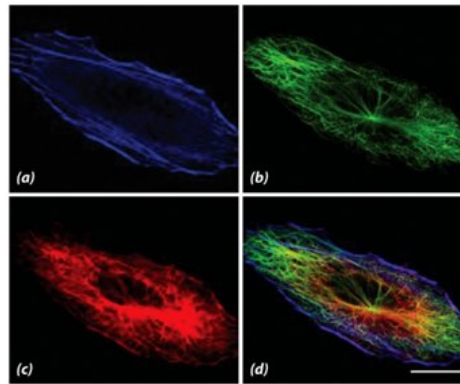


Week 11: Cytoskeleton introduction and microtubules

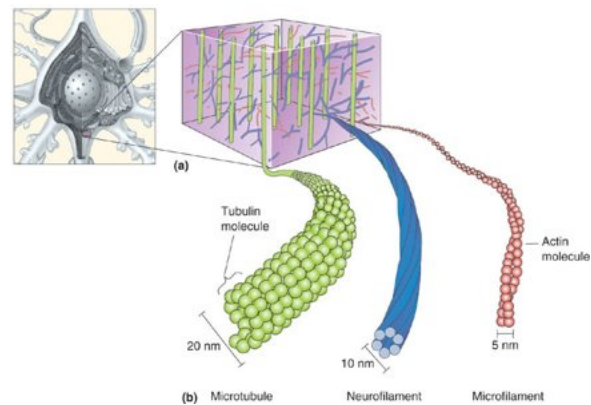


From M. Bister Omary et al., Trends Biochem. Sci. 31:185, 2006. Copyright 2006 with permission from Elsevier Science.

Suggested readings: Karp 9th ed. Chapter 9

Introduction to the cytoskeleton

- **Microtubules (MT)** –
- hollow, rigid cylindrical tubes
- made from tubulin subunits
- **Microfilaments (MF)** - solid, thinner structures
- made of actin
-
- **Intermediate filaments (IF)** - tough, ropelike fibers
- made of a variety of related proteins



The cytoskeleton is a series of long, filamentous protein fibres that are formed within the cell.

Here's a handy-dandy chart to use for studying

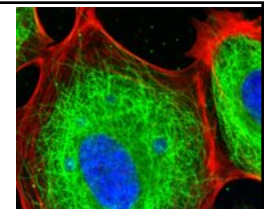
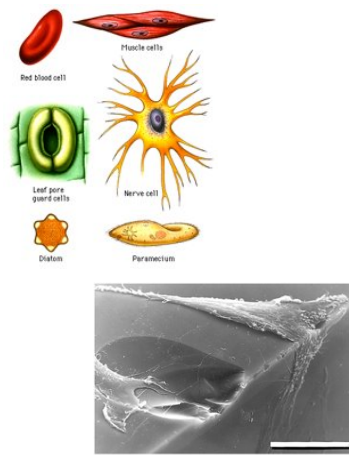
Table 9.1 Properties of Microtubules, Intermediate Filaments, and Actin Filaments

	Microtubules	Intermediate filaments	Actin filaments
Subunits incorporated into polymer	GTP- $\alpha\beta$ -tubulin heterodimer	~70 different proteins, likely incorporated as tetramers	ATP-actin monomers
Preferential site of incorporation	+ End (β -tubulin)	Internal	+ End (barbed)
Polarity	Yes	No	Yes
Enzymatic activity	GTPase	None	ATPase
Motor proteins	Kinesins, dyneins	None	Myosins
Major group of associated proteins	MAPs	Plakins	Actin-binding proteins
Structure	Stiff, hollow, inextensible tube	Tough, flexible, extensible filament	Flexible, inextensible helical filament
Dimensions	25 nm outer diam.	10–12 nm diam.	8 nm diam.
Distribution	All eukaryotes	Animals	All eukaryotes
Primary functions	Support, intracellular transport, cell organization	Structural support, mechanical strength	Motility, contractility, intracellular transport
Subcellular distribution	Cytoplasm	Cytoplasm + nucleus	Cytoplasm

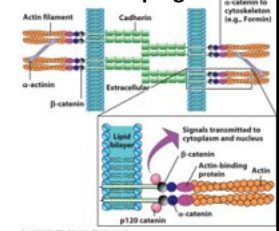
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Functions of the cytoskeleton #1

- The cytoskeleton provides **structural support** that can determine the shape of the cell and resist forces that can deform the cell
- A cell's shape is closely associated with its function
- Cytoskeleton can resist mechanical stresses
- Cytoskeleton can rearrange itself to change the shape of cells



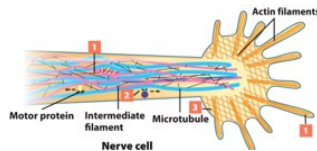
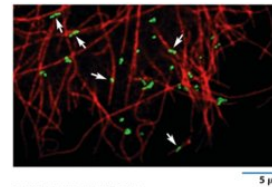
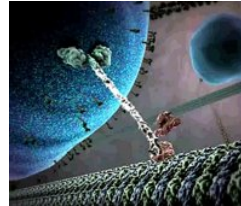
Different cytoskeletal elements labeled... each plays a role in shaping the cell



Functions of the cytoskeleton #2

The **intracellular transport** of organelles and vesicles occurs via the cytoskeleton

- Machinery needed to move materials & organelles within cell; network of highways directs movement
- Movement of mRNA molecules to specific parts of cell
- Movement of vesicles from ER to Golgi complex
- Movement of neurotransmitter-containing vesicles from synthesis site to axon terminal
- Transport of peroxisomes over rails of microtubules; the two are closely associated (microtubules in red, peroxisomes in green)

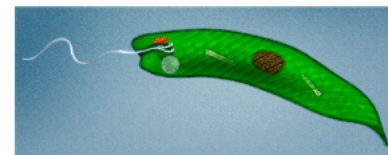


Some tracts are *really* long... some neurons extend from the spinal cord to the end of your fingers! Vesicles are carried along microtubules in the axon.

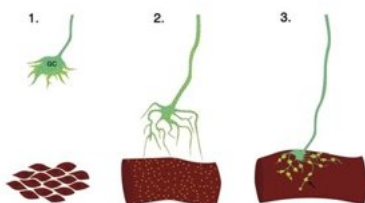
Functions of the cytoskeleton #3

The cytoskeleton is the **force generating apparatus** that moves cells from one place to another (if they're mobile!)

Single-celled organisms move by crawling over surface or propelled by protruding **cilia & flagella**

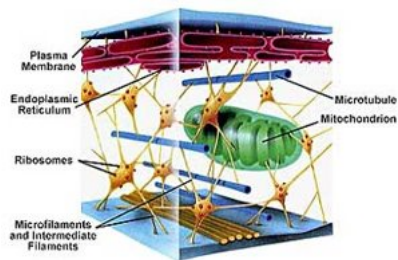


Multicellular organisms have variety of cells capable of independent locomotion - sperm, white blood cells, fibroblasts, highly motile tip of growing axon (movement like crawling white blood cell)



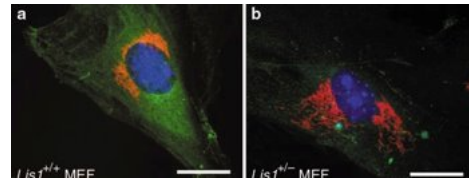
Functions of the cytoskeleton #4

The cytoskeleton provides an **internal framework** for positioning the various organelles within the interior of the cell.



