Movement at the Molecular Level

Diffusion: $\langle r^2 \rangle = 6 D t$ ($D \approx 6 \pi \mu a$)

Typical numbers:

10 nm protein in water D= 10^{-10} m^2/s

….in cells D= 10^{-12} m^2/s (D= 10^{-14} m^2/s lipids)

$[\langle r^2 \rangle]^{1/2}$ =1 μm, t ~0.2 sec in cells

$[\langle r^2 \rangle]^{1/2}$ =10 μm, t ~20 sec in cells

Slow and isotropic.

Image removed due to copyright considerations.

How to generate fast vectorial motion ?

Axonal transport of organelles in giant squids

Directed (Vectorial) Molecular Movement

Polymerization:
Living polymerization of actin/microtubules

Springs:
Conformational changes of molecules

Motor Proteins:
nucleotide (ATP) hydrolysis: chemical energy -> work

Pumps:
Hydrolysis of ATP
Create concentration gradients

Actin Comets Propelling Listeria

Listeria monocytogenes moving in PtK2 cells

These pathogenic bacteria grow directly in the host cell cytoplasm.
The phase-dense streaks behind the bacteria are the actin-rich comet tails.
Actin-based motility is also used in cellular motility; this cell is using it's cytoskeleton to crawl toward the lower right-hand corner. Speeded up 150X over real time. 3

--Julie Theriot & Dan Portnoy

Actin is Transiently Tethered to the Bacteria

Images removed due to copyright considerations.
See Cameron, L.A., T. M. Svitkina, D. Vignjevic, J. A. Theriot, and G. G. Borisy. "Dendritic organization of actin comet tails."
Curr Biol. 2001 Jan 23;11(2):130-5.

Noireaux et al. (2000): it takes about 10 picoN to separate the actin from the comet…

Images removed due to copyright considerations.
See Kuo, S. C and J. L. McGrath.
"Steps and fluctuations of *Listeria monocytogenes* during actin-based motility ."
Nature. 2000 Oct 26;407(6807):1026-9.

Steps of 5.4nm

Elastic Brownian Ratchets and Tethered Filaments

Images removed due to copyright considerations.
See Mogilner, A. and G. Oster.
"Force generation by actin polymerization II: the elastic ratchet and tethered filaments ."
Biophys J. 2003 Mar;84(3):1591-605.

Brownian:
Actin filament tips fluctuate

Some filaments are tethered

Actin Ruffles in Motile Cells

Supramolecular Springs

Energy stored in chemical bonds which act as “latches’

Regulated by Spasmin:
Calcium binding protein

Images removed due to copyright considerations.
See Mahadevan, L. and P. Matsudaira.
"Motility powered by supramolecular springs and ratchets."
Science. 2000 Apr 7;288(5463):95-100.

Horseshoe Crab Sperm

Uncoiling of an actin spring

(unlike the echinoderm sperm - no polymerization!)

Images removed due to copyright considerations.
See Mahadevan, L. and P. Matsudaira.
"Motility powered by supramolecular springs and ratchets."
Science. 2000 Apr 7;288(5463):95-100.

