4/23/08  Cytoskeleton

MICROTUBULE MOVIES
http://www.borisylab.northwestern.edu/pages/movies.html
kinesin motors
http://www.proweb.org/kinesin/KinesinMovies.html

Video of mitosis
http://www.mtholyoke.edu/courses/rfink/Videopages/video7.htm

How are chromosomes moved around?
Figure 18-8. The course of mitosis in a typical animal cell. In these micrographs of cultured newt lung cells, the microtubules have been visualized by indirect immunofluorescence, while chromatin is stained with a blue fluorescent dye. During interphase the centrosome, consisting of matrix associated with a centriole pair, forms the focus for the interphase microtubule array. By early prophase the single centrosome contains two centriole pairs (not visible); at late prophase the centrosome divides and the resulting two asters move apart. The nuclear envelope breaks down at prometaphase, allowing the spindle microtubules to interact with the chromosomes. At metaphase the bipolar spindle structure is clear and all the chromosomes are aligned across the middle of the spindle. The paired daughter chromosomes, called chromatids, all separate synchronously at early anaphase and, under the influence of the microtubules, begin to move toward the poles. By late anaphase the spindle poles have moved farther apart, increasing the separation of the two groups of chromosomes. At telophase the daughter nuclei re-form, and by late telophase cytokinesis is almost complete, with the midbody persisting between the daughter cells. (Photographs courtesy of C.L. Rieder, J.C. Waters, and R.W. Cole.)
Fig. 3. Parallel application of targeting methods and fluorophores. HeLa cells transfected with GFP–tubulin and tetracysteine–ß–actin were stained with ReAsH. After fixation, cells were immunolabeled for the Golgi matrix protein giantin with QDs and for the mitochondrial enzyme cytochrome c with Cy5 as indicated. DNA was stained with Hoechst 33342. Images were acquired from Z planes that best represent each structure using excitation and emission wavelengths as indicated. Individual channels are false-colored (middle) and merged (bottom). Scale bars, 20 µm.

<table>
<thead>
<tr>
<th>Excitation (nm)</th>
<th>488</th>
<th>432</th>
<th>568</th>
<th>637</th>
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<td>Emission (nm)</td>
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<td>500-530</td>
<td>555-565</td>
<td>580-620</td>
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<td>GFP</td>
<td>QD565</td>
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<td>nuclei</td>
<td>microtubules</td>
<td>golgi</td>
<td>stress fibers</td>
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Cytoskeleton:

- complex network of protein filaments that extends throughout the cytoplasm
- cytoskeleton is *not* a rigid static skeleton: highly dynamic structure that reorganizes continuously as the cell changes shape, divides and responds to its environment

**Cytoskeleton is involved in**

- maintaining cell shape
- cell motility: movement of the entire cell
- intracellular movement of chromosomes and other cellular components
Three general types of protein filaments that comprise the cytoskeleton

Each type of filament is a polymer of a specific protein subunit

• Linear filaments form via the polymerization of thousands of protein subunits
• the protein subunits are not covalently linked
• since the protein subunits are not covalently linked, the polymers can assemble and disassemble easily
Microfilaments or actin filaments (actin)
- two-stranded helical polymers of the protein actin
- concentrated below the plasma membrane
- diameter 5-9 nm  

**Functions:**
- maintain cell shape by resisting tension (pull)
- motility via muscle contraction or cell crawling,
- involved in cell division in animal
- movement of organelles and cytoplasm
Intermediate filaments (family of related proteins)
• diameter of 10nm (keratin, lamin and others)

**Functions:**
• *maintain cell shape by resisting tension*
• *anchor nucleus and some organelles*
Microtubules (tubulin filaments)
- hollow cylinders of the protein tubulin
- more rigid than actin filaments
- diameter 25 nm

Functions:
- maintain cell shape by resisting compression
- motility of flagella and cilia
- chromosome movement during cell division
- movement of organelles (and pigment granules
- growth of plant cell walls
Microtubule Cytoskeleton Functions

• mitosis- separation of chromosomes

• movement and positioning of organelles

• intracellular movement of material such as pigment granules

• cell movement - such as sperm cell flagella

• movement of particles outside of cells - such as lung cell cilia
Microtubules

- composed of polymerized dimers of $\alpha$ and $\beta$ tubulin

- polymers are not covalently bound
  so they can assemble and disassemble easily

Microtubules are hollow tubes of protein:
hollow cylinder with walls consisting of tubulin subunits

(this is not electric)