The location of cellular components isn't random... they're all anchored to and moved around the cytoplasm by the cytoskeleton

Intracellular organization is disrupted by drugs or mutations that interfere with normal cytoskeleton structures

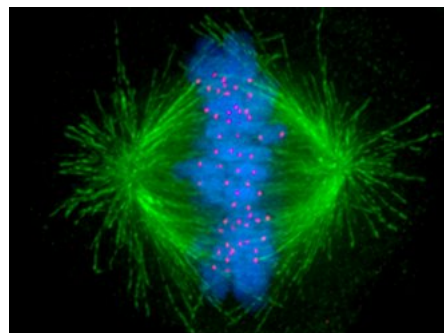


Orange shows normal distribution of Golgi near nucleus (blue)

Mutation in cytoskeleton shows dispersal of Golgi (red)

Functions of the cytoskeleton #5

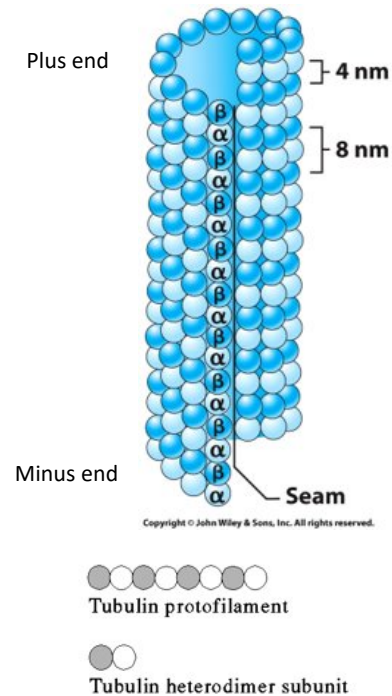
The cytoskeleton is an essential component of the apparatus that divides chromosomes during mitosis and meiosis



Here, the spindle apparatus made of microtubules is dyed green.

Microtubule structure

- Basic unit is a tubulin dimer composed of alpha (α) and beta (β) tubulin subunits
- polymerize to form protofilaments
- protofilaments form a cylinder (typically 13 protofilaments)... not covalently attached!
- protofilaments are asymmetric (α -tubulin at one end, β -tubulin at other)
- all protofilaments in single MT have same polarity - microtubules are therefore asymmetric & polarized (β end is plus; α end is minus)
- The plus ends have higher rates of tubulin addition (thus grow faster); the minus ends are slower growing



Microtubule assembly

- initial polymerization is slow – requires nucleation of 13 dimers, with the help of another tubulin: γ (gamma) tubulin and other proteins
- γ tubulin dissociates from microtubule after polymerization has progressed
- subsequent subunit addition is faster.
- depolymerization also occurs spontaneously, but at a slower rate.
- one end of the MT is slower growing (-) while the other is faster (+) giving each microtubule a polarity.

Microtubules may vary in their rate of assembly and disassembly. Tubulin half life is nearly a full day, however, the half life of a given microtubule may be only 10 minutes.

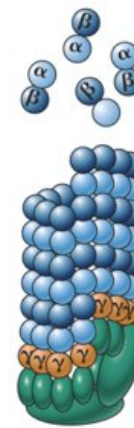
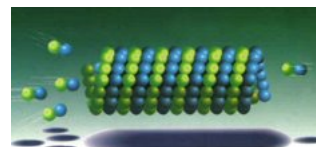
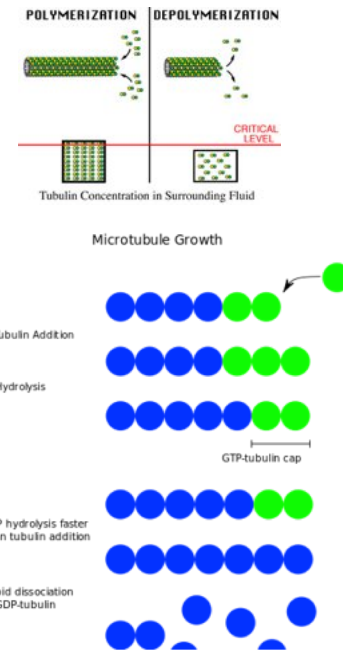


Figure 9-21c Cell and Molecular Biology, 4/e (© 2005 John Wiley & Sons)



Microtubule assembly

- microtubules are in constant flux, polymerizing and depolymerizing
- relative rates of assembly and disassembly determine their growth – dependent on local tubulin concentration
- microtubule's instability is controlled by hydrolysis of GTP bound by tubulin.
- tubulin dimers bind GTP before binding microtubule
- the microtubule dimer hydrolyzes the GTP to GDP.
- this reduces the affinity of the tubulin for its neighbor, thus leading to disassembly.
- To retain some stability, the GDP-dimers can be regularly replaced with more GTP-dimers



Proteins called +TIPs can also affect disassembly rates.

The microtubule cytoskeleton is dynamic

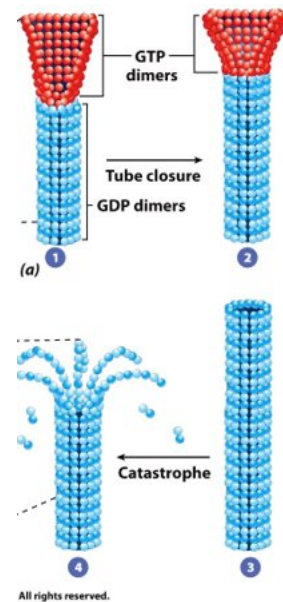
- The array of microtubules helps support entire cell, but it is not static like our skeleton – it changes constantly
- Its ability to assemble and disassemble quickly is what allows cell movement
- For the cell to move or change shape, the microtubule skeleton (and all other skeletal elements) must change length and directions

The first part of this movie is GFP fused to a microtubule binding protein (EB3)

<http://www.dnatube.com/video/4164/Microtubule-Dynamics>

Factors affecting microtubule stability

- Rate of growth: more growth stabilizes the microtubule because added tubulin dimers have GTP bound (forming a GTP cap)
- The rate of growth is largely dependent on the concentration of tubulin dimers available in the vicinity of the growing end
- Rate of GDP to GTP exchange: as the dimers hydrolyze GTP further down the MT (thus destabilizing the microtubule), new GTP can be enzymatically swapped in to stabilize the microtubule
- Association with microtubule associated proteins, some of which help stabilize the microtubule

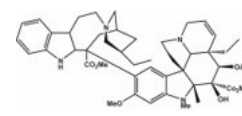


Several chemotherapy drugs target microtubules

- cancer cells, with their high rate of division, require faster cycling of tubulin – drugs can prevent that
- Drugs that interfere with MT dynamics will interfere with the spindle apparatus that divides chromosomes in mitosis
- Normal cells have a 'mitotic checkpoint' that will stop mitosis if something is wrong. Cancer cells often lack these checkpoints... thus will try to divide even with a 'poisoned' spindle. This causes uneven distribution of chromosomes and will often kill the cancer cell.
- colchicine, nocodazole make microtubules disassemble
- taxol, **vinblastine** prevent disassembly



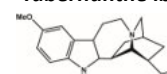
Catharanthus roseus



Vinblastine



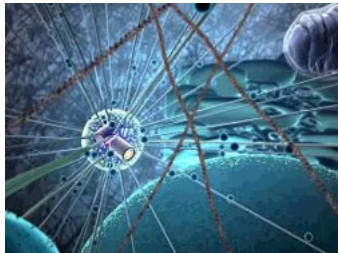
Tabernanthe iboga



Ibogaine

Microtubule organizing centers (MTOCs) can initiate microtubule polymerization

The initiation of microtubules can often occur at specialized structures called MTOCs. The best understood is are centrosomes and basal bodies.



Centrosomes are usually close to the nucleus, and microtubules extend from them.

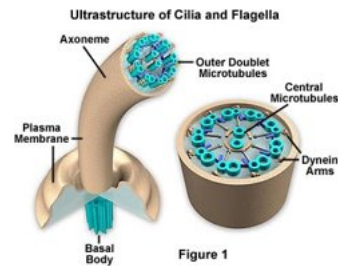


Figure 1

Basal bodies assemble the microtubules that make up cilia and flagella. (we will see this soon!)

These are actually interchangeable... the basal body from a sperm becomes the centrosome of the newly fertilized egg!

