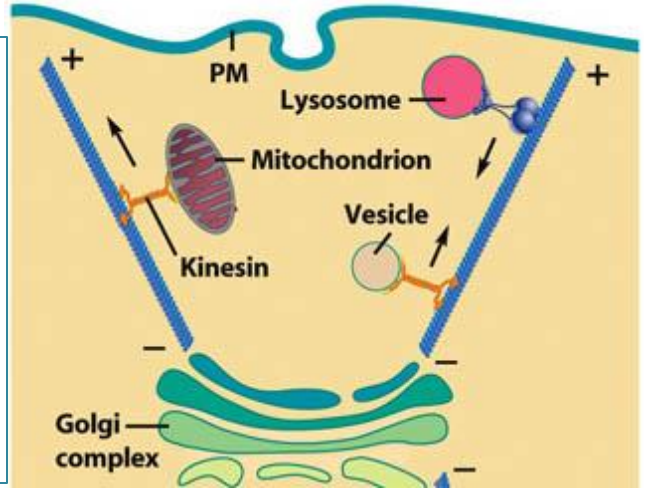
Figure 9-1 Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

(this is an axon)
-minus + plus



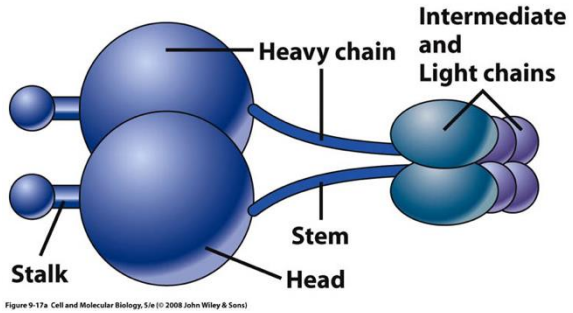
non polarized cell
+ plus

- minus (at MTOC)

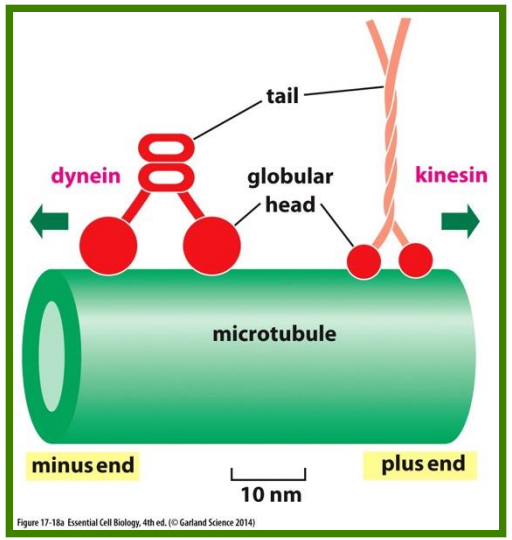


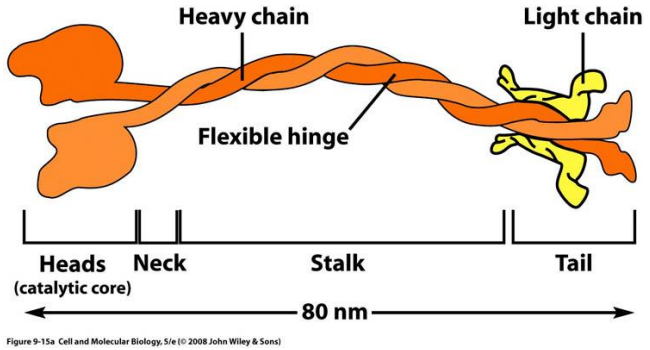
two motor protein families

use energy to move in opposite directions along microtubules



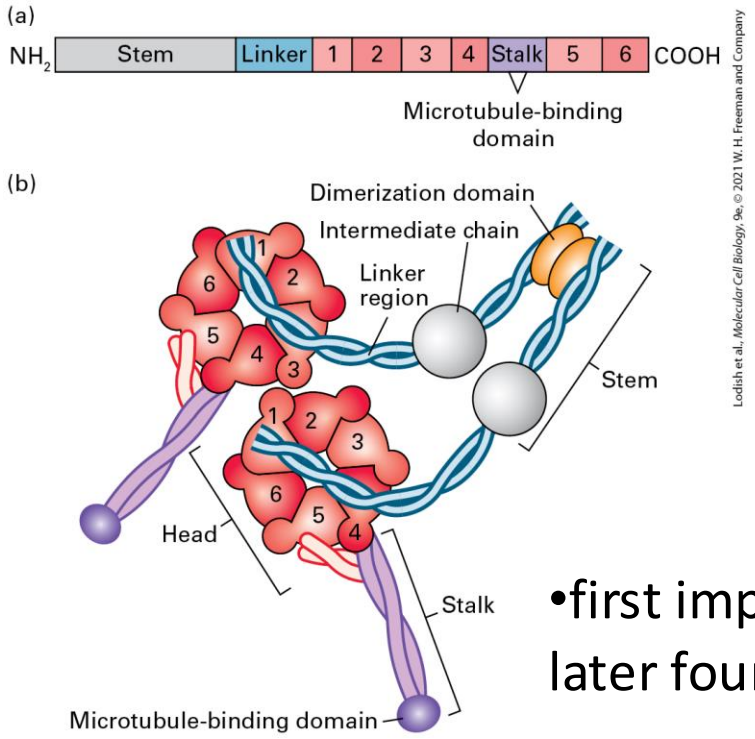
Dynein
(moves along Tub to - end)

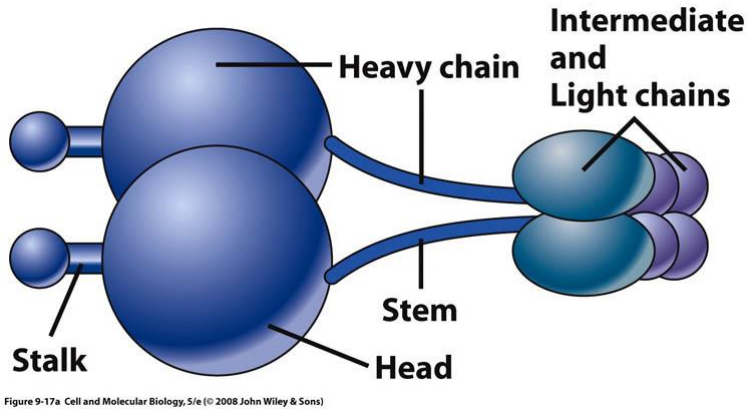


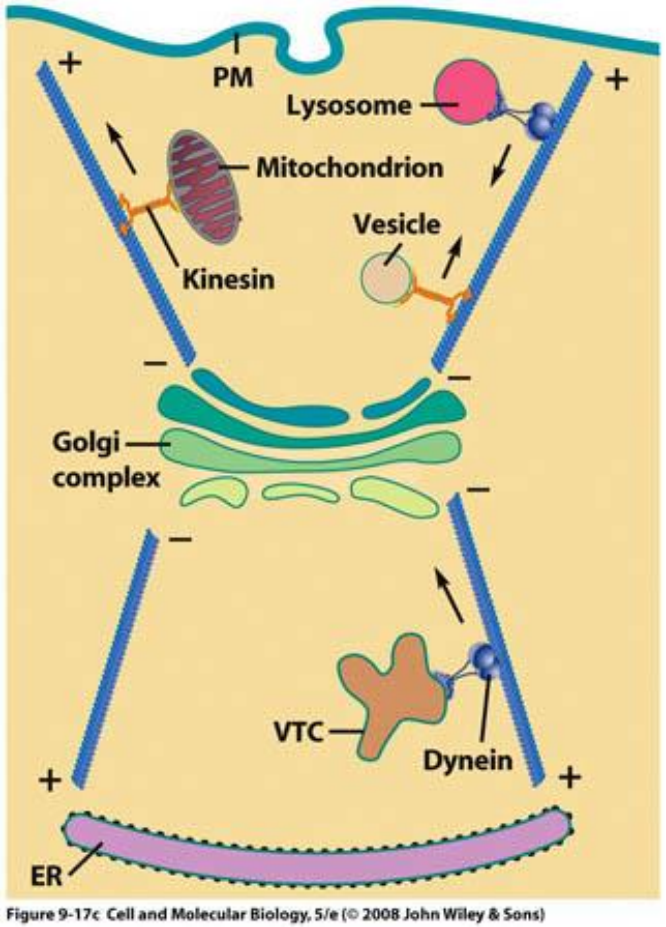


Kinesin
(moves along Tub to + end)

Dynein is a minus-end directed microtubular motor

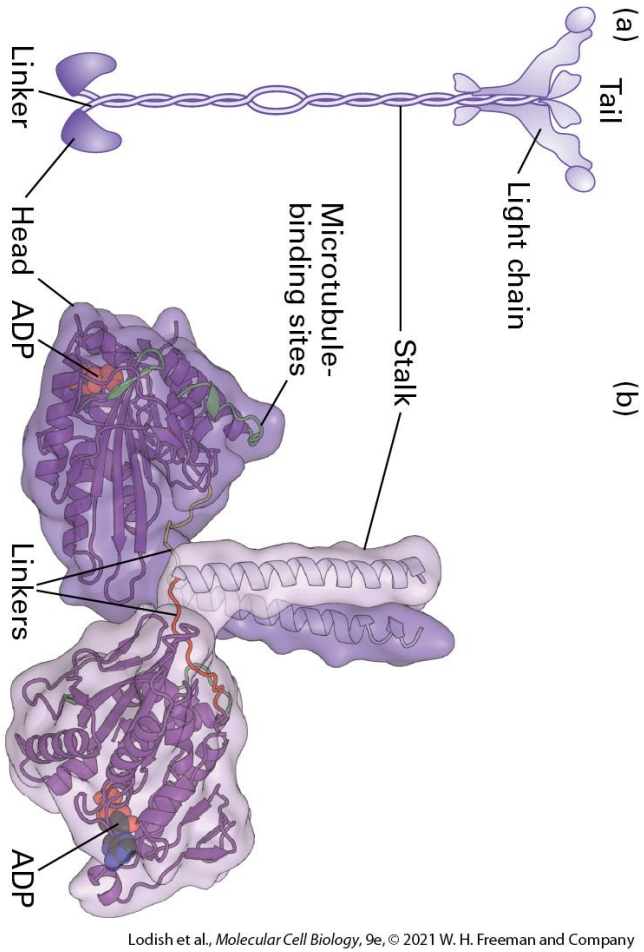






- first implicated in cilia and flagella movement; later found in all animal cells (cytoplasmic)
- stalk binds MTs; head generates force; requires **ATP**
- binds cargo via *dynactin* protein
- moves organelles, vesicles, particles; positions Golgi, centrosomes, mitotic spindle (required for cell division)