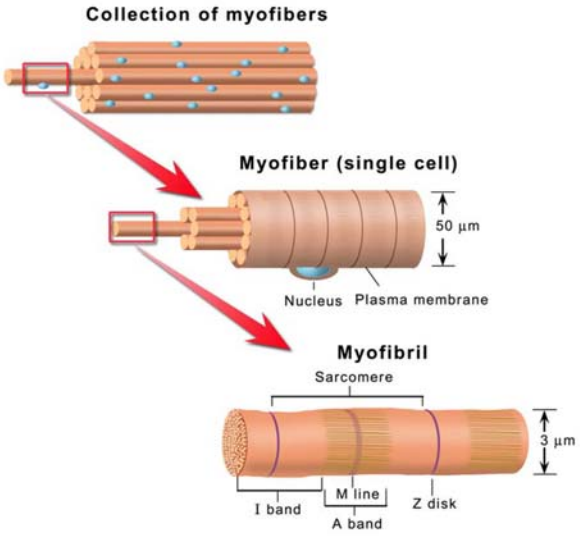
Molecular Motors

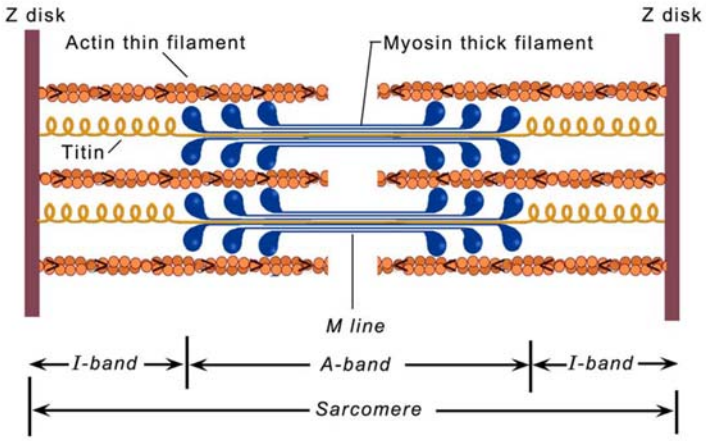
- Molecules that convert chemical energy into mechanical force
- Motors are specialized for specific tasks:
  - cell division
  - cell movement
  - organelle transport
  - synthesis of ATP
- Most move unidirectionally along polymer filaments
- Coupled mechanical and chemical cycles (fuel)

Motor Types

Motor	***Track***	***Functions***
Myosin	F-actin	Cell crawling
Myosin II	F-actin	Muscle contraction Cell division Phagocytosis
Kinesin	microtubule	Organelle transport Mitosis & meiosis
Dynein	microtubule	Flagella & cilia
Polymerases, Helicases	ds and ssDNA	Replication, Repair Recombination