Polymerization or growth of polymer is at + end of microtubule

The minus end tends to lose subunits unless stabilized in some way

In most cells the microtubules are stabilized by being embedded
in a structure called the centrosome or MTOC
The structure of a microtubule and its subunits (tubulin heterodimers) (see legend next page)

The red indicates GTP (guanosine triphosphate) – see next page

(A) The subunit of each protofilament is a tubulin heterodimer, formed from a very tightly linked pair of α- and β-tubulin monomers. The GTP molecule in the α-tubulin monomer is so tightly bound that it can be considered an integral part of the protein. The GTP molecule in the β-tubulin monomer, however, is less tightly bound and has an important role in filament dynamics. Both nucleotides are shown in red. (B) One tubulin subunit (α-β heterodimer) and one protofilament are shown schematically. Each protofilament consists of many adjacent subunits with the same orientation. (C) The microtubule is a stiff hollow tube formed from 13 protofilaments aligned in parallel. (D) A short segment of a microtubule viewed in an electron microscope. (E) Electron micrograph of a cross section of a microtubule showing a ring of 13 distinct protofilaments.
Another example of the role of a small molecule modulating protein function

The GTP molecule in the α-tubulin monomer is so tightly bound that it can be considered an integral part of the protein. The GTP molecule in the β-tubulin monomer, however, is less tightly bound and has an important role in filament dynamics.
Figure 17–13 GTP hydrolysis controls the growth of microtubules. Tubulin dimers carrying GTP (red) bind more tightly to one another than do tubulin dimers carrying GDP (dark green). Therefore, microtubules that have freshly added tubulin dimers at their end with GTP bound tend to keep growing. From time to time, however, especially when microtubule growth is slow, the subunits in this “GTP cap” will hydrolyze their GTP to GDP before fresh subunits loaded with GTP have time to bind. The GTP cap is thereby lost; the GDP-carrying subunits are less tightly bound in the polymer and are readily released from the free end, so that the microtubule begins to shrink continuously.
**Cytoplasmic microtubules**

Polymerize from the Microtubule Organizing Center (MTOC) – NOTE that the minus ends are embedded in this structure

MTOC contains a pair of centrioles (aka centrosome): centrioles consist of microtubules which are sites of polymerization

MTOC localized next to the nucleus in an interphase cell

**Dynamic**
- most cytoplasmic microtubules are not stable
- either growing or shrinking
microtubules exhibit dynamic instability:
• microtubules grow by addition of tubulin subunits to the plus end
• then abruptly a microtubule may undergo a transition that causes it to rapidly lose subunits from the plus end

video of dynamic instability:
http://fire.biol.wwu.edu/trent/trent/17.2-MT_instability.mov

this movie is on the CD that comes with your textbook – look at all of the chapter 17 videos

Various proteins affect microtubule dynamics including a group called catastrophe factors (which will be important during anaphase)
The mitotic spindle is a microtubule based machine

Before mitosis, the centrosome is duplicated to give 2 MTOCs

Spectacular videomicroscopy of a fruitfly embryo

http://fire.biol.wwu.edu/trent/trent/19.3-mitotic_spindles_fly.mov

cytoskeleton of a cultured epithelial cell (prophase)

- green = microtubules
- blue = DNA

anaphase stage of mitosis

- blue = DNA
- green = microtubules
- red = microfilaments
The three classes of microtubules of the mitotic spindle in an animal cell

- **Kinetochore MTs** – connect to the kinetochores of the sister chromatids
- **Interpolar MTs** – from opposite poles interdigitate at the equator of the cell
- **Astral MTs** – help position spindle in the cell

Motor proteins will be introduced later
First: grab the plus end of a microtubule

Each sister chromatid has a microtubule capture device called a kinetochore which is associated with the centromere of chromosome

Kinetochores bind to growing microtubules that are spooled out from the MTOCs
Kinetochores on the sister chromatids are oriented opposite of each other which increases the likelihood that they will attach to MT coming from opposite poles.

Towards the end of prophase the nuclear membrane breaks down – microtubules can now contact the kinetochores on the sister chromatids.
In order to get the sister chromatids into different daughter cells, the sisters must attach to microtubules coming from opposite sides of the cell.
Since the sister chromatids are oriented opposite of each other they will tend to attach to opposite poles **BUT attachment is random and bi-orientation achieved by trial and error**

**Incorrect attachments are highly unstable and do not last while correct attachments are locked into place**

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![Diagram showing alternative forms of spindle fiber attachment](image-url)
How is a correct set of attachments sensed?