Centrosome structure

- In animal cells, an obvious MTOC is the centrosome
- The centrosome is comprised of two **centrioles** surrounded by a cloud of molecules known as the pericentriolar material
- Centrioles are cylindrical structures 0.2 μm in diameter and about 0.4 μm long
- Composed of 9 fibrils, with each fibril composed of 3 fused microtubules
- In centrosome, centrioles usually in pairs, perpendicular to each other
- The centrioles are not actually involved in microtubule nucleation, but probably “recruit” the molecules that do (the gamma tubulin and other proteins)

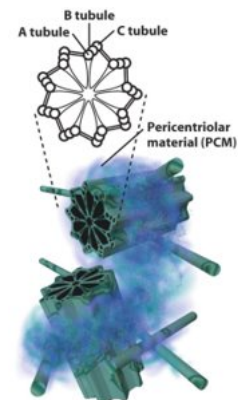
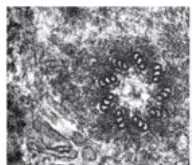


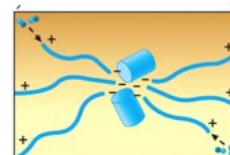
Figure 9-18a Cell and Molecular Biology, 4/e (© 2005 John Wiley & Sons)

(plants lack centrosomes – their MTOCs are typically embedded in the nuclear membrane)



EM of a centriole

Centrosome anchors – ends, + end extend out

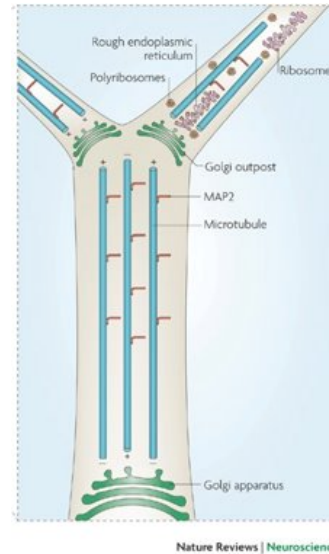


Microtubules usually have microtubule associated proteins (MAPs) attached

MAPs may:

- stabilize microtubules
- alter assembly/disassembly rates
- crosslink adjacent MTs

MAP activity controlled by addition & removal of phosphate groups on particular amino acid residues by protein kinases & phosphatases, respectively

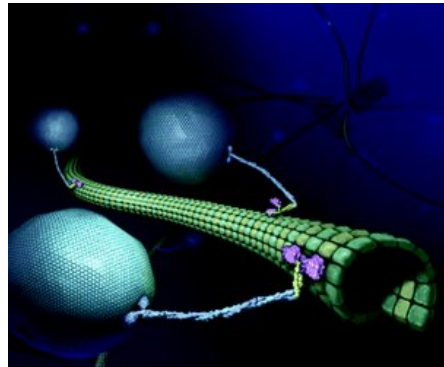


What you should know after this section

- Functions of the cytoskeleton
- Three main components of the cytoskeleton
- Factors that regulate microtubule assembly
- How some drugs target microtubules and their impacts
- Define a MTOC, identify two types, and define the components

Week 11: Cytoskeleton - Part II

Motor Proteins



Suggested readings: Karp 9th ed. Chapter 9

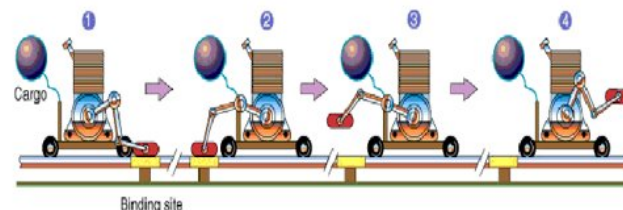
Molecular motors provide movement

Motor proteins of cell convert chemical energy stored in ATP into mechanical energy that is used to generate force or to move cellular cargo attached to the motor

A. Types of cellular cargo transported by these molecular motors include: ribonucleoprotein particles, vesicles, organelles (mitochondria, lysosomes, chloroplasts), chromosomes & other cytoskeletal filaments

B. A single cell may contain dozens of different motor proteins, each specialized for moving a particular type of cargo in a particular cell region

Motion is a bit like rope-climbing or swimming: alternate between power strokes and recovery strokes:

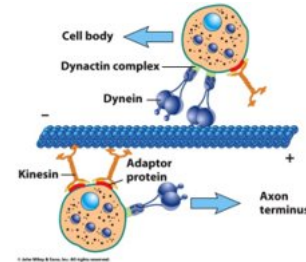


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Cells have three families of motor proteins

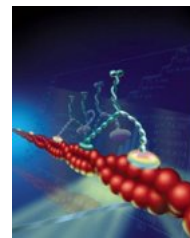
1. **Kinesins** move toward (+) end of microtubules
2. **Dyneins** move toward (-) end of MTs

They can attach to different adapter proteins that then attach to proteins imbedded in vesicle membranes, the cell membrane, or adjacent microtubules to generate movement.



Microfilament motor proteins:

- 3. **Myosins**
-
- (There are no known motor proteins that use intermediate filaments as tracts)



Myosins interacting with a actin microfilament

Kinesin structure

- Kinesins move cargo to the + end of a microtubule
- Kinesins are tetramer proteins constructed of 2 identical heavy chains & 2 identical light chains
- They're the smallest of motor proteins (but still big!)
- There are approx 45 different kinesins in humans, each binds a different subset of cargo

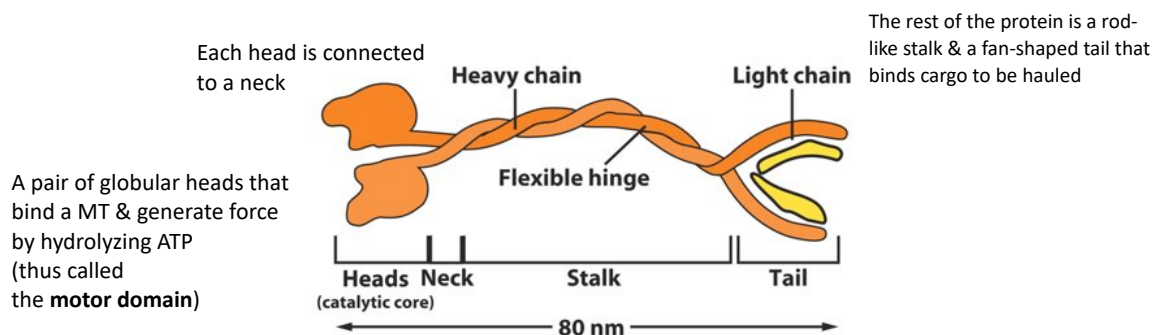


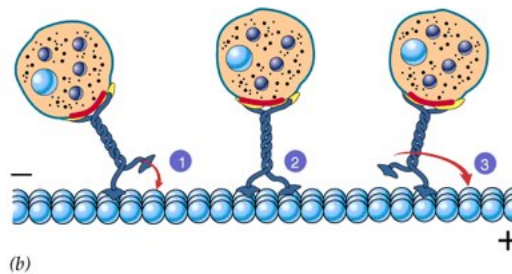
Figure 9-15a Cell and Molecular Biology, 4/e (© 2005 John Wiley & Sons)

How kinesin moves

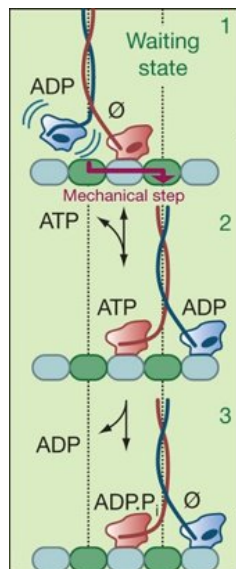
- Kinesin movements mediated by ATP hydrolysis : 1 ATP per “step”
- Moves along single MT protofilament (rate proportional to [ATP]; up to 800 nm/sec)
- Move in distinct steps (1 dimer at time; 8 nm); usually toward plasma membrane (e.g. from RER to Golgi, or from Golgi to plasma membrane)

Kinesin attaches to one of the vesicle's integral proteins (often via Rab proteins).