Kinesin is a plus-end directed microtubular motor



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

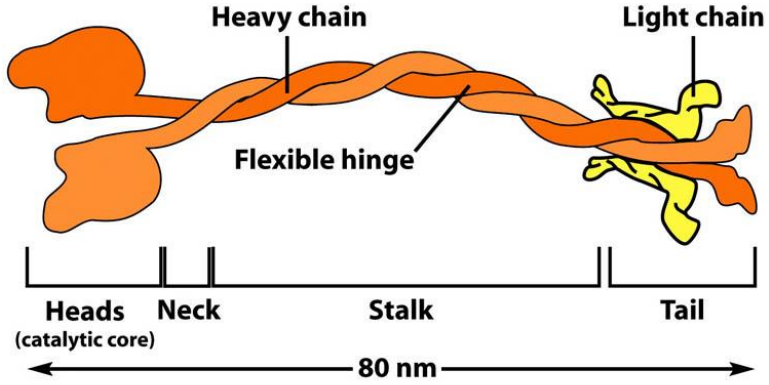


Figure 9-15a Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

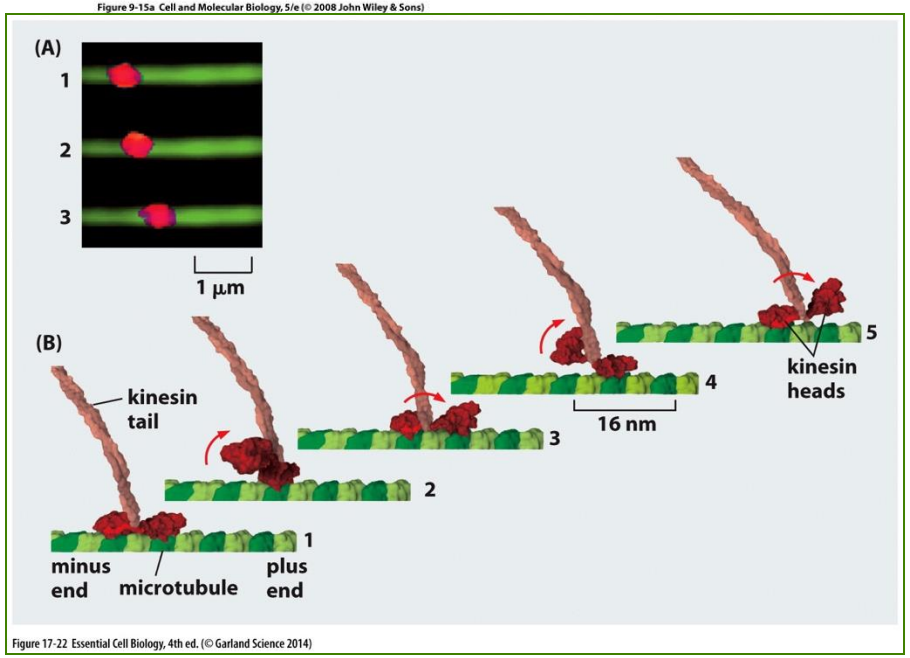


Figure 17-22 Essential Cell Biology, 4th ed. (© Garland Science 2014)

https://www.youtube.com/watch?v=YAva4g3Pk6k

~ 45 different kinesin-like proteins (KLPs) in mammals

• heads "step" along protofilament, 1 tubulin subunit at a time towards **+** end

•[**ATP**] dependent

•**processive**

• tend to move organelles (mitochondria, peroxisomes) and vesicles outward

movie!

Lightning question: Can Kinesin work upside down?

Yes
No

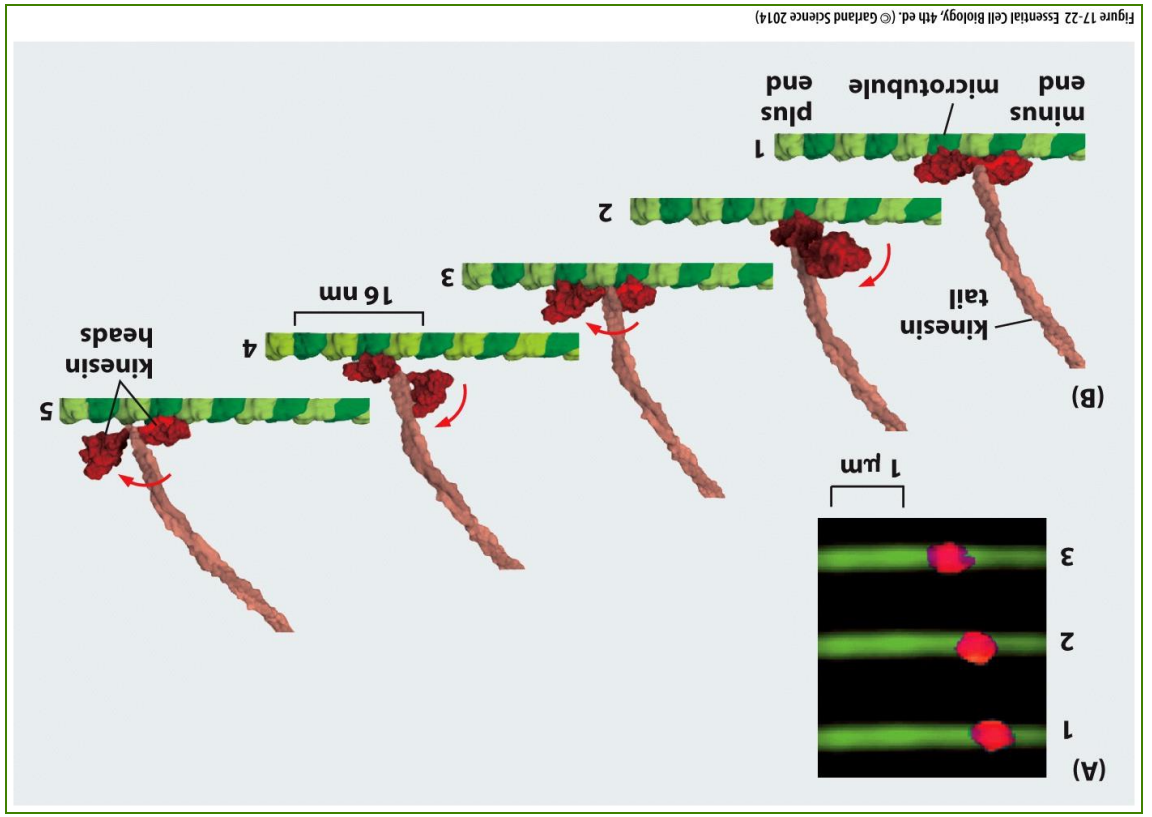


Figure 17-22 Essential Cell Biology, 4th ed. (© Garland Science 2014)

cargo moves along microtubule “tracks”

nerve cell

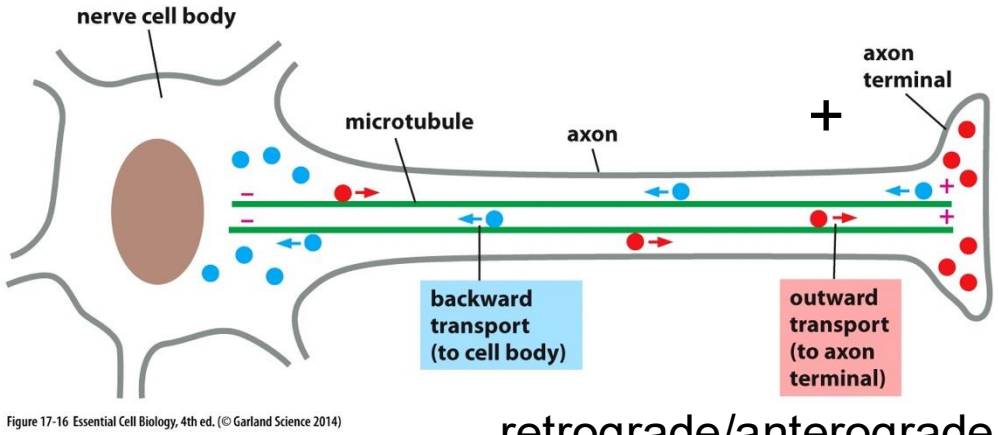


Figure 17-16 Essential Cell Biology, 4th ed. (© Garland Science 2014)

retrograde/anterograde

microtubules provide a path for movement of “cargo” such as vesicles or other protein complexes.

