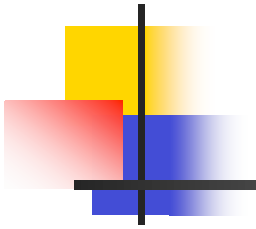
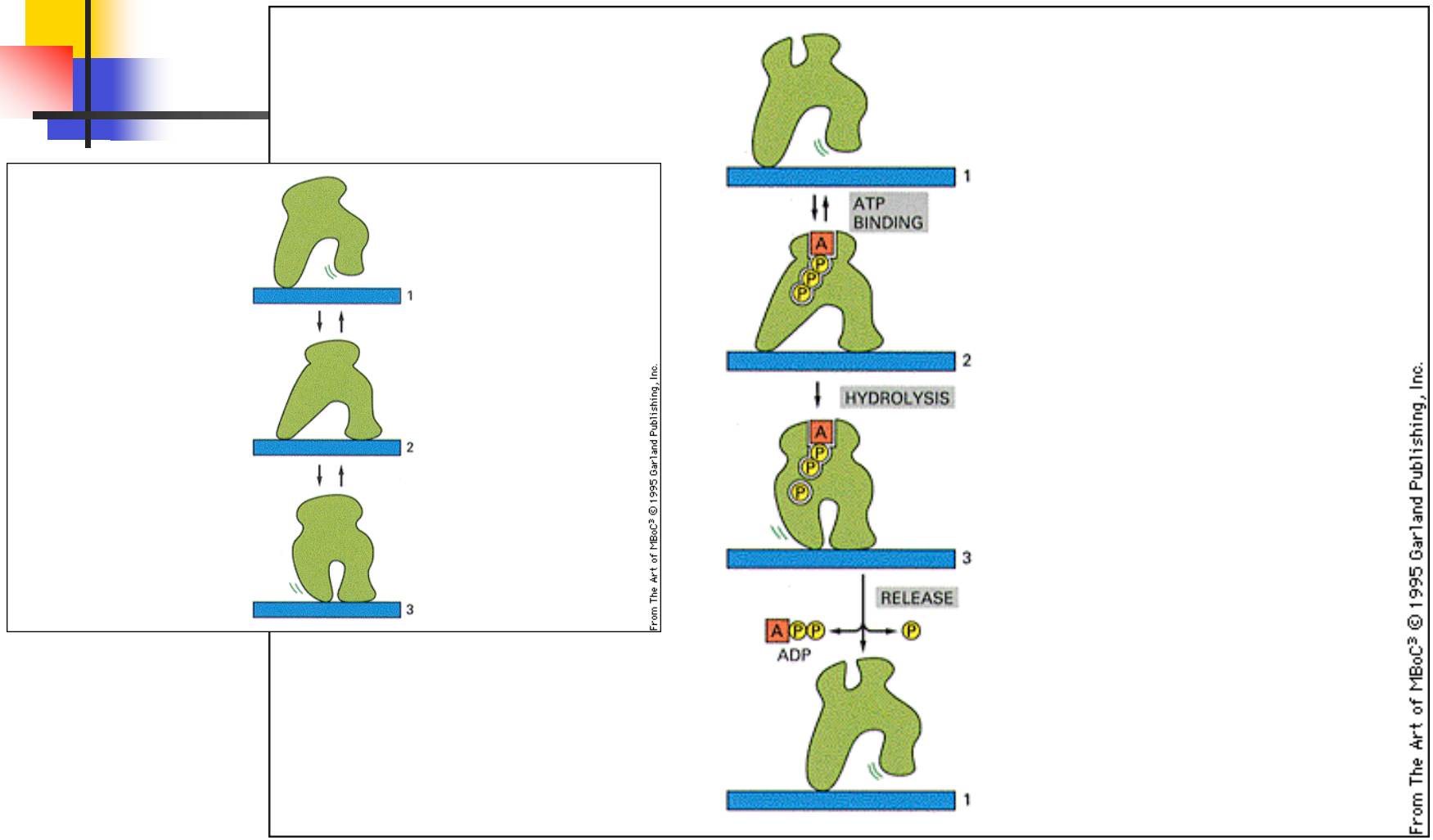


Protein Molecular Motors

- Translational (Walking) Motion
  - Myosin (actin track)
  - Kinesins (microtubule track)
  - Dyneins (microtubule track)
    - Cytoplasm, cilia
- Rotary Motion
  - ATP Synthase
  - Flagellar motor

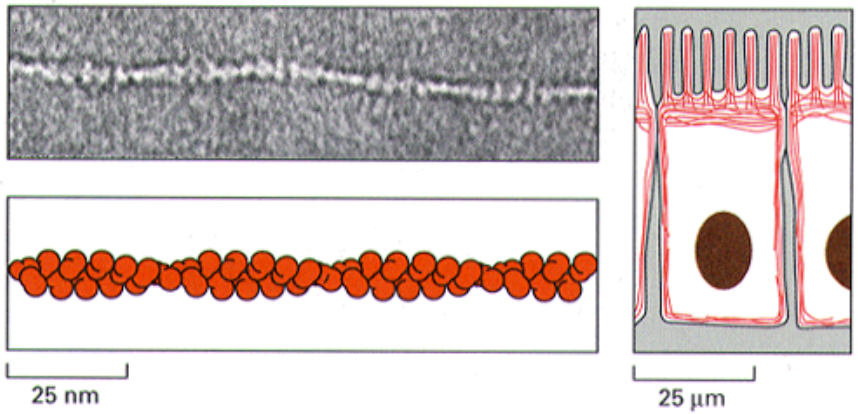
Walking Proteins

- Random diffusion
- Assisted diffusion



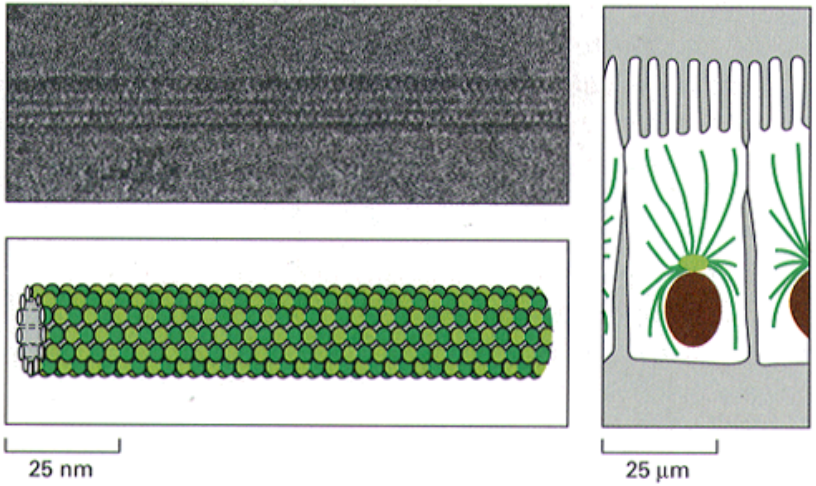
Cytoskeleton

ACTIN FILAMENTS



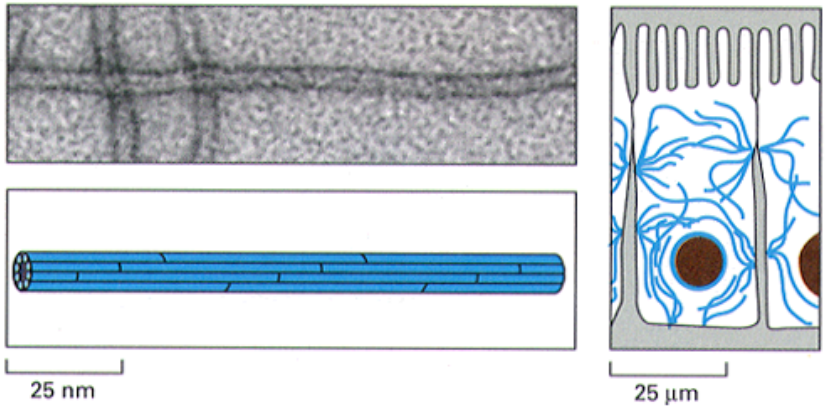
Actin filaments (also known as *microfilaments*) are two-stranded helical polymers of the protein actin. They appear as flexible structures, with a diameter of 5–9 nm, that are organized into a variety of linear bundles, two-dimensional networks, and three-dimensional gels. Although actin filaments are dispersed throughout the cell, they are most highly concentrated in the *cortex*, just beneath the plasma membrane.

MICROTUBULES



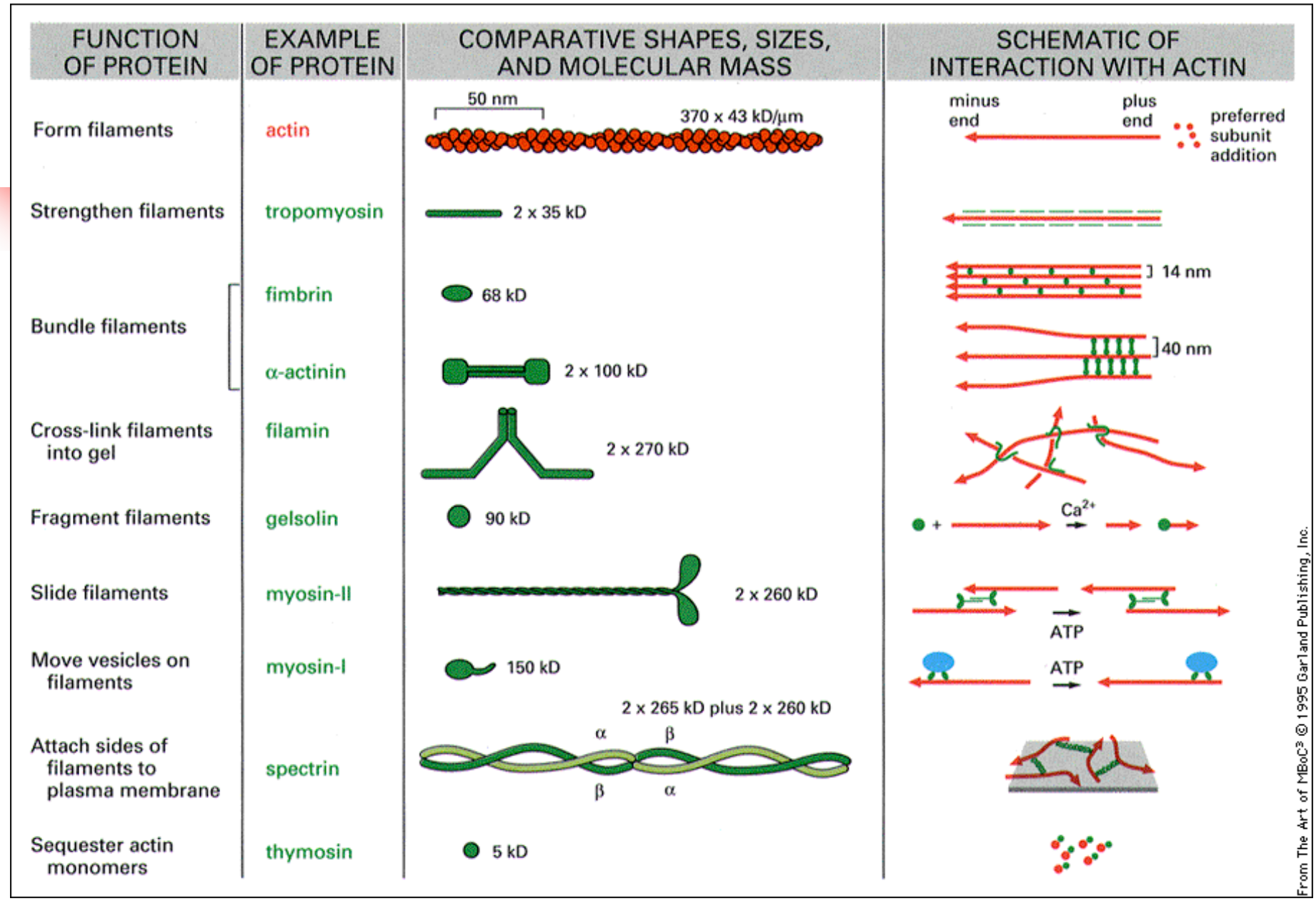
Microtubules are long, hollow cylinders made of the protein tubulin. With an outer diameter of 25 nm, they are much more rigid than actin filaments. Microtubules are long and straight and typically have one end attached to a single microtubule organizing center (MTOC) called a *centrosome*, as shown here.

