(3) Types of cytoskeletal fibers

Microtubule

Actin filament

Intermediate filament

Fibers made of polymerized protein
Cell Shape and Transportation:

<table>
<thead>
<tr>
<th>Microtubule polarity in cells</th>
<th>Fibroblast</th>
<th>Epithelial cell</th>
<th>Neuron</th>
</tr>
</thead>
</table>

(Cells, Figure 7.14)

Organization of the Cell

Organelles are not passive blobs?

(b) Eukaryotic cell
Do these organelles move on microtubules?

A snapshot

(Figure 9.2)
Can we stop it?

Drugs can be used to study the functions of Microtubules

Nocodazole

Taxol

What is the structure?

(13) protofilaments align to form a hollow tube

GTP/GDP

Figure 9.9
What is the structure? Microtubules are polymers

How can we see this?

Self-assembly into polymer/microtubule

Microtubule Organizing Center (MTOC)

Centrosome

PCM

Centrioles
Transportation and Polarity:

<table>
<thead>
<tr>
<th>Microtubule polarity in cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibroblast</td>
</tr>
<tr>
<td>Epithelial cell</td>
</tr>
<tr>
<td>Neuron</td>
</tr>
</tbody>
</table>

Experiment to tell you where the MTOC is?

Colcemid washout experiment

Figure 9.19
(13) protofilaments align to form a hollow tube = microtubule
lateral bonds give tubule strength
(13) protofilaments align to form a hollow tube = microtubule. Lateral bonds give tubule strength.

Microtubule Assembly
Where are subunits added?

Injected with biotin-Tubulin (1 minute)

FRAP
FRAP:

Can tell you about the dynamics of molecules in the polymer

We can watch MT dynamics

Injected with rhod-Tubulin
Dynamic Instability (Speckles)

Inject with GFP-Tubulin

GTP hydrolysis changes conformation and stability of MTs
(+)-end cap regulates stability of MT.

**GTP-cap:**
- GTP-bound structure is different than GDP-bound.

GTP-bound structure is different than GDP-bound.

**Stability of microtubules**

1) GTP vs GDP bound cap

2) Microtubule associated proteins (MAPs)
Stability of microtubules

1) GTP vs GDP bound cap

2) Microtubule associated proteins (MAPs)


http://jcs.biologists.org/content/123/20/3415.full?sid=f97d0f32-c919-4739-a062-0d83b8e8f68b
MAPs can destabilize MTs

How do (+) TIPs stabilize the (+) tip?

XMAP215
CLIP170 (function)
Role of the Cytoskeleton and Motor Proteins in Membrane Trafficking

General principle:
Long-distance transport occurs on MTs
Short-distance on Actin
Transportation and Polarity:

<table>
<thead>
<tr>
<th>Microtubule polarity in cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibroblast</td>
</tr>
<tr>
<td>Epithelial cell</td>
</tr>
<tr>
<td>Neuron</td>
</tr>
</tbody>
</table>

(Cells, Figure 7.14)

Figure 9.13
Which direction do mitochondria travel on microtubules?

Microtubule motor proteins

MOSTLY Plus-end directed motors----kinesins

Minus-end directed motors----dyneins
Motors “walk” along MTs towards the plus or minus end.

dyneins (-, only)  kinesins (+, mostly)
How cargoes are loaded onto motors:

Tail domain binds cargo via adaptor protein AP-1 vesicle

Kinesin:

<table>
<thead>
<tr>
<th>Heads</th>
<th>Neck</th>
<th>Stalk</th>
<th>Tail</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>80 nm</td>
</tr>
<tr>
<td>Side view</td>
<td></td>
<td></td>
<td>16 nm</td>
</tr>
<tr>
<td>Top view</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
What can measure from this movie?

Watching Kinesin-1 move –speed, processive

Figure 9.6
How do you determine which motor is involved?

Motors “walk” along MTs towards the plus or minus end

- dyneins (-, only)
- kinesins (+, mostly)
kinesins:
-MT (+) end
-motor domain at N-terminus
-ATP-dependent

dyneins:
-MT (-) end
-motor domain at C-terminus
-cytoplasmic form (homodimer)
-ATP-dependent

Ron Vale and colleagues
Key to Cytoskeletal Functions

1. Structure and Support
2. Intracellular Transport
3. Contractility and Motility
4. Spatial Organization

(a) Epithelial cell

(b) Nerve cell

(c) Dividing cell

Motor protein
Intermediate filament
Microtubule

Actin filaments

1 Structure and Support
2 Intracellular Transport
3 Contractility and Motility
4 Spatial Organization
General principle 2: Large structural changes occur with microtubules, small changes are with actin filaments

Jonathan Friedman
Role of the Cytoskeleton and Motor Proteins in Membrane Trafficking

General principle 1: long-distance transport occurs on microtubules, short distance on actin filaments

Actin filaments
building block = actin

Functions: structural support, contraction, migration
Rate and direction of growth depends on free actin concentration