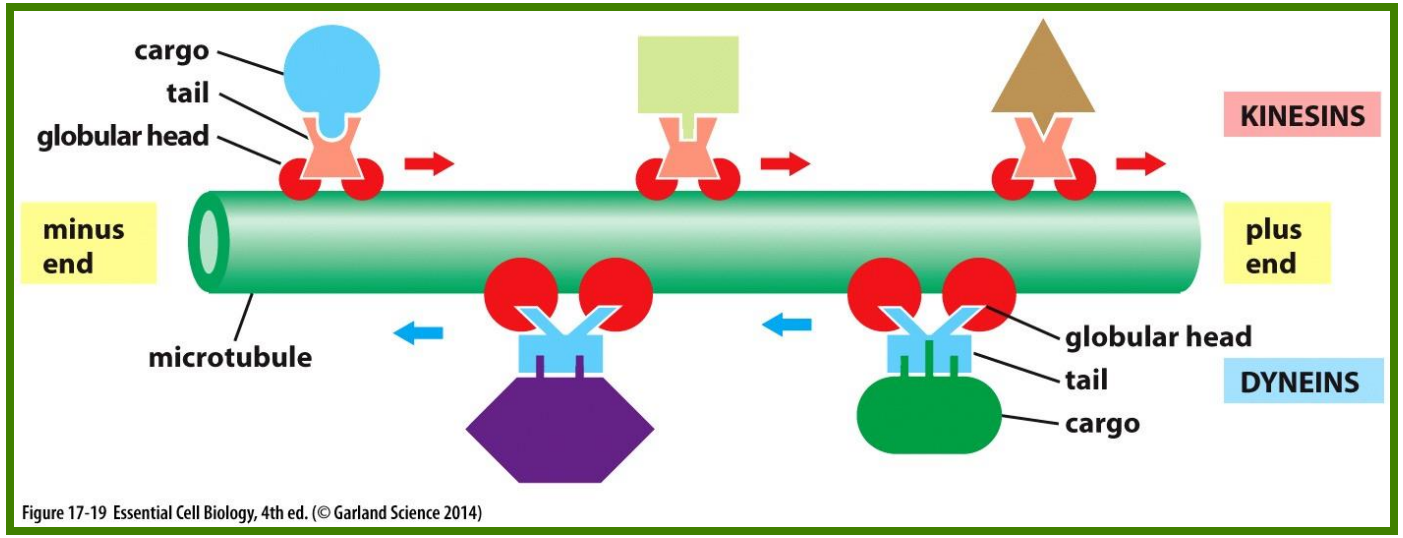
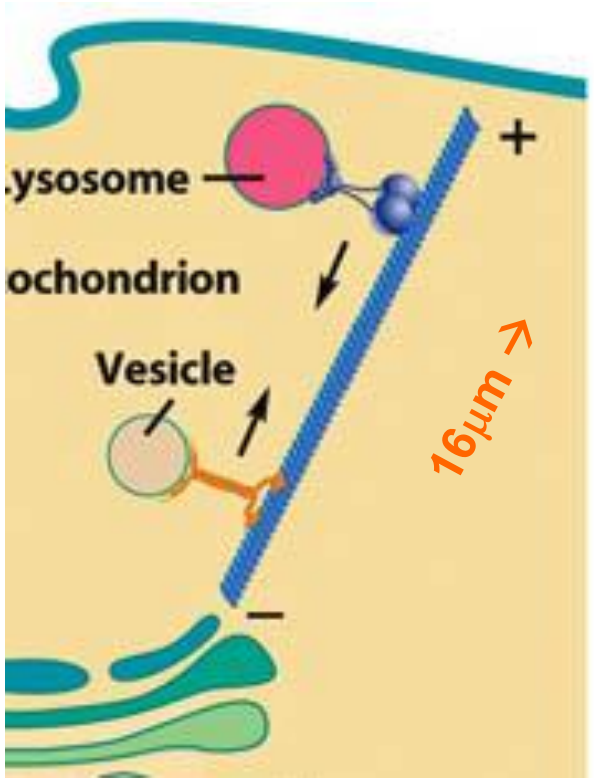


Figure 17-19 Essential Cell Biology, 4th ed. (© Garland Science 2014)

Poll 4: how fast (and expensive) is Kinesin movement?

one step is about 8nm

let's say the distance from Golgi to the plasma membrane of an epithelial cell is 16μm, and that kinesin can carry cargo there in 16 seconds.



each step expends **1 ATP**

How many ATPs does this trip use?

1) 2x10
2) $2x10^2$
3) $2x10^3$
4) $2x10^4$

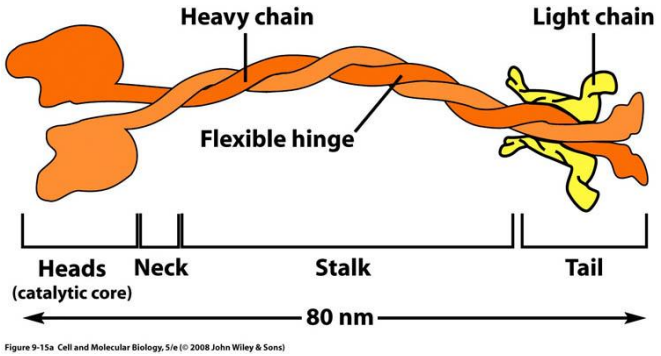
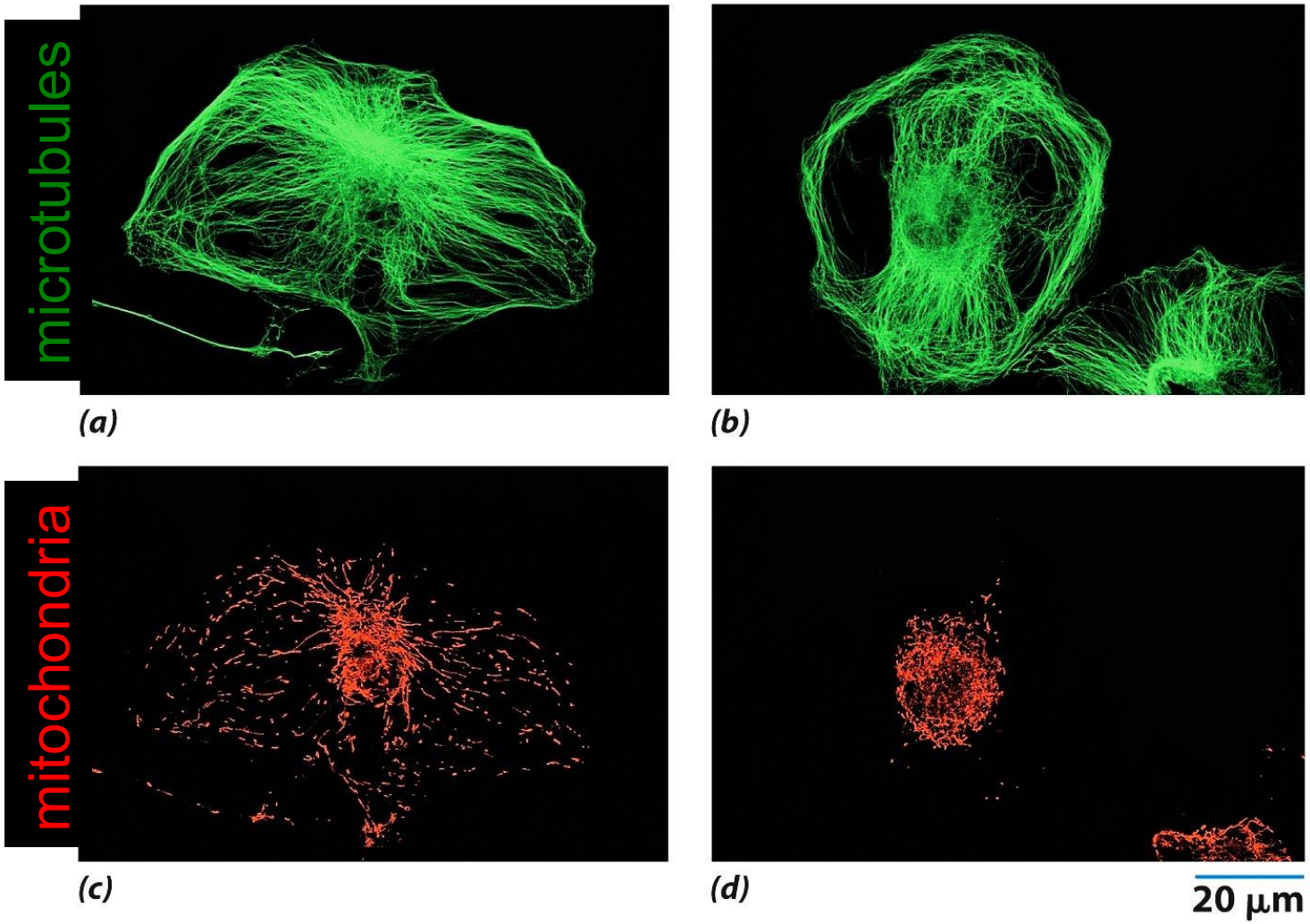
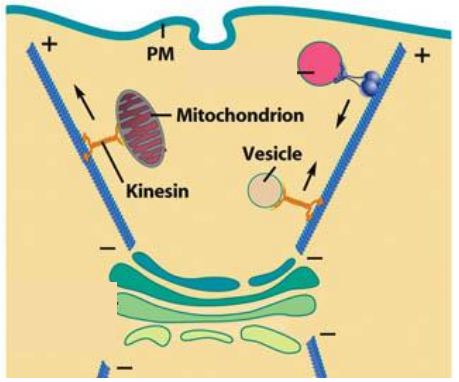


Figure 9-15a Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

What does this result tell us about microtubules and motors?



From Yosuke Tanaka et al., courtesy of Nobutaka Hirokawa, *Cell* 93:1150, 1998; by permission of Cell Press.

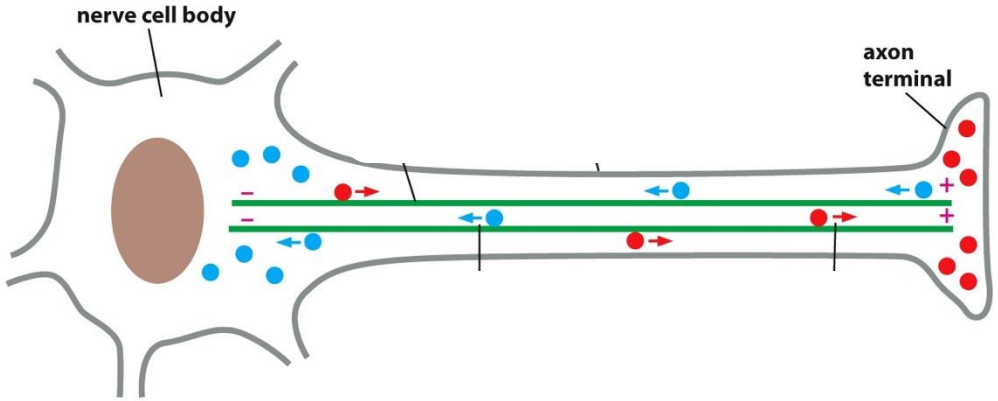


Kinesin KO

experiment

- you isolated a new motor protein and generated a GFP-fusion protein
- yippee! you can see it move when you put it in neuronal cells!
- you treat the cells with nocodozole and you see Green but no movement
- you conclude your motor associates with:
- _____

in cultured neurons, your GFP fusion motor protein tends to move toward the cell body. is your motor protein a plus-end or minus-end directed motor?