INTERMEDIATE FILAMENTS

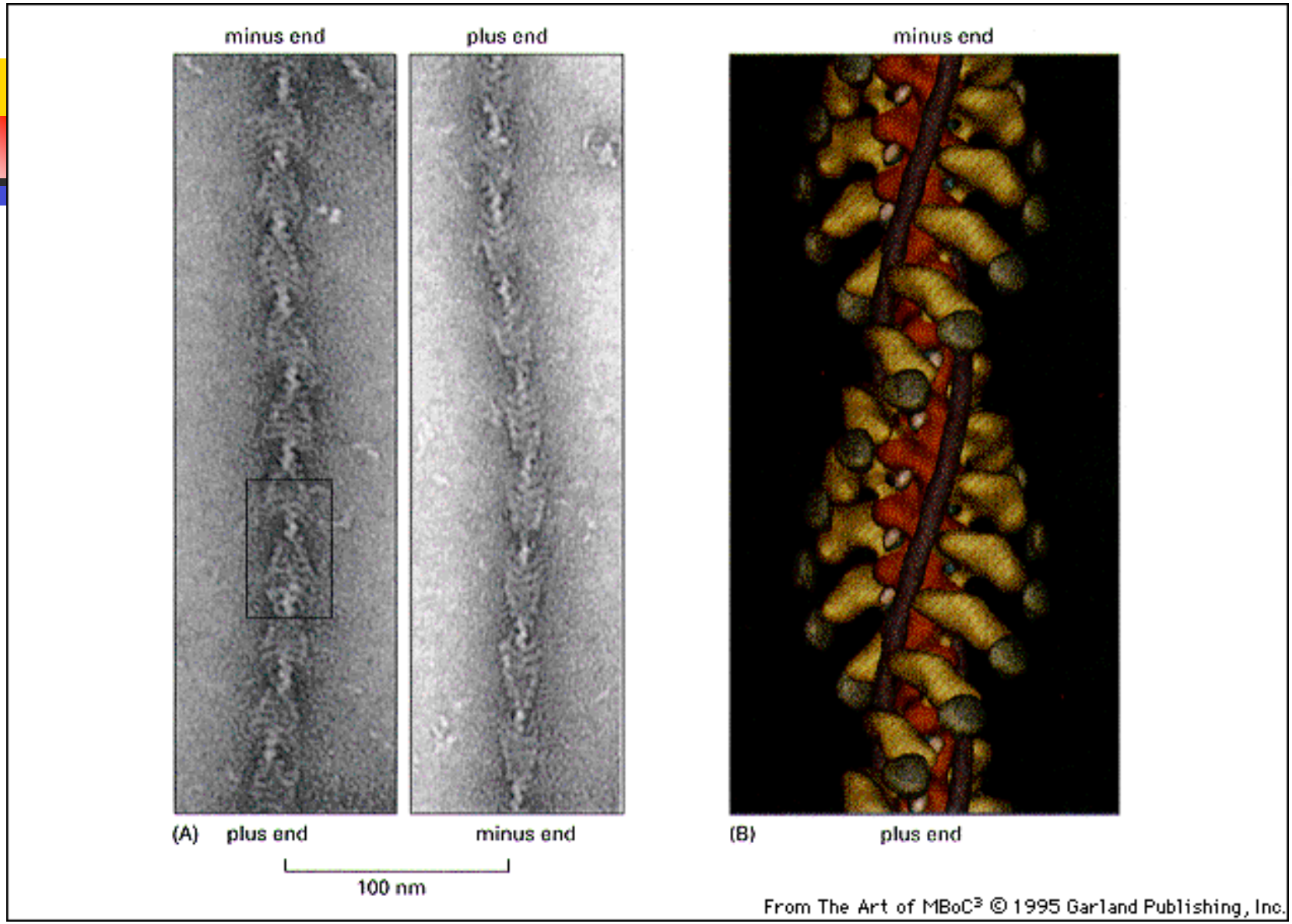


Intermediate filaments are ropelike fibers with a diameter of around 10 nm; they are made of intermediate filament proteins, which constitute a large and heterogeneous family. One type of intermediate filament forms a meshwork called the nuclear lamina just beneath the inner nuclear membrane. Other types extend across the cytoplasm, giving cells mechanical strength and carrying the mechanical stresses in an epithelial tissue by spanning the cytoplasm from one cell-cell junction to another.

- Functions of Cytoskeleton
  - Control cell morphology
  - Cell motility
  - Intracellular transport
  - Placement of organelles
  - Rearrange pigment granules
  - Etc.


FUNCTION OF PROTEIN
EXAMPLE OF PROTEIN
COMPARATIVE SHAPES, SIZES, AND MOLECULAR MASS
SCHEMATIC OF INTERACTION WITH ACTIN
Form filaments
actin
50 nm
370 x 43 kD/μm
minus end
plus end
preferred subunit addition
Strengthen filaments
tropomyosin
2 x 35 kD
Bundle filaments
fimbrin
68 kD
14 nm
α-actinin
2 x 100 kD
40 nm
Cross-link filaments into gel
filamin
2 x 270 kD
Fragment filaments
gelsolin
90 kD
Ca2+
Slide filaments
myosin-II
2 x 260 kD
ATP
Move vesicles on filaments
myosin-I
150 kD
ATP
Attach sides of filaments to plasma membrane
spectrin
2 x 265 kD plus 2 x 260 kD
α
β
β
α
Sequester actin monomers
thymosin
5 kD
From The Art of MBoC3 © 1995 Garland Publishing, Inc.

Actin filaments decorated with myosin II heads



Myosin

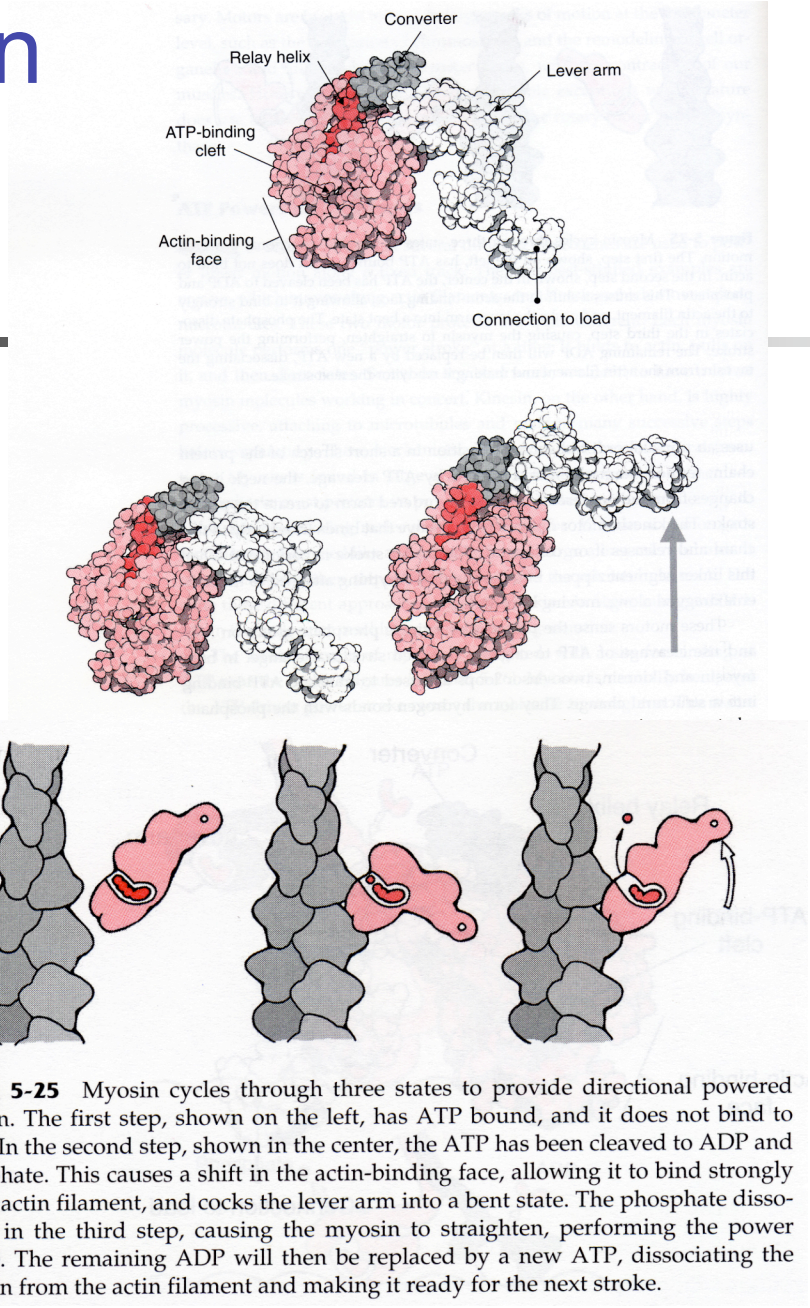
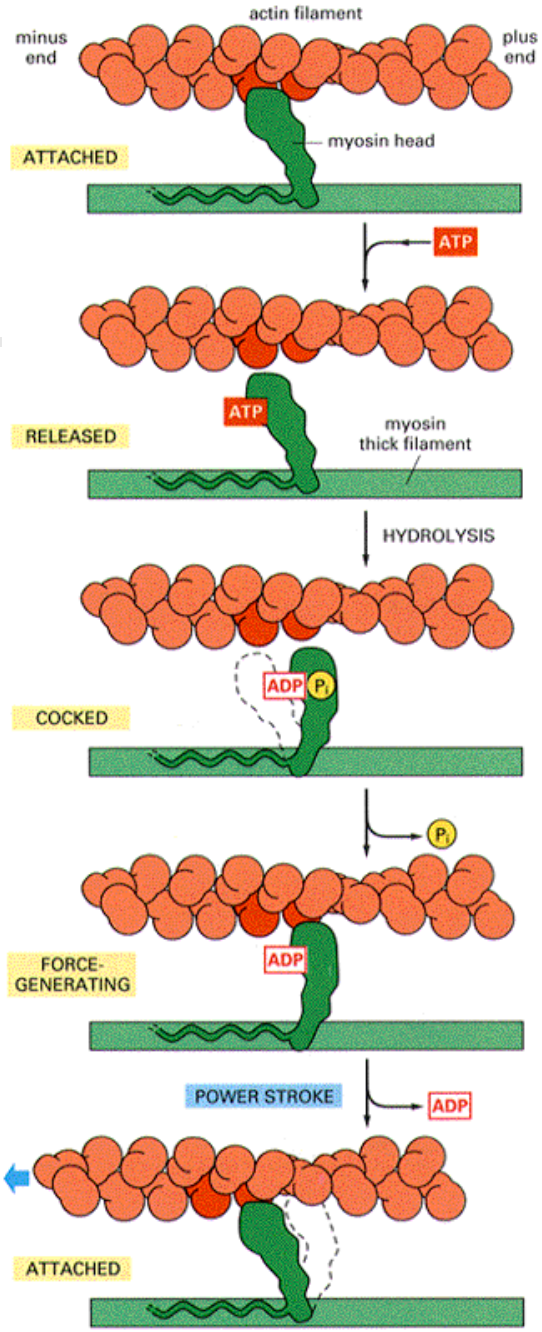
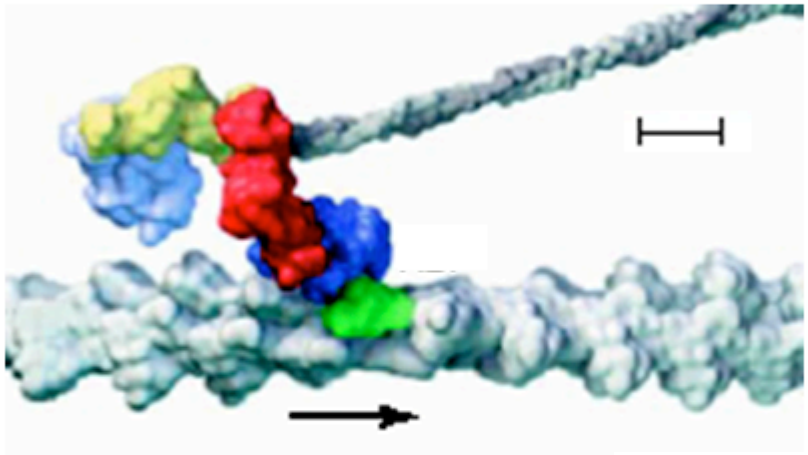


Figure 5-25 Myosin cycles through three states to provide directional powered motion. The first step, shown on the left, has ATP bound, and it does not bind to actin. In the second step, shown in the center, the ATP has been cleaved to ADP and phosphate. This causes a shift in the actin-binding face, 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.



