Myosins: A Superfamily of Actin-Dependent Molecular Motors

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Molecular Motors

Myosins — interact with actin filaments

Kinesins — interact with microtubules
Dyneins — interact with microtubules

Bacterial flagellar motor — rotary motor imbedded in cell membrane

DNA/RNA polymerases — interact with nucleic acid polymers

Translational Elongation Factor G — the motor of the ribosomes — interacts with nucleic acid polymers

F$_1$-ATPase — proton motive rotary motor imbedded in mitochondria matrix
Traditionally, when you think of myosins, this is what you envision

Figure 16–51. Molecular Biology of the Cell, 4th Edition.
These individual molecules polymerize to form a thick filament.

Figure 16–52. Molecular Biology of the Cell, 4th Edition.
Myosin II Powers the Muscle Contraction

I-band
Actin filaments

A-band
Myosin filaments

Z-line
The motor domain of kinesin has structural homology to the core motor region of myosin. Both have homologies to that of the G-proteins. In other words, all three classes of proteins have a switch I, a switch II and a P-loop.
Light Chains Bind to IQ Motifs in the Neck Region of the Heavy Chain

IQXXRGXXXR

I Isoleucine
Q Glutamine
R Arginine
G Glycine
X Others

Light chains may be identical to those found in myosin II, calmodulin, or low molecular weight calmodulin-like proteins.

In myosin II there are 23 amino acids separating the two IQ motifs.

In unconventional myosin the separation varies between 22 and 26 amino acids.

Light chains may be involved in phosphorylation-dependent regulation in some myosin II molecules and in calcium-dependent regulation in other myosins.
Actin binding site
Switch II Relay
SH1 Helix
Nucleotide binding pocket
U50
L50
C
N
ELC
RLC
Near-rigor conformation
Nucleotide-free Scallop S1

From Houdusse et al. *PNAS* 97: 11238, 2000
In Vitro Motility Assay with Rabbit Skeletal Muscle Actin
Diversity of the Myosin Motor Family
Yeast have only 5 myosins:
2 Myosin I genes
1 Myosin II gene
2 Myosin V genes

Humans have 39 myosins
From 12 classes

Most Myosins Have a Head, a Neck and a Tail

**Head**, conserved motor domain

**Neck**, light chain binding domain

**Tail**, may contain functional domains or phosphorylation sites. Responsible for targeting or anchoring the molecule within the cell
Possible Myosin Functions in the Cell

From Post et al.
Myosins clearly perform a diverse set of task within cells.

Some of these functions are common to most cells such as cell division, maintenance of cortical tension, adhesion, Golgi transport.

Other functions may occur only in specialized cells such as maintenance of the structural integrity of stereocelia in the inner ear or in retinal cells.

Myosin XIV may be highly specialized to be involved in the infectious pathway of parasitic cells such as *Plasmodium falciparum*. It is only found in a few such organisms.

To accomplish this diversity, myosins have undergone evolution of both their molecular structure (primarily the neck and tail) as well as their kinetic pathways to give rise to molecules capable of carrying out many different functions.
Three very different myosin functions

Myosin II in muscle contraction

Myosin I in microvilli

Myosin V in cargo transport
Kinetic Evolution of Different Myosins Allow for Different Tasks

\[ K_1' \quad K_2' \quad K_3' \quad K_4' \quad K_5' \]

\[ \text{AM} + \text{ATP} \rightleftharpoons \text{AM(ATP)} \quad \text{AM.ATP} \rightleftharpoons \text{AM.ADP.P_i} \quad \text{AM.ADP} \rightleftharpoons \text{AM} \]

"Strongly Bound" \quad "Weakly Bound" \quad "Strongly Bound"

\[ K_6 \uparrow \quad K_7 \uparrow \quad K_8 \uparrow \quad K_9 \uparrow \quad K_{10} \uparrow \quad K_{10} \uparrow \]

\[ \text{M} + \text{ATP} \rightleftharpoons \text{M(ATP)} \quad \text{M.ATP} \rightleftharpoons \text{M.ADP.P_i} \quad \text{M.ADP} \rightleftharpoons \text{M} \]

"Dissociated States"

