CELL MOTILITY

Historical Development of Cell Motility

In the first half of the 20th century, it was generally accepted that myosin and actin are proteins specific for muscle, and muscle contractility is a specific interaction of actin, myosin and ATP. It came as a surprise when in 1954 Hoffmann-Berling reported that water-glycerol extracted amnion fibroblasts contract upon addition of ATP. Figures CM1a and 1b show that the elongated fibroblast cells shrink to a small globule after addition of ATP.

**Fig. CM1a,** shows the fibroblast without ATP (Reprinted from Biochim. Biophys. Acta, vol. 14, Hoffmann-Berling, H., Adenosintriphosphat als Betriebsstoff von Zellbewegungen, pp. 182-194, Copyright 1954, with permission from Elsevier Science).


Subsequently Hoffmann-Berling showed that glycerinated epithelial cells, hen embryos, or Jensen tumor tissues could be contracted with ATP, similarly to the contraction of glycerinated muscle fibers. The idea that actin and myosin are present in mammalian cells, other than muscle, became the working hypothesis of several laboratories.

Isolated actin-like proteins were identified by SDS gel electrophoresis, polymerization-depolymerization properties, nucleotide content, binding to myosin, activation of myosin ATPase, and tryptic peptide mapping. Actin was identified in cells by electron microscopy of fibers decorated with heavy meromyosin or subfragment-1 of myosin and immunofluorescence.

Isolated myosin-like proteins were identified by ATPase activities, SDS gel electrophoresis, subunit composition, binding to actin and by electron microscopy. Labeled antibodies against myosin provided further proof for the existence of myosin in non-muscle tissues. Thus, by the middle of 1970s actin and myosin were acknowledged as regular components of non-muscle cells.

Contractile proteins were prepared, for example, from brain, oviduct, kidney, blood platelets, liver cells, sympathetic neurons, cultures of fibroblast, plasmodial slime mold,
or Dyctyostelium amoebae. From all these data it appeared that the mechanism of motion follows the same principle in biology.

**Actin-Binding Proteins**

*In situ* the polymerization-depolymerization of actin is controlled by the actin-binding proteins, which combine with actin monomers, cap the ends of the actin filaments, cross-link actin filaments, or attach actin to membranes. Fundamental cellular processes such as, cytokinesis, lamellipodial and growth cone extension, chemotaxis, endocytosis, or exocytosis are regulated by actin-binding proteins.

**Profilin**

The classical actin-binding protein, profilin, was discovered in the middle of 1970s. It is a small (12-15 kDa), soluble protein that is present in a high concentration (20-80 μM) throughout the cytoplasm and has a high affinity to cytoplasmic actin ($K_d = 10^{-6}$ M). Profilin inhibits polymerization of actin by sequestering the monomeric actin (Fig. CM2).

![Fig. CM2. Schematic overview of actin polymerization and its regulation by profilin and related proteins affecting the competence of monomers to assemble into filaments (From Stossel, 1989).](image)

The X-ray structure of profilin (Fig. CM3) reveals that the protein is bisected by an antiparallel beta-pleated sheet. Both termini are alpha-helical and pack against the same side of the central sheet, connecting to it by short loops. The X-ray structure of profilin-beta-actin is presented in Fig. CM4. Profilin forms two major contacts with actin in the crystal. The primary contact comprises a region of profilin defined by helix 3, the amino-terminal portion of helix 4, and strands 4, 5, and 6. This region makes contact with a site on actin spanning the large and the small domains of the molecule at the bases of subdomains 1 and 3.
Fig. CM3. Polypeptide fold of profilin. The four helices (H) and the seven strands (S) are shown (From Schutt et al., reproduced with permission from Nature, 365,810-816, 1993, http://www.nature.com).

Fig. CM4. The profilin-actin ribbon. Profilin red, actin black. For explanation see the text (From Schutt et al., reproduced with permission from Nature, 365,810-816, 1993, http://www.nature.com).

Thymosin β4 is another actin monomer-binding protein. It is a small peptide, 43 residues, and it competes with profilin for binding to actin (Pollard et al., 2000). Thymosin β4 induces a conformational change in actin monomers (Dedova et al., 2006).

**Gelsolin**

Of the many proteins isolated from cell extracts, gelsolin is the most potent to solubilize gelatinous (fibrous) actin (this ability is reflected in the name of the protein). Severing of the actin-gel occurs through the weakening of sufficient bonds between actin molecules within a filament to break the filament. Severing includes binding of gelsolin to actin, structural rearrangement within gelsolin, and changing the conformation of actin. After severing, gelsolin remains attached to the barbed end of the actin filament that cannot reanneal or elongate and, thus, the actin network is disassembled. Ca^{2+} is required to the severing process (Sun et al., 1999).