Movement is *processive* because at least one head is attached to the microtubule at all times. It thus can walk and carry cargo micrometers down a MT.



Kinesin hydrolyses ATP to walk along MTs



(not shown) before attaching to a MT, both heads have ADP bound)

1. The front head binds to a microtubule and releases its ADP.

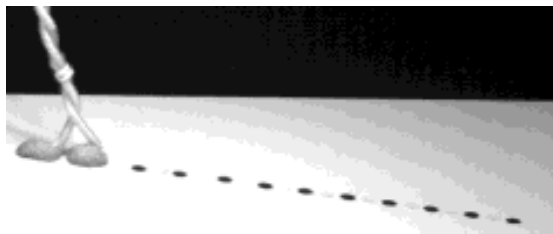
2. ATP binds to the front motor domain and causes a conformational change (the neck region associates with the head region). This causes the back head to swing forward and bind the MT.

3. ADP is released from the (now) forward head. The (now) back head hydrolyses the ATP to ADP. The energy released dissociates the head from the microtubule.

A video depicting the ATP cycle

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

We think that kinesin movement resembles a wasted person walking home from the bar



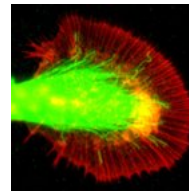
- https://www.youtube.com/watch?v=tMKIPDBRJ1E&ebc=ANyPxKoJi55CjpY3HVgJU6nry1tzA1x8_5rT3hik3mt5Ls_How1bZ8_aaNeNoU_PDz1EQ4htJZ3hf-umqZ5FshvtggMa3sgJjg

Kinesin functions in neuronal transport

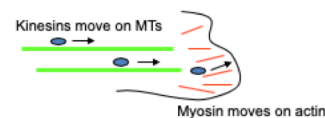
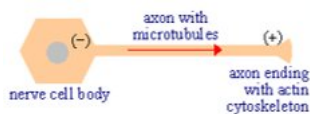
- **Kinesin I** was first isolated in 1985 from squid giant axons
- It is responsible for **axonal flow**, in which vesicles (e.g., precursors of synaptic vesicles formed in the Golgi) are carried along microtubules from the cell body to axon endings.
- **Axon endings** have an extensive **actin** cytoskeleton. **Myosin V** may take over to transport vesicles along actin filaments to near the plasma membrane at the synapse.



You can see these cells!!



tubulin = green; Actin = red



Cytoplasmic dyneins carry cargo to – MT ends

- ubiquitous eukaryotic motor protein, only 2 versions in humans
- Not found in flowering plants but seed free plants
- huge protein (1500 kDa)
- two identical heavy chains: head generates force
- variety of intermediate & light chains (each with different cargo recognition domains)
- cargo attached by dynactin

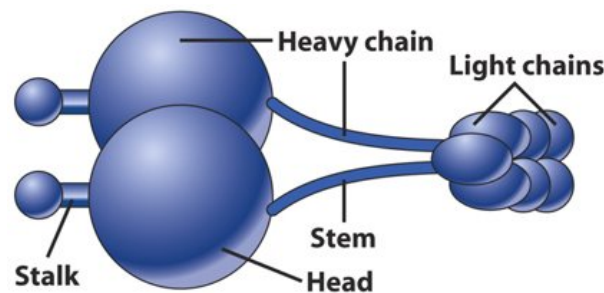


Figure 9-17a Cell and Molecular Biology, 4/e (© 2005 John Wiley & Sons)

Various dynein functions:

- chromosome movement in mitosis
- positioning Golgi complex
- movement of vesicles & organelles through cytoplasm
- involved in axonal retrograde organelle movement
- in fibroblasts & endothelial cells move endosomes, lysosomes, Golgi-derived vesicles toward cell center
- Dynein also 'carries' HIV to the nucleus

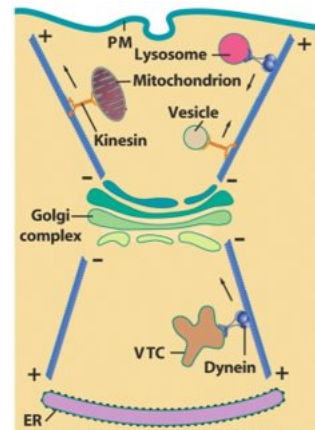
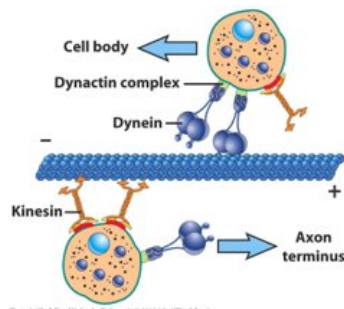
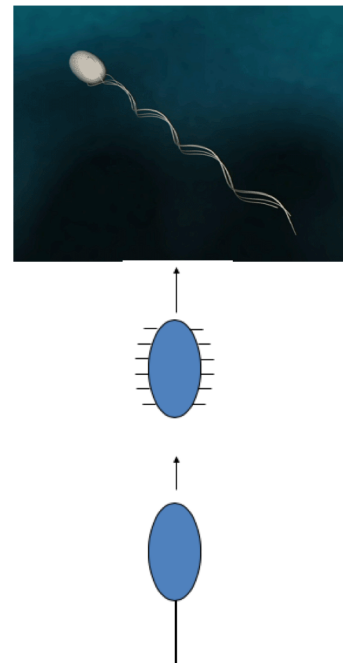


Figure 9-17c. Cell and Molecular Biology, 4/e (© 2005 John Wiley & Sons)

Note that microtubule orientations can be variable in different parts of cell

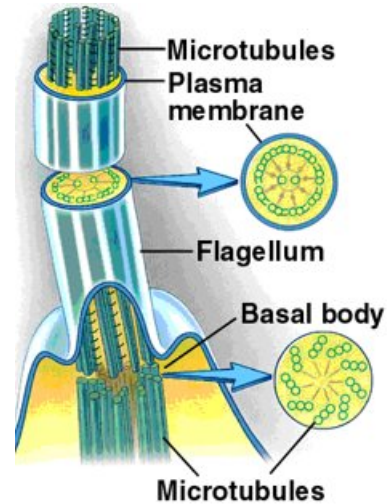
Cilia and flagella

- Hair-like structures that project from cell surface
- both are used for locomotion
- distinction between cilia and flagella is blurred, because both are made of the same components
 - cilia are typically shorter and more numerous
 - cilia are like oars – cell movement is perpendicular to direction of cilia themselves
 - flagella are longer, rarely more than two
 - flagella tend to be aligned in the direction of movement



Structure of cilia and flagella

- both are made of microtubules surrounded by plasma membrane
- core is 9 + 2 array of MTs called the **axoneme**
- 9 peripheral doublet MTs with 2 central single MTs
- all MTs have + ends at tip, - ends at base
- At the base of the flagellum, there is a MTOC called the **basal body** (looks exactly like a centriole)



Cross-section of an axoneme

- each MT doublet in the axoneme has:
 - 1 complete MT (A -13 protofilaments) and
 - 1 incomplete MT (B- with 10 or 11 protofilaments)
- **ciliary dynein** arms and radial spokes are attached to A tubules
- A-tubule is linked to B-tubule of adjacent doublet by **nexin**, which is an elastic protein

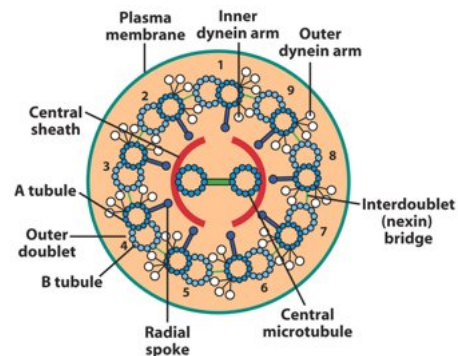


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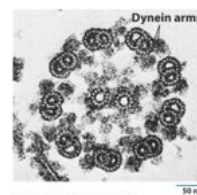