Rapid Equilibrium
Mostly Detached
Attached

**Duty ratio** is the percentage of time myosin spends strongly bound to actin.
Actomyosin working cycle

Duty ratio: fraction of cycle time spent in strongly actin-bound states
Processivity: ability to take multiple steps on actin without detachment

cartoons from De La Cruz et al. JBC 276:32373
Using this same basic kinetic cycle, nature has evolved myosins capable of carrying out a plethora of functions by varying the magnitude of certain key rate constants.
Duty ratio: fraction of cycle time spent in strongly actin-bound states

Processivity: ability to take multiple steps on actin without detachment

Phosphate release rate-limiting in muscle myosins
LOW DUTY RATIO

ADP release rate-limiting in myosin V, VI
HIGH DUTY RATIO

strong binding
weak binding/detached
strong binding
What determines localization in cells

From Post et al.
Migrating *Dictyostelium discoideum* cell

Figure 16–93. Molecular Biology of the Cell, 4th Edition.
Coiled-coil Forming Sequences Are a Means to Dimerize Myosin Heavy Chains

The arrangement of amino acids in a coiled-coil is crucial for dimerization. Amino acids in the “a” and “d” positions are usually hydrophobic.

Myosin II class molecules have the longest coiled-coil sequences. They self-associate via their tails to form filaments.

Other myosin classes appear to use the coiled-coil to make dimers, but do not self-associate to form filaments. Often their coiled-coil regions are much shorter.

Myosin V
What do you want to know about a motor once you’ve discovered it?

• What is its function in cells?
• What is its subunit composition?
• Where is it located in cells/tissues?
• What are the receptors for this localization?
• What regulates the binding to the receptors?
• What are the enzymatic parameters of the motor?
• How is the enzymatic activity regulated?
• What is the directionality of the motor?
• What is the molecular mechanism of the motor?
• Determine the 3D structure of the motor and/or motor-receptor complex.
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- **What is the directionality of the motor?**
- What is the molecular mechanism of the motor?
- Determine the 3D structure of the motor and/or motor-receptor complex.
Motor proteins move unidirectionally on their tracks

In general, myosins move toward the + end of actin filaments. Actin filaments are typically oriented with the + end towards the plasma membrane.

Myosin VI moves in the opposite direction, i.e. towards the – end

Some kinesin isoforms move in the + (antereograde) direction and others move in the – (retrograde) direction.

Dynein moves in the – direction.
Actin binding site

Switch II
Relay
SH1 Helix

Nucleotide binding pocket

Near-rigor conformation
Nucleotide-free Scallop S1
Myosin II

Humans have 14 myosin II genes of which 10 are found in skeletal or cardiac muscle tissue. Some of these fast isoforms and some are slow giving rise to different contractile speeds of muscle. Some are developmentally regulated.

There is one smooth muscle specific myosin genes and three “nonmuscle” myosin II genes. These are closely related in terms of structure and regulation. Different cells may have various combinations of the three nonmuscle isoforms.

Studies demonstrate that the nonmuscle isoforms have differing functions within the cell. They are often located in different regions and have slightly different kinetic properties.
Functions of non muscle myosin II

Cytokinesis
Cell-surface and cell-cell adhesion
Retraction of trailing cell body in motility
Neurite retraction
Maintenance of cortical tension

Regulation of nonmuscle myosin II occurs via phosphorylation of the “regulatory” light chain by myosin light chain kinase or by terminal kinases of various signal transduction pathways such as rho kinase.
Non-muscle myosin II works in minifilaments

Verkhovsky et al. JCB 131:989

Niederman & Pollard JCB 67:72

Yildiz et al. Science 300:2061

>1000 heads

56 heads

2 heads
Duty ratio

muscle myosin II

skeletal muscle

smooth muscle

non-muscle myosin II

vesicle motors

myosin V

myosin VI

weakly bound to actin/detached

strongly bound to actin

NMIIC

NMIIB

NMIIB

NMIIC

NMIIC
Myosin V

May be the only universally expressed myosin

Implicated in cargo transport in melanocytes and in neurons and in mRNA transportation in yeast

Moves processively along actin filaments, taking 36 nm steps

Human disease – Griscelli’s disease

* Dilute* mice have myosin V mutations and severe defects in melanosome transport. Also neurological defects

Expresses well in baculovirus/Sf9 system

Abundant enough to purify from tissue
Myosin V (she1p) is involved in mRNA localization during asymmetric cell division in yeast.
Myosin V As a Cargo Motor

If myosin V had kinetics like myosin II, a large number of molecules would need to be present on the surface.