Actin/Myosin



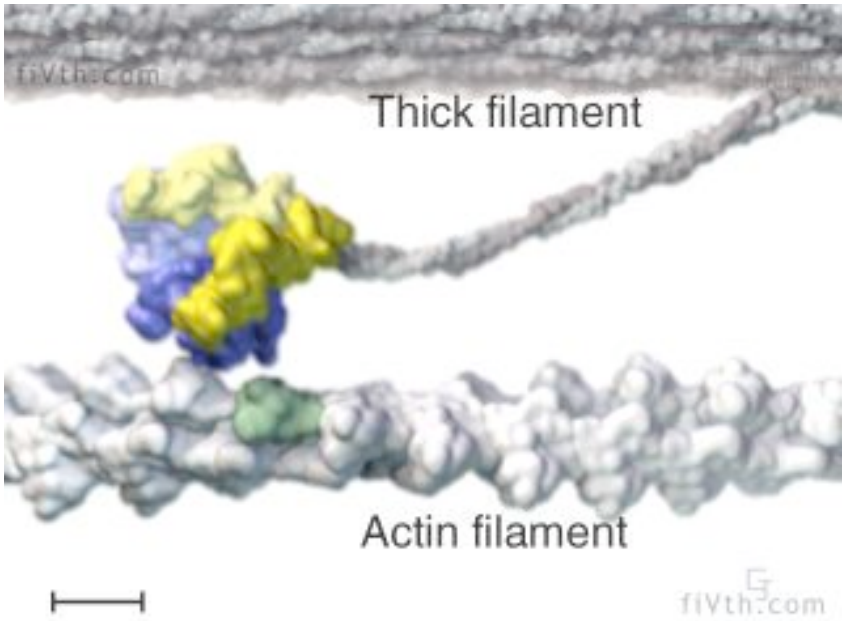
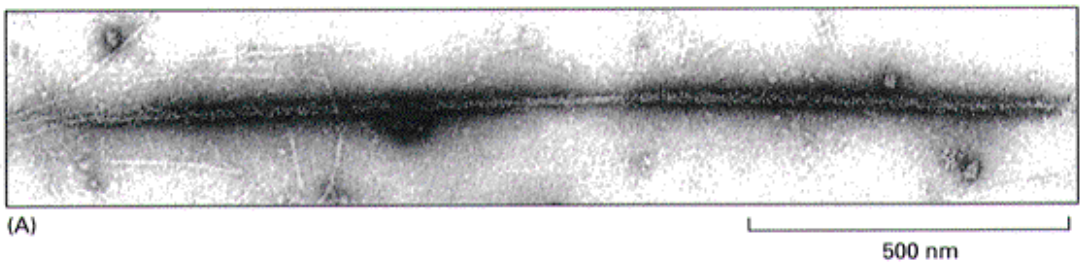
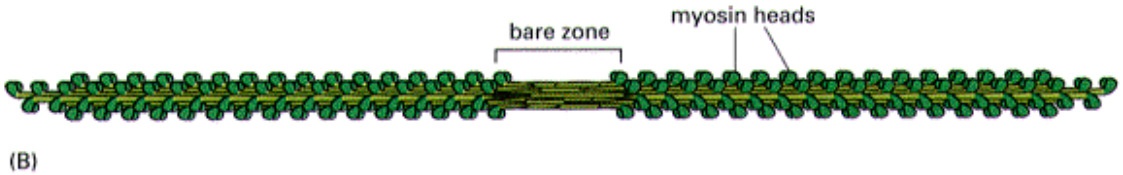
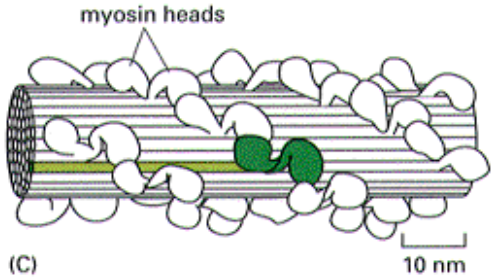


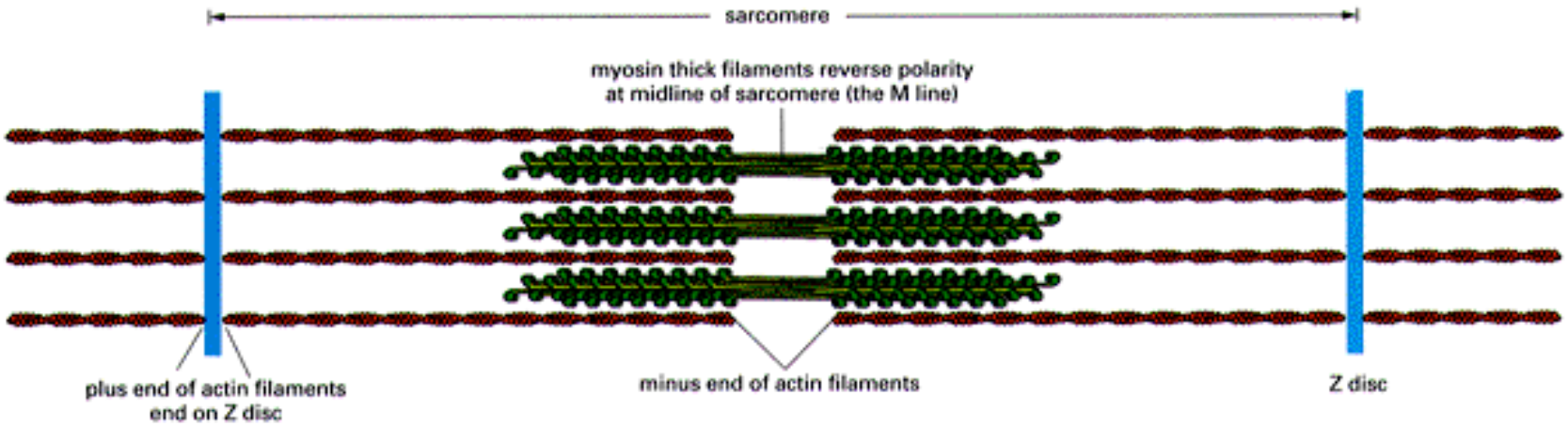
Figure 22.1: During its power stroke, Myosin exerts a force on an actin filament, propelling the motor to the left. Scale bar = 6 nm. (From R. D. Vale and R. A. Milligan, Science **288**, 88 (2000) by permission of the American Association for the Advancement of Science.)

Myosin


(A)
500 nm


bare zone
myosin heads
(B)


myosin heads
(C)
10 nm


sarcomere
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

Actin polymerization

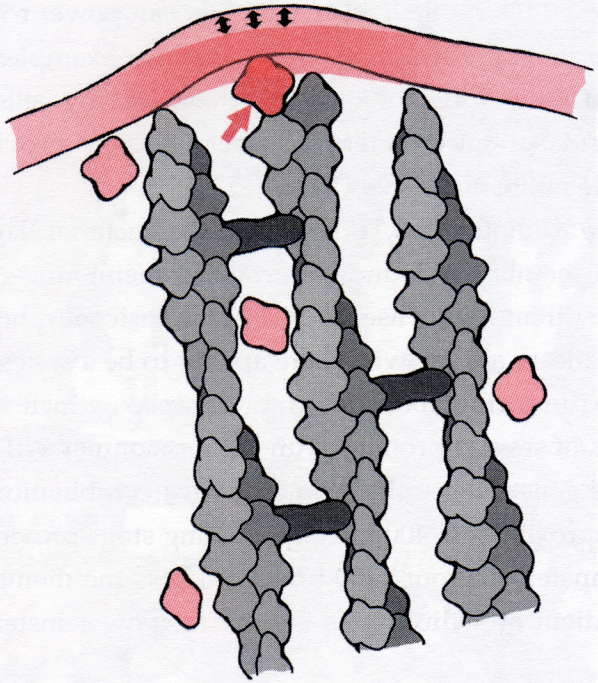
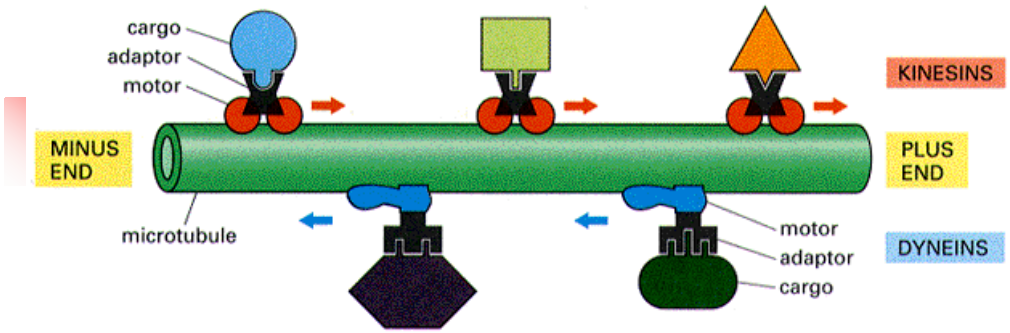
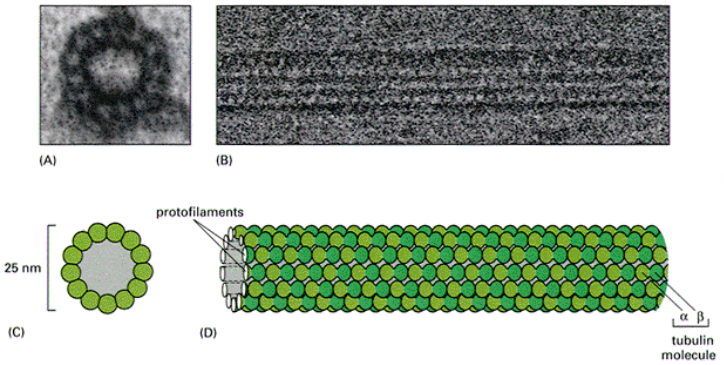


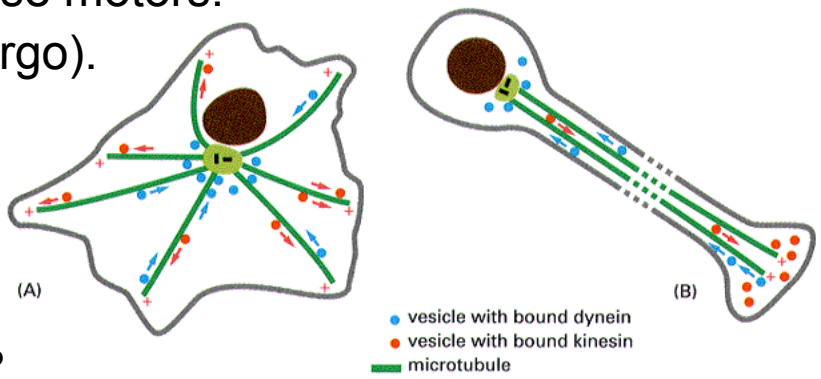
Figure 5-34 Actin acts as a Brownian ratchet to extend membranes. The membrane, shown at the top in pink, undergoes random thermal fluctuations, which transiently open up enough room to add another actin subunit to the growing filament. Cleavage of ATP in the newly added actin subunit glues it in place,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

Kinesins and Dyneins on Microtubules

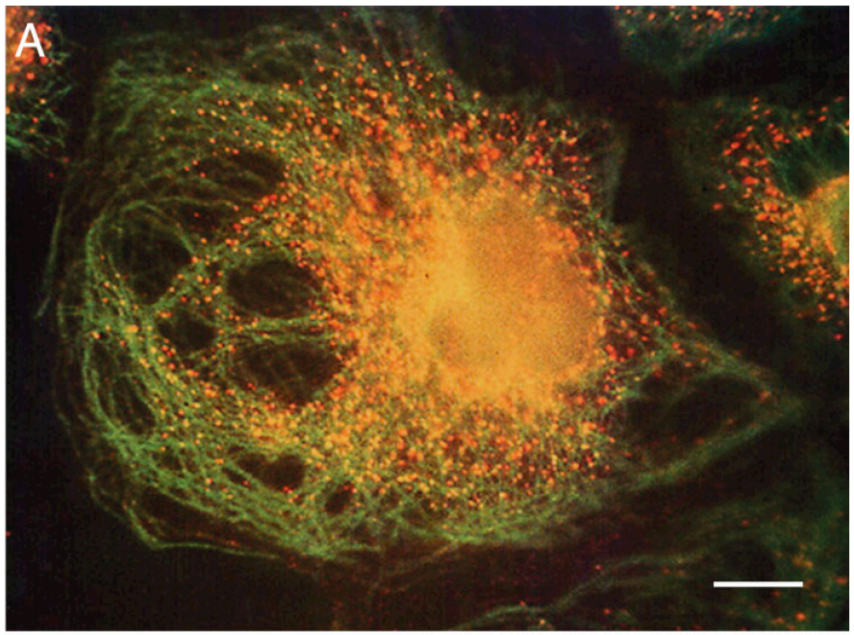


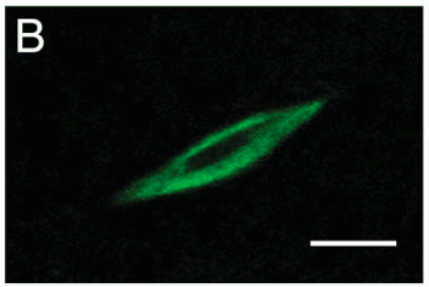


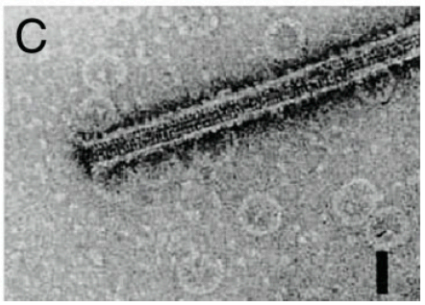
- Organelle movement.
- Mitosis and meiosis.
- Transport of synaptic vesicles along axons.
- Cytoplasmic dyneins and kinesins have 2 heavy chains plus several light chains:
  - Heavy chains have globular, ATP-binding head and rod-like tail domains.
  - Heavy chain head domains are ATPase motors.
  -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