Figure 9-30c Cell and Molecular Biology, 4th (© 2005 John Wiley & Sons)

Mechanism of ciliary and flagellar motion

General model - MT doublets slide past each other

Dynein arms walk along wall of adjacent doublet

Sequence of events

1. Dynein arms anchored on A MT attach to neighboring B MT
2. Dynein molecules undergo conformational change - causes A MT to move slightly toward base of attached B MT; energy comes from ATP hydrolysis
3. Dynein releases B MT
4. Nexin holds MT until dynein reattaches and the dynein arms reattach closer to the base of B MT
5. The cycle repeats

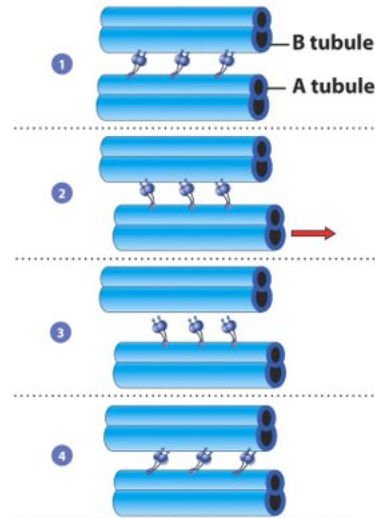


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Alternation of this sliding causes the cilia or flagella to bend

The sliding on one side

of axoneme alternates with sliding on other

- cilium first bends one way, then other
- activity of dynein arms alternates from one side to other
- **Hypothesis:** activity of dynein arms is regulated by central MT pair & radial spokes
- central pair rotates as beating proceeds
- rotation promotes sequential contacts with each radial spoke, making the spoke signal to the dynein arm on the adjacent A MT

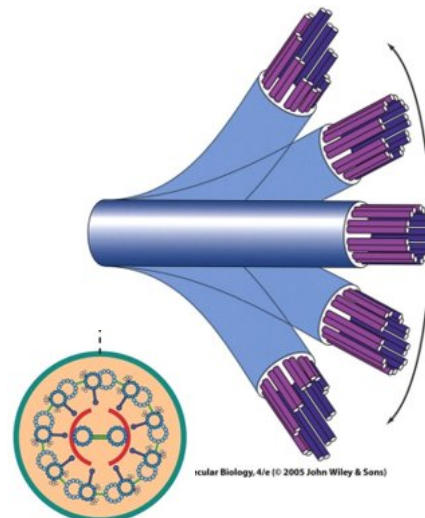
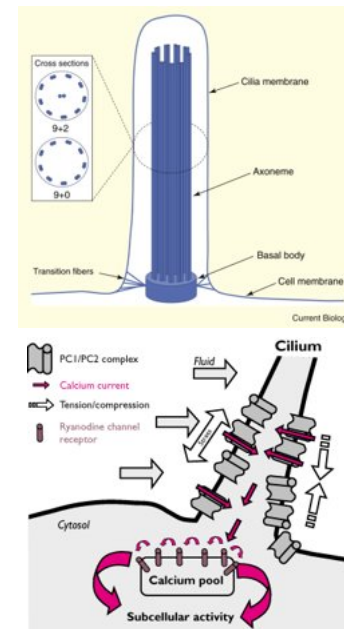


Figure 9-32b Cell and Molecular Biology

The primary cilium

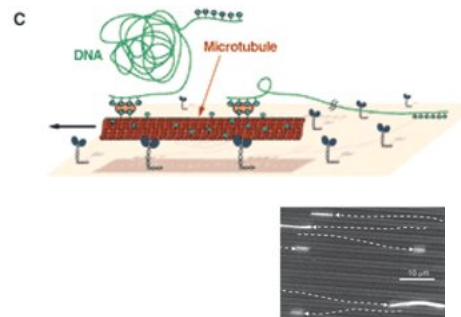
Primary cilia differ from other cilia in that they lack the central pair of microtubules

- they do not have a locomotory function
- there is only one per cell
- they are a type of antenna, picking up chemical signals (they are covered in cell surface receptors), and they carry other signaling molecules up and down the microtubules, into the cell
- they can act as mechanoreceptors (bending send signal), sensing flow outside cell
 - In kidney epithelium, fluid flow causes primary cilia to bend, opening calcium channels, which signals to the cells of kidney ducts to regulate flow
 - Defects in primary cilia can cause kidney disease



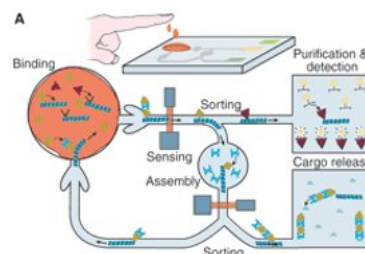
Using protein motors for nanotech

- Motor proteins can be fixed to microchips
- Their heads can still move, and can be used to shuttle microtubules (MTs) across the chip's surface
- E.g. Coated surface with kinesin, added a solution of labeled or tagged MTs, and recorded where the labeled MTs moved



How could we use this technology?

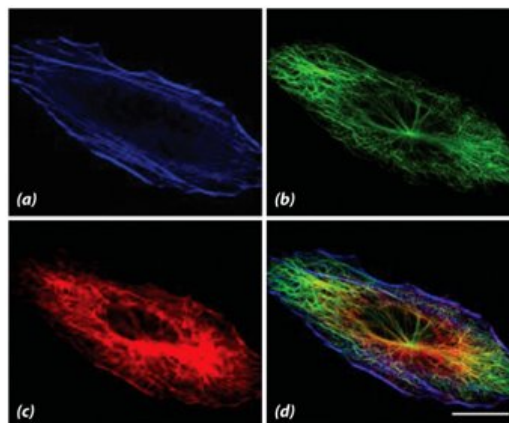
On-chip sorting of materials, assembly of different components, and concentrating components for enhanced detection.



What you should know after this section

- Distinguish kinesins, dyneins, and myosins and know which cytoskeletal fibers they move along
- How do the motor proteins bind the cytoskeleton and move (how does ATP/ADP regulate this)?
- Distinguish between flagella and cilia
- Describe an axoneme and how it facilitates cilium movement
- How does a primary cilium differ from a motory cilium
- How can motor proteins be harnessed for nanotechnology applications?

Week 11: Cytoskeleton - Part II Intermediate Filaments, Actin and Myosin



Fluorescent image of
a liver cell

a) Microfilaments

b) Microtubules

c) Intermediate
filaments

d) Overlay of a-c

Suggested readings: Karp, 9th ed. Chapter 9

Intermediate Filaments

Intermediate in size (10 nm diameter) between MT (25 nm) and microfilaments (8 nm)

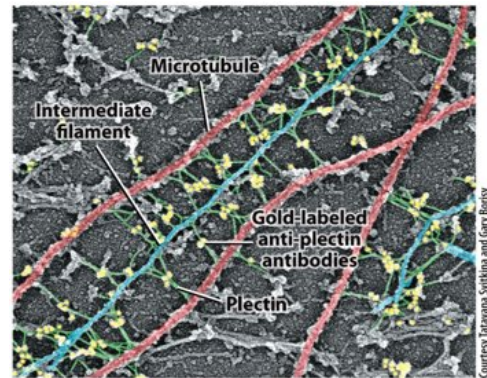
Strongest tensile strength of all cytoskeleton fibers

- (some have been found to tolerate being stretched to 3x normal length)

Made of fibrous proteins that **intertwine** with each other to make dense, rope-like fibers

Especially abundant in **cells that endure physical stresses** such as muscle cells, neurons, and epithelial cells

Connected to other components of cytoskeleton by cross-linking proteins, such as **plectin**



To date, intermediate filaments have only been observed in animal cells. All other lineages, including prokaryotes, have MT-like and actin-like proteins.

