Protein Molecular Motors

- Translational (Walking) Motion
  - Myosin (actin track)
  - Kinesins (microtubule track)
  - Dyneins (microtubule track)
    - Cytoplasm, cilia

- Rotary Motion
  - ATP Synthase
  - Flagellar motor
Walking Proteins

- Random diffusion
- Assisted diffusion
Cytoskeleton

- Functions of Cytoskeleton
  - Control cell morphology
  - Cell motility
  - Intracellular transport
  - Placement of organelles
  - Rearrange pigment granules
  - Etc.
<table>
<thead>
<tr>
<th>Function of Protein</th>
<th>Example of Protein</th>
<th>Comparative Shapes, Sizes, and Molecular Mass</th>
<th>Schematic of Interaction with Actin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Form filaments</td>
<td>actin</td>
<td><img src="image" alt="50 nm x 370 x 43 kD/μm" /></td>
<td>minus end preferred subunit addition</td>
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<tr>
<td>Strengthen filaments</td>
<td>tropomyosin</td>
<td><img src="image" alt="2 x 35 kD" /></td>
<td>plus end</td>
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<tr>
<td>Bundle filaments</td>
<td>fimbrin</td>
<td><img src="image" alt="68 kD" /></td>
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<tr>
<td>α-actinin</td>
<td>filamin</td>
<td><img src="image" alt="2 x 270 kD" /></td>
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<tr>
<td>Cross-link filaments into gel</td>
<td>gelsolin</td>
<td><img src="image" alt="90 kD" /></td>
<td></td>
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<tr>
<td>Fragment filaments</td>
<td>myosin-II</td>
<td><img src="image" alt="2 x 260 kD" /></td>
<td></td>
</tr>
<tr>
<td>Slide filaments</td>
<td>myosin-I</td>
<td><img src="image" alt="150 kD" /></td>
<td><img src="image" alt="14 nm" /> Ca²⁺ ATP</td>
</tr>
<tr>
<td>Move vesicles on filaments</td>
<td>spectrin</td>
<td><img src="image" alt="α β" /> 2 x 265 kD plus 2 x 260 kD</td>
<td><img src="image" alt="ATP" /></td>
</tr>
<tr>
<td>Attach sides of filaments to plasma membrane</td>
<td>thymosin</td>
<td><img src="image" alt="5 kD" /></td>
<td><img src="image" alt="ATP" /></td>
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</table>
Actin filaments decorated with myosin II heads
Myosin cycles through three states to provide directional powered motion. The first step, shown on the left, has ATP bound, and it does not bind to actin. In the second step, shown in the center, the ATP has been cleaved to ADP and phosphate. This causes a shift in the actin-binding face, allowing it to bind strongly to the actin filament, and cocks the lever arm into a bent state. The phosphate dissociates in the third step, causing the myosin to straighten, performing the power stroke. The remaining ADP will then be replaced by a new ATP, dissociating the myosin from the actin filament and making it ready for the next stroke.
Figure 22.1: During its power stroke, Myosin exerts a force on an actin filament, propelling the motor to the left. Scale bar = 6 nm. (From R. D. Vale and R. A. Milligan, Science 288, 88 (2000) by permission of the American Association for the Advancement of Science.)
Myosin

(A) Image showing a section of tissue with labeled components.

(B) Diagram of myosin heads and bare zone.

(C) Enlarged view of myosin heads within the sarcomere.

Diagram annotations:
- myosin heads
- bare zone
- 10 nm scale

Text:
- myosin thick filaments reverse polarity at midline of sarcomere (the M line)
- plus end of actin filaments end on Z disc
- minus end of actin filaments
- Z disc

Date: 2/14/06

LaBean COMPSCI 296.5
Actin polymerization

Figure 5-34  Actin acts as a Brownian ratchet to extend membranes. The membrane, shown at the top in pink, undergoes random thermal fluctuations, which transiently open up enough room to add another actin subunit to the growing filament. Cleavage of ATP in the newly added actin subunit glues it in place, holding the membrane in the extended position.
Kinesins and Dyneins on Microtubules

- Organelle movement.
- Mitosis and meiosis.
- Transport of synaptic vesicles along axons.
- Cytoplasmic dyneins and kinesins have 2 heavy chains plus several light chains:
  - Heavy chains have globular, ATP-binding head and rod-like tail domains.
  - Heavy chain head domains are ATPase motors.
  - Tail bind specific cell components (cargo).
A. Normal kinesin (red) on microtubules (green).

B. Microtubule meiotic spindle.

C. pKinI, a kinesin motor found in Plasmodium falciparum, disassembles microtubules at their ends, forming rings.

Kinesin

Figure 5-27  Kinesin relies on two motor units connected together. The cycle begins with one subunit empty and the other with ADP bound. ATP binds to the empty subunit and causes the neck linker to zip tightly onto the subunit, pulling the lagging subunit off the microtubule and forward to the next position. When it binds, ADP is released. Cleavage of the ATP and release of phosphate in the new lagging subunit releases the neck linker, allowing it to take its unbound, disordered form and readying the complex for the next step. Successive cycles allow kinesin to walk along the microtubule.

