Lecture 10:
The Cytoskeleton (II): Microtubule & Intermediate Filament
The Cytoskeleton (III): Molecular Motors
Outline

- Summary: actin and its associated proteins
- Microtubule and its associated proteins; Centrosome
- Intermediate filament and its associated proteins
- An overview of molecular motors
Outline

• Summary: actin and its associated proteins
  • Microtubule and its associated proteins; Centrosome
  • Intermediate filament and its associated proteins
  • An overview of molecular motors
### Overview of Cytoskeletal Filaments

<table>
<thead>
<tr>
<th>Shape</th>
<th>Diameter</th>
<th>Subunits</th>
<th>Polarized</th>
</tr>
</thead>
<tbody>
<tr>
<td>actin cable</td>
<td>~6 nm</td>
<td>actin monomer</td>
<td>yes</td>
</tr>
<tr>
<td>microtubule tube</td>
<td>~25nm</td>
<td>tubulin heterodimer</td>
<td>yes</td>
</tr>
<tr>
<td>intermediate rope</td>
<td>~10nm</td>
<td>Various dimers</td>
<td>no</td>
</tr>
</tbody>
</table>
Summary: Actin and its Associated Proteins (I)

- Actin is relatively soft (we will study related quantification in later lectures).

- Actin often form bundles; their mechanical strength comes mostly from bundling and crosslinking.

- Actin function mostly to withstand tension rather than compression.

- Actin is relatively stable and easy to work with biochemically.
Summary: Actin and its Associated Proteins (II)

• Different actin associated proteins serve a broad range of functions.

• These proteins generally have multiple functional domains serving multiple functions.

• Some but not all of them are essential.

• Most of the proteins have functional overlap.

• Mathematical models are required to understand complex interactions between these proteins.
Outline

- Summary: actin and its associated proteins
- Microtubule and its associated proteins; Centrosome
- Intermediate filament and its associated proteins
- An overview of molecular motors
Overview of Microtubule Structure

• Microtubule is polarized.
  - plus (β-tubulin) end
  - minus (α-tubulin) end

• The outer diameter of a microtubule is ~25 nm.

• A microtubule typically has 13 protofilaments; Some may have 11, 15, or even 16.

• The GTP bound to the α-tubulin monomer does not hydrolyze.
Microtubule Organization at Different Stages

- **Microtubule in interphase**
  - Organized into a radial pattern centered at the centrosome

- **Microtubule in metaphase**
  - Organized into a bipolar architecture

Fig. 34-1
# Microtubule Dynamic Instability (I)

**Video 1**  
(Figure 1A)  
Microtubules in a PtK1 cell at the edge of an epithelial cell island. Few microtubules rapidly grow into nascent protrusions.  
Elapsed time: 9 min 05 sec

Microtubule Dynamic Instability (II)

- Rapid growth with GTP-capped end
- Accidental loss of GTP cap (CATASTROPHE)
- Rapid shrinkage
- Regain of GTP cap (RESCUE)
- Rapid growth with GTP-capped end
- Etc.

GTP cap

Less stable region of microtubule containing GDP-tubulin dimers

Growing

Shrinking

50 nm
Microtubule Treadmilling vs Dynamic Instability

## Table 1. Comparison of the parameters of microtubule dynamics in vitro and in vivo. Dashes indicate that no microtubules were formed. NA, not applicable.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>In vitro (25 μM tubulin)</th>
<th>In vivo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tubulin alone</td>
<td>+0.8 μM XMAP215</td>
</tr>
<tr>
<td>Growth ($V_g$) (μm/min)</td>
<td>2.56 (±0.75)</td>
<td>6.76 (±1.76)</td>
</tr>
<tr>
<td>Shrinkage ($V_s$) (μm/min)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Catastrophe ($f_{cat}$) (events/min)</td>
<td>0.04</td>
<td>0</td>
</tr>
<tr>
<td>Rescue ($f_{res}$) (events/min)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Xenopus egg extracts | Newt lung cells§ | Mammalian LLCPK-1 cells§ |
Interphase* | Mitotic† | Interphase | Mitotic |
7.10 | 11.40 | 7.20 | 11.50 | 12.80 |
9.40 | 13.50 | 17.30 | 13.10 | 14.10 |
0.69 | 2.44 | 0.84 | 1.56 | 3.48 |
1.08 | 0.70 | 2.64 | 10.50 | 2.70 |