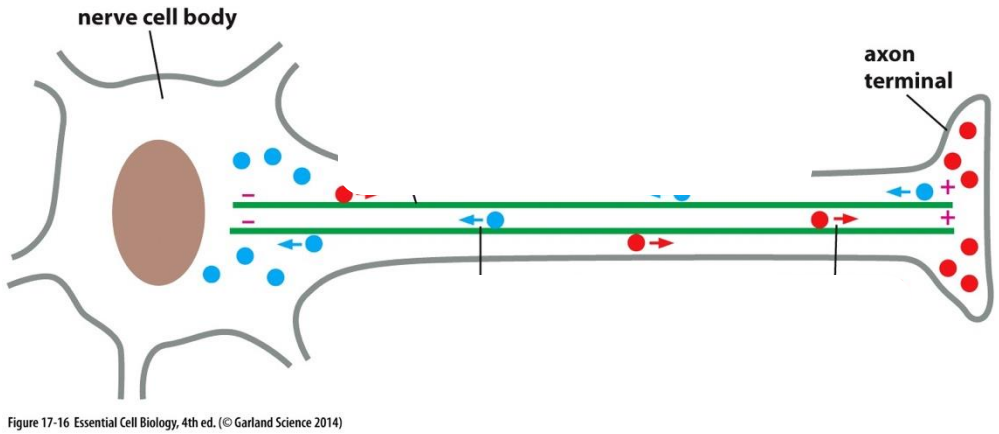


Poll 5

a) plus
b) minus
c) could be either

in cultured neurons, your GFP fusion motor protein tends to move toward the cell body.

what known kind of motor protein does this resemble?



Poll 6

a) Kinesin
b) Myosin
c) Dynein
d) Keratin

experiment

- what experiments might you do to determine what kind of cargo associates with your new motor protein? (there are a lot of answers)

flagella are microtubule-rich structures that beat or wave

- protozoans, algae, sperm use flagella for movement
- not the same molecularly as bacterial flagella
- power stroke is the “push”; recovery stroke swings back into position

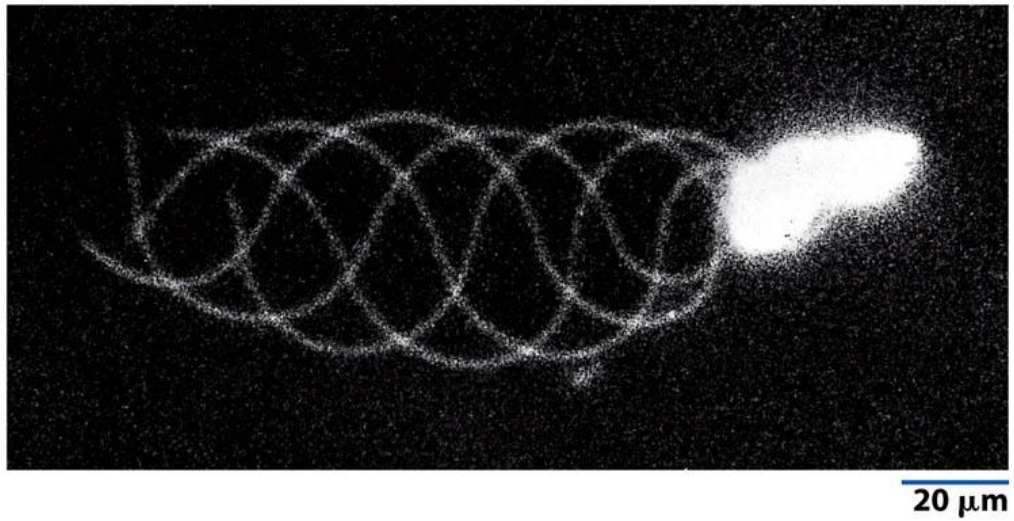


Figure 9-34 Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

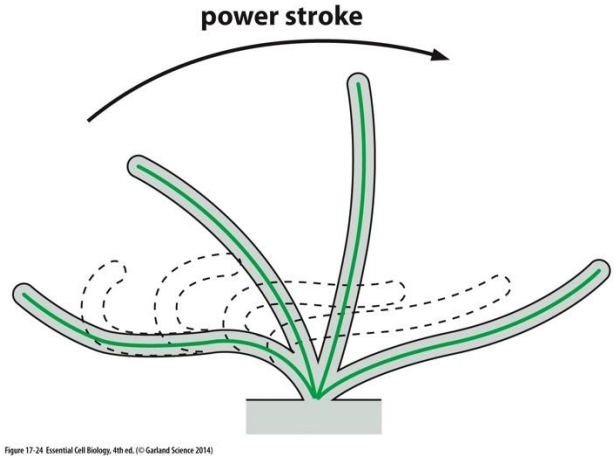
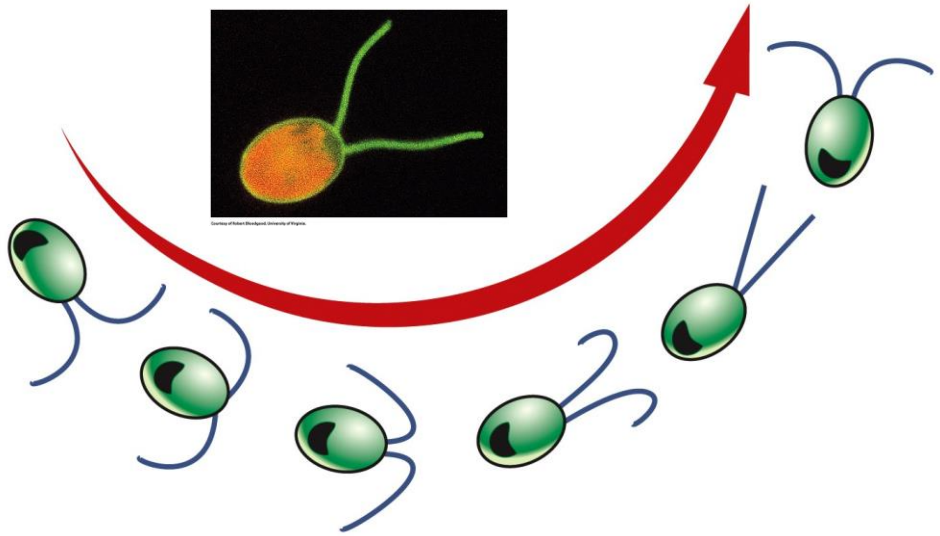
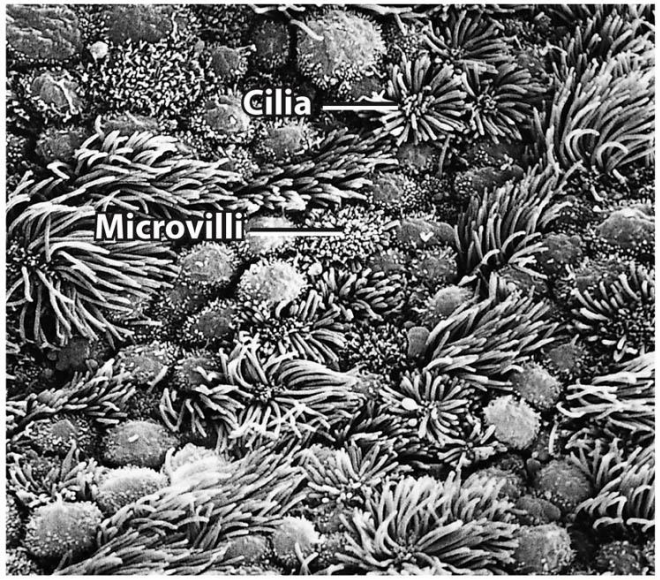
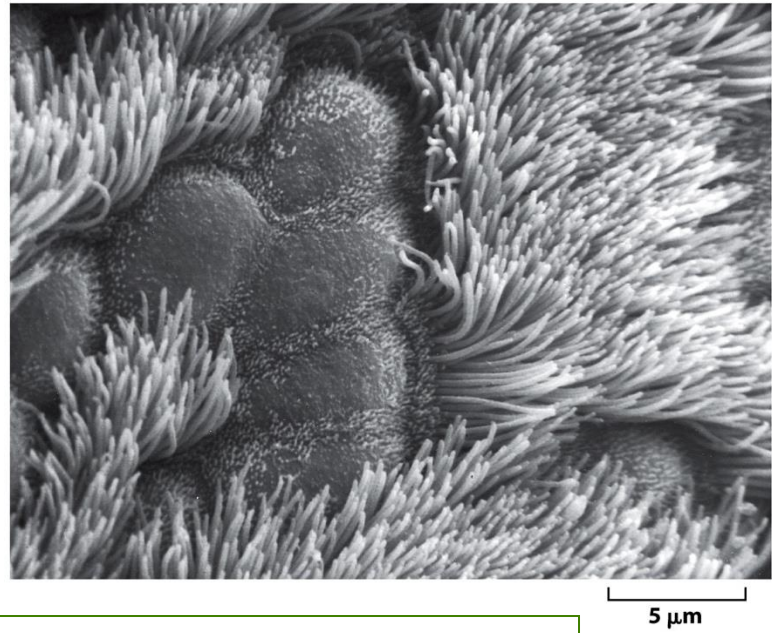


Figure 17-24 Essential Cell Biology, 4th ed. (© Garland Science 2014)

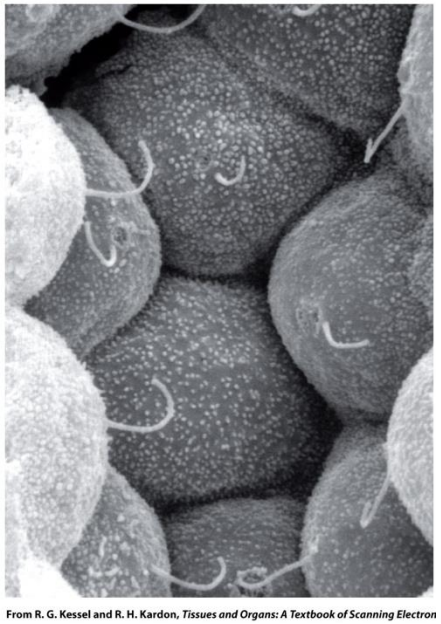


cilia are microtubule rich structures that can wave or be stationary





motile cilia examples



primary cilia

- most cells have one of these structures
- multicellular organisms use cilia to move fluid/particles. "primary cilia" are often non-motile, sense the local environment
- defects in cilia can lead to disease (Bardet-Biedl Syndrome), polycystic kidney disease, and *situs inversus*

flagella/cilia – whip it

Classic cilia film:

