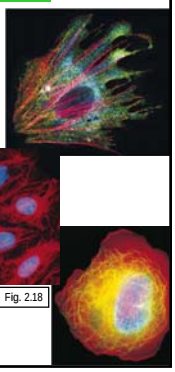
Topic 4 – The cytoskeleton



- Reading
  - Chapter 2.3f, 2.3g, 8.4, 38.1a, 38.1b
  - On-line sources (read selectively)
- Objectives
  - Functions of cytoskeleton
  - Structure and function of microtubules
  - Structure and function of filaments
    - Microfilaments
    - Intermediate filaments
  - Emphasis on the dynamic nature of the cytoskeleton

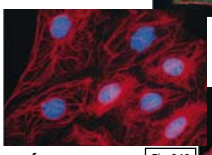


Fig. 2.18

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Functions of the cytoskeleton – an overview

- Functions include:
  - The provision of structure and support (1)
  - Intracellular transport (2)
  - The positioning of organelles within the cell (4)
  - The generation of force for cell movement
  - Contributing to cell division

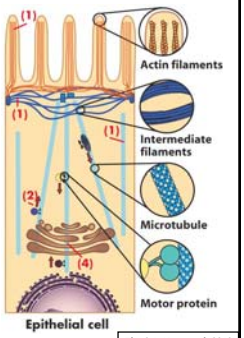

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Fig. 9.1, Karp et al. 2010

1- movement of the cell as well.

-structure: protrusions wouldnt be possible without cytoskeletal support.

2- move things around. eg. organelles, vesicles, etc. organizes contents of cell.

healing relies on cell movement.

Microtubules

- Introduction
  - Hollow, tubular structures of diameter ~25 nm found in all eukaryotic cells
  - Two basic types
    - Cytoplasmic – very dynamic
    - Axonemal – very stable

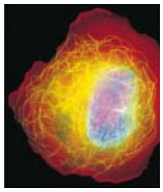

Fig. 2.18

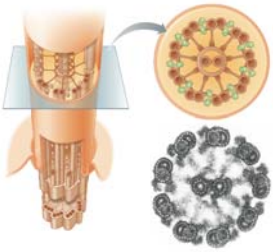

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Fig. 2.21

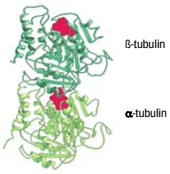
largest of cytoskeletal elements.

change shape a lot. kidna hollow. theyre found in cilia.

theyre very stable.

❑ Structure

- Building block is αß-tubulin
  - globular
  - GTP binding site
  - Only ß-tubulin hydrolyzes GTP
- Protofilament – structural polarity
  - + end = ß-tubulin
  - - end = α-tubulin
- Microtubule = 13 protofilaments



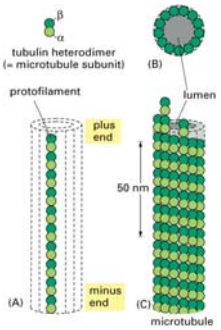


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Fig. 17-10, Alberts et al. 2004

alpha and beta tubulin. they form a heterodimer (2 units with different components).

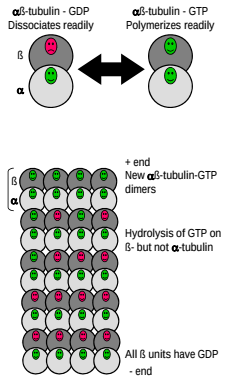
theyre bound by noncovalent bonds, but very stable.

each tubulin protein has a GTP attached/bound. to it

-with a GTP on the beta subunit, the tubulin heterodimers tend to form into microtubules. theyl always form the same orientation. microtubule has a slight polarity. the - end has an alpha unit exposed. the + end has a beta tubulin molecule.

❑ Dynamic character

- Polymerization when GTP bound to ß-tubulin of the αß-dimer (tubulin-GTP)
  - Hydrolysis follows polymerization
- Dissociation more likely when GDP bound to ß-tubulin of the αß-dimer (tubulin-GDP)
  - Treadmilling - growth at + end = shrinkage at - end
- Dynamic instability model
  - Growth or shrinkage at + end depending on rate of addition of tubulin-GTP
- Microtubule-binding proteins regulate the rate of assembly and stability of microtubules
- Useful drugs
  - Colchicine
  - Taxol



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process of polymerization (building microtubule). probabilitiy increases when GTP bound to it.. altho GTP binds to both, only beta is the one that hydrolizing GTP into GDP.

more GDP at the bottom.

when hydrolized to GDP, they tend to disassemble. therefore, at +end, packed together, -end dissasembling. so growing on + and shrinking on - "treadmill effect" since they change at the same rate.