*Verde et al. (9). †Toumebize et al. (10). ‡Cassimeris et al. (5). §Rusan et al. (7).
Overview of Microtubule Associated Proteins

A. Stabilizing
- Minus-end binding: γ-tubulin ring complex
- Side binding: MAP2, tau
- Plus-end binding: Capping (STOP)
  - TIPS (CLIP170, EB1)
  - Dis1/TOG
- Selenium: Katanin
- Plus-end binding: Nucleoplasmin (Nilsen=13)
- Dimer binding: Stathmin

B. Destabilizing
- Filament bundling and cross-linking with tau and MAP2
- Link to intermediate filaments

gamma-tubulin: nucleates assembly and remains associated with the minus end
microtubule: grows as barbed end binds to plus ends
microtubule: severs microtubules
Katanin: stabilizes microtubules
MAP2: stabilizes plus ends and accelerates assembly
tau: remains associated with growing plus ends and can link them to other structures, such as membranes
Katanin: severing catastrophe
Dis1/TOG: Destabilization of microtubules
Stathmin: Destabilization of microtubules

Tubulin dimer
(+) Tubulin dimer
Microtubule Associated Proteins (I)

• Stabilizing MAPs
  - Tau
  - MAP2, MAP4

• Destabilizing MAPs
  - Op18/stathmin
  - Katanin
  - XKCM/MCAK
Microtubule Associated Proteins (II)

Frozen deep-etched, shadowed microtubules with (upper) and without (lower) tau

Fixed, embedded sections of microtubules with (upper) and without (lower) MAP2
Microtubule Associated Proteins (III): Tau

• CLIP170 is the first microtubule plus end tracking protein identified.
  → Links membranes to growing plus ends.
  → Binds the microtubule plus end to reduce catastrophe.

• EB1 is another microtubule plus end tracking protein identified.
  → Binds the microtubule plus end to reduce catastrophe.

Summary: Microtubule

• Microtubule is relatively rigid (quantification in following lectures).

• Microtubule often forms bundles. Mechanical strength of microtubule networks comes mostly from bundling and crosslinking.

• Microtubule can withstand compression.

• Microtubule can denature easily and is therefore difficult to work with biochemically.
Centrosome and Centrioles

A. FIBROBLAST

B. NEURON

C. Centrosome organization

- Distal appendage
- Subdistal appendage
- Ninein, centriolin
- Pericentriolar material (PCM)
- γ-tubulin ring complexes
- pericentrin, GMAP210
- microtubule nucleation

γ-tubulin’s lateral interactions resemble those between subunits in MT

β-tubulin
α-tubulin
SPC57 and SPC98 homologues
Other γ TuRC proteins

Centriole

Pericentriolar material (PCM)

Daughter centriole
Centrioles can Form Basal Bodies for Cilia & Flagella

A. Flagella
- Flagella motion
- Cell motion

B. Cilia
- Cilia motion
- Effective stroke
- Recovery stroke
- Cell motion

C. Metachronal wave

Paramecium

10 µm
Outline

• Summary: actin and its associated proteins

• Microtubule and its associated proteins; Centrosome

• Intermediate filament and its associated proteins

• An overview of molecular motors
Intermediate Filament

- So named because of its diameter in striated muscles (diameter ~10nm).
- Its core structure is an \( \alpha \)-helical coiled coil.
- N- and C-terminal domains vary considerably in size.
# Intermediate Filament Classification