Figure 5-28  The atomic structure of kinesin reveals machinery that is similar to myosin. The surface that binds to the microtubule is along the bottom in this view. When ATP binds, it shifts the position of the relay helix, which creates the long, narrow groove that holds the neck linker. Force is generated when the neck linker zips tightly into this groove, as seen in this structure.

- Diverse family of proteins.
Figure 3. A motor as a machine. A: A general model. The motor consists of binding sites for nucleotide and a cytoskeletal filament, together with proposed mechanical components: a spring-like or elastic element to produce force, a lever to amplify the force, and a latch to regulate nucleotide binding or release. The nucleotide-binding pocket can contain ATP, ADP (shown), or no nucleotide. Modified from Ref. 30 with permission of J Howard. B: Kinesin motors as machines. The model shown in A can now be filled in with the structural elements tentatively identified as mechanical components of the kinesin motors: helix α4 is a putative spring-like element of the motor and the salt bridge between switch I (SWI) and switch II (SWII) may act like a latch to regulate release of ADP. The neck linker of conventional kinesin, possibly together with the stalk, and the stalk/neck of Ncd may act like a lever to amplify force produced by the motor. For Ncd, the conserved neck and motor core residues N340 and K640 may represent a latch that controls movement of the stalk/neck. Movements of the mechanical elements of the motor are thought to occur upon binding to a microtubule and/or ATP, as shown to the right. The movements may involve opening of the switch I-switch II latch, rotation or compression of the spring represented by helix α4, and structural changes in the filament binding site that release the latch controlling movement of the stalk/neck, causing the putative lever to change in position.

Kinesin Walks Hand-Over-Hand

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A

Hand-over-hand

Inchworm

B

1 ATP

0 nm

8.3 nm

16.6 nm

8.3 nm

2/14/06
**Figure 22.2:** Kinesin steps forward, transporting its cargo toward the plus end of a microtubule. Scale bar = 4 nm. Figure from Vale and Milligan (2000). (From R. D. Vale and R. A. Milligan, Science **288**, 88 (2000) by permission of the American Association for the Advancement of Science.)

http://www.scripps.edu/cb/milligan/research/movies/
Building a Monorail at the Nanoscale
to Shuttle Cargo using Motor Proteins:
bringing life to dead matter

H. Hess, G. D. Bachand, V. Vogel,
Chemistry, 10 (2004) 2110-2116

Integration of Motor Proteins into Synthetic
Materials and Devices
Engineering transport systems at the nanoscale
Molecular shuttles: directed motion of microtubules along nanoscale kinesin tracks

John R Dennis†, Jonathan Howard, and Viola Vogel‡

Directed motion of microtubules along kinesin tracks

Figure 22.8: Patterned "ratchets" sort microtubules. Due to asymmetrically patterned photoresist, microtubules entering the triangular area exit to the right irrespective of their side of entry. This results in aligned and oriented microtubules. (From Ref. 42 by permission of the Biophysical Society.)
Cytoplasmic Dyneins

[Image of a diagram showing a microtubule with labels for plus and minus ends, heavy chains, light chains, and kinesin and dynein proteins.]
Ciliary Dyneins

(A) AFTER PROTEOLYSIS: TELESCOPING

(B) INTACT STRUCTURE: BENDING

free doublet (cross-links removed by proteolysis)
doublets slide apart
doublets held in cilium by cross-links
doublet sliding leads to bending
ATP Synthase

- **F₀** powered by proton (or \( \text{Na}^+ \)) electrochemical gradient
- **F₁** powered by ATP

Figure 5.29 ATP synthase is composed of two tethered nanomolecular motors. The **F₀** motor at the bottom is embedded in a membrane and is composed of a rotor, shown in gray, and a stator subunit, shown in pink. An eccentric axle extends up from the rotor and passes through the center of the **F₁** motor, distorting the six subunits in **F₁** as it turns. The large arm connecting the **F₀** rotor to **F₁**, shown schematically here in pink, has been seen by electron microscopy but not in atomic detail.
ATP Synthase Protein Structure

- Individual crystal structures, NMR
- Reconstituted functional assays
- Mutagenesis
- FRET, crosslinking, etc.

ATP Synthase

Figure 5-30  The F\textsubscript{1} rotor of ATP synthase has a binding site for protons that carries a negative charge. Because it is buried in the membrane, it can only turn if the charge is neutralized by a proton. The stator, shown in pink, supplies the protons from one side of the membrane and deposits them on the other side.