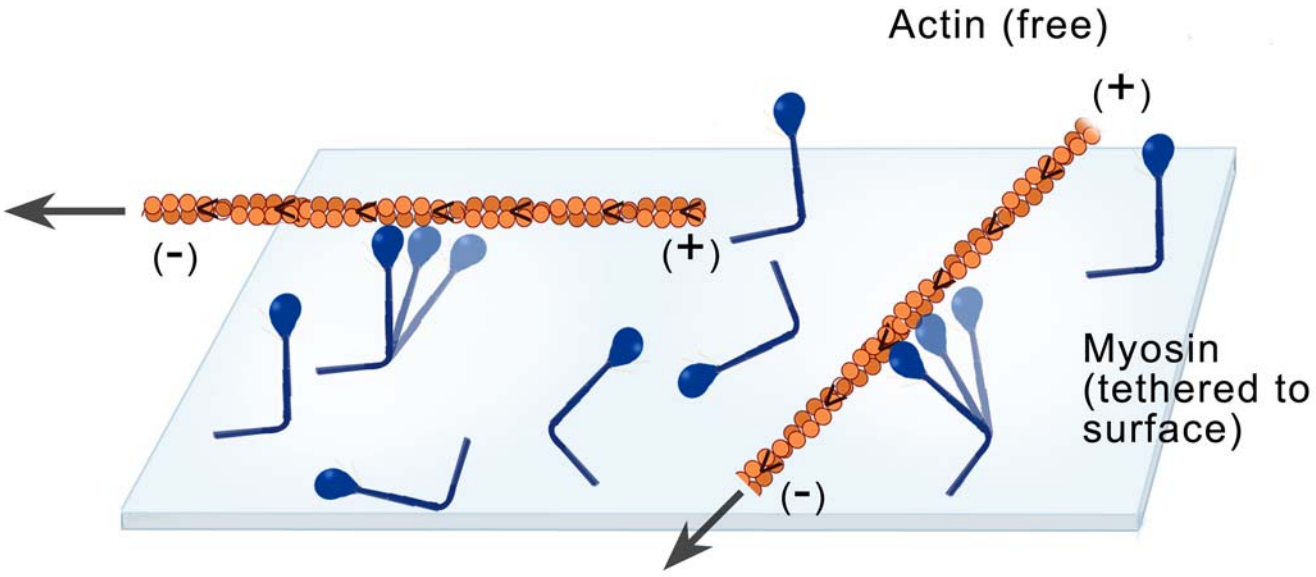
Muscle Anatomy





Muscle Types:
Skeletal: fast
Cardiac: fast
Smooth: slow

Myosin Heads Walk Along Actin Filaments



Polar Biopolymer Molecules : The Tracks Motor Proteins Walk Along

Myosin walks along Actin

G-Actin (globular) F-Actin (microfilaments)

- end

Image removed due to copyright considerations.
See [Lodish 4th ed.] Figure 18-2.

+ end

Diameter:	6-8 nm
Persistence length:	16 μm
Young's modulus:	1.3-2.5 x 10^9 Pa

Myosin: the actin motor protein

All myosins have head, neck, and tail domains with distinct functions

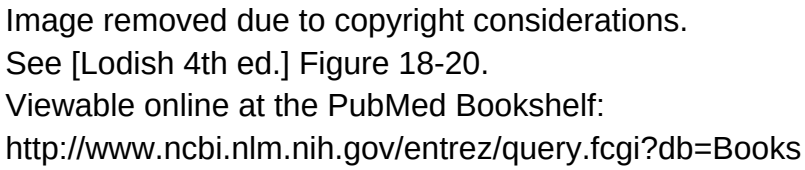


Figure 18-20

Myosin Types

Conventional Type II
- muscle contraction
- cytokinesis
- cell adhesion, migration

Unconventional Types I, III-…
- type I: cell migration

We will concentrate on type II, but other types display similar mechanisms…

Skeletal muscle contains a regular array of actin and myosin II: the sarcomere

Image removed due to copyright considerations.
See [Lodish 4th ed.] Figure 18-27.
Viewable online (Fig. 18-27b only) at the PubMed Bookshelf:
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=Books.

Figure 18-27

Skeletal muscle contains a regular array of actin and myosin

Image removed due to copyright considerations.
See [Lodish 4th ed.] Figure 18-27c.

Figure 18-27

Capping proteins stabilize the ends of actin thin filaments in the sarcomere

Image removed due to copyright considerations.
See [Lodish 4th ed.] Figure 18-28.
Viewable online at the PubMed Bookshelf:
http://www.ncbi.nlm.nih.gov/books/bv.fcgi?rid=mcb.figgrp.5204.

Figure 18-28

Thick and thin filaments slide past one another during contraction

Image removed due to copyright considerations.
See [Lodish 4th ed.] Figure 18-29.
Viewable online at the PubMed Bookshelf:
http://www.ncbi.nlm.nih.gov/books/bv.fcgi?rid=mcb.figgrp.5208.

During contraction the myosin head initially binds tightly the thin filament (actin) to form a cross-bridge

Once in contact the head rapidly bends towards the center of the sarcomere during the power stroke

The thin filament is then displaced towards the center of the sarcomere by about 10 nm

