Microfilament Motors

Myosin motors animate the microfilament cytoskeleton in muscle and other cell types.

http://pleiad.umdNJ.edu/%7Edaw/Cardiomyocytes/HCM-mutations.html cached 040212
GFP-myosin expressed in cardiomyocyte (green) and counterstained with anti-titin mAb (red)
Video loop from http://ipmc.epfl.ch/page23148.html cached 0760213, showing the ability of an isolated myofibroblast to contract (looped to mimic the rhythmic beating observed in cardiac cells in culture)

Skeletal (Striated) Muscle

Skeletal Muscle - lengthwise "striated" array of alternating/interdigitating thick and thin filament arrays; the functional unit is a sarcomere (Z-line to Z-line)

Bipolar thin microfilament array
- Two arrays of microfilaments arranged head-to-head (plus ends) by alpha-actinin/cap z protein at the Z-line
- Protein linkages (costameres/dystrophin) connect the Z-lines to the plasma membranes
- Defects in these linkages cause one form of muscular dystrophy

Bipolar thick filament array
- Two bundles of 300-400 myosins (associated by tails) bundled by M-line proteins

Sliding Filament Model
- Myosin thick filaments slide over actin thin filaments; movement is plus-end directed (toward the z-lines), shortening the sarcomere
- Regulated by troponin/tropomyosin nestled in the helical groove along the microfilaments
- Ca++ release from specialized ER (sarcoplasmic reticulum) binds to troponin, shifts tropomyosin so that myosins engage
- In smooth muscle the contractile apparatus is not as ordered, and Ca++ regulation is effected by caldesmon

Building a muscle involves generating a regular array of filaments of identical length. Nebulin extends
Myosin II is a dimer; each 230kDa head contains microfilament and ATP binding sites. The neck region converts head motions to the tail and has “IQ” binding sites for a variety of regulatory light chains [the essential light chains (ELC, 25kDa) and regulatory light chains (RLC, 20kDa) are shown in this structure and gel]. Note the S1 fragment is a 120kDa section of the heavy chain released by papain protease digestion and contains both the ATP and microfilament binding sites.

Tail is a lever arm, a 130 nm rigid alpha helical coiled-coil involved in dimerization and higher order bipolar filament assembly.

Light chain phosphorylation by myosin light chain kinase (MLCK) and Rho Kinase (ROCK - a target of Rho) increases myosin activity. Certain light chains include calmodulin-like light chains that confer Ca$^{+2}$ regulation.

AFM [http://www.people.virginia.edu/~zs9q/zsfig/myosin.html](http://www.people.virginia.edu/~zs9q/zsfig/myosin.html) cached 060208

BW and gel images [http://www.cytoskeleton.com/products/motor/my03.html](http://www.cytoskeleton.com/products/motor/my03.html) 080116


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This animation of myosin II, based on structural and biochemical studies, literally “brings to life” the previous sequence.

[http://www.scripps.edu/milligan/projects.html](http://www.scripps.edu/milligan/projects.html) cached 040202

Information at: [http://www.scripps.edu/milligan/research/movies/myosin_text.html](http://www.scripps.edu/milligan/research/movies/myosin_text.html)
The Swinging Lever Arm

Note that the position in (1) is analogous to S1 decoration, where many S1 fragments generate the arrowhead appearance “pointing” toward the minus end of the microfilament. This ATP-free state is also called the Rigor state and its tight association with the microfilament results in rigor mortis in death.

Binding of ATP in (2) causes release of the microfilament (relaxation), and hydrolysis to ADP+Pi causes the lever arm to swing forward (in a plus end direction). Upon rebinding to the microfilament, the release of ADP and Pi result in force production and stepping of the cargo toward the plus end of the microfilament track, completing the cycle.

http://www.esrf.fr/UsersAndScience/Publications/Highlights/2003/MX/MX03 cached 060208

Despite the apparent completeness of this model (a flaw of a good diagram can be that we think we know everything!) there is still debate about exact form of the motor cycle!