Fig. 3. Structure of ATP synthase showing proposed proton transport path. Residues cAsp61 and aArg210 lie in the center of the bilayer, at the α/ε interface. Their concerted interaction is required for proton movement. Putative access channels for ingress/egress of protons are shown. The c-ring carries protons around on protonated cAsp61 as it rotates.
ATP Synthase, $F_1$

- Reversible
- Mechanoenzymatic mechanism
- Large protein motions
- Allosteric control

*Figure 5-31* The rotary cycle of ATP synthase has includes two types of rotary steps. In step one, ATP binds, causing a 90° rotation. In the second step, ATP and phosphate from an adjacent site leave, causing an additional 30° rotation. By repetition of these steps three times, ATP synthase makes a full revolution.
ATP Synthase

**ATP SYNTHESIS**
- Matrix Space
- Inner mitochondrial membrane
- $nH^+$
- $H^+$
- $H^+$
- $F_0$
- $F_1$
- $ADP + P_i$
- ATP

**ATP HYDROLYSIS**
- Matrix Space
- $nH^+$
- $F_0$
- $F_1$
- $ADP + P_i$
- ATP

**Summary:** ATP Synthase is responsible for the synthesis and hydrolysis of ATP, which is a crucial process in cellular metabolism. The enzyme complex consists of two main parts: $F_0$ (the membrane-embedded part) and $F_1$ (the soluble part). The synthesis process involves the transfer of protons ($H^+$) from the matrix space to the intermembrane space, driving the synthesis of ATP from ADP and P_i. During hydrolysis, ATP is cleaved back into ADP and P_i, releasing the protons back into the matrix space.
Figure 22.9: Left: Depiction of an assembled hybrid biomolecular nanodevice based on the rotary motor F1-ATPase (not to scale). Right: Exploded view showing all structural and linking components. The device self-assembles in multiple steps.
Flagellar motor

- Motion coupled to ion flow.
- Switchable - can rotate either direction.
- 100,000 rpm.
- Membrane bound stator and membrane spanning rotor.
- Multiple stator and rotor subunits provide ~400 force generating interactions per rotation and require transfer of ~1200 ions across the membrane.

Figure 5-33 The flagellar motor of Escherichia coli spans the two-layered cell wall of the bacterium and turns the long corkscrew-shaped flagellum. The other rotary motor of the cell, ATP synthase, is also found spanning the cell wall, shown in darker pink in this illustration.
Fig. 1. A: Diagram of the flagellum in a Gram-negative bacterium. Gram-positive species lack the LP-ring assembly. Only a fraction of the full filament length is shown, as it is quite long on this scale (ca. 10 μm). OM, outer membrane; PG, peptidoglycan; IM, inner membrane. B: Electron micrographic reconstruction of the flagellar basal body – side view. The image was obtained by averaging micrographs of single particles embedded in vitreous ice. The cytoplasm is down and the hook is up; only the bottom-most portion of the hook is visible. C: En face view of the C-ring, viewed from the cytoplasmic side. Subunit structure is clearly visible. Rotational averaging and Fourier transforms demonstrate a 34-fold rotational symmetry for this specimen [24] (panels B and C from D.J. DeRosier, with permission). D: Circular array of membrane-embedded particles, thought to be MotA/MotB protein complexes, that is the stator. The larger particle in the center is the cell-proximal part of the basal-body rod. The inner diameter of this particle ring is about 30 nm. The image is from Salmonella but similar structures have been seen in several species (from S. Khan, with permission). E: Central protrusion within the C-ring that is probably the export apparatus essential for assembly of exterior structures of the flagellum. The view is from inside the cell (from S.-I. Aizawa, with permission).
Flagellar motor

Fig. 2. Proteins that function in rotation. The MotA and MotB proteins form the stator complexes, anchored to the cell wall by a putative peptidoglycan-binding motif in the periplasmic domain of MotB. Each motor contains several (as many as eight) stator complexes, each with composition MotA₄MotB₂. FliF does not function directly in rotation, but forms the MS-ring that is the mounting surface for the 'switch complex' comprising FliG, FliM, and FliN. FliG is known to contact the MS-ring directly, whereas FliM and FliN are somewhere farther down in the C-ring. Exact protein locations are not known, and so details of the pictured arrangement are speculative.
Some notes:

Lessons from Nature

- Power strokes of ATP-fueled molecular motors are powered by the binding of ATP and/or the release of ADP and phosphate. The cleavage reaction provides an irreversible step that makes the process directional.
- Multi-nanometer scale motions can be powered by linking an ATP-cleavage site to a protein conformational change. Examples include a series of articulated motions, as in myosin and ATP synthase F₁, or motions that drive specific order/disorder transitions, as in kinesin.
- Thermal motion can be rectified by a Brownian ratchet. These require one-way barriers to provide rectification. Examples include the charge-neutralization gate used in the ATP synthase F₀ motor and ATP used in actin polymerization.
PRESTIN, A NEW TYPE OF MOTOR PROTEIN

Peter Dallos* and Bernd Fakler†

Prestin, a transmembrane protein found in the outer hair cells of the cochlea, represents a new type of molecular motor, which is likely to be of great interest to molecular cell biologists. In contrast to enzymatic-activity-based motors, prestin is a direct voltage-to-force converter, which uses cytoplasmic anions as extrinsic voltage sensors and can operate at microsecond rates. As prestin mediates changes in outer hair cell length in response to membrane potential variations, it might be responsible for sound amplification in the mammalian hearing organ.