BME 42-620 Engineering Molecular Cell Biology

Lecture 12:

The Cytoskeleton (III): Molecular Motors
Mechanical Properties of Cytoskeletal Polymers
Course Administration Notes (I)

• **Correction**: Midterm exam: **October 27, 2011**; Take-home exam; Instructions handed out at the end of the class.

<table>
<thead>
<tr>
<th>Date</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>October 21</td>
<td>F  Undergraduate Mini-1 Exam Day</td>
</tr>
<tr>
<td>October 21</td>
<td>F  Mid-Semester Break; No Classes (Mini-1 exams will take place)</td>
</tr>
<tr>
<td>October 24</td>
<td>M  Mid-Semester Grades Due by 9 p.m.</td>
</tr>
<tr>
<td>October 24</td>
<td>M  Mini-2 Classes Begin (except for Tepper)</td>
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<tr>
<td>October 26</td>
<td>W  Mini-1 Final Grades Due by 6 p.m.</td>
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<tr>
<td>October 26</td>
<td>W  Mini-2 Classes Begin (Tepper only)</td>
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</tbody>
</table>

• Midterm exam format:

1) conceptual and factual questions based on lectures and the textbook;
2) a literature-based research project;
3) Due **October 31** Monday 12:00Noon at Mellon Institute 403;
4) Regular lectures will continue as scheduled.
Outline

- Overview of molecular motors
- The myosin superfamily; Myosin motility
- The kinesin superfamily; Kinesin motility
- The dynein family; Dynein motility
- Mechanical properties of cytoskeletal polymers
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Overview of Molecular Motors

- Myosin walks on actin filaments.
- Kinesin and dynein walks on microtubule.
- Motor (head) domain
  - Produces force and motion
- Tail domain
  - Adapts to different cargoes

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.
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 actively interact with cytoskeletal polymers. For example,
  - Molecular motors transport cytoskeletal polymers, e.g. in neurons.
  - Molecular motors, e.g. MCAK, regulate cytoskeletal dynamics.
Example: Active Transport of Neurofilaments


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Myosin Family

A. Phylogenetic tree of myosin family members:

- **Acanthamoeba MII**
- **Dictyostelium MII**
- **Drosophila striated muscle MII**
- **Chicken smooth muscle MII**
- **Drosophila cytoplasmic MII**
- **Budding yeast Myo1**
- **Fission yeast Myo2**
- **Drosophila 95F**
- **Pig MVI**
- **Cow MX**
- **Human M7A**
- **Human M9A**
- **Drosophila Nina C**
- **C. elegans M12**
- **Arabidopsis ATM1**
- **Acetabularia M2**

B. Myosin structures:

1. **Dictyostelium MyoB**
2. **Bovine BB myosin-I**
3. **Chicken skeletal m. myosin-II**
4. **Dictyostelium MII**
5. **Human brush border Myo**
6. **Rat MII**
7. **Rat M1**
8. **Human M1c**
9. **Acanthamoeba MIB**
10. **Budding yeast Myo3**
11. **Dictyostelium MIA**
12. **Acanthamoeba HMW**
13. **Toxoplasma-A**
14. **Drosophila Nina C**

**Heavy chain domains**:
- Head
- Mem band
- G1/G2
- SH2
- SH3

**Architecture**:
- = 100 amino acids
Example: Structure of Head of Myosin-II

- Muscle myosin (Myosin II): 2 heavy chains, 2 light chains

- Light chain stabilizes the heavy chain α-helix.
  - ELC: essential light chain
  - RLC: regulatory light chain
Protein Analysis Using Gel Electrophoresis

- Proteins can be separated based on their molecular weights and analyzed using gel electrophoresis.

- Gels (e.g. polyacrylamide-gel) are used to generate a viscous matrix through which protein molecules move.

- Ionic detergent such as SDS (sodium dodecyl sulfate) binds to hydrophobic regions of proteins so that they unfold and negatively charged.

- SDS-PAGE: SDS polyacrylamide-gel electrophoresis
Identification of Heavy Chain & Light Chain

A. Gel electrophoresis
- Samples placed in wells
- Run gel
- Process to reveal molecules

B. DNA gel
- Stained
- Size in kD

C. Protein gel
- Immuno blots
- ARPC2
- ARPC1
Mechanical Parameters of Myosin

- **Velocity**
  - Varies substantially between different families
    - Myosin II: 6000 nm/sec
    - Myosin V: 200 nm/sec

- **Force**
  - Ranging between 1~10 pN

- **Step**
  - Myosin II: 5 nm
  - Myosin V: 36 nm

- **Run-length**
  - up to several hundred nm
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# Kinesin Families