<table>
<thead>
<tr>
<th>Class</th>
<th>Type</th>
<th>Genes</th>
<th>Molecule</th>
<th>Distribution</th>
<th>Diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Acidic keratin</td>
<td>&gt;15</td>
<td>40–65 kD, obligate heterodimer with class II</td>
<td>Epithelial cells</td>
<td>Blistering skin, corneal dystrophy, brittle hair and nails</td>
</tr>
<tr>
<td>II</td>
<td>Basic keratin</td>
<td>&gt;15</td>
<td>51–68 kD, obligate heterodimer with class I</td>
<td>Epithelial cells</td>
<td>Similar to class I</td>
</tr>
<tr>
<td>III</td>
<td>Desmin</td>
<td>1</td>
<td>53 kD, homopolymers</td>
<td>Muscle cells</td>
<td>Cardiac and skeletal myopathies</td>
</tr>
<tr>
<td></td>
<td>GFAP</td>
<td>1</td>
<td>50 kD, homopolymers</td>
<td>Glial cells</td>
<td>Alexander disease; mouse null viable</td>
</tr>
<tr>
<td></td>
<td>Periplakin</td>
<td>1</td>
<td>57 kD</td>
<td>Peripheral &gt; CNS neurons</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Synemin</td>
<td>1</td>
<td>190 kD, interacts with other class III IFs</td>
<td>Muscle cells</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vimentin</td>
<td>1</td>
<td>54 kD, homopolymers and heteropolymers</td>
<td>Mesenchymal cells</td>
<td>Mouse null viable</td>
</tr>
<tr>
<td>IV</td>
<td>Neurofilament</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>NFL</td>
<td>1</td>
<td>Obligate heteropolymers with NFH, NFH</td>
<td>Neurons</td>
<td>Mouse null viable; neuropathies</td>
</tr>
<tr>
<td></td>
<td>NFM</td>
<td>1</td>
<td>Obligate heteropolymers with NF, NFH</td>
<td>Neurons</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NFH</td>
<td>1</td>
<td>Obligate heteropolymers with NF, NFM</td>
<td>Neurons</td>
<td>Mutations a risk factor in amyotrophic lateral sclerosis</td>
</tr>
<tr>
<td></td>
<td>α-Internexin</td>
<td>1</td>
<td>55 kD, homopolymers</td>
<td>Embryonic neurons</td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>Lamins</td>
<td>4</td>
<td>7 Isoforms, 62–72 kD, homodimers</td>
<td>Animal, plant nuclei</td>
<td>Cardiomyopathy, lipodystrophy, one form of Emery-Dreifuss muscular dystrophy, two forms of progeria plus many others</td>
</tr>
<tr>
<td>VI</td>
<td>Nestin</td>
<td>1</td>
<td>230 kD, homopolymers</td>
<td>Embryonic neurons, muscle, other cells</td>
<td></td>
</tr>
</tbody>
</table>

IF, intermediate filament; NFH, neurofilament heavy; NFL, neurofilament light; NFM, neurofilament medium.

Intermediate Filament

• Biochemically most stable among the three cytoskeletal filaments.
• Assembly disassembly regulated by phosphorylation
  - **Phosphorylation**: reaction in which a phosphate group is covalently coupled to another molecule.
  - **Kinase**: an enzyme that catalyzes the addition of phosphate groups
  - **Phosphatase**: an enzyme that catalyzes the removal of phosphate groups
• Phosphorylation may stabilize or destabilize intermediate filaments.
### Table 35-2