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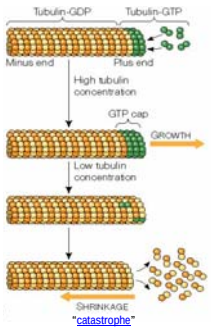

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Fig. 15-7, Becker et al. 2006

as long as there are enough tubulins added at = end, it'll be fine. if its added too slowly, the + end wil start shrinking and end up collapsing in process of catastrophe.

cell wants control. so they have microtubule proteins to regulate it.

colchicine binds to tubulins avoiding them to bind so they cant make microtubule, causes them to fall apart

taxol binds to microtubule preventing it from growing, shrinking, or falling apart.

- Microtubule-organizing centres (MTOC)
  - Initiate assembly and anchor microtubules; establish orientation
  - γ-tubulin acts as template to initiate assembly
  - e.g. centrosome = centrioles + pericentriolar material (animal cells)

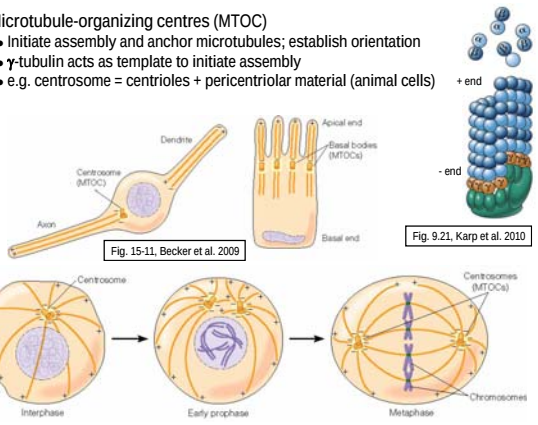

Fig. 15-11, Becker et al. 2009

Fig. 9.21, Karp et al. 2010

to increase microtubule formation. they control where microtubules occur. gamma tubulin helps initiate microtubule formation. basal body at end of cilia eg. - end on basal body. + end in cilia / flagella

- Test your understanding...

  According to the dynamic instability model...

  a. The rate of polymerization at the plus end of the microtubule is exactly matched by the rate of depolymerization at the minus end such that the microtubule turns over but neither grows nor shrinks.
  b. Stability at the - end of a microtubule can achieved by replacing αß-tubulin dimers with γ-tubulin.
  c. The plus end of the microtubule either grows or shrinks depending on the cellular availability of tubulin dimers.
  d. Catastrophe occurs when the two GTP bound to the αß-dimer both become hydrolyzed, causing depolymerization at the + end of the microtubule.
  e. None of a, b, c, or d is true of the dynamic instability model.
  - Explain your answer.

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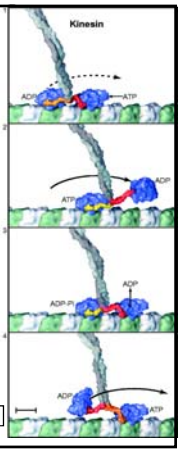
b) this is not possible

d) alpha part of dimer cant hydrolyze GTP

so C) is the right answer.

- Functions
  - Intracellular motility via motor proteins
    - Motor proteins = ATP-dependent "motors", "mechanoenzymes"
      - Motor domain – hydrolyzes ATP, conformation change
      - Tail – hauls cargo
    - Use cytoskeleton as "highway"

	Kinesin	Car engine
Size	10^{-8} m	1 m
Fuel	ATP	gasoline
Speed	0.004 m h^{-1}	100,000 m h^{-1}
	4 x 10^5 lengths h^{-1}	10^5 lengths h^{-1}
Efficiency	~60%	~10%