**Structure and function:** Gelsolin has two tandem homologous halves, each of which contains a 3-fold segmental repeat, segments S1-S3 and S4-S6, respectively (Fig. CM5).
Fig. CM5. Gelsolin structure-function domain. Amino acid residues are numbered and the 6 segments are indicated. Actin, PIP₂, and Ca²⁺-binding segments, and the caspase-3 protease site are shown (From Sun et al., 1999).

The crystal structure of gelsolin shows that in the absence of Ca²⁺ gelsolin has a compact quaternary structure. Its two halves are held together by a C-terminal S6 tail which latches onto S2 (Fig. CM6). It was predicted from this structure that "Ca²⁺ must induce major conformational changes in each half and in the relation between the halves to accommodate actin binding". Indeed new X-ray diffraction studies revealed domain movement in gelsolin (Robinson et al., 1999). Upon Ca²⁺-binding the S6 domain moved by about 40 angstroms resulting in a major structural reorganization in gelsolin, i.e. the actin-binding site on S4 became exposed enabling the severing and capping of actin filaments to proceed. As a continuation of this work, the Robinson laboratory reported Ca²⁺ exchange in crystalline gelsolin (Chumnarsilpa et al., 2006). The various steps of gelsolin action are illustrated in Fig. CM7.

Fig. CM6. Structural model of gelsolin in the absence of Ca²⁺ (From Sun et al., 1999).
Phosphoinositides, particularly PIP$_2$, dissociate gelsolin from actin (Fig. CM7), thus reverse the capping of the filaments. Severing of the long actin filaments by gelsolin increases the number of the filaments. Uncapping of gelsolin from these filaments generates many polymerization-capable ends from which actin can grow to rebuild the cytoskeleton to a new specification.

The barbed ends of the actin filaments are also interacting with the capping protein, called CapZ in muscle. The protein exists at micromolar concentration in the cytoplasm, and it has a high affinity to the barbed ends, $K_d = 0.1$ nM (Pollard et al., 2000).

**ADF/cofilin**

The actin-depolymerizing factor (ADF), also called cofilin, has been recognized early as a widespread, small (15-18 kDa) actin binding protein that plays an important role in cytokinesis, endocytosis, and in the development of all embryonic tissues (Carlier et al., 1999). ADF/cofilins from different organisms present a high degree of sequence homology and the general mechanism of action of the different ADF/cofilins is conserved. The three-dimensional structure determined by X-ray crystallography is shown on Fig. CM8A. Five central beta-sheets are flanked by three to four alpha-helices. Image reconstruction (Fig. CM8B) shows that ADF interacts with two actin subunits along the long pitch helix, bridging subdomain 1 of the actins with subdomain 2 of the second subunit. As a result of the ADF binding to F-actin, there is a twist of 5° per subunit and, therefore, the long pitch helices crossover every 27 nm on average instead of 36 nm for standard F-actin filaments.
Importantly, the stimulus-responsive function of ADF/cofilin is regulated by phosphorylation of a single serine residue. In response to stimuli, ADF is dephosphorylated. The stimuli, such as growth factors, chemotactic peptides, or agents increasing the levels of $[\text{Ca}^{2+}]_{i}$ and cAMP, promote the reorganization of the actin cytoskeleton. In quiescent cells, ADF/cofilin appears diffusely distributed in the cytoplasm, the activated (dephosphorylated) protein translocates to regions of the cells where actin filaments are highly dynamic like the leading edge of ruffled membranes, the cleavage furrow of dividing cells, or the neuronal growth clone. Dephosphorylation correlates with increased motility and extension of cellular processes (Carlier et al., 1999). ADF/cofilin increases the turnover of actin filaments which powers actin motility (Fig. CM9).

Chhabra and dos Remedios (2005) used fluorescence resonance energy transfer and confocal microscopy to analyze the interactions of cofilin and G-actin within the nucleus; almost all the G-actin in the nucleus was bound to cofilin. The presence of actin and myosin I in the nucleus was reviewed by de Lanerolle et al., (2005), it is believed that β-actin is involved in transcription by RNA polymerase II.