IF proteins are a heterogeneous group

Table 9.2 Properties and Distribution of the Major Mammalian Intermediate Filament Proteins

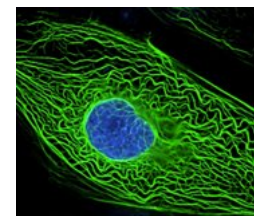
IF protein	Sequence type	Primary tissue distribution
Keratin (acidic) (28 different polypeptides)	I	Epithelia
Keratin (basic) (26 different polypeptides)	II	Epithelia
Vimentin	III	Mesenchymal cells
Desmin	III	Muscle
Glial fibrillary acidic protein (GFAP)	III	Astrocytes
Peripherin	III	Peripheral neurons
Neurofilament proteins		Neurons of central and peripheral nerves
NF-L	IV	
NF-M	IV	
NF-H	IV	
Nestin	IV	Neuroepithelia
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.

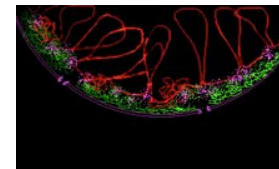
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Approx. 65 different IF genes in humans.

The primary amino acid sequence of these can be quite different. They all however exhibit similar structural properties.



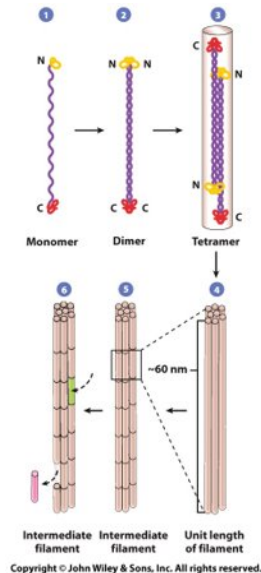
GFP-keratin



Lamins (green) inside nucleus

Images acquired free from:
<http://www.cellimagelibrary.org/>

The basic structure of intermediate filaments



Despite differences in type of protein, all IFs share similar structure

Each has conserved central, α -helical domain

Differences arise due to globular domains of variable size & sequence that flank the helical core

Two monomers wrap around each other in coiled-coils to form dimers with both subunits aligned parallel to each other

Dimers bind to each other to form tetramers

Dimers are aligned in opposite orientations & are staggered: tetramers are not polar (no plus or minus end)

Tetramers aggregate in a staggered manner to form intermediate filaments

IFs continuously assemble and disassemble (like MTs), but subunits can insert in the middle, not the ends of the filaments

Functions of intermediate filaments

Main function is providing mechanical support because they are highly resistant to tensile forces

Hair, fingernails are made of keratin IFs
skin is mainly a mat of keratin IFs

Nuclear lamins form network lining inside of nuclear envelope

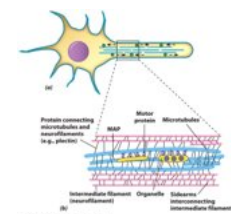
Neurons are filled with neurofilaments

IFs are useful in cancer diagnosis--

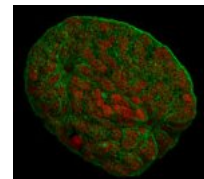
Many cell types have specific IF profile. Keratins have subtypes that are unique to different epithelial cells (bladder, skin, etc) or even different subsets of one cell type (like basal epidermal cells). This is useful in detection of the origin of cells in a tumor, especially cells that have metastasized



Hipsters would be lost without keratin.

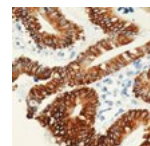


Neuron axons supported by neurofilaments



Nucleus surrounded by

Science 320 1332



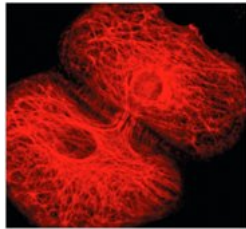
Antibodies (brown stain) bind a keratin subtype in colon cancer cells

Image from: <http://www.cellimagelibrary.org/>

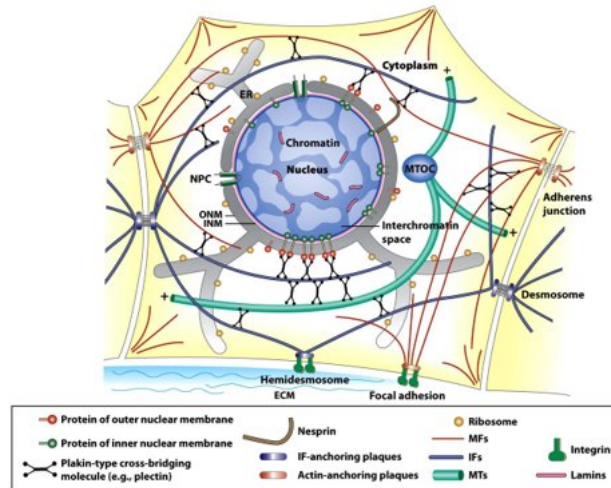
Intermediate filaments help hold cells together

In epithelia, keratin intermediate filaments form junctions that hold cells together (desmosomes), or attach cells to matrix (hemidesmosomes).

The intermediate filaments loop into the plaques and spread out into the cytoplasm. This links two cells together structurally.



Keratin-containing IFs stained red in a cultured skin cell.



The final cytoskeleton fiber: microfilaments

Thinnest of the 3 cytoskeletal fibers (8 nm)

Composed of actin - monomers of the protein actin polymerize to form long, thin fibers

Actin needs ATP to become part of the growing filament; ATP is hydrolyzed once actin is part of the microfilament

Some functions of actin filaments:

- forms a band just beneath the plasma membrane that provides mechanical strength to the cell
- links transmembrane proteins (e.g. cell surface receptors) to cytoplasmic proteins
- anchors the centrosomes at opposite poles of the cell during mitosis pinches dividing animal cells apart during cytokinesis
- generate cytoplasmic streaming in some cells
- generate locomotion in cells such as white blood cells and the amoeba
- interact with myosin filaments in skeletal muscle fibers to provide the force of muscular contraction



Each actin subunit is made of 4 sub-domains

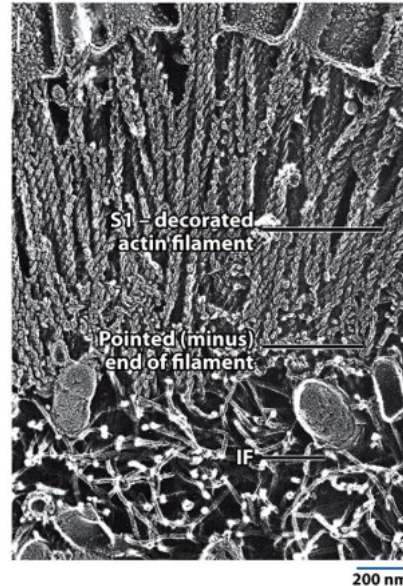
“barbed” end

“pointed” end

Note that all of the actin subunits are made of the same actin (i.e. it's a homopolymer). This figure is colored so that you can see the rotation of the units as they're assembled into the filament.

Microfilaments have polarity

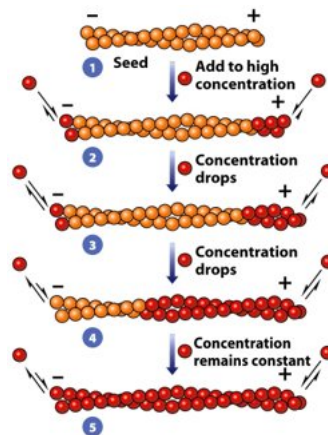
- One of the micro-filaments appears *pointed*, and the other appears *barbed*.
- **Pointed end = minus end**
- **Barbed end = plus end**
- Orientation of the arrowheads formed by actin provides information about direction of the microfilament movement.
- Much like microtubules, motor proteins can 'read' the polarity of the microfilament tract



Microfilament assembly

Actin assembly/disassembly *in vitro* depends upon concentration of actin monomers. Filament assembly leads to drop in ATP-actin. Actin subunits are added to plus end and removed from the minus end (**steady state**). Microfilament cytoskeleton is organized by controlling equilibrium between assembly and disassembly of microfilaments.