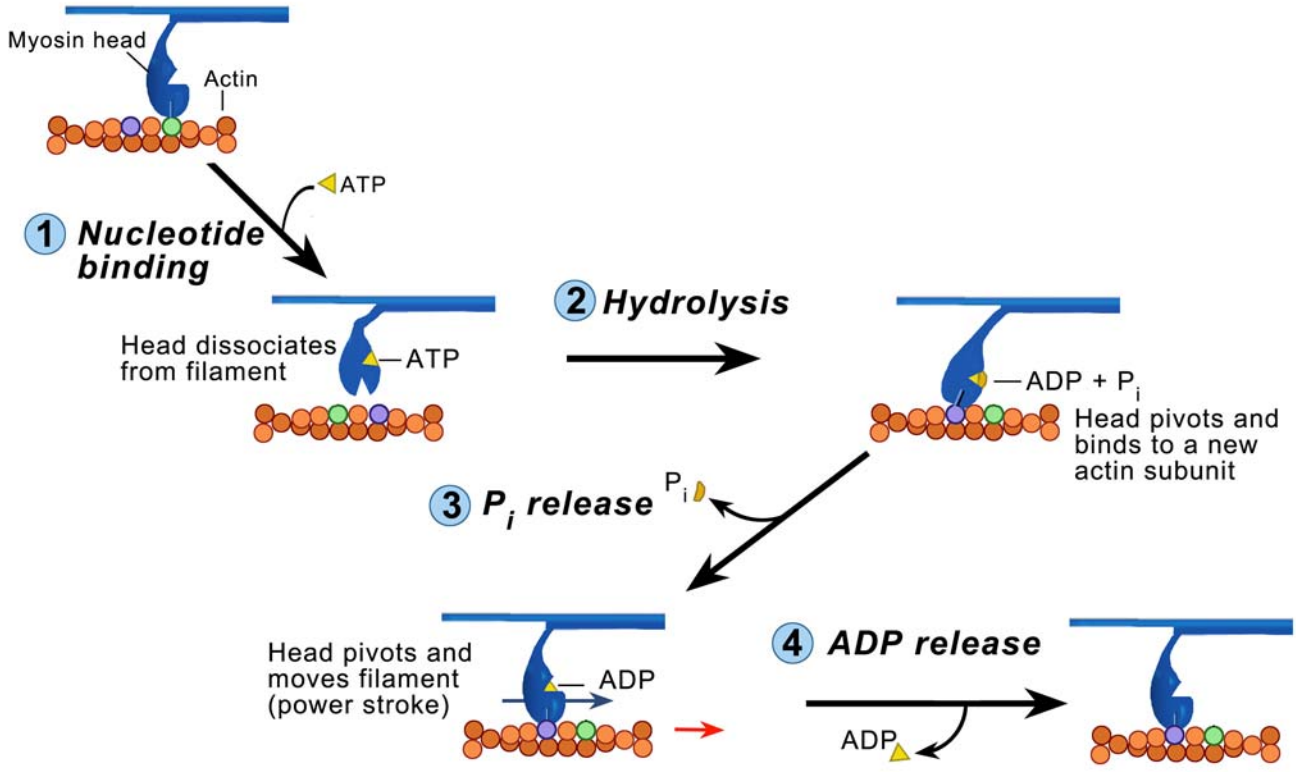
The head releases from the thin filament, reverts back to the initial conformation and the cycle repeats