**Arp2/3 complex and WASp/Scar proteins**

Actin related proteins (Arps) participate in a diverse array of cellular processes (Schafer and Schroer, 1999). Wiskott-Aldrich syndrome proteins (WASp/Scar proteins) stimulate the formation of new actin filaments by Arp2/3 complex (Higgs and Pollard, 1999). Fig. CM10 shows that the Arp2/3 complex contains seven protein subunits, Arp2 and Arp3...
are actin related proteins, the other five subunits are novel. The Arp2/3 complex cross-links filaments in an end to side manner, with the slow growing end of one filament attached to the side of another at an angle of 70 degrees. Fig CM11 shows the binding of WASp/Scar to an actin filament and to Arp2/3, creating new filaments and cross-linking them into a branching meshwork.

**Fig. CM10.** Arp2/3 complex structure. Based on nearest neighbor relationship of the subunits from chemical cross linking. Molecular masses in kDa are indicated (From Higgs and Pollard, 1999).

**Fig. CM11.** Dendritic nucleation model. Arp2/3 complex (green) binds to the side of a preexisting actin filament (yellow), and WASp/Scar (red) bound to an actin monomer binds to Arp2/3, forming a nucleus for barbed end growth from the side of the filament (From Higgs and Pollard, 1999).

The crystal structure of the Arp2/3 complex has been determined at 2.0Å resolution (Robinson et al., 2001). Arp2 and Arp3 are folded like actin with distinctive surface features. The structure of the five subunits has also been elucidated. Subsequently, the structure of the Arp2/3 complex in its activated state and in actin filament branch junctions has been described (Volkmann et al., 2001). Internal reflection fluorescence microscopy was used for direct real-time observation of actin filament branching mediated by Arp2/3 complex (Aman and Pollard, 2001). Furthermore, it was shown that the binding of ATP to Arp2 is required for filament branching (Le Clainche et al., 2001; Dayel et al., 2001).

External stimuli drive the assembly of the actin filament network, acting through receptors and multiple signal transduction pathways, several of which converge on WASp/Scar proteins and Arp2/3 complex. Diverse signals including those carried by the Rho family GTPases, Rac, and Cdc42 are involved (Fig. CM11A). Zhang et al., (2005) made the important discovery that WASp-mediated activation of Arp2/3 complex regulates actin polymerization and tension development initiated by muscarinic stimulation in canine tracheal smooth muscle.
Dendritic nucleation model

This is a model for molecular mechanisms controlling actin filament dynamics in nonmuscle cells (Fig. CM11B).
There are 10 steps in the figure. In the first step all the ATP-actin monomers are bound to profilin, there are no free barbed ends; the actin cytoskeletal components are held in a metastable state, poised for assembly. Activation of WASp family proteins (Step 2) leads to activation of the Arp2/3 complex and this creates new barbed ends and subsequently new filaments (Step 3). These filaments grow rapidly (Step 4) and push the membrane forward (Step 5). After a short while, growth of the barbed ends is terminated by capping (Step 6). ATP hydrolysis and Pi dissociation (Step 7) triggers severing and depolymerization of actin filaments by ADF/cofilins (Step 8). LIM kinase inhibits ADF/cofilins (Step 9). Nucleotide exchange catalyzed by profilin recycles ADP-actin to ATP-actin monomer in the pool (Step 10).

Accordingly, the molecular mechanism of motion in nonmuscle cells is a very complex cycle that converts the energy of the hydrolysis of actin-bound ATP into mechanical force through the polymerization and depolymerization of actin filaments (treadmilling of actin filaments). In a continuously moving cell, assembly and disassembly of actin filaments are balanced.

Reviews of Pollard et al., (2000), Higgs and Pollard, (2001), and Pantaloni et al., (2001) are recommended for a further understanding of the molecular mechanism of motion in nonmuscle cells.

**Role of WASp homology domain 2 in actin filament nucleation**

The WASp homology domain 2 WH2 is a small actin-binding motif, it plays a role in actin filament nucleation by Arp2/3 complex (Chereau et al., 2005). The crystal structure of WH2 complexed with actin has been determined (Chereau et al., 2005). WH2 shares structural similarity with thymosin (Tβ); WH2 is shorter than Tβ but binds actin with 10-fold higher affinity. Owing to their shorter length, WH2 domains connected in tandem by short linkers can coexist with intersubunit contacts in F-actin and are proposed to function in filament nucleation by lining up actin subunits along a filament strand. The WH2-central region of WASp-family proteins is proposed to function in an analogous way by forming a special class of tandem repeats whose function is to line up actin and Arp2 during Arp2/3 nucleation. The structures also suggest a mechanism for how profiling-binding Pro-rich sequences positioned N-terminal to WH2 could feed actin monomers directly to WH2, thereby playing a role in filament elongation.(Figure CM11C).