- **Step 1:** Add a preformed actin filament to a solution of actin subunits and ATP.
- Yellow beads = filament
- Red beads = actin subunits
- **Step 2:** increased concentration of ATP-actin, actin is added at both ends.
- **Step 3:** Drop in ATP-actin, addition of monomers at plus end (higher affinity for ATP-actin).
- **Step 4:** subunits continually added to plus end, and now being lost from minus end.
- **Step 5:** both ends are now balanced, gaining and losing ATP-actin. Process is called 'treadmilling'.



Myosins are the motor proteins of microfilaments

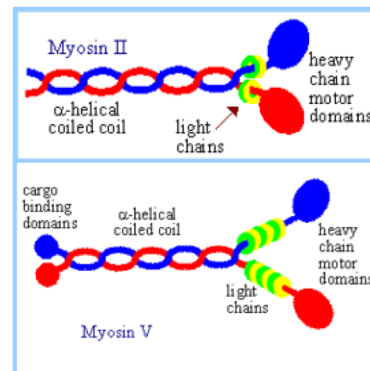
The **myosins** are a large family of motor proteins that move along **actin** filaments, while hydrolyzing ATP.

All myosins share a characteristic motor head for binding actin and hydrolyzing ATP.

The myosin tail is divergent.

Myosins can be divided into two groups:

- **Conventional (type II) myosin**
- **Unconventional myosins (I, V, VI, VII, XV)**



Myosin II includes two heavy chains, each with a globular motor domain that includes a binding site for ATP and a domain that interacts with actin.

Myosin V has two heavy chains like myosin II, but myosin V has a longer neck region

Conventional Type II myosin

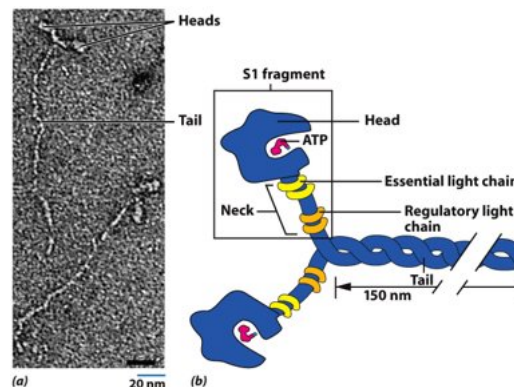
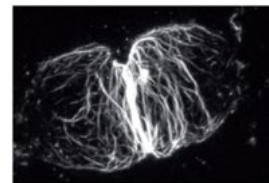
Generates force in muscles and some non-muscle cells.

Required for cell splitting during cytokinesis.

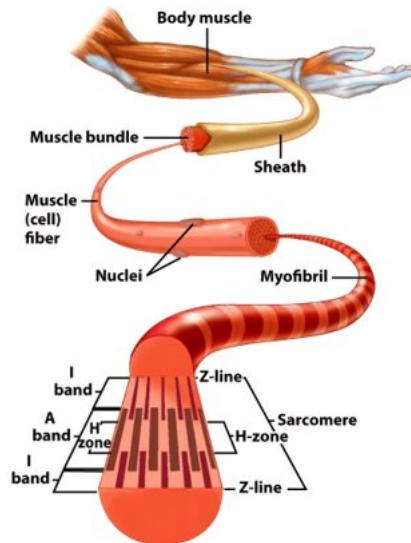
Each myosin II is composed of two heavy chains, two light chains, and two globular heads (catalytic sites).

All of the machinery required for motor activity is contained in a single head.

The tail portion plays a structural role allowing the protein to form filaments.



Muscle anatomy



Muscle as an organ is comprised of many muscle cells (fibers) bundled together.

- Myoblasts are mononucleated muscle progenitor cells
- They fuse together to form polynucleated muscle fibers
- Mostly made of cytoskeletal elements, other organelles pushed to the periphery

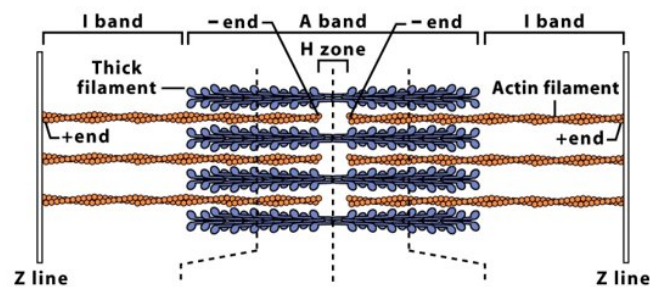
A muscle fiber is composed of multiple **myofibrils**

A myofibril is composed of multiple **sarcomeres**

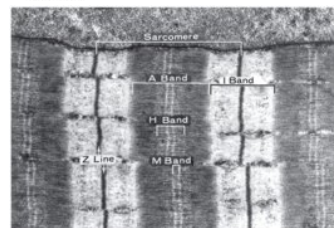
The sarcomere is the functional unit of skeletal muscle

Sarcomere structure

The main proteins in the sarcomere are actin microfilaments and type II myosins.



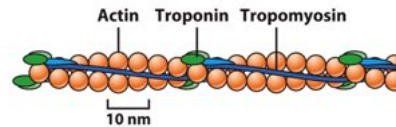
What a stained muscle fiber looks like under a light microscope.



Myofilament structure

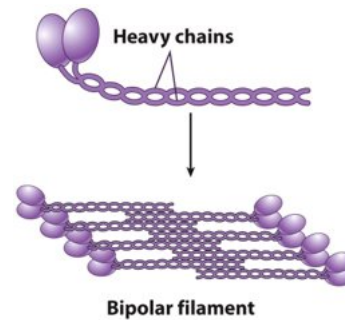
THIN FILAMENT

- **Actin** is the monomer that makes up most of the thin filament
- Helix of actin units
- **Tropomyosin** associated with the helix
- **Troponin** – 3 subunits:
 - Troponin I – binds to actin
 - Troponin T – binds to tropomyosin
 - Troponin C – binds to Ca^{2+}



THICK FILAMENT

- **Myosin II** proteins
- **Bipolar** – each myosin has head and tail regions
- The head has an actin-binding site and an ATP-binding site



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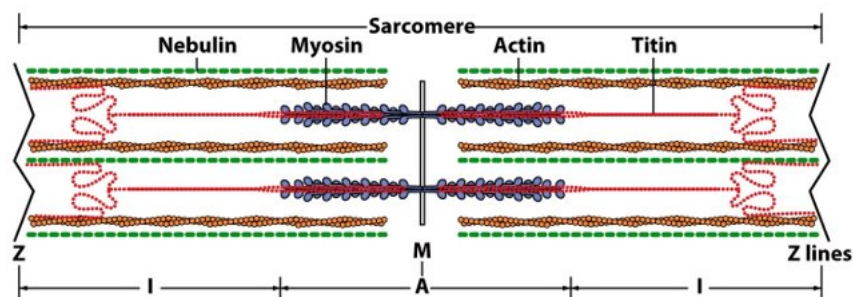
Other proteins of the sacromere

TITIN

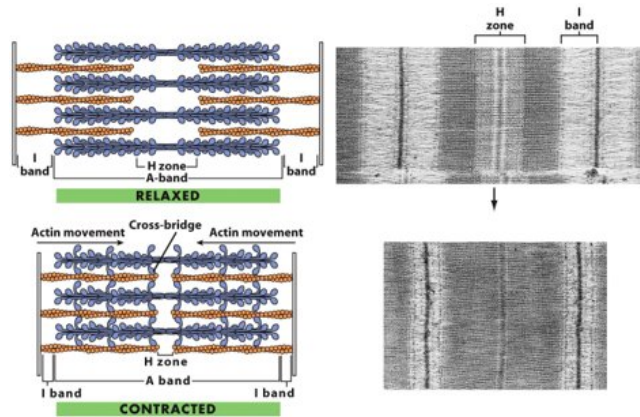
- Largest protein known (3500 kDa)
- Suspends the thick filaments in place
- Elastic – prevents sarcomere from being pulled apart when stretched