<table>
<thead>
<tr>
<th>Standardized Name</th>
<th>Example Sequences</th>
<th>Other Names for this Group of Sequences</th>
<th>Other Names</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kinesin-1</td>
<td>KHC (J09265) KIF5A (A700719) KHC (J47796)</td>
<td>KHC</td>
<td>N-I</td>
</tr>
<tr>
<td>Kinesin-2</td>
<td>KRP85/96</td>
<td>N-V</td>
<td>Kinesin-II</td>
</tr>
<tr>
<td>Kinesin-3</td>
<td>Unc-104/Kif1</td>
<td>N-III</td>
<td>Unc104</td>
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<tr>
<td>Kinesin-4</td>
<td>Chromokinesin/Kif4</td>
<td>N-V</td>
<td>Chromokinesin (upper clade)</td>
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<tr>
<td>Kinesin-5</td>
<td>BimC</td>
<td>N-II</td>
<td>BMC</td>
</tr>
<tr>
<td>Kinesin-6</td>
<td>MKLP1</td>
<td>N-VI</td>
<td>MKLP (lower clade)</td>
</tr>
<tr>
<td>Kinesin-7</td>
<td>CENP-E</td>
<td>N-VII</td>
<td>CENP-E</td>
</tr>
<tr>
<td>Kinesin-8</td>
<td>Kip3</td>
<td>N-IX</td>
<td>Kip3</td>
</tr>
<tr>
<td>Kinesin-9</td>
<td>MKLP (middle clade)</td>
<td>Chromokinesin (lower clade)</td>
<td>Kid</td>
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<td>Kinesin-10</td>
<td>Ncd (AF140589) KIF18 (J002410) KIF22 (C84194)</td>
<td>Uvengrit</td>
<td>Kinesin-I</td>
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<td>Kinesin-11</td>
<td>MKI P (Upper clade)</td>
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<tr>
<td>Kinesin-12</td>
<td>Nmd (M10684) HIF4 (14259)</td>
<td>C-terminal Motor</td>
<td>O-Type</td>
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<tr>
<td>Kinesin-13</td>
<td>Orphans</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kinesin-14</td>
<td>Orphans</td>
<td>Ungrouped</td>
<td></td>
</tr>
</tbody>
</table>
Structure of Different Kinesin Families

Mechanical Parameters of Kinesin

- **Velocity**
  - typically ~ 1 \( \mu m/sec \)

- **Stall force**
  - up to 7 pN

- **Step**
  - 8nm (size of tubulin heterodimer)

- **Run length**
  - typically ~1 \( \mu m \)
Kinesin and Myosin are Structurally Similar

- Kinesin head is less than half of the size of a myosin head.
- Kinesin and myosin lack similarity in amino acid sequence.
- Kinesin head is folded in a way similar to the ATP binding core of the myosin head.
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Dynein

- **Two classes**
  - axonemal dynein: cilia and flagella
  - cytoplasmic dynein

- **Subunits**
  - DLC
  - DLIC
  - DIC
  - DHC
Axonemal and Cytoplasmic Dynein

Dynein: Basic Parameters

• **Function in vivo requires dynactin**
  

• **Velocity**
  
  axonemal: can be up to 7 μm/sec
  
  cytoplasmic: typically ~ 1 μm/sec

• **Stall force**
  
  up to 7 pN

• **Step:** multiples of 8nm

Dynein: Processivity

- Processivity of dynein is relatively poor.
- Dynein can undergo lateral and backward motion on microtubule.

The Rotating Crossbridge Model

- Motors cycle through attached and detached states.
- Motors undergo amplified conformational change during attached state.
- Motors undergo conformational recovery during detached state.

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Polymer Mechanics (I)

• Rationale: characterizing mechanical properties of individual filaments as a starting point for understanding mechanical properties of tissues and organs.

• Polymer mechanics is an established research field.

• Investigating the mechanics of biopolymers in cells is a very active research field.
Polymer Mechanics

- We will take a simplified approach here.
- Theory of elasticity holds at the scale of single filaments.
- Cytoskeleton polymers are modeled as thin and slender beams.
Basic Properties

- Bending rigidity
- Drag coefficient
- Buckling force
- Persistence length
Bending Rigidity

• Basic equation

\[ M = EI \frac{1}{R} \]

- \( R \): Radius of curvature
- \( M \): Torque; bending moment
- \( E \): Young's modulus
- \( I \): second moment of inertia
- \( EI \): bending (flexural) rigidity

• Bending rigidity of cytoskeletal filaments is generally independent of bending direction since cytoskeletal filaments have approximately circular or helical symmetry.
Cantilever Beam Under Small-Angle Bending

- **Deflection**

\[ y(x) = \frac{F}{EI} \left( \frac{Lx^2}{2} - \frac{x^3}{6} \right) \]

- **Spring constant**

\[ k = \frac{F}{y(L)} = \frac{3EI}{L^3} \]

Examples: cantilever beam models

- **Glass cantilever beam**
  \[ r = 0.25\mu m, \ L = 100 \mu m \]
  \[ E=70 \text{ GPa}, \ I=(\pi/4)r^4=3 \times 10^{-27} \]
  \[ k=0.64 \text{ pN/nm} \]

- **Microtubule**
  \[ EI=30 \times 10^{-24} \text{N⋅m}^2, \ L = 10 \mu m \]
  \[ k = 0.00009 \text{ pN/nm} \]

- **Coiled coil**
  \[ EI=400 \times 10^{-30} \text{N⋅m}^2, \ L = 8 \text{ nm} \]
  \[ k = 2.34 \text{ pN/nm} \]
Questions ?