Part A of Fig. CM11C shows how WH2 could play a role in both nucleation and elongation. The WH2 domains are short and are connected by short linkers, which has two important implications. First, the actin-binding interface of these WH2 domains does not interfere with intersubunit contacts in F-actin and can thereby coexist with the actin filament. Second, consecutive WH2 domains must bind actin subunits along the same filament strand. Part B of Fig. CM11C shows that WH2 binds in the cleft between subdomains 1 and 3, accounting for most of the actin-binding affinity and nucleotide exchange inhibition of WH2. Part C of Fig. CM11C suggest that profilin bound to the consensus Pro-rich sequence immediately N-terminal to WH2 could deliver its actin directly to WH2, contributing to barbed end elongation.
Fig. CM11C. Structural basis for the role of WH2 in filament nucleation and elongation. (A) WH2 occurs in the form of tandem repeats C-terminal to Pro-rich sequences that support the binding of profilin-actin. The structures of WH2-actin suggest that this basic arrangement may allow WH2 to function in filament nucleation and elongation. (B) Tandem repeats of short WH2 domains connected by short linkers can function to line up actin monomers along a filament strand, thereby playing a role in filament nucleation. (The numbers 1, 2, 3, and 4, correspond to the subdomains of actin). The WH2-C region may represent a specialized form of tandem repeat whose role is to add an actin subunit at the barbed end of Arp2 during Arp2/3 nucleation. (C) A superimposition of the structure of profilin-actin and profilin-polyPro with the structures of WH2-actin illustrates how profilin bound to the last consensus profilin-binding site could deliver in actin directly to WH2, playing a role in filament elongation. (PDB means Protein Data Bank) A partial overlap between the actin-binding sites of profilin and WH2 may then help release profilin from the barbed end of the growing filament. (Reprinted with permission from Chereau, D., Kerff, F., Graceffa, P., Grabarek, Z., Langsetmo, K., and Dominguez, R. (2005). Actin-bound structures of Wiskott-Aldrich syndrome protein (WASP)-homology domain 2 and the implications for filament assembly. Proc. Natl. Acad. Sci. USA, 102, 16644-16649, 2005. Copyright, 2005, National Academy of Sciences, USA).

Structural basis of actin filament nucleation

Actin nucleation is very slow and there are special proteins which accelerate this process (Otoma et al., 2005). Formin proteins nucleate unbranched actin filaments that elongate from their fast growing barbed ends. Formins contain a conserved FH2 domain that
mediates interaction with actin. The FH2 domain nucleates actin filaments and remains bound to the barbed end of the growing filament. The yeast Bni1p FH2 domain in complex with tetramethylrhodamine-actin was crystallized; each of the two structural units in the FH2 dimer binds two actins in an orientation similar to that in the actin filament. This suggests that the structure could function as a filament nucleus.

**Actin in the Cytoskeleton**

Living cells have the ability to change their shape and move upon stimuli in the environment. There is a network, called the cytoskeleton, a complex of filamentous proteins, which is the engine of biological adaptation. Actin is the key component of the cytoskeleton, because it exists in two forms, globular and fibrous, and it can also form a gel; these properties are ideal for restructuring the cytoskeleton in response to a variety of signals. For instance, growth factor stimulation promotes actin assembly at the plasma membrane to generate movement, whereas apoptotic signals cause cytoskeletal destruction to elicit characteristic membrane blebbing and morphological changes.

The dynamic role of actin in modifying the cytoskeleton is regulated by the actin binding proteins. Fig. CM 12 illustrates the multiple pathways involved in the regulation. Cellular movements are initiated by extracellular stimuli and the signals transduced through the cell membrane couple the messenger response to actin assembly in the cell. This is illustrated in Fig. CM13.

**Fig. CM12.** Regulation of actin polymerization by the actin-binding proteins. Symbols used: "C" for monomeric binding proteins; bracket for capping and severing proteins; squiggle for cross-linking proteins (From Pollard and Cooper, with permission from the Annual Review of Biochemistry, vol. 35, 1986, by Annual Reviews, [http://www.AnnualReviews.org](http://www.AnnualReviews.org)).
The understanding of the mechanism of actin-based motility in cells has been greatly advanced by the work of Loisel et al., (1999), who were able to reconstitute bacterial motility from pure protein components. In addition to actin, only three other components were absolutely needed: the Arp2/3 complex, ADF, and capping protein, all which are actin-binding proteins. These results demonstrate that actin-based propulsion is driven by the free energy released by ATP hydrolysis coupled to actin polymerization, and does not require myosin. The same conclusion was drawn by Stewart and Roberts (2005) in their review on cytoskeleton dynamics on nematode sperm motility.