If the heads of myosin V worked cooperatively, such that at least one head was bound at any time, then a single molecule could move the cargo.

Actin is a helical polymer. There are advantages to being able to step across the helical pitch, i.e. to be able to take 36 nm steps.

Myosin V: Strides = helical repeat → linear walk
Evidence that Myosin V is a Processive Motor Capable of Moving in a Linear Fashion on Actin

Kinetics suggest that it should be strongly bound to actin for most of its kinetic cycle

Is able to move actin filaments at very low density in the in vitro motility assay

Has multiple steps per diffusional encounter in the optical trap. Steps are 36 nm apart.

Can bind to myosin via two heads even in the presence of ATP at dilutions required for electron microscopy. The two heads are bound 36 nm apart.

Single fluorescently labeled myosin V molecules can move on actin filaments
Kinetic Evolution of Different Myosins Allow for Different Tasks

Rate limiting step
For myosin V. Means that steady products bind actin strongly. Well adapted for a

Rapid Equilibrium
Mostly Detached

Myosin V is a high duty ratio motor
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Single fluorescently labeled myosin V molecules can move on actin filaments
Myosin V Has a High Duty Cycle

- Myosin II requires a high surface density
- Myosin V moves actin filaments at a very low density in the in vitro motility assay
- In some instances the actin filaments appear to be moving about a single point
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Two types of Optical traps

Single Bead Trap

Three Bead Trap
Amplitude of “single events” indicates working stroke is smaller than stepsize.

TPMV

100 (nm)

2nd to 4th step in staircases mean 34.5+/-0.6nm
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Single fluorescently labeled myosin V molecules can move on actin filaments.
Structure of myosins bound to actin in the presence of ATP

- Polar structure: like telemark skiing stance
- Telemarks on one filament all point one way
- Correlate with actin polarity

In collaboration with Peter Knight and John Trinick
Myosin V Motor Separation on Actin in ATP

Major peak 13 subunits apart

= helical repeat for 13/6

Subsidiary peaks:
11 & 15 subunits apart
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Single fluorescently labeled myosin V molecules can move on actin filaments.
Single myosin V molecules can be observed using TIRF Microscopy
Movement of Single Molecules of Myosin V on Actin Visualized by Total Internal Reflectance Microscope
Lesson for today: Make use of the unique properties of a particular myosin to understand how myosins in general work
In Search of the Holy Grail of Muscle Research

Which is, of course, the direct visualization of the myosin powerstroke in action

To do this we collaborated with John Trinick, Stan Burgess and Peter Knight of the University of Leeds

In the absence of nucleotide or in the presence of ADP the binding of myosin II to actin is very tight \((K_d=\text{nanomolar range})\). The problem is that myosin with ATP bound has a \(K_d\) of around 20 \(\mu\text{M}\).

To do EM on the isolated proteins they must be diluted down to less than 1\(\mu\text{M}\) which means that virtually all of the myosin II rapidly dissociates.

Myosin V has a submicromolar \(K_d\) for actin in the presence of ATP, suggesting that it may work for this experiment.
Singly-attached Molecules on Actin in ATP

Variety of angles of attachment: some beyond 90°

Seeing the start of the working stroke for the first time!
Motion of detached heads

Attached head pre-power stroke

Detached head:
• cannot reach next attachment site

Attached head post-power stroke

Detached head:
• can reach next site
• cannot reach previous site
Animated Walking Cycle
MYOSIN Va (*DILUTE*)

**Domain**
- Head/Neck (~100 kDa)
- Stalk (~55 kDa)
- Globular Tail (~45 kDa)

**Function**
- Processive Motor
- Dimerization
- Vesicle Binding Kinesin Interaction

Cheney et. al., *Cell* (1993)
Using Mouse Coat Color Mutants to Investigate Organelle Transport and Distribution
The Cooperative/Capture Model

The myosin Va-dependent interaction of melanosomes with F-actin in the periphery prevents a fraction of melanosomes delivered there by centrifugal microtubule-dependent movements from being returned to the cell center by centripetal microtubule-dependent movements, thereby causing their distal accumulation.