- http://www.youtube.com/watch?v=QGAm6hMysTA

- human sperm:

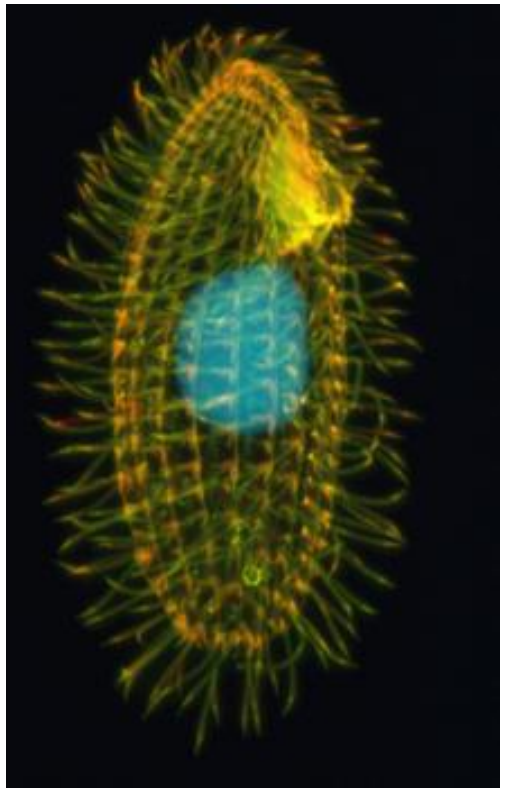
http://www.youtube.com/watch?v=vvnEsOaKxuw&feature=related

- soothing cilia on a frog embryo

http://www.youtube.com/watch?v=-UAgL6GxcS0&feature=related

airway cilia: (in culture)

https://www.youtube.com/watch?v=SDweiaW7fXs



tetrahymena pic from wikipedia

HOW DOES THIS WORK?

the axoneme of cilia/flagella is made up of microtubules and dynein

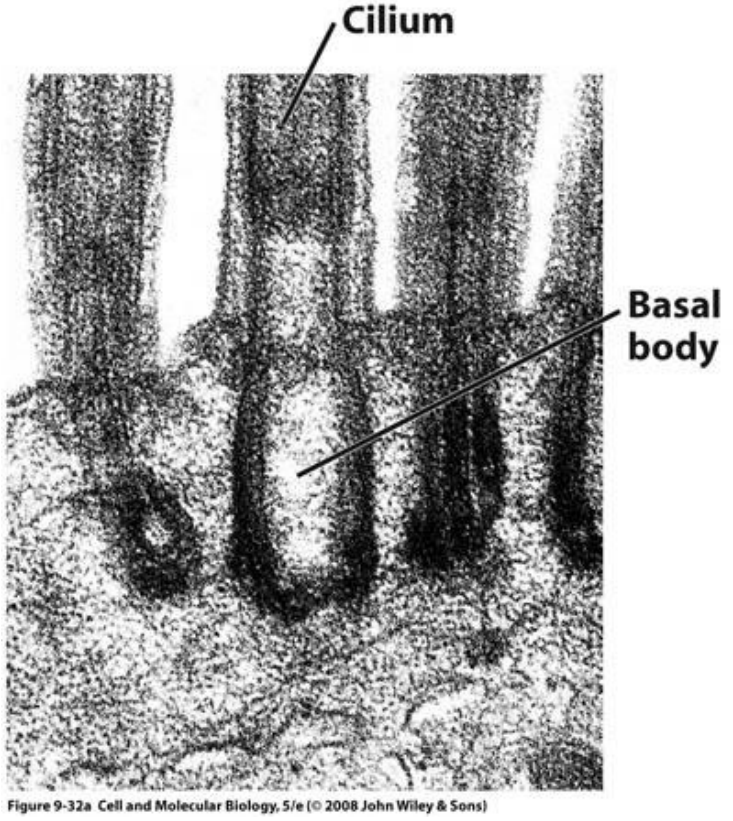


Figure 9-32a Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

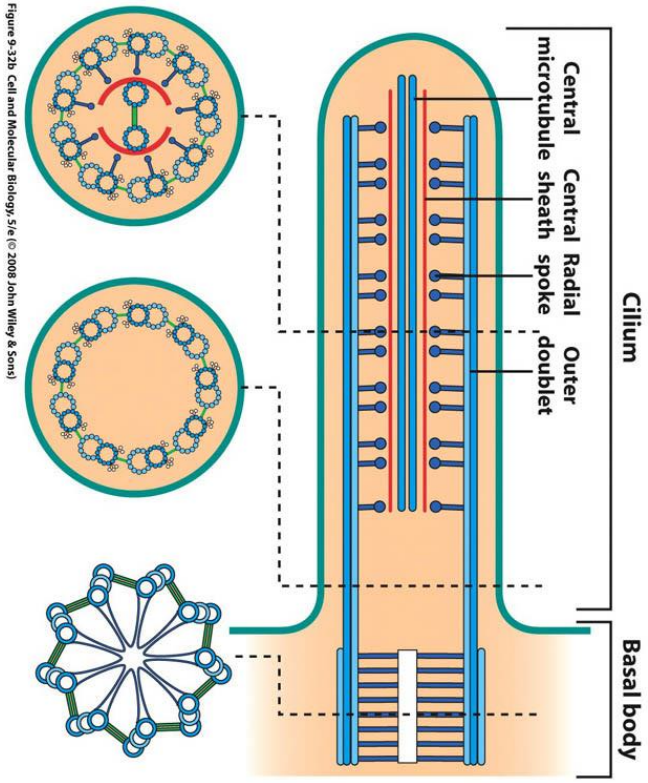
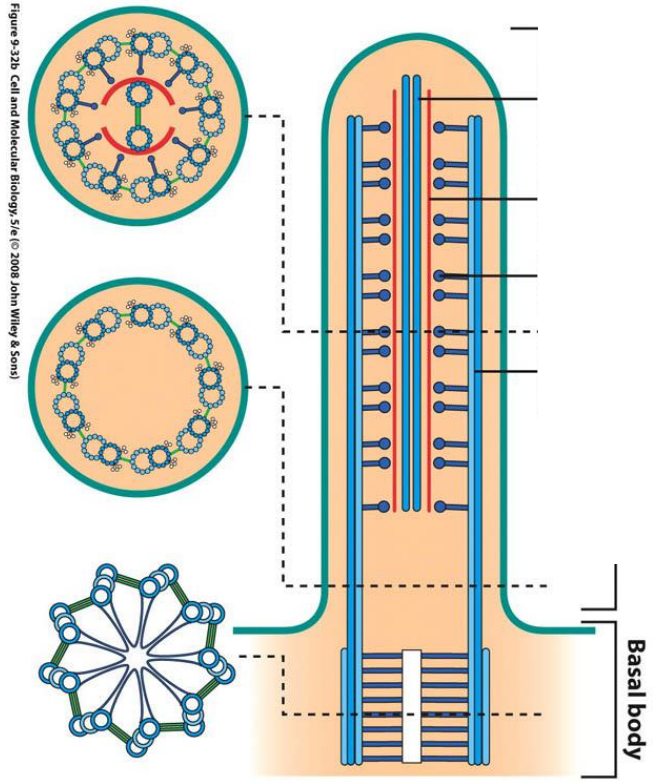
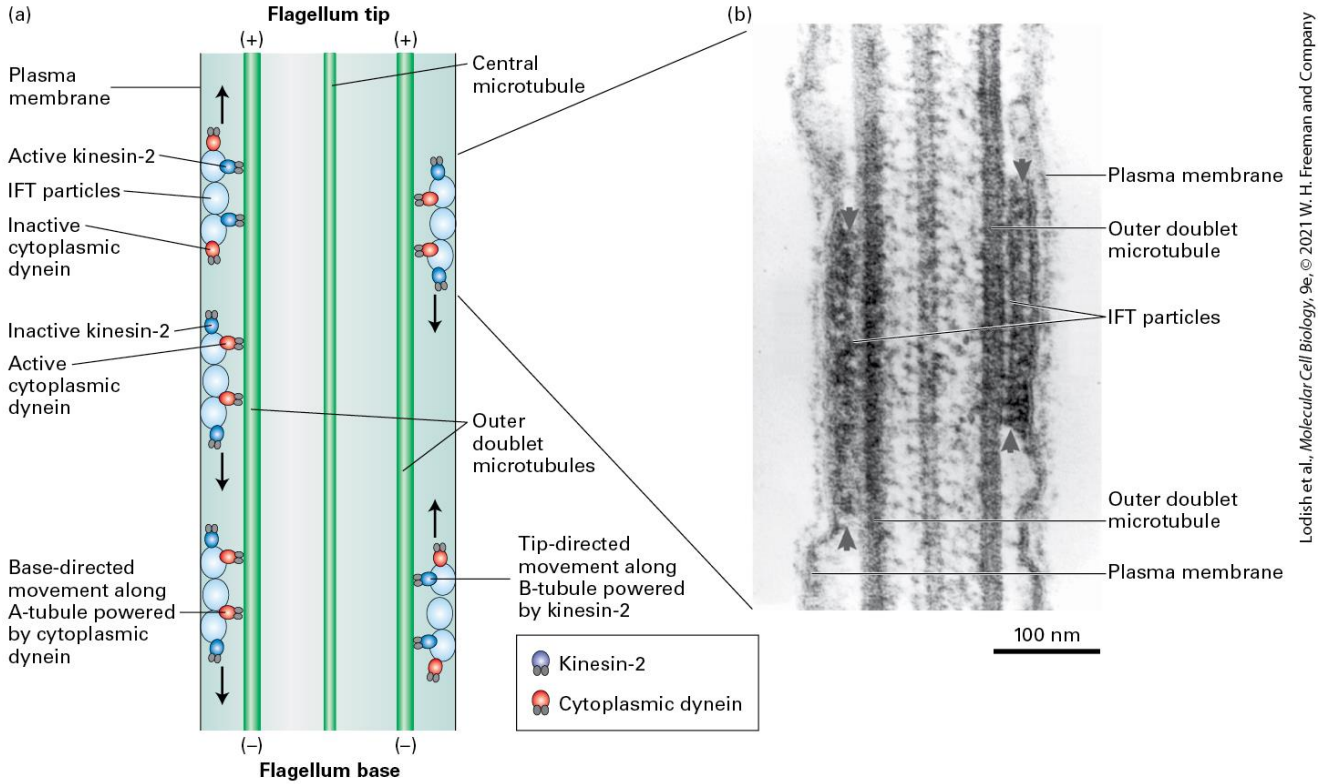