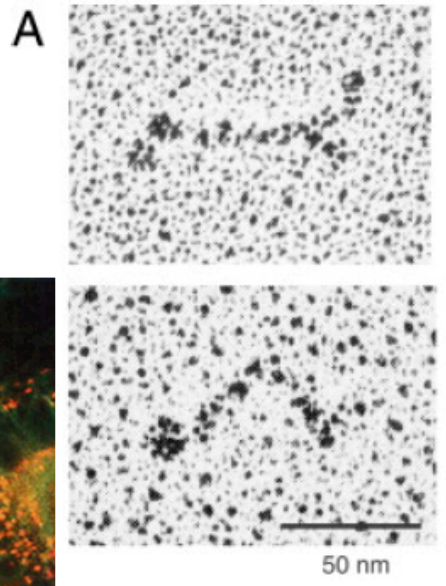


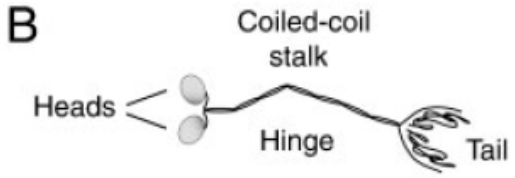
Kinesin











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.

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Kinesin

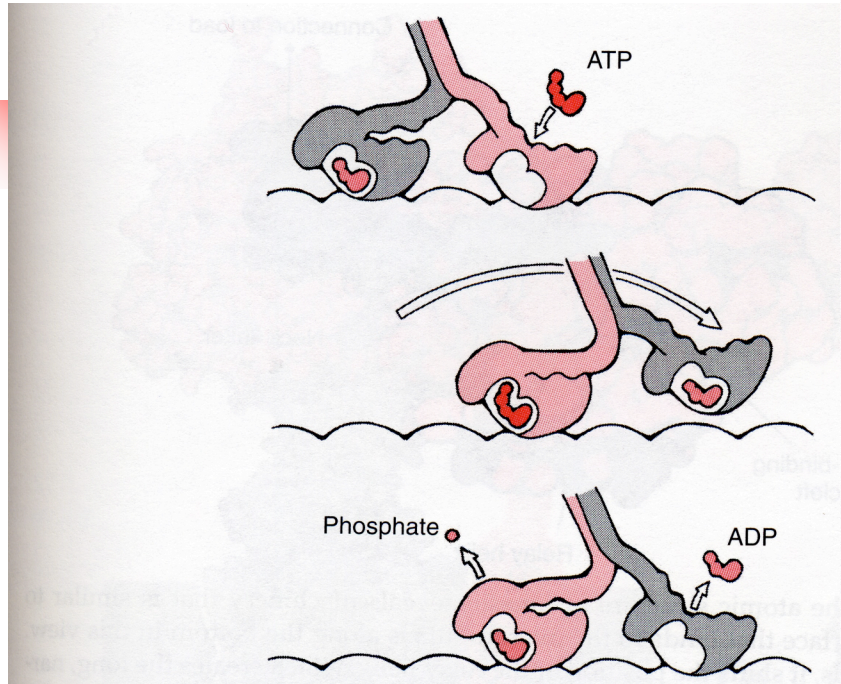


Figure 5-27 Kinesin relies on two motor units connected together. The cycle begins with one subunit empty and the other with ADP bound. ATP binds to the empty subunit and causes the neck linker to zip tightly onto the subunit, pulling the lagging subunit off the microtubule and forward to the next position. 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.

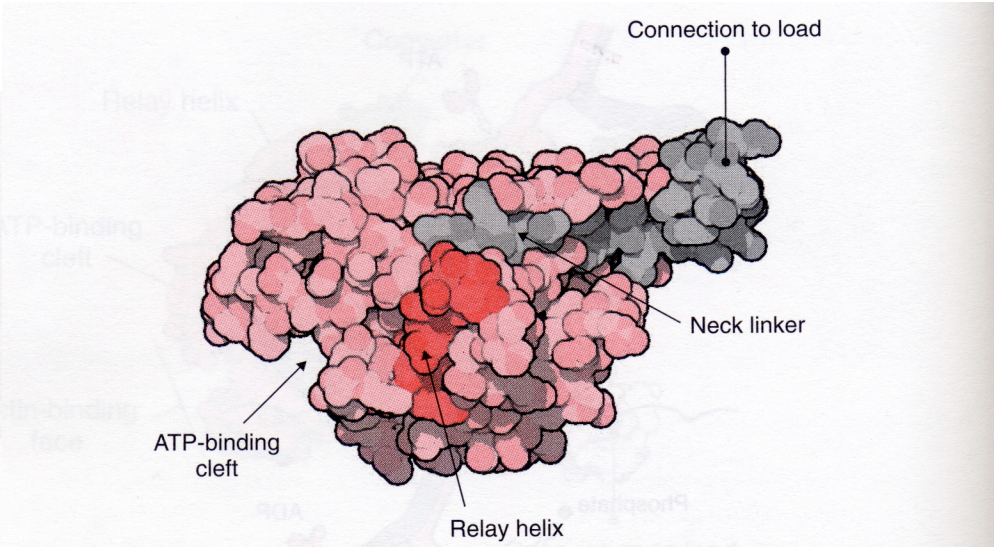
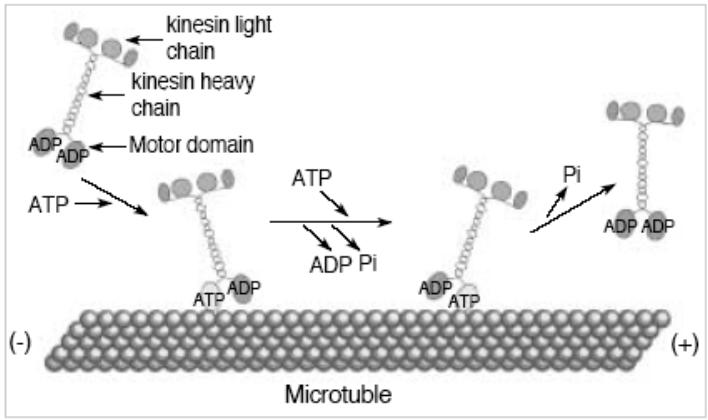


Figure 5-28 The atomic structure of kinesin reveals machinery that is similar to myosin. The surface that binds to the microtubule is along the bottom in this view. When ATP binds, it shifts the position of the relay helix, which creates the long, narrow groove that holds the neck linker. Force is generated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



- Diverse family of proteins.

Kinesin

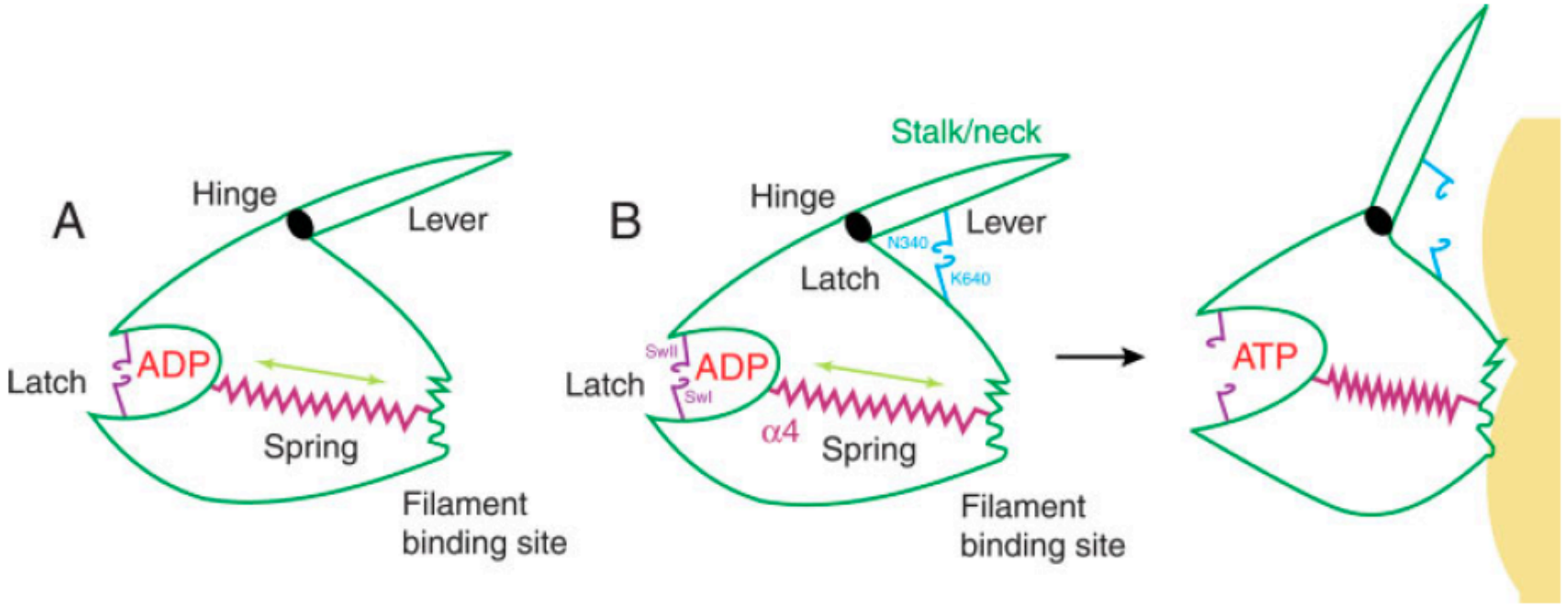


Figure 3. A motor as a machine. **A:** A general model. The motor consists of binding sites for nucleotide and a cytoskeletal filament, together with proposed mechanical components: a spring-like or elastic element to produce force, a lever to amplify the force, and a latch to regulate nucleotide binding 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.

