Mitosis vs. microtubule

**Interphase**
- Chromosome duplication and cohesion
- Centrosome duplication

**Prophase**
- Breakdown of interphase microtubule display and its replacement by mitotic asters
- Mitotic aster separation
- Chromosome condensation

**Prometaphase**
- Nuclear envelope breakdown
- Chromosomes captured, bi-oriented and brought to the spindle equator

**Metaphase**
- Chromosomes aligned at the metaphase plate

Figure 18-34a part 1
Molecular Cell Biology, Sixth Edition
© 2008 W. H. Freeman and Company
Anaphase-promoting complex/cyclosome (APC/C)

- **Anaphase**
  - APC/C activated and cohesins degraded
  - Anaphase A: Chromosome movement to poles
  - Anaphase B: Spindle pole separation

- **Telophase**
  - Nuclear envelope reassembly
  - Assembly of contractile ring

- **Cytokinesis**
  - Reformation of interphase microtubule array
  - Contractile ring forms cleavage furrow

*Figure 18-34a part 2
Molecular Cell Biology, Sixth Edition
© 2008 W. H. Freeman and Company*
Duplicated centrosomes align and begin separating in prophase

Relation of centrosome duplication to the cell cycle

Parent centrioles

Daughter centrioles

Grow complete

G1 Phase → 1st growth phase
S Phase → DNA duplicated
G2 Phase → Final growth phase
Mitosis
Cytokinesis
The mitotic spindle contains three classes of microtubules:

1. Astral microtubule, from spindle poles to the cell cortex
2. Kinetochore microtubule, attaches to chromatid
3. Polar microtubule, pushing the duplicated centrosomes, and maintaining the structure
Electron microscopy visualizes components of the mitotic apparatus in a metaphase mammalian cell.

Chromosomes align at the equatorial plane.

Prophase signals must convert interphase array to mitotic apparatus - increase in short dynamic microtubules - **mitotic MT turnover 5-10 fold faster** than interphase MT - less polymer and more monomer tubulin during M phase than at other times.
Shortening at the (+) end of kinetochore MTs moves chromosomes poleward in anaphase A.

Shortening at + end (attach kinetochores) of microtubule by disassembly.
In vivo fluorescent-tagging experiment
Kinetochore associated kinesin, MCAK $\rightarrow$ promotes disassembly at the + end, CENP-E (also at kinetochore) binds to progressively shortening end.
Chromosome move toward to “-”

Shortening at the + end of kinetochore microtubules moves chromosomes poleward in anaphase A.
Microtubule dynamics increase dramatically in mitosis

XMAP215 (xenopus MAP of 215 kDa): stabilizing microtubule

Only kinesin-13 $\rightarrow$ unstable

Microtubule dynamic increases in mitosis due to loss of a stabilizing MAP
Microtubules treadmill during mitosis

GFP-tubulin

Different colors were shown different velocity
Microtubules in mitosis treadmill toward the spindle poles
The kinetochore captures and helps transport chromosomes

Kinetochore is a centromere (著絲點)-based protein complex that mediates attachment of chromosomes to MTs.

Centromere: a constricted region of the condensed chromosome defined by centromeric DNA
Kinetochore is a centromere-based protein complex that mediates attachment of chromosomes to MTs.

**MACK:** plus ends of spindle microtubules attach to chromosomes

**CENP-E:** keeps the kinetochore tethered to the kinetochore microtubule

**CENP-E**

**MCAK**

**Dynein**

**Fibrous corona**

**Kinetochore microtubule**

**Cytosol**
Spindle poles (星狀體)
→ Microtubule very dynamic → chromosomal attachment (1a, 1b) → capture by kinetochores selectively → motor protein (dynein/dynactin) → move toward to spindle pole (-), 2 → chromosome pair bi-oriented (3) → two kinetochores opposite → pulled and separate

PUSH AND PULL

Disassembly >> assembly
Chromosome movement and spindle pole separation in anaphase

Anaphase A moves chromosomes to poles by microtubules shorting

Anaphase B separates poles by the combined action knesins and dynein

Chromosome movement is powered by microtubule-shorting kenesin-13 at kinetochore and spindle pole

Chromosome arms point away spindle pole due to chromokinesin/kinesin-4 → depolymerization force → overcome the force pulling the arms toward the center of spindle

Anaphase B:
1. sliding of antiparallel polar microtubule powered by kinesin-5
2. dynein/dynactin located at cell cortex
Additional mechanisms contribute to spindle formation

Centrosomes is not the only way a spindle can form
Other factor cooperate to make a spindle.

Mitotic spindles can form in the absence of centrosome
Plant cells reorganize their MTs & build a new cell wall in mitosis.