Figure 9-32b Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

- the basal body is a microtubule organizing center (MTOC)
- microtubules are organized with the plus end away from the cell body

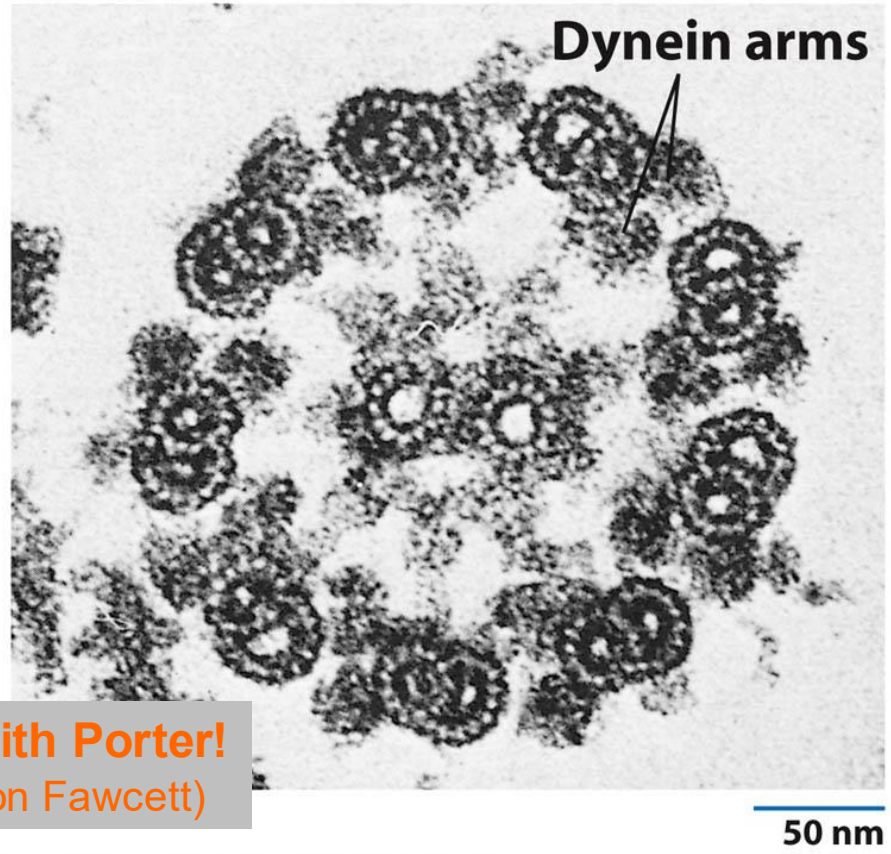
motor proteins are also needed to build/maintain the axoneme of cilia/flagella





Kinesin 2 and cytoplasmic dynein transport cargo in and out

the axoneme of cilia/flagella is made up of microtubules and dynein (cross section)



Keith Porter!
(Don Fawcett)

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

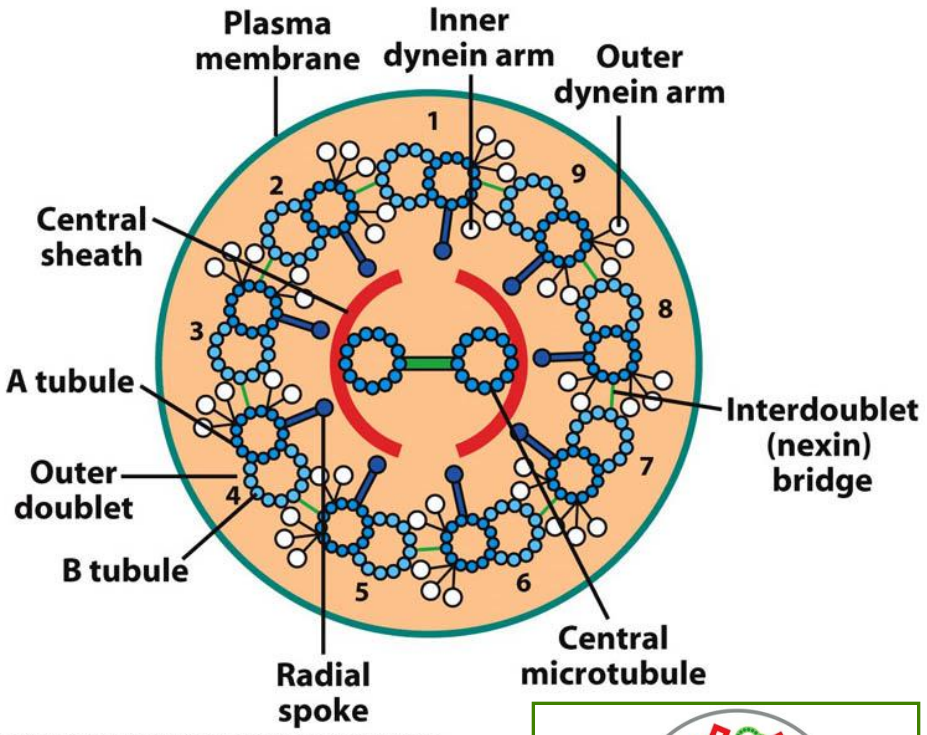
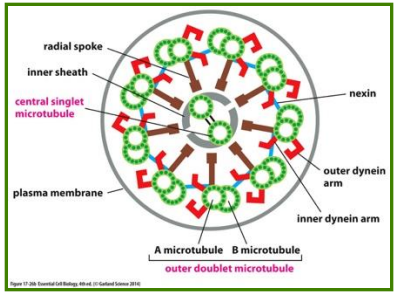


Figure 9-30b Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)



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

the axoneme of cilia/flagella is made up of microtubules and dynein

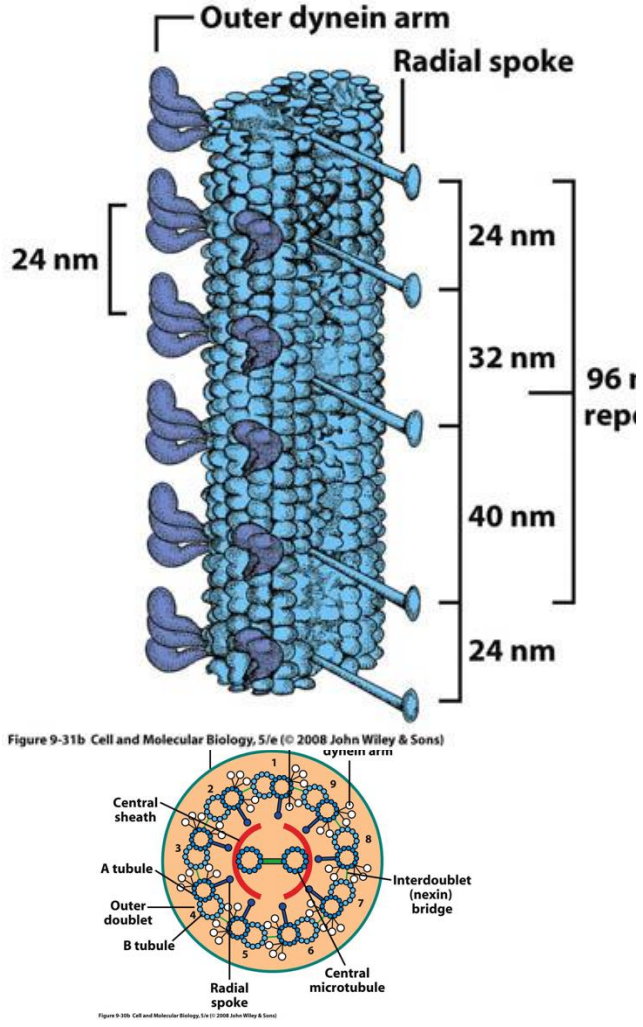
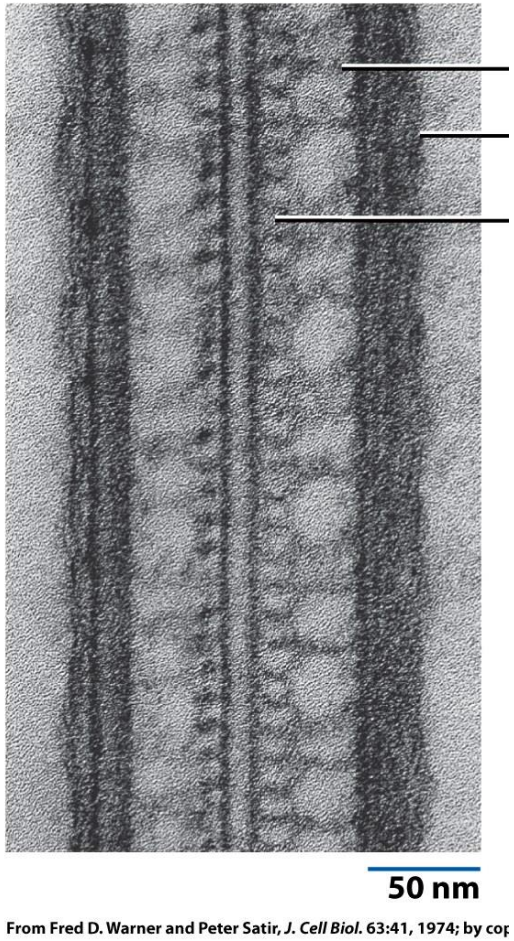


Figure 9-31b Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)