BioEssays **25:1212–1219, 2003.**

Kinesin Walks Hand-Over-Hand

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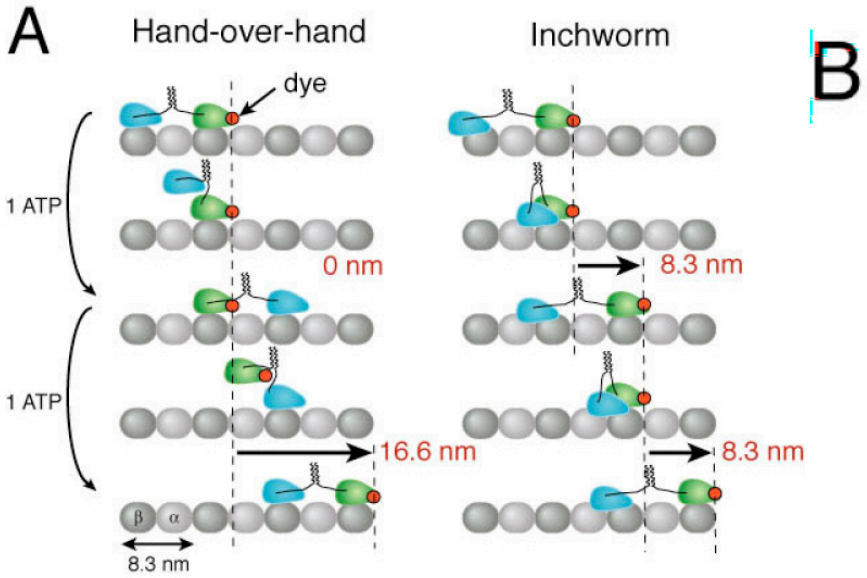
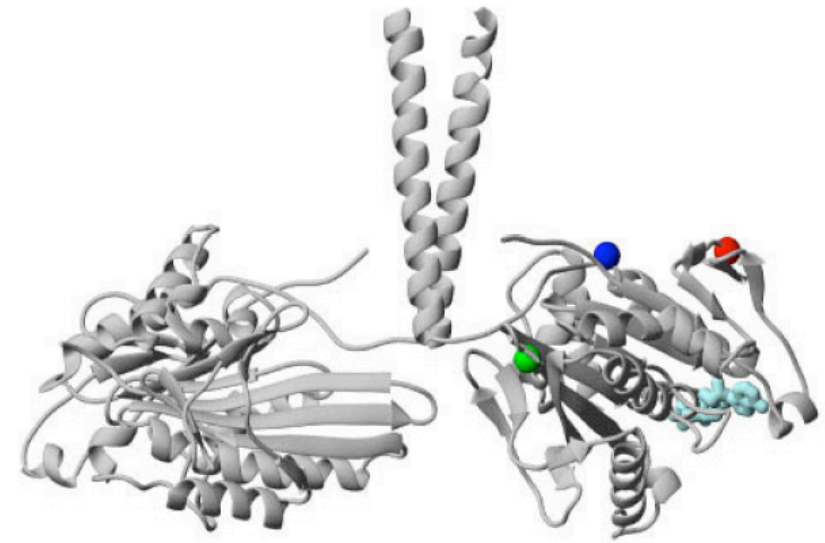
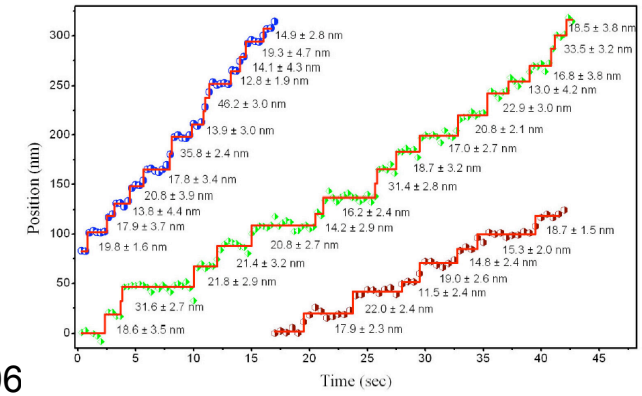
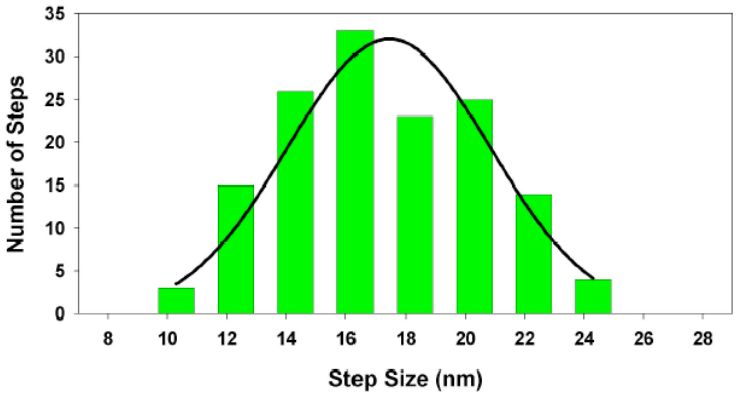
Sciencexpress / www.sciencexpress.org / 18 December 2003 / Page 2/ 10.1126/science.1093753

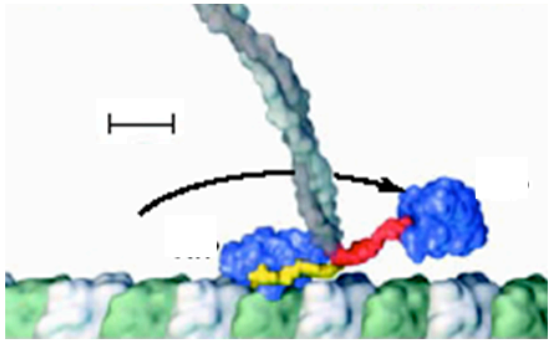








http://www.scripps.edu/cb/milligan/research/movies/



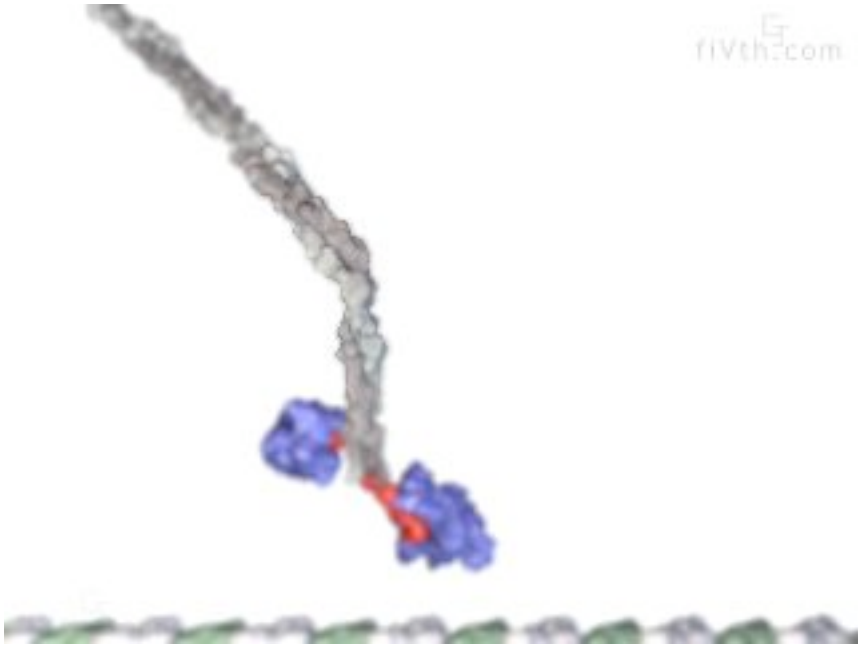
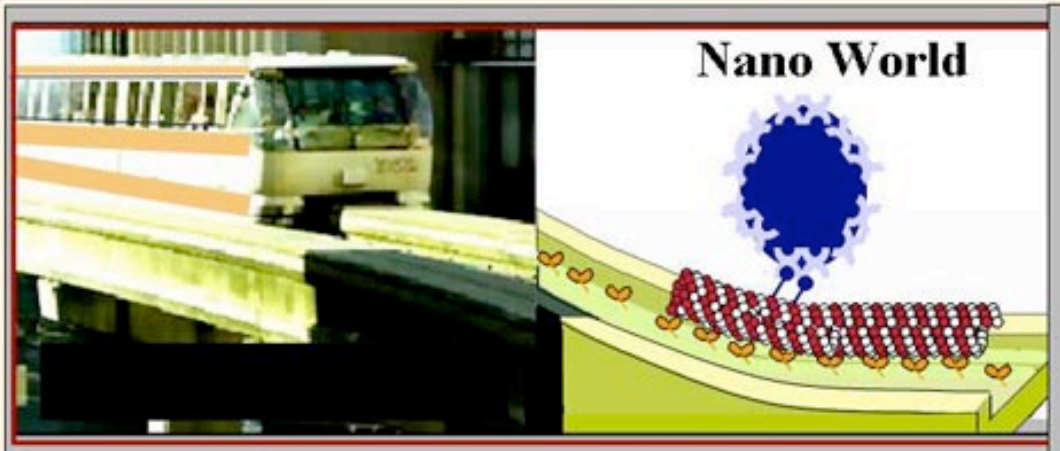


Figure 22.2: Kinesin steps forward, transporting its cargo toward the plus end of a microtubule. Scale bar = 4 nm. Figure from Vale and Milligan (2000). (From R. D. Vale and R. A. Milligan, Science **288**, 88 (2000) by permission of the American Association for the Advancement of Science.)

Building a Monorail at the Nanoscale to Shuttle Cargo using Motor Proteins:

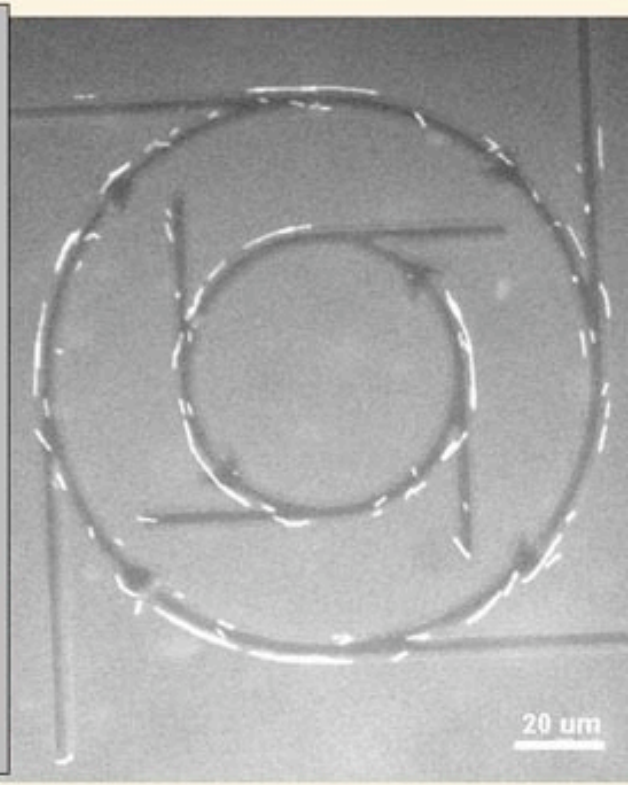
bringing life to dead matter



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

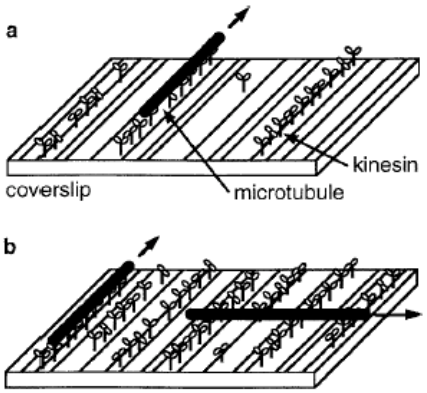


Nanotechnology 10 (1999) 232–236. Printed in the UK PII: S0957-4484(99)97176-3

Molecular shuttles: directed motion of microtubules along nanoscale kinesin tracks

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

Directed motion of microtubules along kinesin tracks



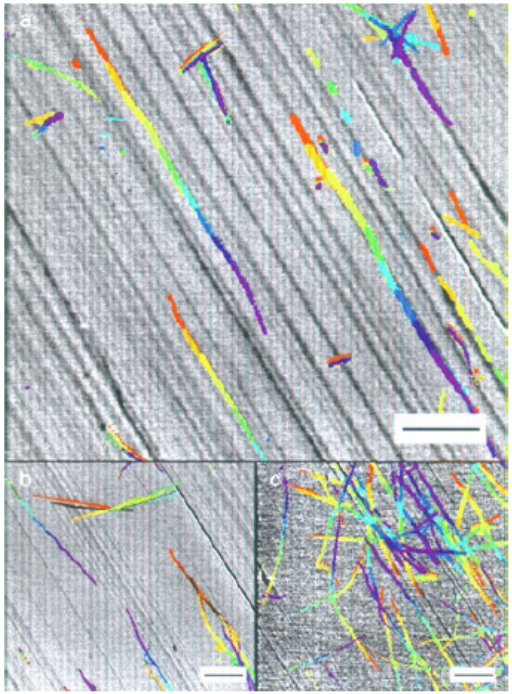


Figure 1. Motility of microtubules on shear-deposited PTFE films. Fluorescence images of photolabelled microtubules taken at 5 s intervals have been coloured (advancing from red to violet) and superimposed on a differential interference contrast (DIC) microscope image of the underlying shear-deposited PTFE film which is nonfluorescent. The kinesin surface density was varied by changing the concentration of the solution from which kinesin was adsorbed; concentrations used were: (*a*) 3 μg ml^{-1}, (*b*) 0.5 μg ml^{-1}, (*c*) 18 μg ml^{-1}. Scale bars are 10 μm.