**PROTEINS ASSOCIATED WITH INTERMEDIATE FILAMENTS**

<table>
<thead>
<tr>
<th>Name</th>
<th>Molecule</th>
<th>Distribution</th>
<th>Partners</th>
<th>Diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plakins</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BPAG-1</td>
<td>Multiple splice isoforms (a, b, c, n) with ABDs and plakin domains ± spectrin and plakin repeats</td>
<td>a: Hemidesmosomes, b: Muscle, cartilage, c: Epithelial hemidesmosomes, n: Neurons</td>
<td>IFs, MTs, actin</td>
<td>Autoimmune bullous pemphigoid</td>
</tr>
<tr>
<td>Desmoplakin</td>
<td>Two splice isoforms with plakin and coiled-coil domains and plakin repeats</td>
<td>Desmosomes</td>
<td>IFs; cadherin and other desmosome proteins</td>
<td>Autoimmune pemphigus; genetic striate palmoplantar keratoderma</td>
</tr>
<tr>
<td>Plectin</td>
<td>Multiple splice isoforms; ABD, plakin domain and plakin repeats</td>
<td>Most tissues except neurons</td>
<td>IFs, actin, MTs, spectrin, β4 integrin</td>
<td>Autoimmune pemphigus; genetic epidermolysis bullosa with muscular dystrophy</td>
</tr>
<tr>
<td><strong>Epidermal</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Filaggrin</td>
<td>Ten 37-kD filagrins cut by proteolysis from profilaggrin</td>
<td>Cornified epithelia</td>
<td>Aggregates keratin</td>
<td></td>
</tr>
<tr>
<td><strong>Lamin Associated</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LAP1</td>
<td>57-70 kD isoforms</td>
<td>Integral nuclear membrane proteins</td>
<td>Binds laminin to nuclear envelope</td>
<td></td>
</tr>
<tr>
<td>LAP2</td>
<td>50 kD</td>
<td>Integral nuclear membrane protein</td>
<td>Binds laminin to nuclear envelope</td>
<td></td>
</tr>
<tr>
<td>LBR</td>
<td>73 kD</td>
<td>Integral nuclear membrane protein</td>
<td>Binds laminin to nuclear envelope</td>
<td>Pelger-Huët anomaly; Greenberg skeletal dysplasia</td>
</tr>
<tr>
<td>Emerin</td>
<td>34 kD</td>
<td>? Peripheral protein of the inner nuclear membrane</td>
<td>? Nuclear and binds actin filaments to the nuclear envelope</td>
<td>Emery-Dreifuss muscular dystrophy</td>
</tr>
</tbody>
</table>

ABD, actin binding domain; IFs, intermediate filaments; MTs, microtubules.
Plectin Connects IFs to Actin & Microtubules

Microtubule: red
Intermediate filament: orange
Intermediate Filaments

• Intermediate filaments are flexible but stable.

• Primary assumed function of intermediate filaments is to prevent excessive stretching.

• In general, much less is known about intermediate filaments compared to actin and microtubule.
An Example: Microtubule, Intermediate Filament & Organelles in a Frog Axon

Cross-linker System between Neurofilaments, Microtubules, and Membranous Organelles in Frog Axons Revealed by the Quick-freeze, Deep-etching Method


Bar: 0.1μm
Outline

• Summary: actin and its associated proteins

• Microtubule and its associated proteins; Centrosome

• Intermediate filament and its associated proteins

• An overview of molecular motors
Overview of Molecular Motors

- Myosin binds and walks on actin.
- Kinesin and dynein binds and walks on microtubule.
Molecular Motors and Motility Assays

- **Actin motor**
  - myosin
  - Usually for short-distance movement

- **Microtubule motors**
  - kinesin
  - dynein
  - Usually for long-distance movement

- **In vitro motility assays**
  - bead assay
  - microtubule sliding assay
Bead Motility Assay Video

kinesin-coated bead moves along a microtubule
Block Lab, Stanford
Many Molecules Generate Intracellular Force & Motion

<table>
<thead>
<tr>
<th>ATPase</th>
<th>Track</th>
<th>Direction</th>
<th>Cargo</th>
<th>Energy</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Myosins</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muscle myosin</td>
<td>Actin</td>
<td>Barbed end</td>
<td>Myosin filament</td>
<td>ATP</td>
</tr>
<tr>
<td>Myosin II</td>
<td>Actin</td>
<td>Barbed end</td>
<td>Myosin, actin</td>
<td>ATP</td>
</tr>
<tr>
<td>Myosin I</td>
<td>Actin</td>
<td>Barbed end</td>
<td>Membranes</td>
<td>ATP</td>
</tr>
<tr>
<td>Myosin V</td>
<td>Actin</td>
<td>Barbed end</td>
<td>Organelles</td>
<td>ATP</td>
</tr>
<tr>
<td>Myosin VI</td>
<td>Actin</td>
<td>Pointed end</td>
<td>Endocytic vesicles</td>
<td>ATP</td>
</tr>
<tr>
<td><strong>Dynabins</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Axonemal</td>
<td>Microtubule</td>
<td>Minus end</td>
<td>Microtubules</td>
<td>ATP</td>
</tr>
<tr>
<td><strong>Kinesins</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conventional</td>
<td>Microtubule</td>
<td>Plus end</td>
<td>Membranes, intermediate filaments</td>
<td>ATP</td>
</tr>
<tr>
<td>Ncd</td>
<td>Microtubule</td>
<td>Minus end</td>
<td>? Microtubules</td>
<td>ATP</td>
</tr>
</tbody>
</table>