Control by Troponin/Tropomyosin

Engagement of the myosin II motors with the actin microfilaments in the contractile array is governed by a protein system of troponin hinges and tropomyosin “guardrails”. Tropomyosin is an extended protein that sits in the groove of the actin thin filaments, covering the myosin binding sites. When Ca++ is released from a specialized form of the ER called the sarcoplasmic reticulum, it binds to the Troponin trimeric complex, driving a shape change that swings the tropomyosin aside permitting myosin II action. Later, reconcentration of Ca++ back into the SR (by calcium pumps) reversed these changes, resetting the cycle.

Images from http://www.embl-heidelberg.de/CellBiophys/LocalProbes/motorproteins/myosin.html cached 070219
Myosin Diversity

The myosin family tree - over 35 subfamily classification groups and growing; a conserved feature is the highly conserved motor domain.

Most myosins are plus-end directed motors; Myosin VI is an exceptional minus-end directed myosin.

Most take 5 nm steps along a microfilament, stepping from monomer to monomer along the track and process around the filament as they move (generating 3-5 picoNewtons (pN) of force); a few, like Myo V, take much longer steps that step along the helical repeat of the microfilament and can walk quickly in a straight line.

Myo I - monomers, membrane cargo (Golgi maintenance, vesicles, plasma membrane)
Myo II - dimers to thick filaments, muscle myosin; also involved in the contractile ring for cytokinesis (all cells)
Myo V - dimers involved in vesicle transport, Golgi organization, melanosome transport (dilute mouse mutant affects coat color)
Myo VI, VII, XV are involved in stereocilia of the ear; mutations (like the mouse Snell’s Waltzer) have auditory and balance problems.
Myo VIII and XI are only found in plants

Structural Themes

Different myosin family members come in many shapes and forms. Note that some myosins are monomeric (Myo I, IV, XIV and XV) and may act in teams, whereas others are dimers and can move progressively on their own.

http://www.umassmed.edu/physiology/faculty/ikebe.cfm?start=0&
In this dividing *Dictyostelium* cell, Myosin I localizes to the leading edges of the lamellipodia, whereas Myosin II participated in the cleavage furrow.

http://faculty-web.at.northwestern.edu/med/fukui/04-Cytoskeleton.html cached 060208

There are at least 39 myosin genes in the human genome - myosins everywhere!

http://www.bio.ic.ac.uk/research/tps/images/fig2large.gif cached 040212
Myosin VI broke the plus-end stereotype when it was found that it moves in a minus-end direction! Myo VI may be involved in the internalization of endosomes (and delivery of endocytosed material to lysosomes).

Significantly, in addition to relatively slow progression toward the minus end, Myo VI torques (rotates) microfilaments in motility assays. More recently, Myo VI has been joined by Myo IX in “backward” motility.

Myosin VI mutations are associated with deafness, and the protein is found in stereocilia (microfilament based projections) of the ear.

Top diagram from http://www.bio.umass.edu/vidali/web/cell_motil/oct_3_long1.htm cached 080217; bottom from http://www.nature.com/nrm/journal/v11/n2/fig_tab/nrm2833_F5.html cached 141110

Polarity marked microfilament motor assays (red end is + addition of red labeled actin) http://www.youtube.com/watch?v=a4k7vcTm_rw and http://www.youtube.com/watch?v=FKoeOq6fFz8 cached 141110

These examples are nano-engineered constructs, but they provide nice examples of how polarity marked microfilaments can be used to determine motor polarity in vitro.
A comparison of Myo V and Myo VI shows the difference between the converter domains (in green) and how that positions the coiled-coil backbone differently after the power step. On the right side both motors are docked into the Myo VI density map to show that the Myo Vi structure fits well whereas the MyoV structure is different. In Myo VI there is a novel light chain (shown in magenta) that literally turns the coiled-coil about face so that the complex is poised to step in a minus-end directed fashion.

http://www.nature.com/nature/journal/v435/n7043/fig_tab/nature03592_F1.html#figure-title cached 060209