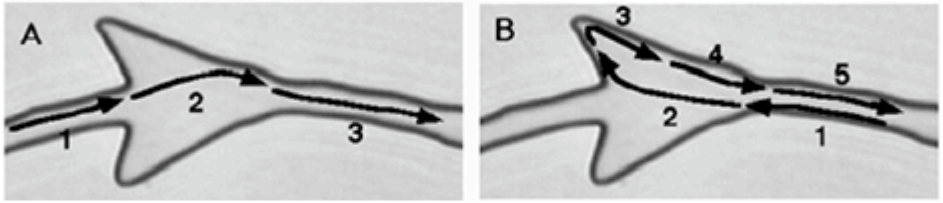
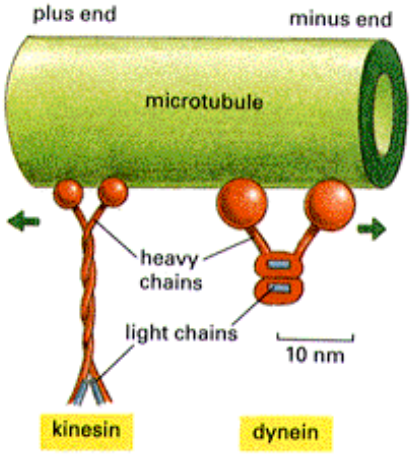
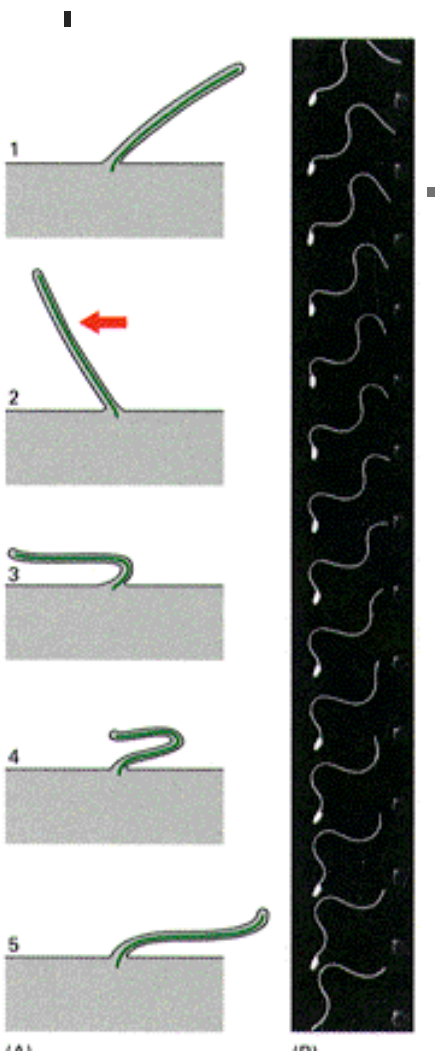


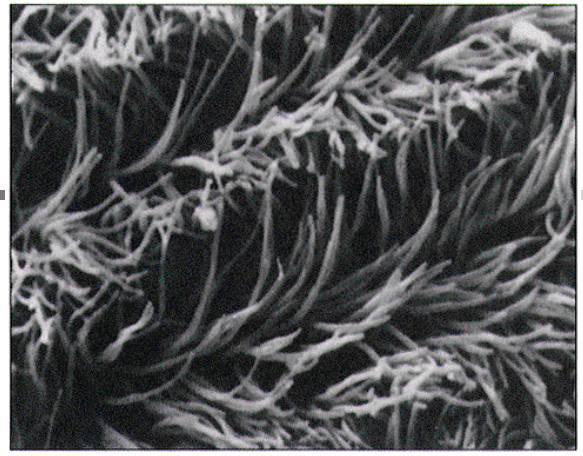
Figure 22.8: Patterned "ratchets" sort microtubules. Due to asymmetrically patterned photoresist, microtubules entering the triangular area exit to the right irrespective of their side of entry. This results in aligned and oriented microtubules. (From Ref. 42 by permission of the Biophysical Society.)

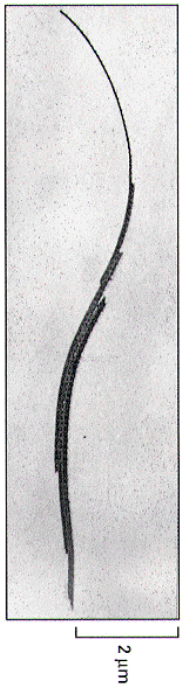
Cytoplasmic Dyneins

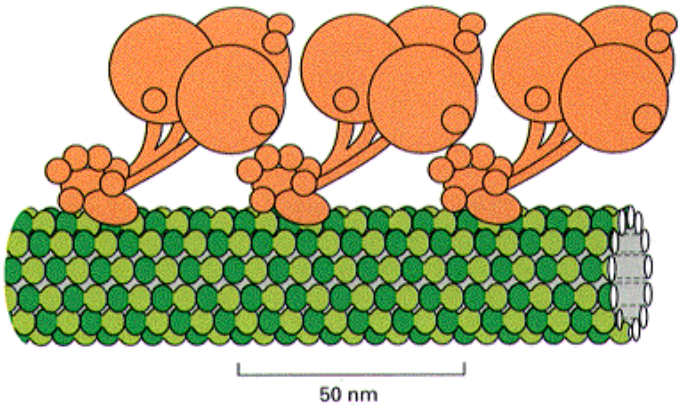


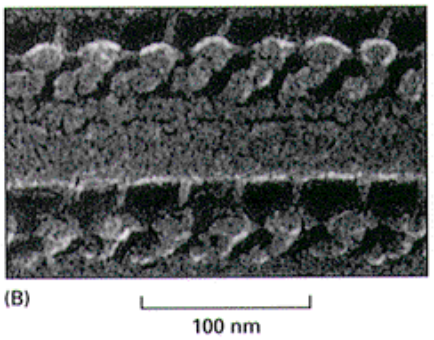
Ciliary Dyneins

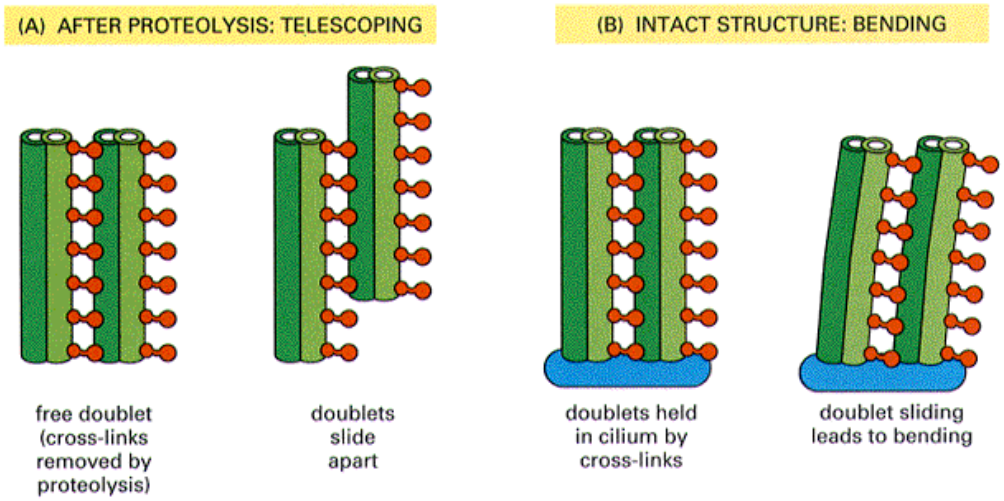




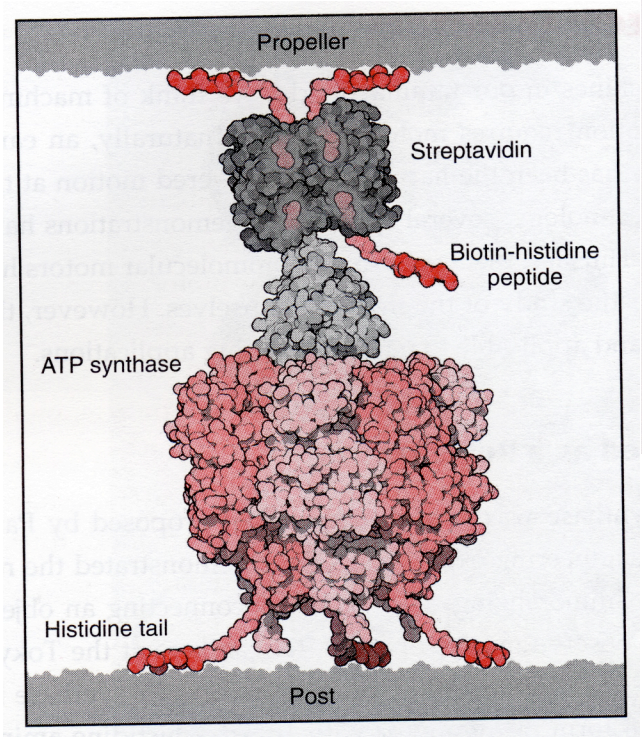








ATP Synthase



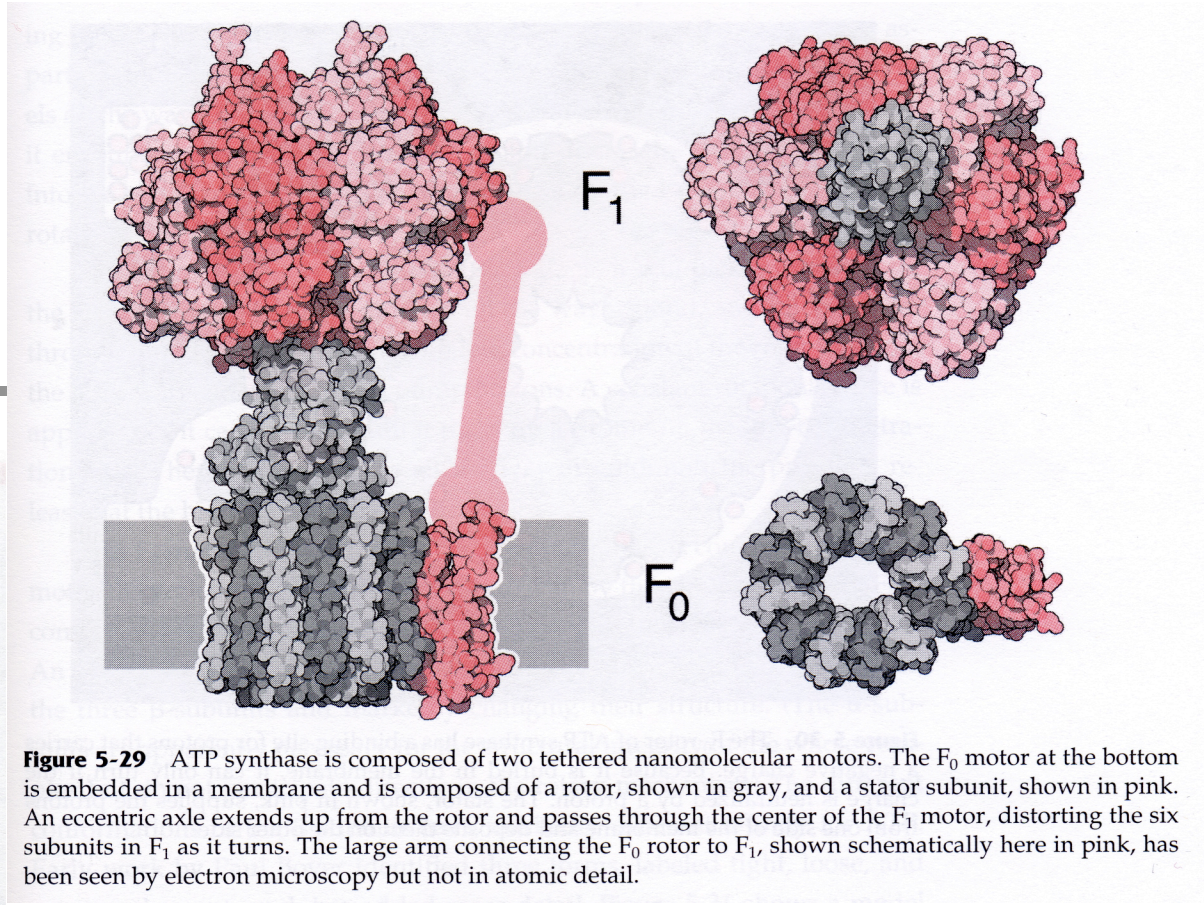
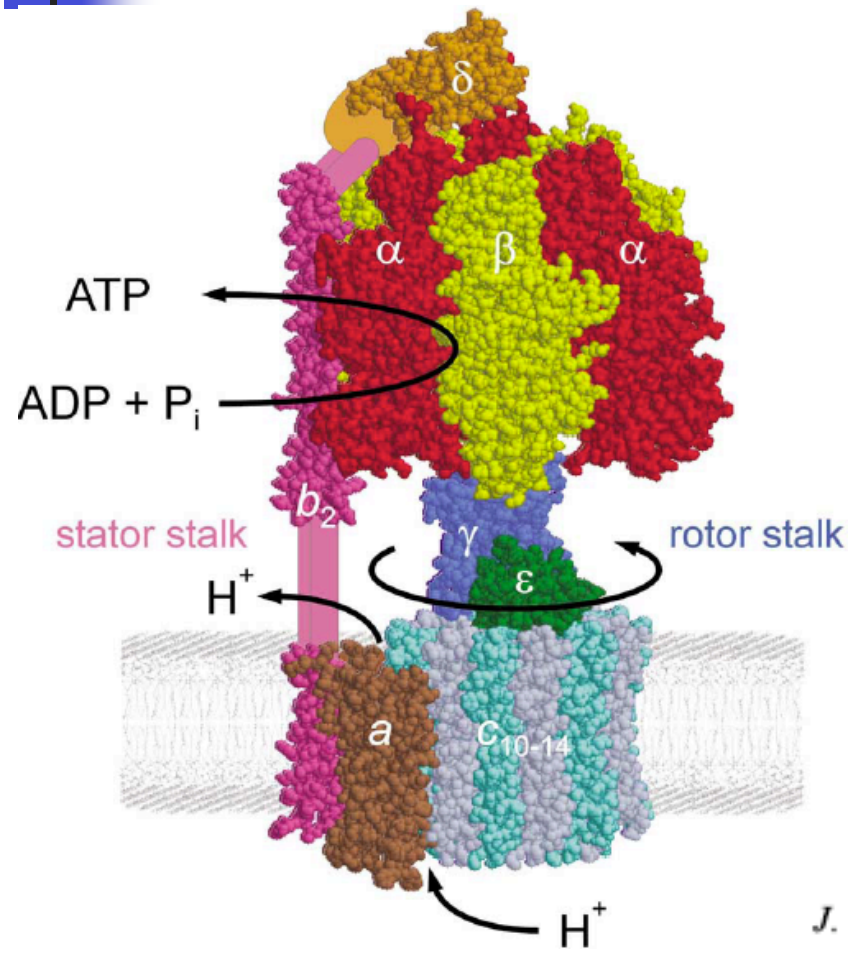


Figure 5-29 ATP synthase is composed of two tethered nanomolecular motors. The F_0 motor at the bottom is embedded in a membrane and is composed of a rotor, shown in gray, and a stator subunit, shown in pink. An eccentric axle extends up from the rotor and passes through the center of the F_1 motor, distorting the six subunits in F_1 as it turns. The large arm connecting the F_0 rotor to F_1, shown schematically here in pink, has been seen by electron microscopy but not in atomic detail.