Vale & Milligan 2000 Science 288, 88-95

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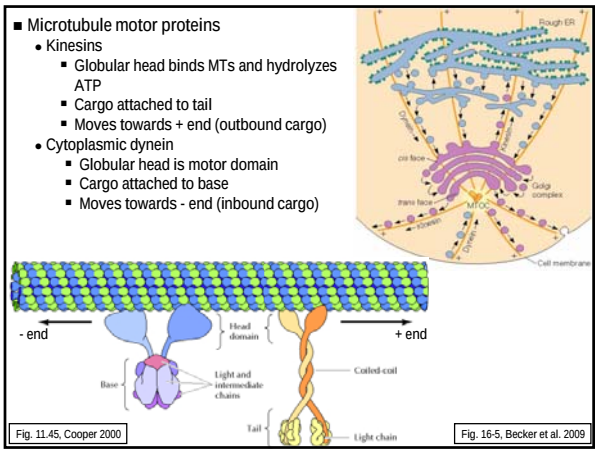
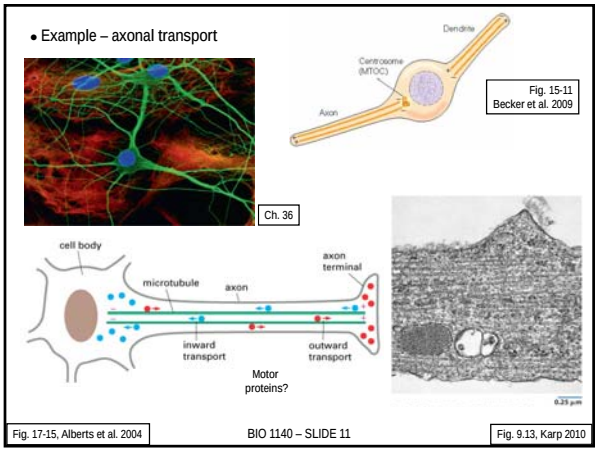
"highways" need "cars": motor proteins. they can hydrolize ATP and use that E to move. they have a motor domain. this hydrolizes ATP . releases E. changes shape to mechanically move (mechanoenzyme). Has a motor domain and a "tail" that hauls the cargo. always come in pairs, so there are 2 motor proteins wound together so they can help each other walk. otherwise, they would fly off if they had to hop.

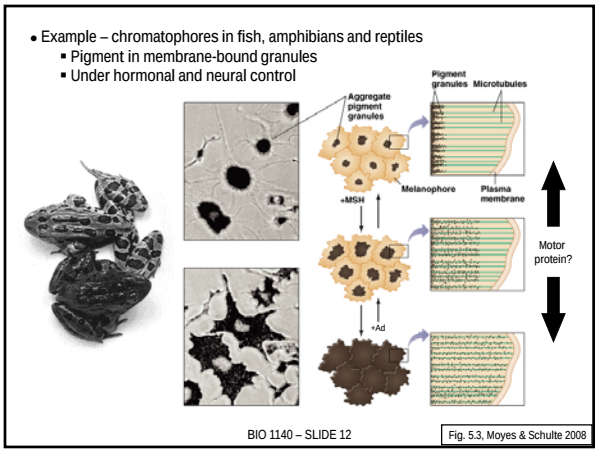


2 families of motor proteins are the ones that are wound around each other.

difference b/w kinesin and dyenin: kinesin moves to + end. dyenin moves to - end. kinesins are moving things to outside of cell, and dyenins move things towards the centrosome.

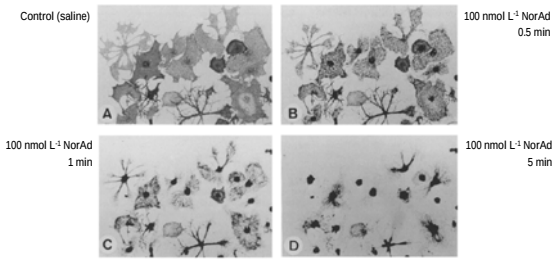


axons of some neurons can be up to 1m long. microtubules act as "highways" to help move something from one end of the neuron to the other. the centrosome is at the centre of the neuron. the+end is at the end of the axon. dyenin brings things in (from -ve to +ve end) and kinesin from center to +end. movement would be rlly slow if movement along cell depended on diffusion, so thats why microtubules and their motor proteins are so useful.



pigments are dispersed all over the cell by motor proteins. when scared, theyre pulled to the middle because of adrenalin by dyenin motors, making the animal look pale, when they are relaxed, they disperse it all over the cell (using kinesin motors).