The heads are only in contact with the filament about 5% of the time

Continuous movement because several heads are marching along the filament

Muscle Contraction

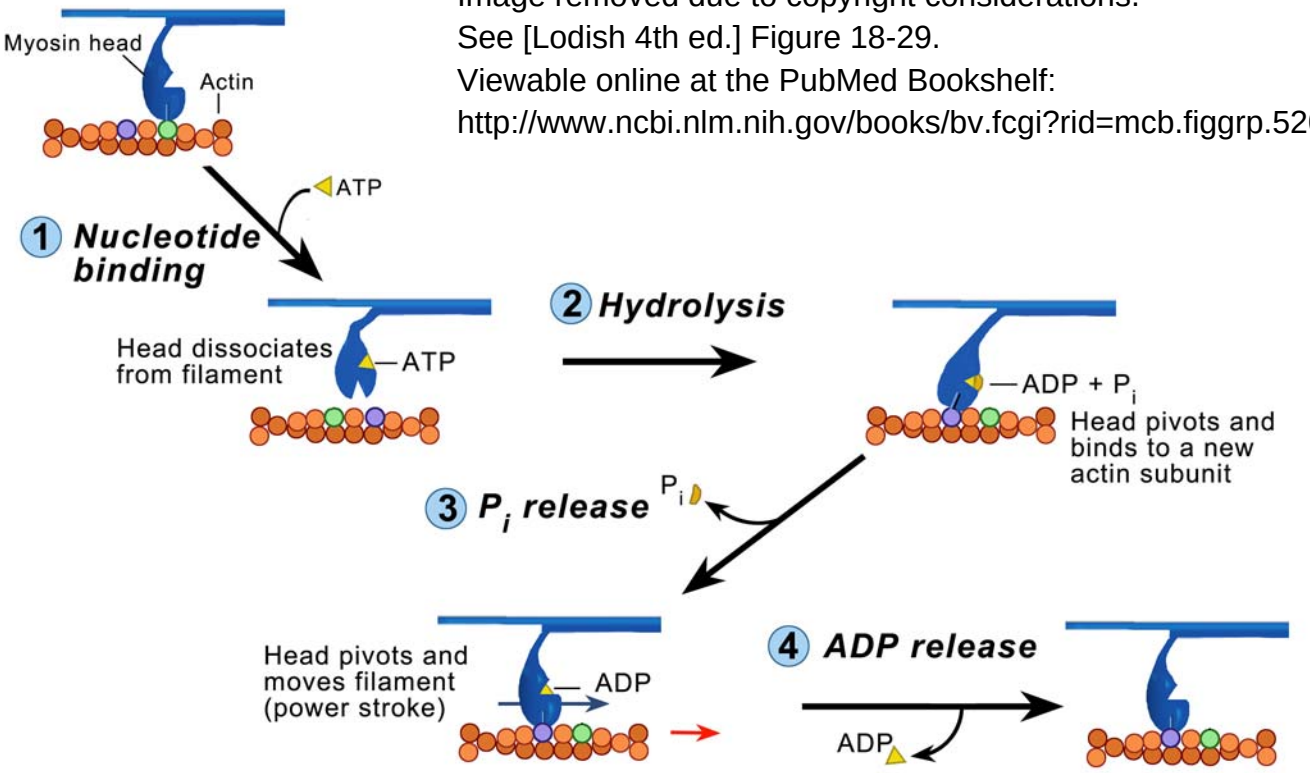
Conformational changes in the myosin head couple ATP hydrolysis to movement



Sliding Filament Model

Huxley & Huxley 1954

Image removed due to copyright considerations.
See [Lodish 4th ed.] Figure 18-29.
Viewable online at the PubMed Bookshelf:
http://www.ncbi.nlm.nih.gov/books/bv.fcgi?rid=mcb.figgrp.5208.



The Myosin ‘Power Stroke’

pre-stroke

Small conformational change
In head is amplified by swinging
movement of the neck.

Light chains increase
rigidity of the neck.

post- stroke

Images removed due to copyright considerations.
See Figures 4 and 6 in Geeves and Holmes.
"Structural mechanism of muscle contraction."
Annu Rev Biochem. 1999;68:687-728.

ATP: Cellular Fuel

Image removed due to copyright considerations.
Chemical bond diagram of ATP.

ATP Hydrolysis:

$$ATP + H_20 \text{ - > } ADP + P_i$$

Strongly favored

$$K_{eq} = 4.9 \times 10^5$$

Depends on conditions

- ***Large activation barrier w/o a catalyst= stable fuel***
- ***Free energy change at cellular conditions: -25 kT***

Mechanochemical Coupling : Myosin II

Motor and no actin: low activity ~ 0.1 s^{-1}

$$M \rightleftharpoons MT \rightleftharpoons MDP \rightleftharpoons MD + P$$

Rate limiting step

M = motor
T = ATP
D = ADP
P = phosphate
A = actin

Motor and with actin: increased activity ~ 25 s^{-1}

$$MT \rightleftharpoons MDP \rightleftharpoons AMDP \rightarrow AMD \rightleftharpoons AM \rightleftharpoons AMT \rightleftharpoons MT$$