Critical concentration
Regulation by actin-binding proteins

Actin polymerization

The ARP 2/3 complex and formins nucleate different actin arrays

<table>
<thead>
<tr>
<th>ARP2/3</th>
<th>Formins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actin monomers</td>
<td>ARP2/3 complex recruits actin monomers</td>
</tr>
<tr>
<td>ARP2/3 complex</td>
<td>Formin recruits actin monomers</td>
</tr>
<tr>
<td>Complex binds to actin filament</td>
<td>Barbed end</td>
</tr>
<tr>
<td>Elongation occurs at the barbed (+) end</td>
<td>Elongation occurs at the barbed (+) end</td>
</tr>
<tr>
<td>Barbed (+) end</td>
<td>Barbed (+) end</td>
</tr>
<tr>
<td>70° Pointed (-) end</td>
<td></td>
</tr>
</tbody>
</table>
Proteins that regulate actin polymerization

Therefore, the direction of growth is regulated

Proteins that depolymerize actin filaments
Small G protein activation regulates actin organization

- Rac
- Rho
- Cdc42

- lamellipodia
- Stress fibers
- Filopodia

Actin

Nerve cell

Dividing cell
Actin

(a) Dorsal Ectoderm
   Ventral

(b) Neural plate

(c) Neural tube

(d) Microtubules

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Directed cell motility

Figure 9.71

Actin

- Myosin Va
- Kinesin
- Microtubules
- Actin filaments
- Pigment granule
Myosin: variety of tail domains

Structure of myosin proteins

- Binds tightly in the absence of ATP
- ATP hydrolysis - power stroke - lever arm
Regulation of myosin by phosphorylation

Rab proteins on vesicles are linked to cytoskeleton
Also regulated by GTP-Rab state
Myosin: variety of tail domains
Myosin: mostly (+) end directed

Kinesin

(b) Courtesy Hugh Huxley

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Heavy chains

Bipolar filament

Courtesy Hugh Huxley
Myosin: variety of tail domains

Myosin II

Figure 9.49

Kinesin

Myosin: mostly (+) end directed
Myosin: variety of tail domains

Regulation of myosin by phosphorylation
The Cytoskeleton: Intermediate Filaments
<table>
<thead>
<tr>
<th>IF protein</th>
<th>Sequence type</th>
<th>Primary tissue distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Keratin (acidic)</td>
<td>I</td>
<td>Epithelia</td>
</tr>
<tr>
<td>(28 different polypeptides)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Keratin (basic)</td>
<td>II</td>
<td>Epithelia</td>
</tr>
<tr>
<td>(26 different polypeptides)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vimentin</td>
<td>III</td>
<td>Mesenchymal cells</td>
</tr>
<tr>
<td>Desmin</td>
<td>III</td>
<td>Muscle</td>
</tr>
<tr>
<td>Glial fibrillary acidic protein (GFAP)</td>
<td>III</td>
<td>Astrocytes</td>
</tr>
<tr>
<td>Peripherin</td>
<td>III</td>
<td>Peripheral neurons</td>
</tr>
<tr>
<td>Neurofilament proteins</td>
<td>IV</td>
<td>Neurons of central and peripheral nerves</td>
</tr>
<tr>
<td>NF-L</td>
<td>IV</td>
<td>Neurons of central and peripheral nerves</td>
</tr>
<tr>
<td>NF-M</td>
<td>IV</td>
<td>Neurons of central and peripheral nerves</td>
</tr>
<tr>
<td>NF-H</td>
<td>IV</td>
<td>Neurons of central and peripheral nerves</td>
</tr>
<tr>
<td>Nestin</td>
<td>IV</td>
<td>Neuroepithelial</td>
</tr>
<tr>
<td>Lamin proteins</td>
<td>V</td>
<td>All cell types</td>
</tr>
<tr>
<td>Lamin A</td>
<td>V</td>
<td>(Nuclear envelopes)</td>
</tr>
<tr>
<td>Lamin B</td>
<td>V</td>
<td></td>
</tr>
<tr>
<td>Lamin C</td>
<td>V</td>
<td></td>
</tr>
</tbody>
</table>


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The Cytoskeleton: Intermediate Filaments

epidermolysis bullosa simplex

Mutant  Normal
Keratins form Intermediate Filaments that organize tissues.

Intermediate filaments
building block = variable
(keratin, vimentin, nuclear lamins, others)

Functions: mechanical integrity of tissue, cell, or subcellular organelle
Laminopathies (HGPS, progeria)

Some IFs are found in all cells. Nuclear lamins, a special type of IF, form a basket underlying the nuclear membrane, giving it strength and organization.

*Phosphorylated at head and tail during mitosis*
From Werner W. Franke, et al., J. Cell Biol. 91:475, Figure 8, 1981.
Reproduced with permission of Rockefeller University Press.

From H. Ma, A.J. Siegel, and R. Bereczky, J. Cell Biol. 146:535, Figure 2, 1999.
Reproduced with permission of The Rockefeller University Press.
Cell Cycle NE/Lamin Changes

Regulation of Nuclear Lamins

CDK1/cyclinB (kinase)

LBR

Lamin B

PP1 (phosphatase)
Phosphorylation of Tyrosine Residues

![Diagram](http://www.soi.wide.ad.jp/class/20050028/slides/03/img/19.png)