- Example – chromatophores in fish, amphibians and reptiles
  - Pigment in membrane-bound granules
  - Under hormonal and neural control



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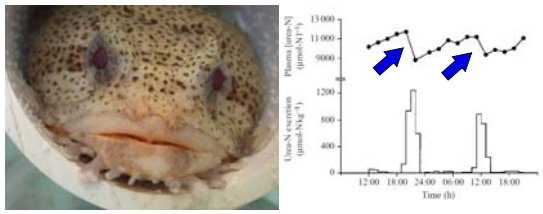
Morishita et al, 1993

Noradrenalin affect the scale of the cell and the colour pigments were brought into the middle of the cell, draining the scale of colour

- Example – urea excretion in toadfish
  - Urea transporter (tUT) insertion into the gill cell membrane
  - Experimental test?

Gulf toadfish, *Opsanus beta*



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Wood et al. 1997 JEB 200, 1039-1046

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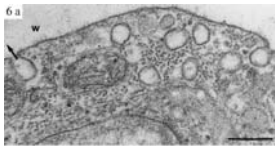

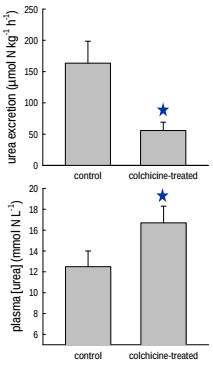

Laurent et al. 2001 Cell Tissue Res 303, 197-210

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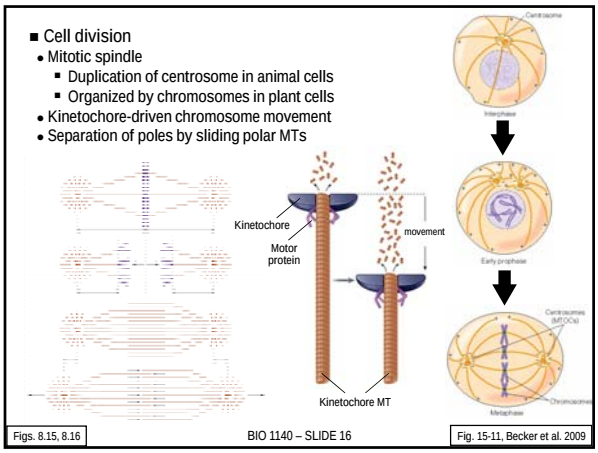
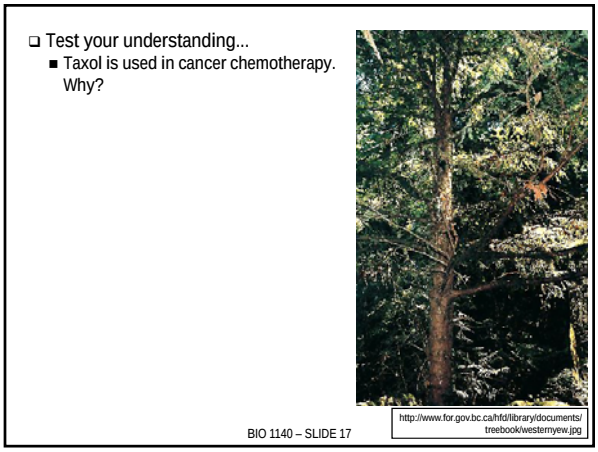
Gilmour et al. 1998 PZ 71, 492-505

when the animal pulses urea out of it, the vesicles go to outside of cells and move along microtubules maybe (? if it does, if we treat it with colchisine, there will be a reduction in urea excretion). in fact, yes. this is exactly what happened

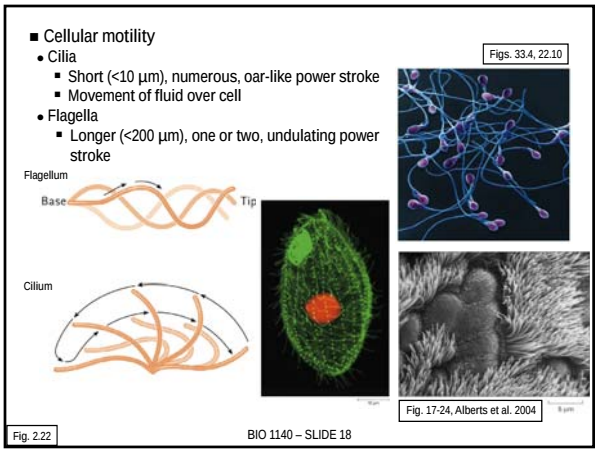
in CELL DIVISION: role of microtubules.