Interphase plant cells: lack a single perinuclear microtubule-organizing center

Similar to animal:

Prophase: Bundle their cortical microtubules and reorganize, without centrosomes

Metaphase: *golgi-derived vesicles* are transported into the mitotic apparatus along microtubules

Telophase: vesicles line up near the center of the dividing cell and to form the phragmoplast (microtubule formation), a membrane structure similar to animal cell contractile ring → the plasma membrane of daughter cells; vesicles contains cellulose pectin for cell wall
Figure 18-43
Molecular Cell Biology, Sixth Edition
© 2008 W.H. Freeman and Company
Intermediate Filaments (IF)

Differ in stability, size, and structure from other cytoskeletal fibers

Intermediate diameter ~10 nm

Subunits are fibrous

Almost all subunits are incorporated into stable intermediate filaments

No hydrolysis of ATP or GTP is required for polymerization

No known polarity of the filament

The formed fibers are not easily soluble

No direct participation in cell motility

Two types of intermediate filaments

Lamin intermediate filaments: blue; nucleus

Cytoplasmic keratin cytoskeleton: red
Intermediate filament assembly

spontaneous assembly

→ No need of chaperone proteins or energy (no hydrolysis of nucleotides) actin polymerization need energy

the filament has no polarity ≠ from actin or microtubule filaments

Intermediate filaments: cytoplasmic and nuclear
non-polar, tough, rope-like, less than 5% in soluble form, no nucleotide

provide protection against mechanical stress,
withstand stretching forces

IF protein-specific antibodies or cDNA used for cell typing and tumour diagnosis

REGULATION:
phosphorylation by PKC of the N-terminal Ser induces disassembly of IF
(particularly in nuclear lamins during mitosis)

IF associated proteins (IFAPs)
All IF proteins have a conserved core domain & are organized similarly into filaments.
<table>
<thead>
<tr>
<th>CLASS</th>
<th>PROTEIN</th>
<th>DISTRIBUTION</th>
<th>PROPOSED FUNCTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Acidic keratins</td>
<td>Epithelial cells</td>
<td>Tissue strength and integrity</td>
</tr>
<tr>
<td>II</td>
<td>Basic keratins</td>
<td>Epithelial cells</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>Desmin, GFAP, vimentin</td>
<td>Muscle, glial cells, mesenchymal cells</td>
<td>Sarcomere organization, integrity</td>
</tr>
<tr>
<td>IV</td>
<td>Neurofilaments (NFL, NFM, and NFH)</td>
<td>Neurons</td>
<td>Axon organization</td>
</tr>
<tr>
<td>V</td>
<td>Lamins</td>
<td>Nucleus</td>
<td>Nuclear structure and organization</td>
</tr>
</tbody>
</table>
Keratins: epithelial
Vimentin is the major IF in cells of mesenchymal and neuronal origin

Glial Fibrillary Acidic Protein forms IF in glial cells and some Schwann cells

Peripherin is a rare IF, occurring in some types of neurons

Desmin is the predominant IF in skeletal and cardiac muscle sarcomers and in smooth muscle myofibrils
Intermediate filaments are anchored in cell junctions.

Figure 16–18. Molecular Biology of the Cell, 4th Edition.

Intermediate filaments are resistant to bending or stretching forces

staggered long subunits: lateral contacts dominate

Intermediate filaments are dynamic.

(a) 20 minutes after injection

(b) 4 hours after injection

Figure 18-46
Molecular Cell Biology, Sixth Edition
© 2008 W.H. Freeman and Company
Disruption of keratin networks causes blistering.

Epidermis

Dermis

Normal mouse

Keratin gene mutant
Separation between epidermis and dermis

Mutated
Blistering of the skin caused by mutant keratin genes

Epidermolysis bullosa simplex EBS: the skin blisters in response to very slight mechanical stress

Other blistering diseases:
mouth, esophageal lining and cornea of the eye--mutations of different keratins

Truncated keratin (missing both the N- C- domains) Tg mice

Figure 16–19. Molecular Biology of the Cell, 4th Edition.
Disruption of a single keratin filament can create fragile epithelia that is genetically inherited.

Epidermolysis Bullosa Simplex (EBS)
- a single point mutation in keratin 14 or in keratin 5
- results in change in amino acid composition
- produces a conformational change in protein structure
- results in incomplete keratin heterodimerization
- produces weak protein in basal cells
- a little friction produces blisters

Glutamine to Lysine
IFAPs cross-link IFs to one another and to other cell structures (microtubules, actin filaments, membranes).

Intermediate filament associated protein (IFAPs): cross link intermediate filaments with one another, forming a bundle or a network and with other cell structures, including the plasma membrane.

Fibroblast cell. Microtubules are red, intermediate filaments-blue, short connecting fibers is green.
Cdc42 coordinates microtubules and microfilaments during cell migration

Figure 18-49
Molecular Cell Biology, Sixth Edition
© 2008 W.H. Freeman and Company
Chapter 20 summary

I. Microtubule structure
   1. tubulins and microtubule structure
   2. Microtubule-organizing center (MTOC)

II. Microtubule dynamics & associated proteins
   1. Assembly/disassembly of microtubule
   2. Dynamic instability
   3. Temperature influences microtubule stability
   4. Drugs involved in microtubule dynamics
   5. Microtubule associated protein (MAP)

III. Motor proteins and intracellular transport
   1. Motor proteins
      --microtubule motor proteins: Kinesin family, Dynein family
   2. Multiple motor proteins are associated with membrane vesicles
IV. Microtubules & motor proteins during mitosis

1. Mitotic apparatus
2. Centromere and kinetochore
3. Centrosome duplication
4. Microtubule dynamics during mitosis
5. Centrosome movement during mitosis
6. Formation of spindle poles and capture of chromosomes
7. Chromosome separation & spindle elongation
8. Cytokinesis