Mechanics of Motor Proteins and the Cytoskeleton

Jonathon Howard: Dept. of Physiology and Biophysics University of Washington and Max Plank Institute for Molecular Cell Biology and Genetics

Chapter 14: ATP Hydrolysis
Part 2: Kinesin & Kinesin vs Myosin

Presented by:
Chris Johnson & Rami Amro
Kinesin hydrolysis reaction’s are Microtubule dependent.

<table>
<thead>
<tr>
<th>Without Microtubules</th>
<th>With Microtubules</th>
</tr>
</thead>
<tbody>
<tr>
<td>• ADP product release is very slow $\rightarrow$ “Rate Limiting”</td>
<td>• ATPase rate increases 5000-fold (~50 s$^{-1}$ per head) $\rightarrow$ ADP release rate increases by the same factor.</td>
</tr>
<tr>
<td>• A slow ATPase rate (0.01 s$^{-1}$) reflects the high affinity of Kinesin to ADP, $\sim$1nM.</td>
<td>• $K_m$ for ATP increases (3nM to 40μM)</td>
</tr>
<tr>
<td>• Kinesin has a low affinity for ATP ($\sim$10μM)</td>
<td>• Hydrolysis rates increase due to microtubule binding.</td>
</tr>
</tbody>
</table>
More on Kinesin & Microtubules: One head or two???

- Nucleotide dependence is present in Kinesin, just as myosin, but:
  - Detachment of K & KT is very slow relative to the KDP & KD states.
  - Hydrolysis catalyzes microtubule unbinding, while microtubule binding catalyzes ADP release.

- Observations suggest a “hand-over-hand” mechanism:
  - Kinesin can spend no more than 1 μs detached from the microtubule.
  - Kinesin’s processivity cannot be accounted for with the one-head model.
The release of Kinesin’s trailing head is contingent on the binding of the leading head?

Consider a Kinesin with ADP bound to both heads:

• What happens with no ATP???
  – ADP release rates vary per head: 100 \text{s}^{-1} \text{ vs } .01 \text{s}^{-1}.
  → An attached head w/o a nucleotide bound prevents the other head from going to conformation that promotes ADP release.

• Now introduce some ATP...
  – The second releases ADP quickly now: \text{~}100 \text{s}^{-1}
  → ATP binding to the attached head encourages conformational changes and accelerate the binding of the second head.
The release of Kinesin’s trailing head is contingent on the binding of the leading head? (cont)

- Observations show that one-headed Kinesin’s have slower rates (in both microtubule-detachment and ATPase) relative to the active dimer cases:

\[ k_{\text{detach}}^{1\text{-head}} \approx 3 \text{ s}^{-1} \ll 50 \text{ s}^{-1} \approx k_{\text{detach}}^{2\text{-heads}} \text{ (for 100 steps/s)} \]

\[ k_{\text{atp}}^{1\text{-head}} \approx 3 \text{ s}^{-1} \ll 50 \text{ s}^{-1} \approx k_{\text{atp}}^{2\text{-heads}} \]

- Perhaps binding of the second head:
  - accelerates the detachment of the first head: How?
    - Intramolecular strain catalyzes release of the trailing head???
    - Influencing detachment in the ADP*P_i state???
Hence, the mechanical and chemical cycles or Kinesin are coupled.

C₁: ATP binds to head #1 \( \rightarrow \) M₁: Head #2 attaches to MT \( \rightarrow \)
C₂: ADP releases from head #2 \( \rightarrow \) M₂: Head #1 detaches from \( \rightarrow \)
C₃: Pᵢ releases from head #1 \( \rightarrow \) C₁

One nucleotide hydrolysis cycle results in an **8nm step**.
Kinesin vs Myosin: High duty ratio vs Low duty ratio

• Kinesin is attached during it’s rate-limiting step; Myosin is not.

• Biochemical Evidence for Kinesin’s Processivity.

• Biochemical Evidence that Myosin has a Low Duty ratio.
Kinesin is attached during it’s rate-limiting step, while Myosin is detached.

- The ATPase rates of both motors increase for increasing filament concentration, however:
  - Kinesin: [MT] required for half-maximal ATPase is similar to the [MT] required for physical binding of the motor to tubulin.
    - Rate-limiting step occurs when motor is bound.
  - Myosin: ATPase saturates at an [Actin] much lower than that needed for binding saturation.
    - Rate-limiting step occurs when motor is detached.
Biochemical Evidence for Kinesin’s Processivity.

• For a low Microtubule concentration (16nM):
  – \( t \approx 25 \text{ sec} \) for Kinesin to diffuse & bind to MT.
    • This corresponds to a \( 2^{\text{nd}} \) order rate constant of \( 2.5 \times 10^6 \text{ M}^{-1}\text{s}^{-1} \) (“a fast, diffusion-limited protein-protein interaction”)
  – \( k_{\text{ATPase}} \approx 5 \text{ s}^{-1} \rightarrow “one \text{ ATP hydrolyzed per 0.2 sec}” \)
    • Ie. Kinesin must hydrolyze 125 ATP (25s/0.2s) per average processivity length: \( L \approx 1\mu\text{m} = 125 \times 8\text{nm} \).

• NOTE: This is in contrast to myosin, which hydrolyzes one ATP per encounter with an Actin filament.
Biochemical Evidence that Myosin has a Low Duty ratio.

Let $k_{+1}$ be the 2\textsuperscript{nd} order rate constant for the binding of ATP to the motor. 
$	au_{on}$ is the attached time (high [ATP]). 
$	au_{total}$ is the total hydrolysis cycle time (high [ATP]). 
$K_{speed} = [ATP]$ that the motor spends time $\tau_{on}$ waiting for ATP to bind. 
$K_{ATPase} = [ATP]$ that the motor spends time $\tau_{total}$ waiting for ATP to bind.
Biochemical Evidence that Myosin has a Low Duty ratio.

\[
k_{+1} \cdot K_{\text{speed}} = \frac{1}{\tau_{\text{on}}}
\]

\[
k_{+1} \cdot K_{\text{ATPase}} = \frac{1}{\tau_{\text{total}}}
\]

\[
\tau = \frac{\tau_{\text{on}}}{\tau_{\text{total}}} = \frac{K_{\text{ATPase}}}{K_{\text{speed}}}
\]

\[
r_{\text{myosin}} = \frac{10 \mu M}{200 \mu M} = 0.05
\]

\[
r_{\text{kinesin}} = \frac{10 \mu M}{24 \mu M} = 0.42
\]