during cell division, the centrosomes double and one goes to each end of the cell. the + ends extending into body of cell while - end is attached to the centrosome. some of the microtubules attaches to the structure that holds the chromosomes together "kinetochore"/centromere and are moved along microtubules. microtubule gets desintegrated as it moves along. the microtubules are overlapping in the beginning. to get separated, theres a kinesin motor with one motor foot on a microtubule of one centromere and one of the other, so they get pushed apart.



taxol stables microtubules in the form theyre in. prevents growth/ decomposition. this inhibits cell division everywhere. it's unselective. since cancer cells are the ones that reproduce the quickest, this is why they're so good, but there are side effects too. with taxol, the mitotic spindle cant form. .



theyre major microtubule structures that help with the cell movement.

theyre basically identical on the inside. their differences are: cilia are short, stubby protrusions. they're numerous. they COULD move the cell, but they could also move things over/past the cell (think airway cells)

flagella are long, and usually only 2 or two. they always move the cell

using length, number and whether they move things or not, you can differentiate cilia and flagella because of their different movements. flagella are whip-like (long ondulating movement). cilia are more like a row boat

- Structure
  - Enclosed in cell membrane
  - Axoneme – 9 + 2 arrangement of MTs
    - A = 13, B = 10-11 protofilaments
    - Radial spokes + nexin links
  - Basal body anchors minus end of MTs
    - centriole (9 triplet MTs)
  - Axonemal dynein
    - Associated with A tubule
    - Moves along adjacent B tubule (in minus direction)
    - Results in sliding tubules
    - Coordinated

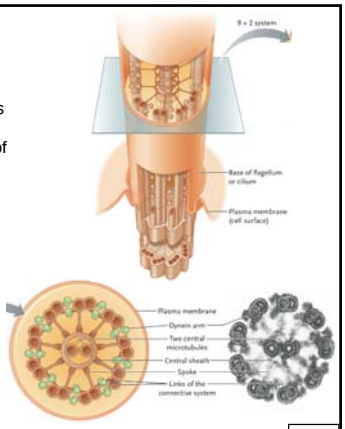


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Fig. 2.21

within plasma membrane: axoneme arrangement. this is where the microtubules are in a 9+2 arrangement.
consits of 1 complete tubule is an A tubule and an incomplete tubule (B tubule-- contains less filaments). theyre connected by nexin. there are protein connections b/w the doublets and center of axoneme.
at the base is the basal body. it's like a centriole (microtubule organization center). +end near tip of cilia/flagella.

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Fig. 2.22

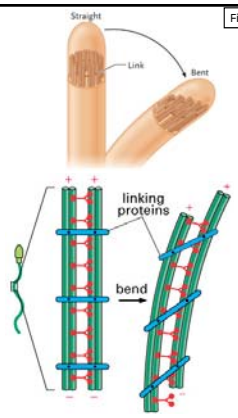

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Fig. 17-28, Alberts et al. 2004

Motor protein: axonemal dynein. it's anchored onto the A doublet but walks along B doublet. if they weren't connected, they'd slide way past each other, like in mitotic division.
What happens is that there are protein connections between the doublets. when the motor protein tries to move the doublets past each other, the structure bends. the motors coordinate in such a way that the other side opposes it and it becomes a back and forth motion of the cilia/flagella.
.
*radial spokes: from doublets to center of axoneme
*nexin links: between A and B doublets.

- ❑ Consolidate your knowledge...
  - ■ Distinguish between:
    - Cytoplasmic and axonemal microtubules
    - Dynein and kinesin
    - A cilium and a flagellum

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*****

Can you meet these objectives?

- By the end of this lecture you will be able to...
  - List the components of the cytoskeleton
  - Describe the major functions of the cytoskeleton
  - Discuss the basis and significance of the dynamic nature of cytoplasmic microtubules
  - Define a motor protein and provide examples
  - Contrast and compare kinesins and dyneins, distinguishing between cytoplasmic and axonemal dynein
  - Explain how vesicles are moved around the cytoplasm
  - Explain how microtubules are used to power cell movement
  - Comment on structure/function relationships in cilia or flagella