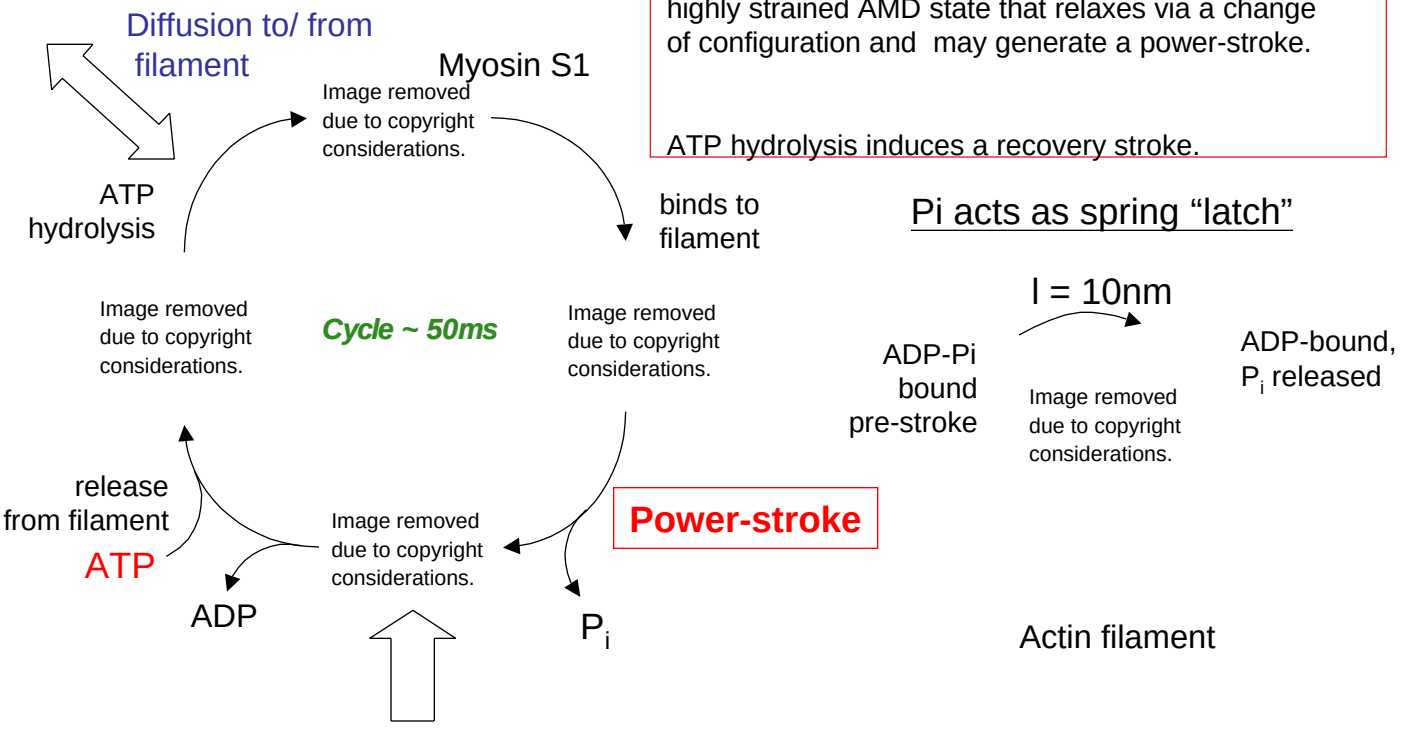
Key ideas:

- ***release of P (chemical) is catalyzed by binding to actin (mechical)***
- ***w/o ATP myosin bonds strongly to actin***
- ***release of myosin (mechanical) is catalyzed by ATP binding (chemical)***

The actin-myosin ATPase cycle

Allostery: release of P after MDP binds to actin creates a highly strained AMD state that relaxes via a change of configuration and may generate a power-stroke.

ATP hydrolysis induces a recovery stroke.



Images adapted from
Vale and Milligan, Science **288**, 88 (2000).