Mouse Coat Color Mutants

Melanoblast Migration / Differentiation

• (endothelin B receptor)
• *Microphthalmia* (MITF transcription factor)
• *Dominant spotting* (Kit oncogene)

Pigment Synthesis

• *Albino* (tyrosinase)
• *Brown* (TRP-1)
• *Slaty* (TRP-2)

Membrane Traffic

• *Pearl* (AP3 β3a subunit)
• *Mocha* (AP3 δ subunit)

Melanosome Movement and Distribution

• *Dilute* (myosin Va)
• *Ashen* (Rab27a)
• *Leaden* (melanophilin)
BRAIN MYOSIN Va
1828 a.a.

Exon “B”
(3 a.a.)


MELANOCYTE MYOSIN Va
1877 a.a.

Exon “D”
(27 a.a.)

Exon “F”
(25 a.a.)
DOMINANT NEGATIVE

GTD
NO
NO

MC STK
NO
NO

MC STAD
YES
YES

MC ST
YES
YES

Exon B
Exon D
Exon F

Dominant Negative: YES
Colocalization: YES
Mouse Coat Color Mutants

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Melanosome Movement and Distribution

• Dilute (myosin Va)
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• Leaden (melanophilin)
Rab GTPases

Reside on the surface of organelles/vesicles in the endocytic and secretory pathways.

Play critical roles in the targeting and fusion of these vesicles with their appropriate acceptor membrane.

Participate in the formation and/or function of SNARE complexes.
Rab Cycles

“Off”

GDP

“On”

GTP

v/t SNARES

Tethering / docking proteins

GEFs/GAPs

Vesicle motors

GTP

GDI

vesicle
Does myosin Va directly interact with Rab27a?

Can look at this using myosin V affinity columns?

The answer is…

No… There must be some intermediate protein that interacts with both myosin Va and Rab27a
Mouse Coat Color Mutants

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Melanosome Movement and Distribution

• Dilute (myosin Va)
• Ashen (Rab27a)
• Leaden (melanophilin)
Organization of the melanosome receptor for myosin Va
Regulation of myosin V

In the absence of calcium myosin V forms a folded, inactive complex in which the globular tail domain interacts with the heads. In vitro, we can activate the enzymatic activity of myosin V by adding calcium, but it is likely that in cells myosin V is activated by binding of cargo.
Myosins Linked to Human Diseases
Myosins involved in familial deafness

<table>
<thead>
<tr>
<th>Myosin</th>
<th>DFNA</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myosin IA</td>
<td>DFNA48</td>
<td></td>
</tr>
<tr>
<td>Myosin IIA</td>
<td>DFNA17</td>
<td></td>
</tr>
<tr>
<td>Myosin III</td>
<td>DFNB30</td>
<td></td>
</tr>
<tr>
<td>Myosin VI</td>
<td>DFNA22; Snell’s Waltzer mouse</td>
<td></td>
</tr>
<tr>
<td>Myosin VIIA</td>
<td>Usher’s IB Syndrome,DFNB1, Shaker1 Mouse</td>
<td></td>
</tr>
<tr>
<td>Myosin XV</td>
<td>DFNB3, Shaker2 mouse</td>
<td></td>
</tr>
</tbody>
</table>

Interestingly, the Snell’s Waltzer mouse is deaf and has circling behavior, but is relatively normal in other respects. It reproduces, looks normal and has no other obvious defects. Myosin VI, however is present in all cell types and has been implicated in endocytosis and Golgi transport. Its functions in these processes must not be essential.
Differential Localizations of Myosins in the Inner Ear

Myosin I\(\beta\) may be involved in adaptation, by moving the insertion point of the tip link which connects one stereocelia to another. The stereocelia contain a core of actin filaments.

Myosin V is only involved in neurotransmission, not in sensory perception, itself.

Myosin VI mutations are associated with deafness in mice and humans. It is found in the region in the cuticular plate region which lies beneath the stereocelia and in the cell body. Mutations in myoVI result in degeneration of stereocelia.

Myosin VII mutations are associated with deafness and, in humans, also with blindness. It is located near the base of the stereocelia and in the cell body.

Nonmuscle Myosin IIA is associated with human deafness. It is located in the terminal web region.

Myosin XV is associated with deafness in humans and mice. It is located at the tip of the stereocelia.

Figure from Hasson et al. J.Cell Biol. 137: 1287, 1997
Figure 16-74. Molecular Biology of the Cell, 4th Edition.
Myosins Linked to Human Diseases
Figure 16-59. Molecular Biology of the Cell, 4th Edition.
INACTIVE STATE:
(light chains not phosphorylated)

ACTIVE STATE:
(light chains phosphorylated)