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

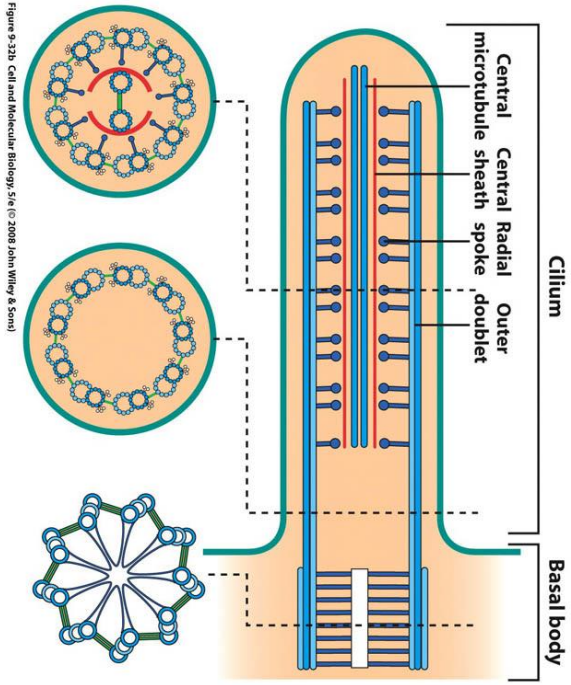


Figure 9-32b Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

intraflagellar/ciliary transport also occurs - via dynein and kinesin

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

cilia move by microtubule sliding via ciliary Dynein

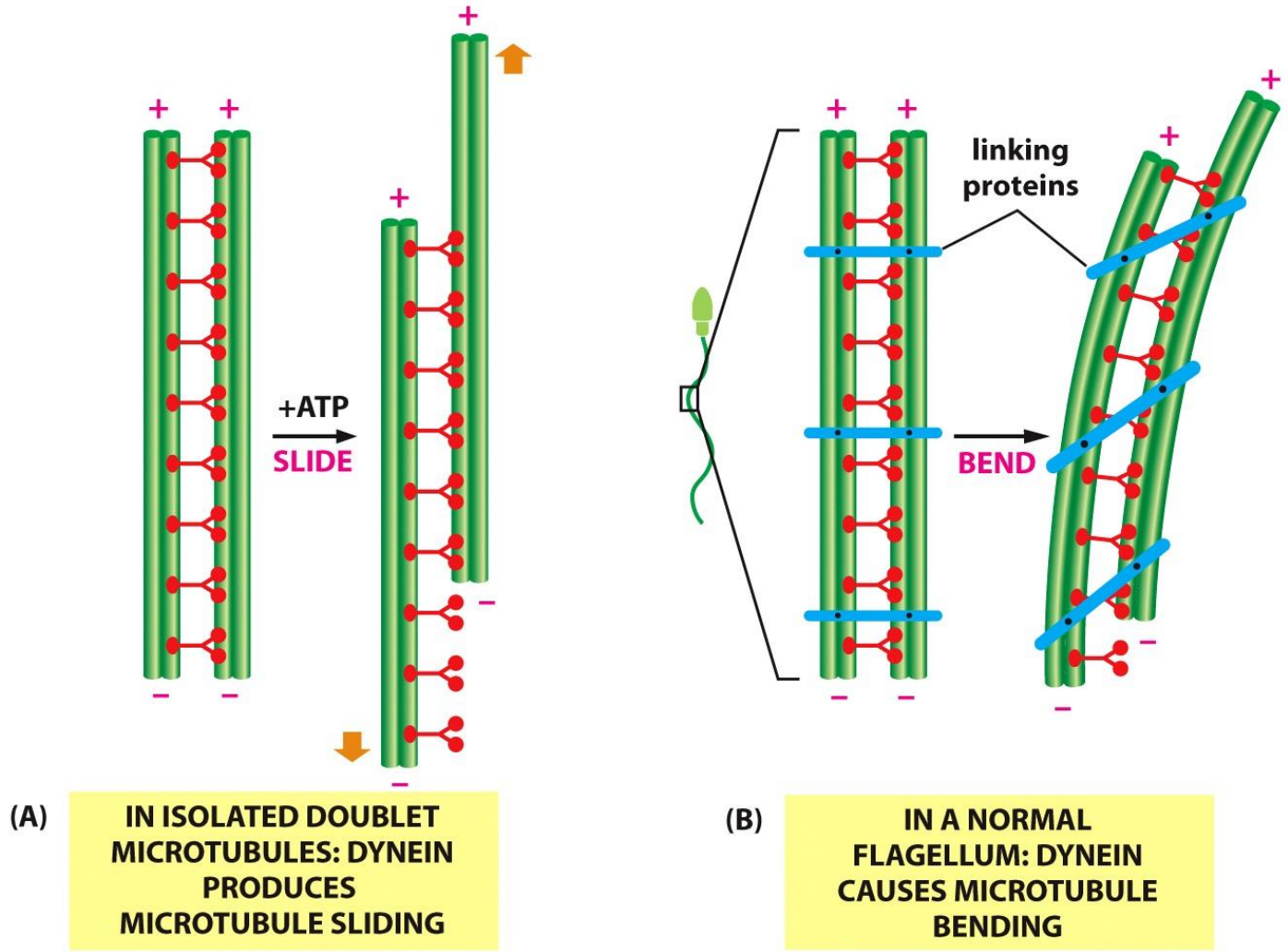
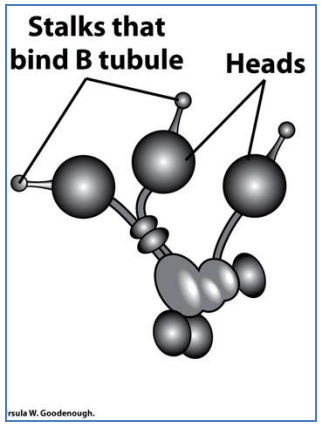


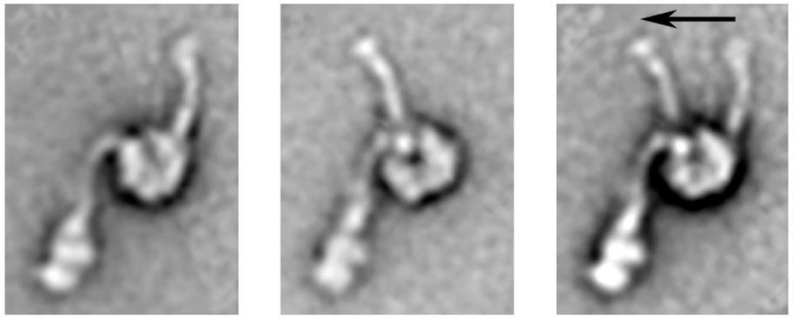
Figure 17-27 Essential Cell Biology, 4th ed. (© Garland Science 2014)

cilia/flagella move by microtubule sliding via dynein

3 heavy chains –

separately encoded





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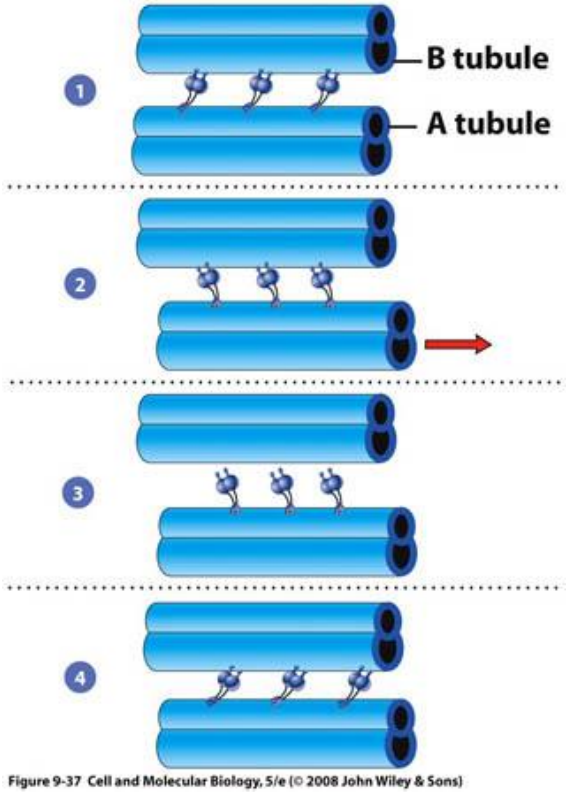
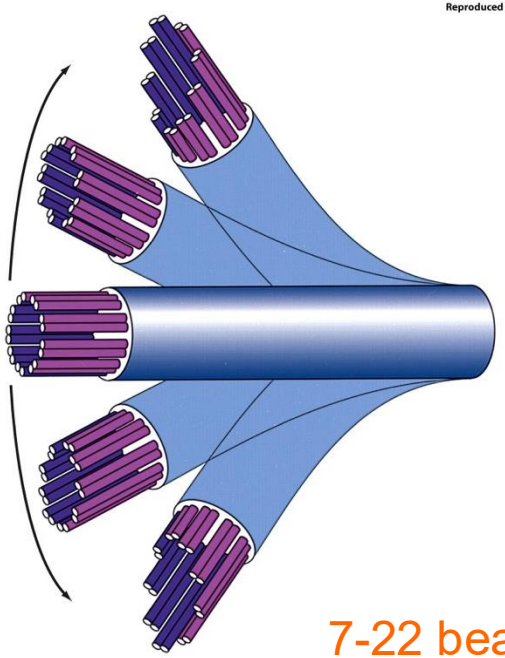
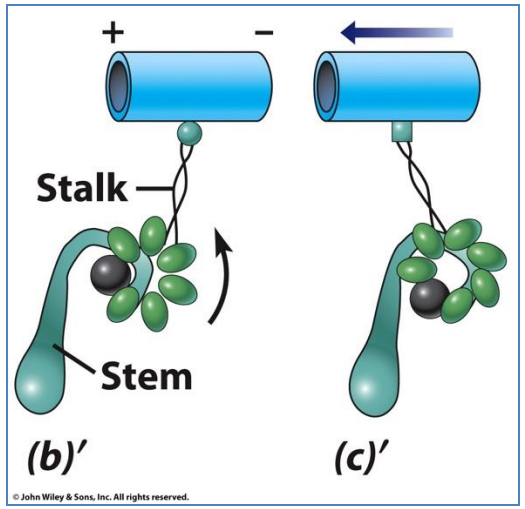


Figure 9-37 Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)