Mechanochemical Coupling : Myosin Power-Stroke

Tropomyosin and troponin regulate contraction in skeletal muscle

Ca2+ influences the position of
TP & TN on the actin filament.

Image removed due to
copyright considerations.

Binding sites: closed open

Increasing the Working Stroke Distance in Myosin

Image removed due to copyright considerations.
Diagram with caption Figure 4.

Processive

J. Howard 1997

Assays to Study Motor Proteins *in vitro*

Image removed due to
copyright considerations.

Polar Biopolymer Molecules : The Tracks Motor Proteins Walk Along

Microtubules

Image removed due to copyright considerations.

Diameter:	24 nm
Persistence length:	60,000 μm
Young's modulus:	1.9×10^9 Pa

The Role of Kinesin & Dyneins

Image removed due to copyright considerations.

MTOC: Microtubule-organizing center

Bidirectional transport of organelles

Structure of Motor Proteins

Myosin Kinesin

Image removed due to copyright considerations.
See [Lodish].

The head contains ATP and filament binding sites.

Mechanochemical Coupling :Conventional Kinesin

Key Points:

-***with ATP kinesin is bound tightly to microtubules***

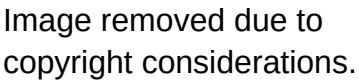
-***unbinding of kinesin from microtubule***
requires hydrolysis of ATP

- ***in the absence of microtubule kinesin dissociates***
P quickly

-***release of ADP from kinesin is catalyzed by binding to microtubule***

- 2 heads coordinate movement

Hand-over-Hand Model for Conventional Kinesin



http://www.current-opinion.com/jcel/mov1.mov

Image removed due to copyright considerations.

Release of trailing head (K1) is contingent on the binding of the leading head (K2)…

…binding of ATP to K1 catalyzes attachment of K2 to microtubule…

…this catalyzes release of ADP from K2…

…which catalyzes detachment of K1 from the microtubule…

…release of P from K1…

Schief & Howard 2001

Motor Proteins: Power Strokes

Image removed due to copyright considerations.

Common Themes

- *Filaments are polar and motor binding is stereospecific.*
- *This leads to movement in one direction (+ or -).*
- *Stall forces ~ few (6-10) pN*
- *Cyclic motors*
- *Nucleotides roles:*

    1. *regulates attachment/detachment.*
    2. *drives working/recovery strokes.*
    3. *chemical steps are contingent on the completion of mechanical steps.*

Differences

Myosin II	*Conv. Kinesin*
non-processive	*processive (~100 steps of more)*
5-15 nm step sizes	*8 nm*
slips	*no slipping*
walks towards + end of filament	*walks towards -end of filament*
found in large assemblies	*works well alone or low #'s*
'rower'	*porter*

Motor Speeds (assemblies)