A current model of this process suggests that the sensor is that an equal and opposite tension across the kinetochore which stabilizes microtubule attachment:

*aurora* is a protein that is involved in sensing tension across the kinetochores and generating an inhibitory signal that reduces the strength of the microtubule attachment in the absence of tension (left).  see your text for info on cohesion
At anaphase, the sister chromatids synchronously separate to form two daughter chromosomes, and each is pulled slowly toward the spindle pole it faces. The kinetochore microtubules get shorter, and the spindle poles also move apart; both processes contribute to chromosome segregation.
Forces that move chromosomes around during metaphase and anaphase

1. and I quote: “By an uncertain mechanism, depolymerization at the plus end of the microtubule somehow generates a force that pulls the kinetochore poleward” during anaphase

2. Chromosomes are also “reeled in at minus end by depolymerization” (loss of tubulin subunits) during anaphase
and I quote: “By an uncertain mechanism, depolymerization at the plus end of the microtubule somehow generates a force that pulls the kinetochore poleward”

A microtubule attachment site in a kinetochore. Each site is thought to contain a collar structure (yellow) that surrounds the microtubule plus end, allowing polymerization and depolymerization to occur at the exposed plus end while the microtubule remains attached to the kinetochore.

How depolymerization may pull the kinetochore toward the spindle pole. When depolymerization occurs, the protofilaments of the microtubule curl outward (see Figure 16-16) and push against the collar structure that surrounds the microtubule plus end. In principle, this will move the kinetochore toward the microtubule minus end at the spindle pole.
The major forces that separate sister chromatids at anaphase in mammalian cells. Two separate forces are thought to be responsible for anaphase B: the elongation and sliding of the interpolar microtubules past one another in the central spindle push the two poles apart, and motor proteins attached to the plasma membrane near each spindle pole act on astral microtubules to pull the poles away from each other, toward the cell surface.

- **Spindle pole separation depends on molecular motors**
- **The sliding of the polar microtubules is dependent on molecular motors**
MICROTUBULE MOTORS CAN WALK ALONG MICROTBULES

Need to add the another player to the picture
Convert energy from ATP hydrolysis to movement

http://aimediaserver.com/studiodaily/harvard/harvard.swf
Microtubule motors can move from $+\ to\ -$ or from $-\ to\ +$ on a microtubule

**Kinesin** (one type of molecular motor) minus end to plus end movement on microtubules

**Dynein** (another type of molecular motor) plus end to minus end movement on microtubules
Energy source drives change in conformation of the kinesin subunits

Adenosine triphosphate (ATP)
Figure 17–22 A single molecule of kinesin moves along a microtubule.
(A) Electron micrograph of a single kinesin molecule showing the two head domains (red arrows). (B) Three frames, separated by intervals of 1 second, record the movement of an individual kinesin-GFP molecule (green) along a microtubule (red) at a speed of 0.3 µm/sec. (C) Series of molecular models of the two heads of a kinesin molecule, showing how they are thought to processively walk their way along a microtubule in a series of 8 nm steps.

kinesin animation -- kinesin moving along a microtubule
http://www.scripps.edu/milligan/projects.html
With respect to mitosis **MOLECULAR MOTORS**:

*Position the centrosomes during prophase*

*Push chromosome arms towards the metaphase plate during prometaphase/metaphase* (plus-end directed motors)

*Move polar spindle fibers apart during anaphase:*
*Consider: one of the MTs is the track and the other is the cargo* (red motors below)

- which type of motor (plus or minus end-directed) would move the poles apart?
- Which type of motor would bring the poles closer together?
Molecular MOTORS are involved in some way (not clear) with kinetochore motility

- (minus-end directed) motor proteins may also drives chromosome poleward
Here is another cargo-carrying, motor-protein-filament system:

*Myosin* (green), a cellular motor protein, carries cargo within cells by moving along *actin* filaments. It takes 37 nanometer "steps" by placing one foot over the other.

Actin and myosin drive comprise the contractile system in muscles.
This month's winning image was submitted by Paul Andrews (University of Dundee (p.d.andrews@dundee.ac.uk)). It shows two telophase cells from a stable HeLa cell line expressing green fluorescent protein (GFP)-tagged human Aurora B. Microtubules are shown in red, INCENP in blue, Aurora B–GFP in green and DNA in white. The image is a maximum-intensity projection of a deconvolved 3D data set acquired on a DeltaVision Restoration microscope (Applied Precision LLC). Bar, 5 μm.
pigment granule aggregation

Current Biology 8: R186
Frog skin cell
green: tubulin   red: pigment granules

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