**Other Mechanochemical Systems**

<table>
<thead>
<tr>
<th>Polymerases</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Ribosome</td>
<td>mRNA</td>
<td>5’ to 3’</td>
<td>None</td>
<td>GTP</td>
</tr>
<tr>
<td>DNA polymerase</td>
<td>DNA</td>
<td>5’ to 3’</td>
<td>None</td>
<td>ATP</td>
</tr>
<tr>
<td>RNA polymerase</td>
<td>DNA</td>
<td>5’ to 3’</td>
<td>None</td>
<td>ATP</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Conformational System</th>
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</thead>
<tbody>
<tr>
<td>Spasmlin</td>
<td>None</td>
<td>None</td>
<td>Cell, basal body</td>
<td>Ca^{2+}</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Polymerizing Systems</th>
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</thead>
<tbody>
<tr>
<td>Actin filaments</td>
<td>None</td>
<td>Barbed end</td>
<td>Membranes</td>
<td>ATP</td>
</tr>
<tr>
<td>Microtubules</td>
<td>None</td>
<td>Plus end</td>
<td>Chromosomes</td>
<td>GTP</td>
</tr>
<tr>
<td>Worm sperm MSP</td>
<td>None</td>
<td>Not polar</td>
<td>Cytoskeleton</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Rotary Motors</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacterial flagella</td>
<td>None</td>
<td>Bidirectional</td>
<td>Cell</td>
<td>H^+ or Na^+ gradient</td>
</tr>
<tr>
<td>F-type ATPase</td>
<td>None</td>
<td>Bidirectional</td>
<td>None</td>
<td>H^+ or ATP</td>
</tr>
<tr>
<td>V-type ATPase pump</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>ATP</td>
</tr>
</tbody>
</table>

mRNA, messenger RNA; MSP, major sperm protein.
Relations Between Molecular Motors and Cytoskeleton Polymers

- Interactions between motors and cytoskeletal polymers are dynamic and complex.

- Cytoskeletal polymers provide dynamic tracks for molecular motors to walk on.

- Molecular motors active interacts with cytoskeletal polymers. For example,
  - Molecular motors transport cytoskeletal polymers, especially in neurons.
  - Molecular motors, e.g. MCAK, regulate cytoskeletal polymer dynamics.
Molecular Motors Are ATP-Hydrolysis Enzymes

- Molecular motors convert chemical energy derived from ATP hydrolysis directly into mechanical work.

- ATP (adenosine triphosphate) hydrolysis

\[ \text{ATP} + \text{H}_2\text{O} \rightleftharpoons \text{ADP} + \text{P}_i \]
Motor Behavior Parameters

• Parameters that characterizing motor behaviors
  - processivity: run-length, number of steps
  - step size
  - stall force

• Myosin is nonprocessive.

• Kinesin and dynein are both processive. Processivity of dynein is weaker.

• Motors walk nano-meter scale steps of specific lengths.

• Stall force is on the pico-Newton level.
Analyzing Motor Movement at Nanometer Resolution

• Nanometer-resolution measurement of step sizes
  - First implemented in late 1980's
  - Based on fitting of point spread function


• Further improved by many others

  - Up to 1nm resolution

Analyzing Motor Force at Piconewton Resolution

- Optical tweezer is used to generate and measure motor stall force.

From Steven Block Lab webpage
http://www.stanford.edu/group/blocklab/
Laser Force Trap Video

kinesin-coated bead moves in a force trap
Block Lab, Stanford
General Motor Structure

- **Motor (head) domain**
  - Produces force and motion

- **Tail domain**
  - Adapts to different cargoes
Different Motility Schemes

**Generic motor with stretched spring**
- Force
- Motor
- Spring
- ATP
- ADP + P_i
- Cytoskeletal fiber

**Resulting movement with anchored fiber**
- Motor and cargo move
- Cargo

**Resulting movement with anchored motor**
- Fiber moves
- Support

**Result with anchored fiber and anchored motor**
- Spring stretched, force transmitted through fiber to anchoring sites, no movement, energy lost as heat

Support or cargo
Required Reading

- Chapters 2 & 16
Questions ?