NEBULIN

- Binds to the actin of the thin filament
- Thought to influence thin filament length during sarcomere assembly



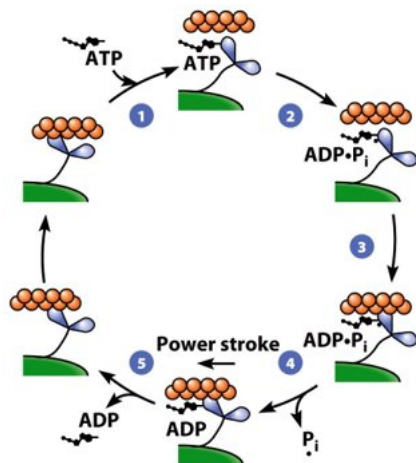
Muscles contract based upon a 'sliding filament' model



When a sarcomere contracts:

- The distance between Z lines *decreases*
- The H zone and I bands *shorten*
- The A band remains the *same* length

Myosin ATP hydrolytic cycle

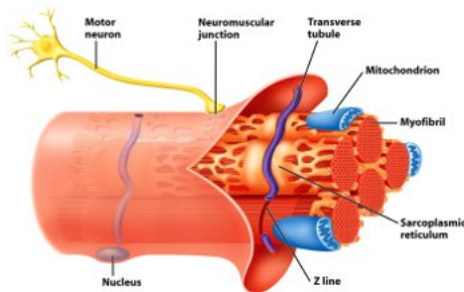


ATP is converted from chemical to physical energy.

1. ATP binds to the myosin head, causing it to detach from the actin
2. ATP is hydrolyzed in the myosin head
3. Energy is used to bind the myosin head with the actin
4. Release of P_i creates a tighter bind and the **power stroke** that moves the thin filament closer to the M line
5. ADP is released

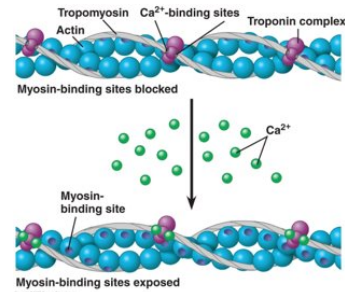
Note that a lack of ATP in a corpse is why *rigor mortis* occurs.

The role of Ca^{2+} in triggering muscle contraction



Remember that the sarcoendoplasmic reticulum Ca^{2+} -ATPase (SERCA) pumps of the SR pumps Ca^{2+} into the SR lumen until it is needed for muscle contraction.

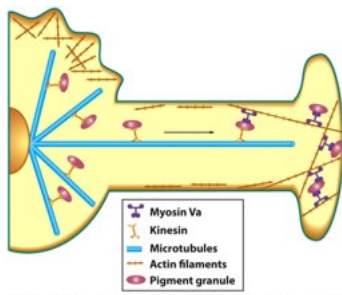
The nerve impulse from the motor neuron goes through the transverse tubules and triggers the release of Ca^{2+} into the muscle fibres.



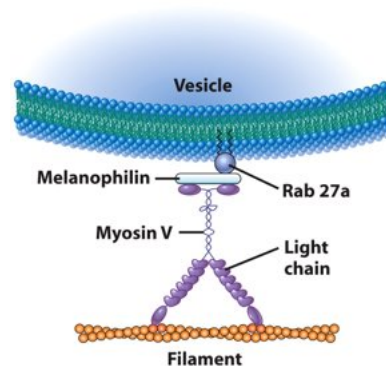
In a resting muscle cell, tropomyosin blocks the myosin binding sites on the actin microfilament. The influx of Ca^{2+} binds to the troponin, causing tropomyosin to move and revealing the binding sites. Myosin can now do its thing and contract the sarcomeres.

Myosin V and vesicle transport

- Myosin V is a dimeric, two headed myosin.
- Length of Myosin V neck is much longer than myosin II and can take large steps in a single direction.
- Walks in a similar fashion as kinesin (processively).
- Rab27A is an adaptor protein linking the molecular motor to the vesicle.



In neurons, myosin V does the 'final delivery' of vesicles filled with neurotransmitters to the axonal terminus.

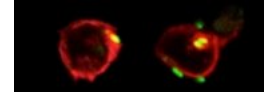
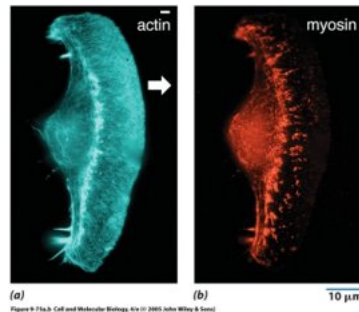


Note that actin is a helix – by stepping every 13th residue, myosin V can walk along one side of the filament

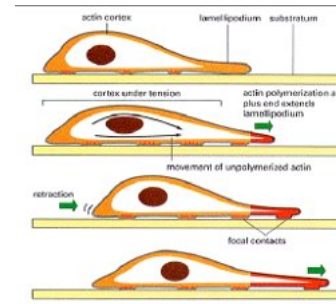
Microfilaments are involved in non-muscle motility

- forming the growing extensions of cells undergoing ingestion (phagocytosis)
- constricting the cell during cytokinesis
- forming the cellular extensions during cell movement:

Note that actin extends to the plasma membrane and myosin is concentrated in a band behind the actin, helping to pull the rest of the cell forward (myosin obviously needs to attach to other cellular components to “pull” them forward)



Dense accumulation of actin at point of formation of phagosome



Growing actin filaments cause lamellipodium growth myosin, attached to trailing ends of actin, pull rest of cell along

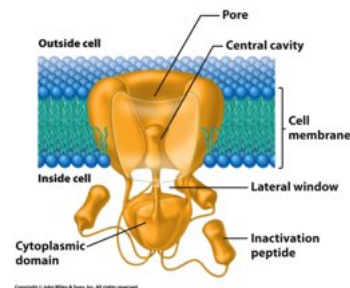
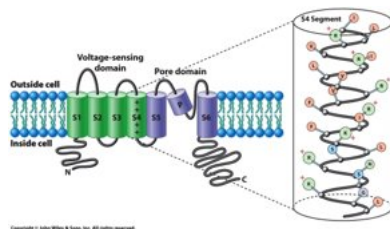
What you should know after this section

- What are the functions of intermediate filaments?
- How does intermediate filament structure and assembly differ from that of structure and assembly of other cytoskeletal fibers?
- How are microfilaments structured and assembled?
- What are the similarities and difference between all of the cytoskeletal structures we've discussed? (MTs, IFs and MFs)
- Describe the structure of muscle fibers, and know which proteins they're made of.
- Describe the sequence of events from the initiation of a nerve impulse to the contraction of muscle fibers.
- Describe the motor proteins that interact with microfilaments in a) intracellular transport and b) muscle contraction

Midterm review: question 2

In the potassium (K⁺) ion channel, which of the following does NOT occur in its reaction cycle?

- a) At rest, the S4 helix keeps the ion channel closed
- b) When a charge state crosses the membrane, S4 moves and open the pore
- c) The pore is open for some time
- d) The S4 helix then immediately moves to block the ion channel
- e) All of the above occurs in the reaction cycle



It's not the S4 helix that immediately blocks the ion channel, but rather the inactivation peptide. The S4 helix does eventually move to block the channel as the protein 'resets'.

A defective MAP is a possible cause of Alzheimer's disease

- Abnormally high phosphorylation of a MAP (specifically, the tau protein) implicated in fatal neurodegenerative diseases like AD; **hyperphosphorylated tau** protein sticks together into neurofibrillary tangles in neurons; causes microtubules to disintegrate;
- short microtubules prevent normal intracellular transport and therefore kill nerve cells, resulting in brain deterioration