- F_0 powered by proton (or Na^+) electrochemical gradient
- F_1 powered by ATP

ATP Synthase Protein Structure



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

J. Weber, A.E. Senior/FEBS Letters 545 (2003) 61–70

ATP Synthase

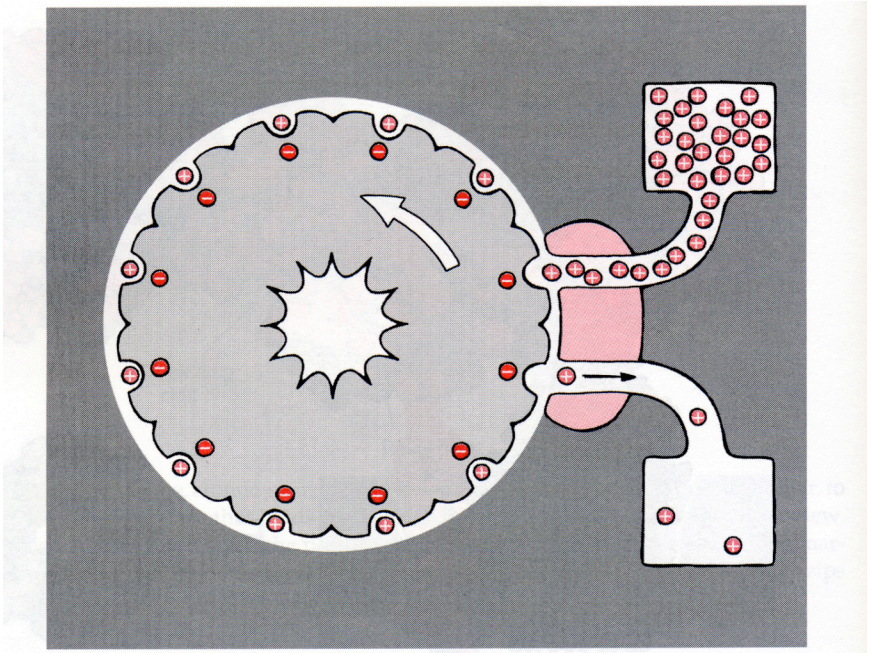


Figure 5-30 The F_0 rotor of ATP synthase has a binding site for protons that carries a negative charge. Because it is buried in the membrane, it can only turn if the charge is neutralized by a proton. The stator, shown in pink, supplies the protons from one side of the membrane and deposits them on the other side.

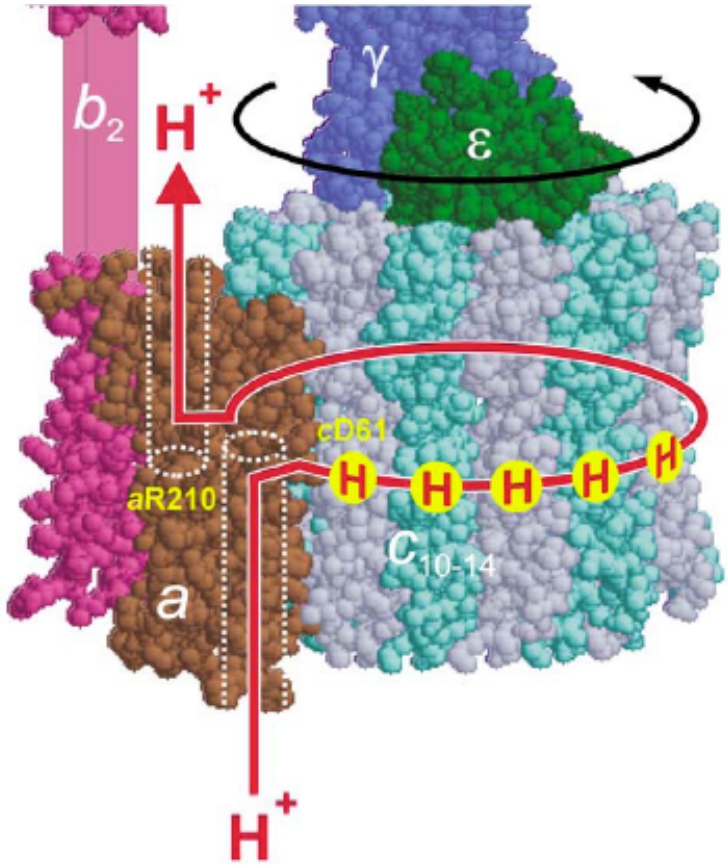
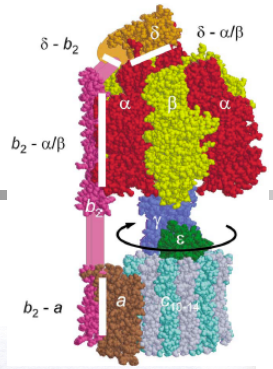


Fig. 3. Structure of ATP synthase showing proposed proton transport path. Residues *c*Asp61 and *a*Arg210 lie in the center of the bilayer, at the *a*/*c* interface. Their concerted interaction is required for proton movement. Putative access channels for ingress/egress of protons are shown. The *c*-ring carries protons around on protonated *c*Asp61 as it rotates.

ATP Synthase, F_1



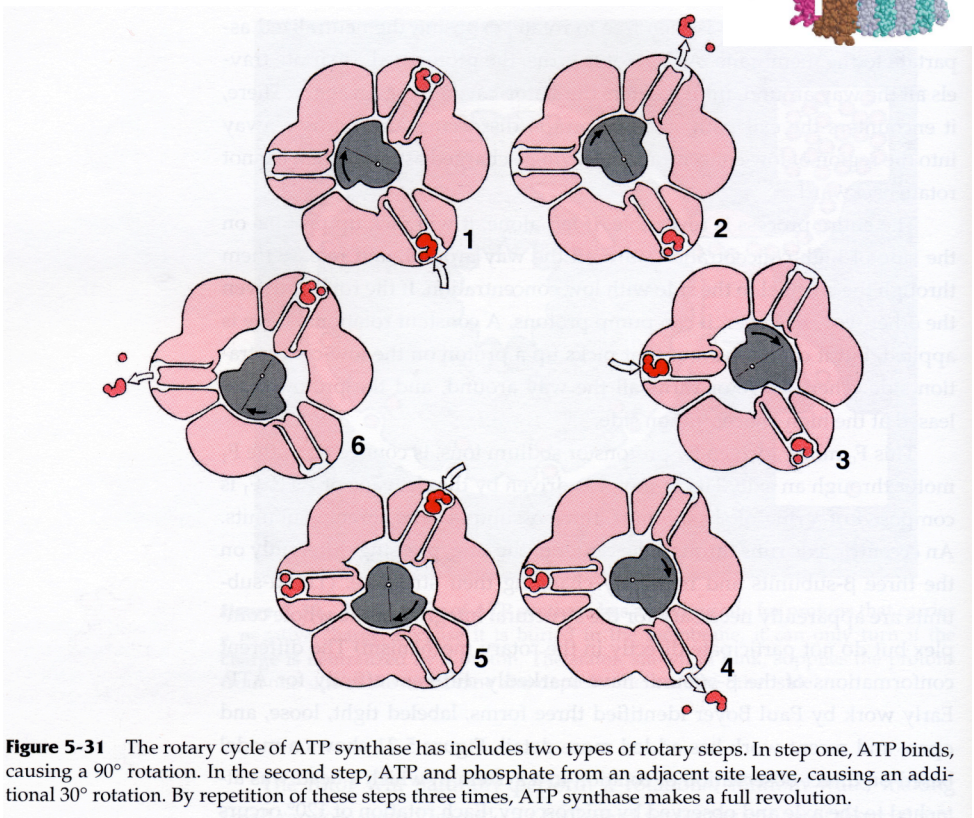
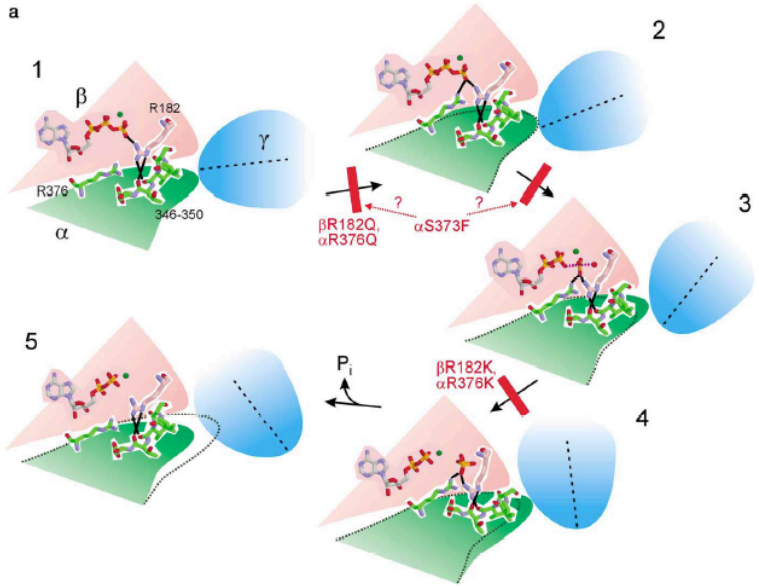
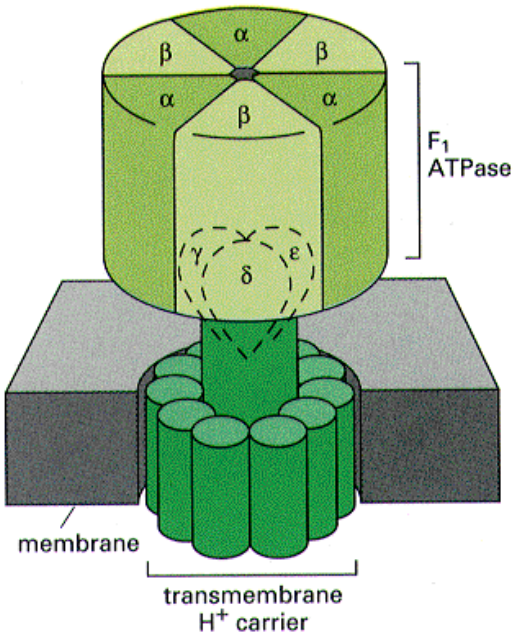


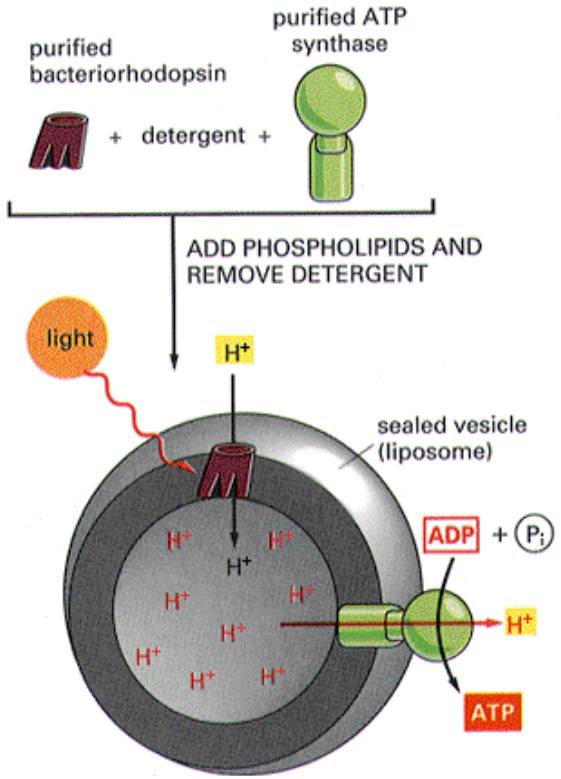
Figure 5-31 The rotary cycle of ATP synthase has includes two types of rotary steps. In step one, ATP binds, causing a 90° rotation. In the second step, ATP and phosphate from an adjacent site leave, causing an additional 30° rotation. By repetition of these steps three times, ATP synthase makes a full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