Work on nonmuscle cells has been applied to muscle as well. Thus, it was reported that actin dynamics at pointed ends regulates thin filament length in striated muscle (Littlefield et al., 2001).
It was thought for a long time that bacteria lacked the actin network that organize eukaryotic cytoplasm. The \textit{mreB} gene is involved in determining cell shape in rod-like bacteria; van den Ent et al., (2001) showed that bacterial MreB protein assembles into filaments with a subunit repeat similar to that of F-actin in eukaryotic cells. In addition, the MreB crystal structure revealed a shape similar to that of actin. Accordingly, prokaryotes possess homologues of both tubulin and actin, suggesting that the building blocks for the actin cytoskeleton originated in prokaryotes before becoming the mainstay of eukaryotic cells.

Recently, it was reviewed that cortactin is an important scaffold for actin assembly and organization (Lua and Low, 2005) The regulation of actin cytoskeletal networks by cortactin is mediated by its multi-domains binding to F-actin and Arp2/3 complex and various SH3 targets. Furthermore, its role in actin remodeling is subjected to regulation by tyrosine and serine/threonine kinases. Thus, cortactin phosphorylation serves as a switch for actin cytoskeletal network and cell dynamics control.

The article of Tilney and DeRosier (2005) “How to make a curved \textit{Drosophila} bristle using straight actin bundles” is an educational masterpiece for studying the actin cytoskeleton.

\textbf{Non-Muscle Myosins}

Ten years ago, beside the conventional myosin-2, at least six more unconventional myosins, myosins-I, -V, -VI, -VII, -IX, and -X, existed (Hasson and Mooseker, 1996). A schematic comparison of conventional and unconventional myosins is shown in Fig. CM14. At the N terminus, each vertebrate unconventional myosin contains a Head, a conserved motor domain that includes both the ATP- and actin-binding sites. Following the Head is the Neck domain, a light chain binding regulatory part of the structure, that contains a 24-30-amino acid repeat termed the IQ motif. The neck domain also serves as a binding site for calmodulin. Each class of myosin has a distinct Tail domain that ends at the C terminus, which provides sequences that can serve to target the myosin to its particular subcellular location.
Conventional Myosins

**HEAD**

**NECK**

**TAIL**

II Chicken Skeletal Muscle Myosin, 223 kD

COILED COIL (CC)

Unconventional Myosins

I Human Myosin-IC, 127 kD

MEMBRANE BINDING

II Bovine Brush Border Myosin-I, 119 kD

MEMBRANE BINDING

III Chick Myosin-VIb/h, 212 kD

CC

VI Porcine Myosin-VI, 145 kD

CC

VII Human Myosin-VIIa, 250 kD

MYTH4 TALIN MYTH4 TALIN

IX Rat ncl3, 225 kD

GAP FOR RHO PROTEINS

XI Bovine Myosin-X, ~230 kD

ZINC BINDING

PIT DOMAINS

Fig. CM14. Schematic showing the Head, Neck, and Tail domains of vertebrate myosins. Molecular masses and specific binding sites are indicated (From Hasson and Mooseker, 1996).

Thus myosin tails may have a membrane-binding site and/or a site that binds to a second actin filament independently of the head domain. Depending on its tail, the myosin molecule may move a vesicle along an actin filament, or attach an actin filament to the plasma membrane.

A few years later, it was shown that functional activities of many nonmuscle myosin isoforms are regulated by heavy chain phosphorylation (Redowicz, 2001). For instance, organelle transport by myosin-V was down-regulated by this phosphorylation (Karcher et al., 2001). Mass spectrometry phosphopeptide mapping showed that the tail of myosin-V was phosphorylated in mitotic *Xenopus* egg extract on a single serine residue localized in the carboxyl-terminal organelle-binding domain. Phosphorylation resulted in the release of the myosin motor from the organelle. The phosphorylated site matched the consensus sequence of calcium/calmodulin-dependent protein kinase II (CaMKII), and inhibitors of CaMKII prevented myosin-V release.

Recent studies on the above described class of unconventional myosins I – X revealed:

A myosin I isoform was found in the nucleus (Pestic-Dragovich et al., 2000). A unique 16-amino acid amino-- terminal extension characterizes this myosin. This isoform of myosin I appears to be in complex with RNA-polymerase II and may affect transcription. Human MYO18B, a novel unconventional myosin heavy chain expressed in striated muscles (identified as class XVIII) moves into the myonuclei upon differentiation (Salamon et al., 2003). De Lanerolle and collaborators (2005) reviewed myosin I in the nucleus.
Mechanosensitive hair cells of the inner air contain the myosin-1c isozyme that is a component of the hair cell’s adaptation-motor complex (Gillespie and Cry, 2004). This complex carries out slow adaptation, provides tension to sensitive transduction channels, and may participate in assembly of the transduction apparatus.