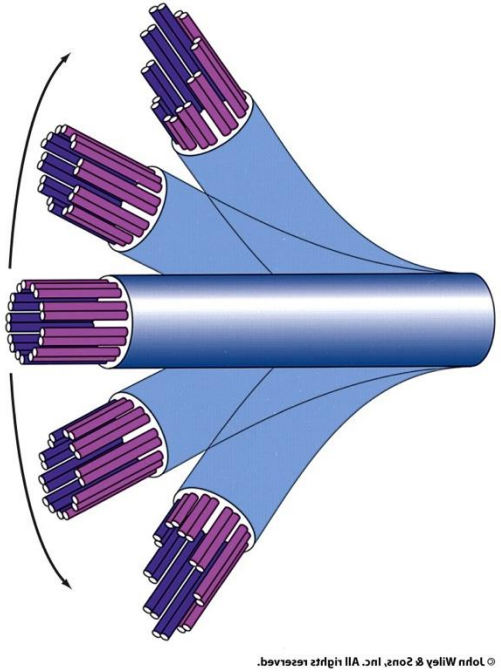
7-22 beats per second in human cilia

stems of dyneins (and intermediate and light chains) are anchored to the A tubule

more molecular movies…

- https://www.youtube.com/watch?v=9nZYlyFGm50

- Fan favorite:
- https://www.youtube.com/watch?v=tMKlPDBRJ1E



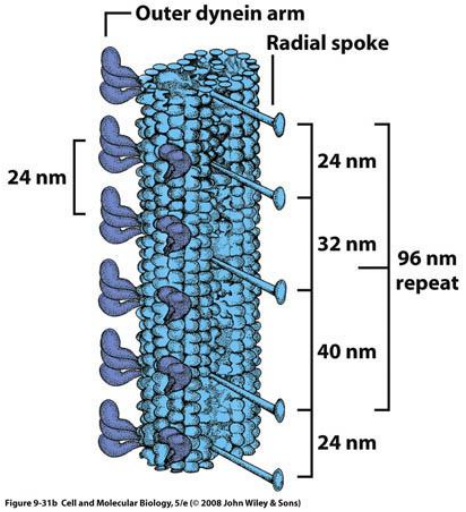
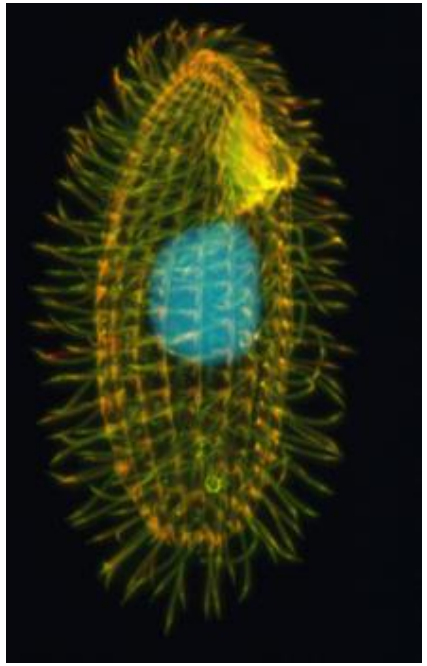
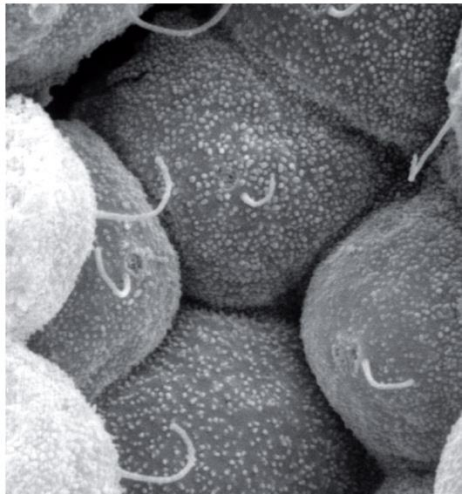


Figure 9-31b Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)



tetrahymena pic from wikipedia

some diseases are attributable to cilia defects



- may affect motile cilia or primary cilia
- cilia may act as antenna or signal organizing centers
- Bardet-Biedl syndrome – mutations affect cilia/basal bodies, result in polydactyly, situs inversus, congenital defects, retinal degeneration etc…
- left-right asymmetry of organs due to abnormal fluid flow in developing embryo
- Polycystic kidney disease (Ca++ channel) disrupts cell division

Case study: Kartagener Syndrome

- Primary ciliary dyskinesias (PCD) affects 1 in 30,000 (Kartagener's in a subset)
- Rare, autosomal recessive genetic ciliary disorder (ciliopathy)
- Symptoms: situs inversus, chronic sinusitis, and bronchiectasis àear, nose, throat, chestinfections, infertility

Kartagener Syndrome

cilia exist but have defective or no movement

poll 7. 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

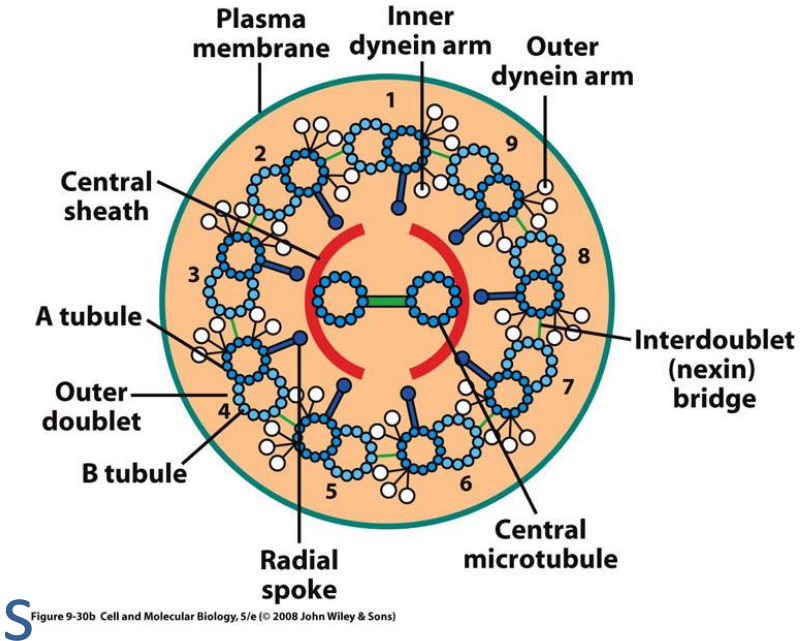


Figure 9-30b Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

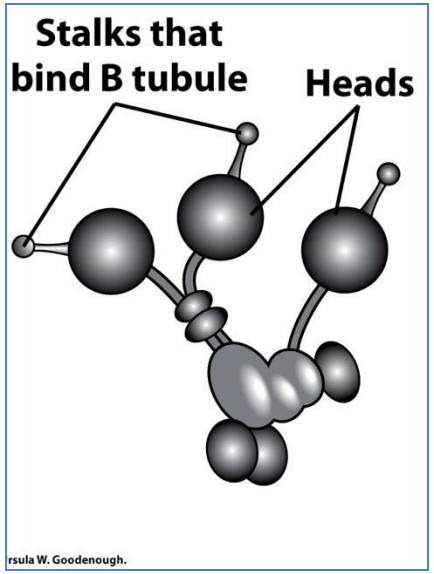
Kartagener Syndrome

cilia exist but 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?

a) heavy chain motor (head) domain
b) heavy chain stem domain
c) light chain

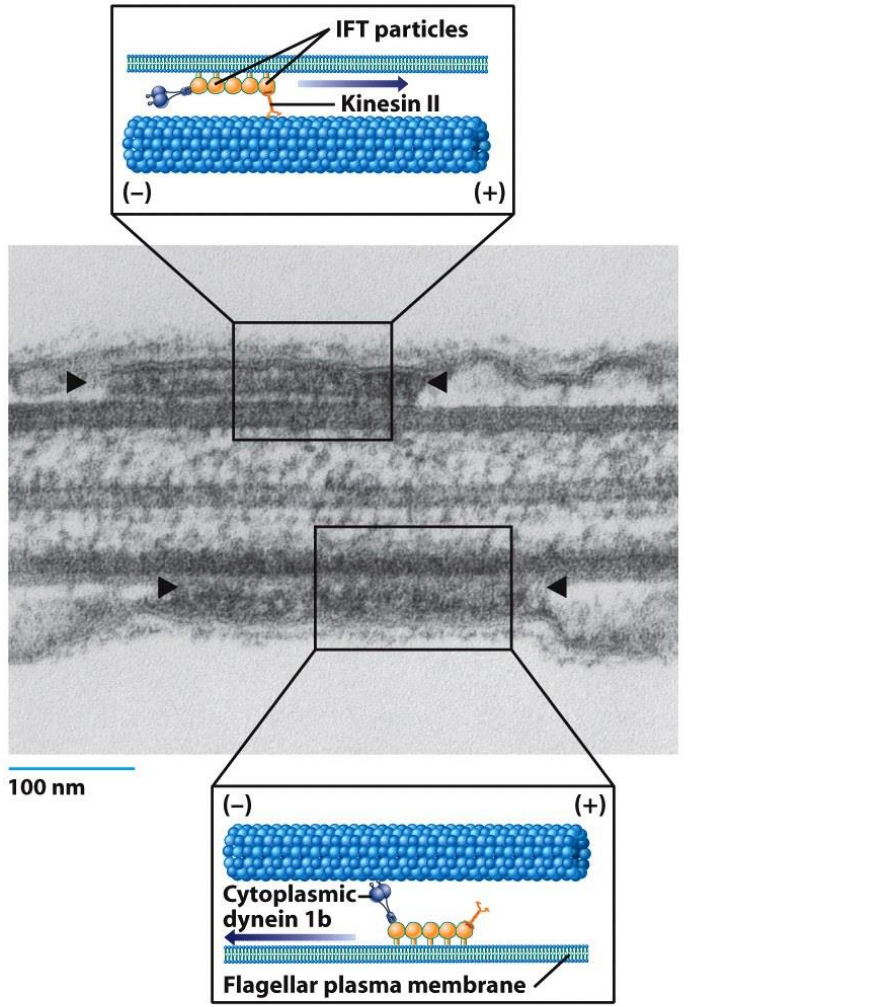


poll

Kartagener Syndrome mutations

- cilia have defective or no movement
- you decide to also make th mutation in the cytoplasmic d is found in the epith the airways. do yo see any defect in cilia whe this dynein is mutated?

We didn't get to this



discussion- microtubule dynamics

lecture thursday: actin filaments and sarcomeres

(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

(b)