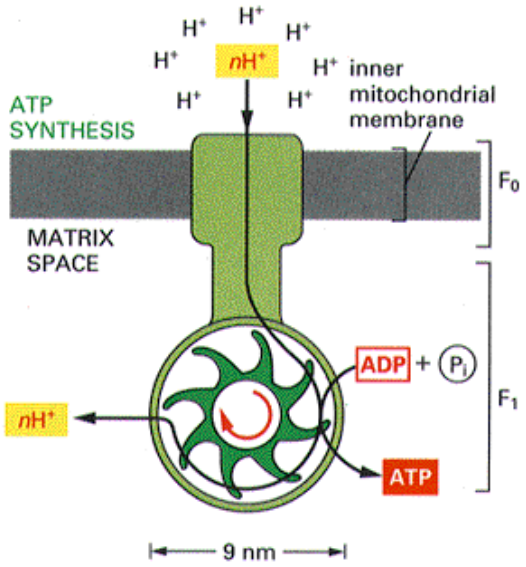


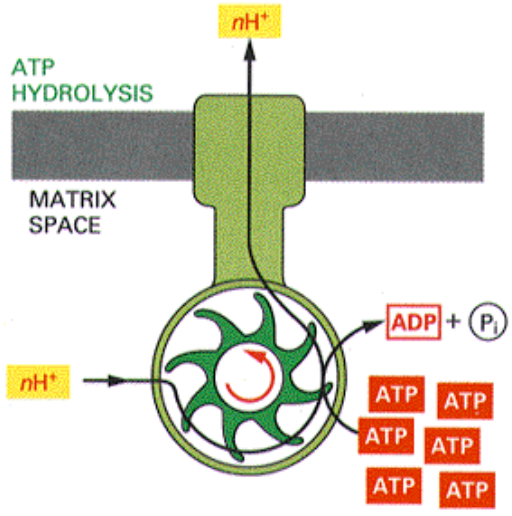
- Reversible
- Mechanoenzymatic mechanism
- Large protein motions
- Allosteric control

ATP Synthase









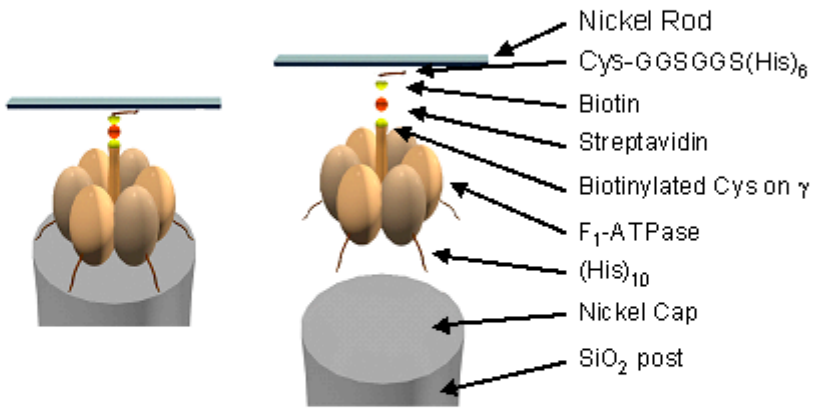


Figure 22.9: Left: Depiction of an assembled hybrid biomolecular nanodevice based on the rotary motor F1-ATPase (not to scale). Right: Exploded view showing all structural and linking components. The device self-assembles in multiple steps.

Flagellar motor

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.

- 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.

Flagellar motor

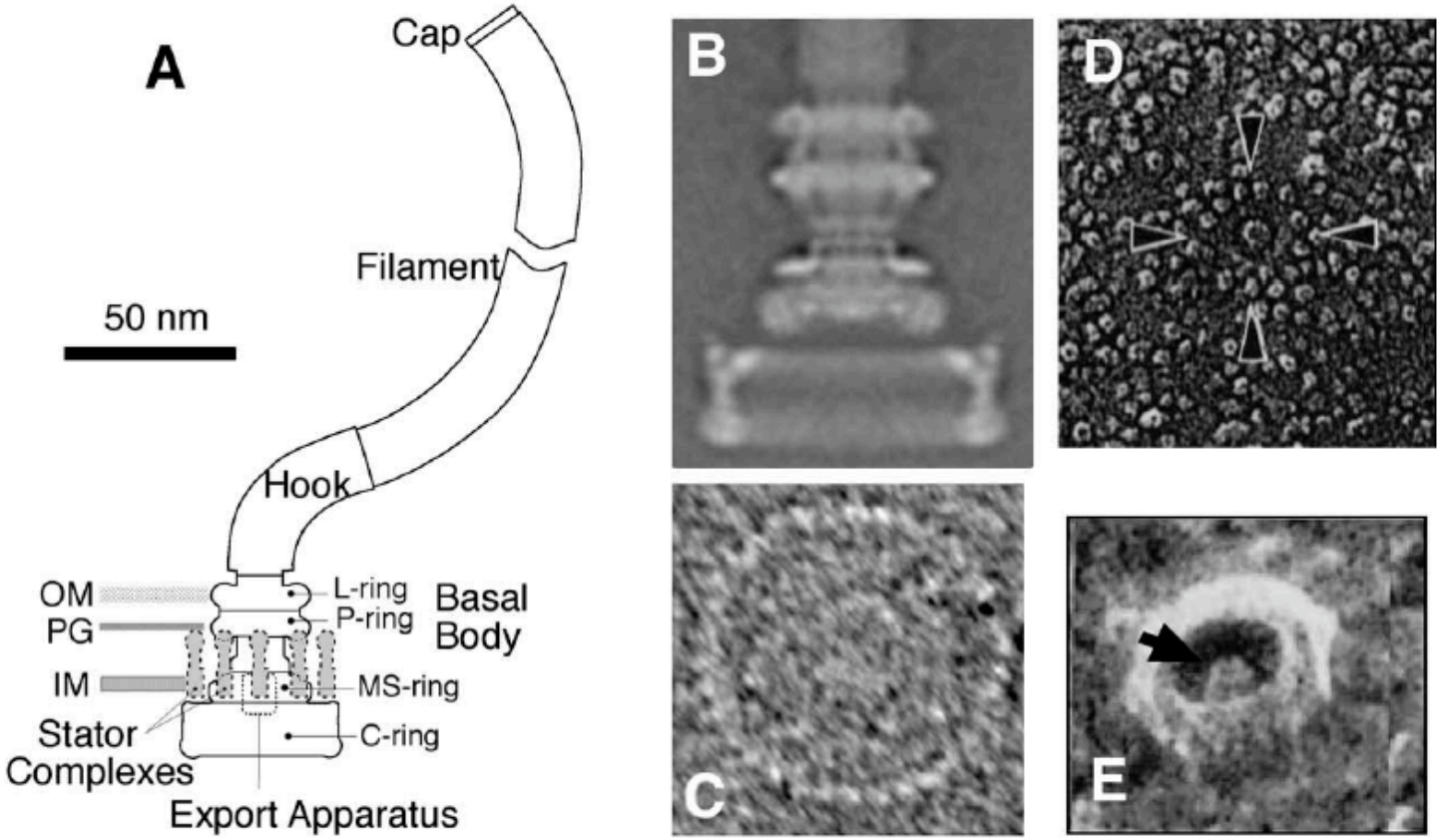


Fig. 1. A: Diagram of the flagellum in a Gram-negative bacterium. Gram-positive species lack the LP-ring assembly. Only a fraction of the full filament length is shown, as it is quite long on this scale (ca. 10 μm). OM, outer membrane; PG, peptidoglycan; IM, inner membrane. B: Electron micrographic reconstruction 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

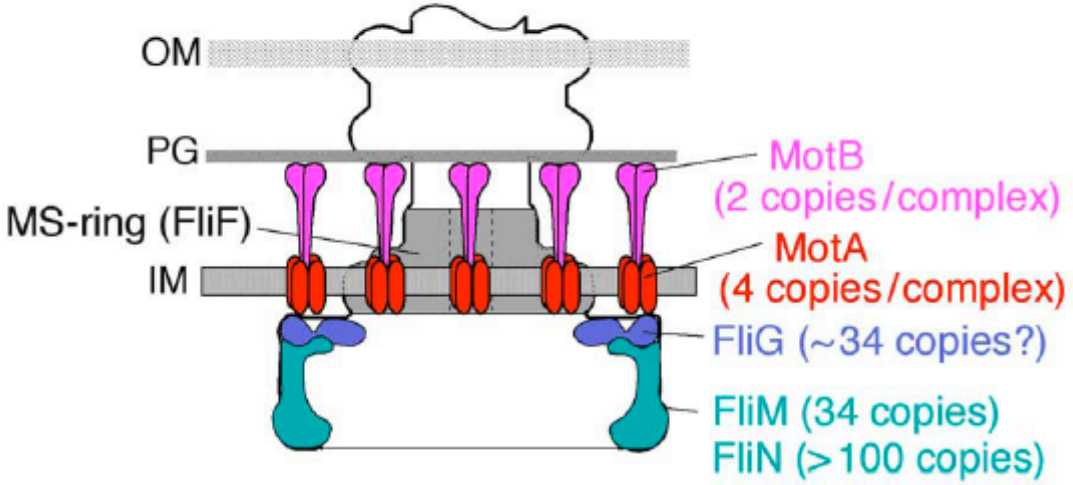


Fig. 2. Proteins that function in rotation. The MotA and MotB proteins form the stator complexes, anchored to the cell wall by a putative peptidoglycan-binding motif in the periplasmic domain of MotB. Each motor contains several (as many as eight) stator complexes, each with composition $MotA_4MotB_2$. 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 F_1, 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 F_0 motor and ATP used in actin polymerization.

PRESTIN, A NEW TYPE OF MOTOR PROTEIN

NATURE REVIEWS | MOLECULAR CELL BIOLOGY
VOLUME 3 | FEBRUARY 2002 | 105

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.

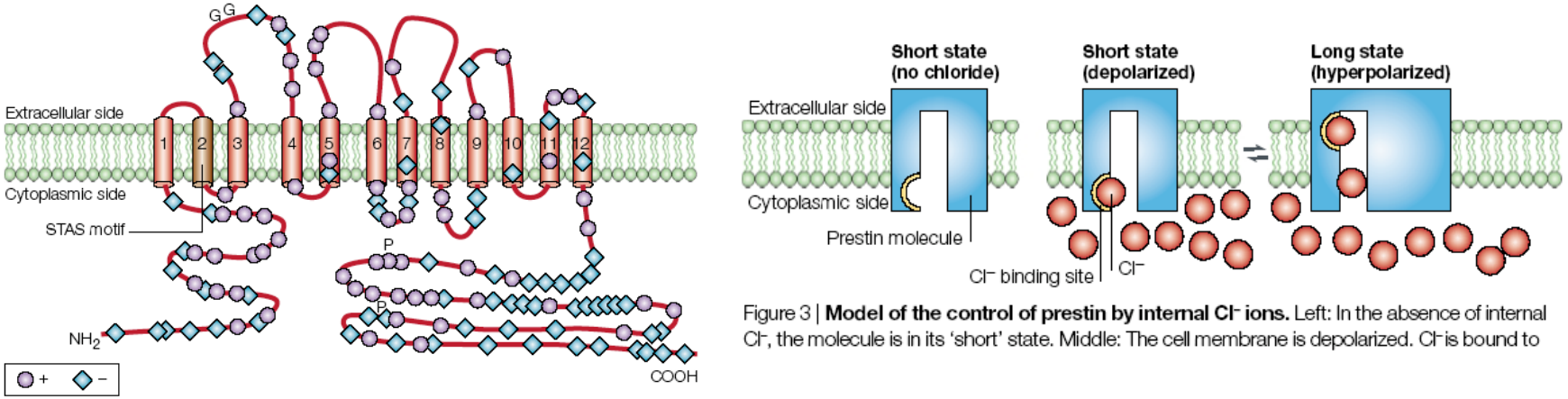


Figure 3 | **Model of the control of prestin by internal Cl^- ions.** Left: In the absence of internal Cl^-, the molecule is in its 'short' state. Middle: The cell membrane is depolarized. Cl^- is bound to