Vertebrate class myosin VIIb is expressed in polarized cells and localizes to highly ordered actin filament bundles such as those found in the microvilli of the intestinal brush border and kidney (Henn and DeLaCruz, 2005). This myosin is a high duty ratio motor adapted for generating and maintaining tension.

Myosin IXb is a single-headed motor consisting of a single heavy chain and associated light chains (Post et al., 2002). A few years later it was found (Kambara and Ikebe, 2006) that myosin IXb has a rate limiting ATP hydrolysis step unlike other known myosins, thus populating the prehydrolysis intermediate (M.ATP). M.ATP has a high affinity for actin, and, unlike other myosins, the dissociation of M.ATP from actin is extremely slow, thus preventing myosin from dissociating away from actin.

By 2006 the number of previously categorized myosin heavy chain classes reached the number of 18, and through the comprehensive phylogenetic examination of many previously unclassified myosins by Foth et al., (2006) the number of named myosin classes increased from 18 to 24. Clearly the biochemical characterization of the myriad myosin isoforms “will keep researchers busy for a long time to come” (Goodson and Dawson, 2006).

Of the many myosin isoforms myosin V and VI got the greatest attention:

**Myosin V**

Myosin V is a member of the myosin family recognized to have special significance. It is a double-headed myosin that walks along actin filaments toward their barbed ends while transporting cargos for long distances without dissociating from the track. That is myosin V moves processively on actin, taking 36-nm steps that coincide with the helical repeat of actin, and thereby avoids spiraling around the actin filament. This allows the myosin V molecule to “walk” across the top of the actin filament, necessary for moving large vesicles along the actin structure bound to the cytoskeleton. Myosin V was the first myosin shown to be processive (reviewed by Rosenfeld and Sweeney, 2005) and because of the molecular mechanism of its movement is of central interest, it has been studied extensively in several laboratories.

Single fluorophore imaging with 1.5-nm localization and 0.5-sec temporal resolution established that myosin V walks hand-over-hand (Yildiz et al., 2003). One year later the same laboratory showed that kinesin walks hand-over-hand as well (Yildiz et al., 2004), indicating that different molecular motors satisfy the requirement for processive movement in a similar way. Three-dimensional structural dynamics studies of myosin V by single-molecule fluorescence polarization (Forkey et al., 2003) provided evidence for lever arm rotation of the calmodulin-binding domain in myosin V and has supported a
hand-over-hand mechanism for translocation of the double-headed myosin V molecules along actin filaments.

Myosin V has an extremely long neck containing six calmodulin-binding IQ motifs. Sakamoto et al., (2005) altered the length of the neck by adding or deleting IQ motifs in myosin V and measured the step size of these individual IQ mutants with nanometer precision and subsecond resolution. The step size was proportional to neck length for constructs containing 2, 4, 6, and 8 IQ motifs, thus supporting the swinging lever arm model for myosin V motility.

Like other molecular motors, myosin V works directionally, with forward steps being favored over backward steps (Cross, 2006). Sustained directional stepping for any motor against a load requires an energy source, which is ATP hydrolysis for myosins. Coupling the mechanical stepping cycle to ATP turnover allows the myosin-V motor to step continuously against a load of up to 2-3 pN, consistent with its using one ATP per 36-nm step. Above 2-3 pN, the motor stalls, pausing with both heads attached to the actin filament but unable to step forward because the work involved exceeds the energy available from ATP hydrolysis. Importantly, in this stalled state, ATP turnover is halted, so that the myosin V motor only consumes energy when it is actively stepping. The question is raised what happens if one pulls backward on a walking myosin V molecule with greater force than the stall force? This question was answered by Gebhardt et al., (2006) who have found that pulling backward on a walking myosin V molecule causes the motor to reverse its mechanical action, again taking 36-nm steps, but without a requirement of ATP-binding. Backstepping continues, at the rate that depends on the load, until the load diminishes to the point where the myosin V motor resumes its natural movement, stepping in an ATP-dependent manner toward the barbed end of the actin filament. This pronounced mechanical asymmetry was explained by a model in which the strength of actin binding of a motor-head is modulated by the lever arm conformation (Fig. CM15).
Fig. CM15. A model for the asymmetric stepping of myosin V. The double-headed molecule experiences an asymmetric energy landscape where the conformation of the lever arm determines the affinity of the respective head to actin. The head in post-power stroke lever arm conformation (dark blue, trailing head) is strongly bound to actin. The leading head can adopt a weakly and a strongly bound pre-power-stroke conformation (blue and light blue). Backward force applied to the motor can induce detachment of the leading head, whereas a forward force does not lead to detachment of the trailing head.