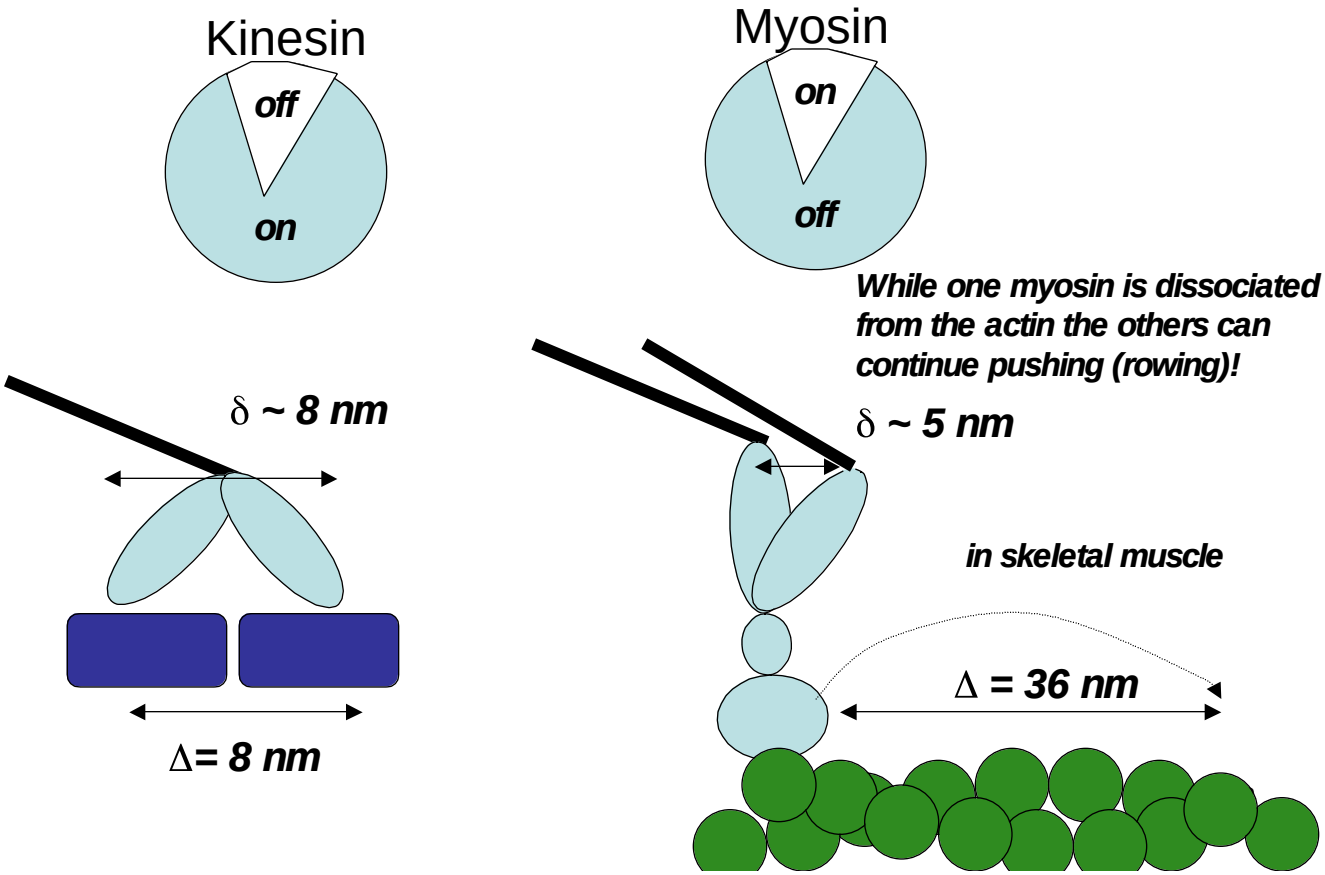
Motor type	*speed in vivo (nm/s)*	*in vitro(nm/s)*	*in vitro ATPase (s-1)*
***Myosin II** (skeletal muscle)*	***6000***	***8000***	***20***
***Myosin II** (smooth muscle)*	***200***	***250***	***1.2***
***Myosin V** (vesicle transport)*	***200***	***350***	***5***
***Conv. Kinesin** (axonal transp)*	***1800***	***840***	***44***
***Nkin** (sec. Vesicle transp.)*	***800***	***1800***	***78***
***BimC/Eg5** (Mitosis/meisos)*	***18***	***60***	***2***
***Dyneins** (cytoplasmic)*	***-1100***	***-1250***	***2***

Speed in vivo = cell/extracts, motion of motor relative to filament w/o a load. Positive values Indicate movement toward positive end of filament.

Speed in vitro = purified motors at high ATP concentrations.

ATPase = max rate of hydrolysis per head per sec, measured at high ATP, filament concentrations.

Kinesin is attached during it's rate limiting step while myosin is detached during it's rate limiting step- ATP hydrolysis.



Mechanical Models for Motor Proteins

Myosin

Kinesin

Common Themes for Molecular Motors

- ATP hydrolysis drives the motors.
- The motors walk along polar tracks (polymers).
- Type of motor fits the function: processive/non.