Prior to the work of Gebhardt et al., (2006), Purcell et al., (2005) investigated of how two identical heads of myosin V coordinate their activity to produce efficient hand-over-hand stepping. They have used a laser-trap microscope to apply a 2-pN forward or backward force on a single-headed myosin V molecule, hypothesized to stimulate forces experienced by the rear or lead head, respectively. They found that pulling forward produced only a small change in the kinetics, whereas pulling backward induced a large reduction in the cycling of the head. In their model the coordination of myosin V stepping is mediated by strain-generated inhibition of the lead head.

Myosin VI

Like myosin V, myosin VI is also a special member of the myosin family. Myosin VI was the first identified in the family to move toward the pointed (-) end of the actin...
filament, whereas all other members of the family move toward the barbed (+) end (reviewed in Yildiz et al., 2004a). In addition to its unusual directionality, myosin VI has a number of unusual features. Like myosin V, the double-headed myosin VI is capable of taking multiple steps on actin filament without detachment (processive movement). Myosin V (containing 6 calmodulins, CaM, per head) moves along the actin filaments in 36-nm steps, whereas myosin VI (containing 2 CaMs) moves on average in 30-nm steps. Yildiz et al., (2004a) labeled myosin VI with a single fluorophore on either its motor domain or on the distal where the 2 CaMs are located on its putative lever arm. When observing the step size with a <1.5 nm space and 0.5-s temporal resolution Irrespective of the probe position, the average step size of a labeled head was ~60 nm, supporting the hand-over-head model of motility. However, the CaM probe displayed a large special fluctuation around the main position, whereas the motor probe domain did not. This supports a model of myosin VI motility in which the lever arm is either mechanically uncoupled from the motor domain or is undergoing reversible isomerization for part of its motile cycle on actin.

Phosphorylation of the motor domain of the single-headed (S1) and double headed (HMM) constructs of myosin VI does not alter the rate of actin-filament sliding or the maximal actin-activated ATPase rate of S1 or HMM constructs (Morris et al., 2003). Increasing Ca^{2+} above 10 μM slows both the rate of ADP release from S1 and HMM actomyosin VI and the rates of \textit{in vitro} motility. Furthermore, high Ca^{2+} concentrations appear to uncouple the two heads of myosin. Thus, phosphorylation and Ca^{2+} are not on/off switches for myosin VI.

A truncated version of myosin VI, that contains the motor domain and binding sites for 2 CaM molecules has been crystallized and the structure solved at 2.4-Å resolution (Ménétrey et al., 2005). The structure reveals only minor differences in the motor domain from that in plus-end directed myosins, with the exceptions of two unique inserts. The first is near to the nucleotide-binding pocket and alters the rates of nucleotide association and dissociation. The second insert forms an integral part of the myosin VI converter domain along with a CaM bound to a novel target motif within the insert. This serves to redirect the effective lever arm of myosin VI towards pointed (minus) end of the actin filament. This repositioning largely accounts for the reverse directionality of myosin VI.

**Microtubules**

Microtubules based motility is somewhat different from the actin-myosin contractile system. Microtubules are hollow tubes formed from tubulin subunits; there are two kinds of tubulins, alpha and beta, each with a molecular mass of 50-kDa. The structure of the microtubules is built from 13 protofilaments, each is composed of alternating alpha and beta tubulin subunits and bundled in parallel to form a cylinder (Fig. CM16). Since the 13 protofilaments are aligned in parallel with the same polarity, the microtubule itself is a polar structure, and it is possible to distinguish a plus, fast growing, and a minus, slow growing, end (similarly to those in actin filaments). Brain tissue is very rich in tubulin (10 -20% of the total soluble proteins) but virtually every eukaryotic cell contains tubulin.
Fig. CM16. Illustration of the structure of a microtubule (From Alberts et al., Copyright 1994, from Molecular Biology of The Cell 3rd ed., by Alberts et al., reproduced by permission of Routledge, Inc., part of The Taylor and Francis group).

The assembly of tubulin subunits into microtubules is associated with the hydrolysis of the tubulin-bound GTP into GDP (similarly to the hydrolysis of the bound ATP during polymerization of actin). Microtubules depolymerize and repolymerize continually in animal cells (Alberts et al., 1994), and this is central to cell morphogenesis. Thus, microtubules play a major role in separating daughter chromosomes in mitosis. Microtubules are major components of eukaryotic cilia and flagella. These hairlike organelles protrude from the surface of many cells and are involved in motility. The long microtubules extend throughout the cytoplasm and govern the location of organelles and other cell components.

It has been proposed that microtubules might generate force through their unusual mechano-chemistry, since each microtubule polymer is a store of chemical energy that can be used to do mechanical work. Grishchuk et al., (2005) reported force production by disassembling microtubules. A single depolymerizing microtubule can generate about ten times the force that is developed by a motor enzyme; thus, this mechanism might be the primary driving force for chromosome motion.

A recent review about the structures and function of microtubules is available (Amos and Schlieper 2005). For previous important references see Hackney, 1996, Vallee and Sheetz, 1996; Samsó et al., 1998, and Rice et al., 1999.

Kinesin and dynein: There are two classes of motor proteins, kinesin and dynein that direct organelle and particle movement along microtubules. Kinesins are a family of proteins that are involved in organelle transport, in mitosis, in meiosis, and in the transport of synaptic vesicles along axons. Cytoplasmic dyneins are involved in organelle transport and mitosis. Kinesins and dyneins are myosin-like proteins composed of two heavy chains plus several light chains. Each heavy chain contains a conserved, globular, ATP-binding head and a tail composed of a string of rodlike domains. The two head domains are ATPase motors that bind to microtubules, while the tails generally bind to specific cell components and thereby specify the type of cargo that the protein transports. Organelles and vesicles containing kinesin move from the minus end of a microtubule (at a microtubule organizing center, such as centrosome) to the plus end (Fig. CM17). Hence, kinesin produces movement from the center of a cell to its periphery, called anterograd transport. In contrast, cytoplasmic dynein moves the
particles from the plus end to the minus end of the microtubules (Fig. CM17), called retrograd transport.

A structural view of the kinesin movement was revealed by Kikkawa et al., (2001). The monomeric form of the kinesin motor was crystallized and the X-ray structure obtained was combined with cryo-electron microscopy. The motor was revealed in its two functionally critical states - complexed with ADP and with a non-hydrolysable analogue of ATP. Analysis of the force generating conformational changes could be done at atomic resolution. The authors suggest that a roughly 20-degree rotation of the motor region of kinesin helps to tighten its grip on the surface of the microtubular tracks it moves along.

Whether kinesin moves by a “hand-over-hand” or an “inchworm” model has been disputed. In the hand-over-hand model, the heads exchange leading and trailing roles with every step, whereas no such an exchange is postulated for inchworm models, where one head always leads. Two different research groups came to the same conclusion: kinesin walks hand-over-hand (Asbury et al., 2003; Yildiz et al., 2004). Carter and Cross (2005) made the interesting observation that under very high backward loads, kinesin’s stepping can be reversed such that the motor walks sustainedly backwards. Both the forward and backward 8-nm steps occur on the microsecond timescale. Tsiavallaris et al., (2005) succeeded in engineering of an artificial backwards-moving myosin from three pre-existing molecular building blocks: 1) a forward-moving class I myosin motor domain, 2) a directional inverter formed by a four-helix bundle segment of human guanylate binding protein-1, 3) and an artificial lever arm formed by two α-actinin repeats. The results of these authors suggest that the reverse-direction movement of myosin can be achieved simply by rotating the direction of the lever arm by 180°.

Ciliary dynein is a much larger protein than the cytoplasmic dynein: its mass is about 2 million daltons, composed of either 2 or 3 heavy chains and 10 or more smaller polypeptides. The dynein motor protein in cilia and flagella has a head which hydrolyzes ATP and interacts with the adjacent microtubules to generate a sliding force between the microtubules. However, the microtubules are linked together, therefore, they can not slide but must bend. This local bending of the microtubules is the mechanism of ciliary movement, the beating. Kural et al., (2005), used fluorescence imaging with 1 nanometer accuracy to analyze organelle movement by conventional kinesin and cytoplasmic dynein
in a cell. Within 1.5 nanometers in 1.1 milliseconds, they could measure an average step size of ~8 nanometers for both dynein and kinesin. Dynein and kinesin do not work against each other in vivo, but rather work together, producing up to 10-times the in vitro speed.

A comprehensive review of kinesin and dynein is available (Müller and Mandelkov, 2005).

**Suggested readings:** Schliwa and Woehlke, 2003; Buss et al., 2004; Selvin, 2005.

**References**


