Background and Significance

The Myosin Superfamily

A crucial achievement of cells is the development of sophisticated mechanisms for motility. One mechanism for cell motility involves mechanochemical enzymes, called motor proteins. Motor proteins convert chemical energy into mechanical energy by coupling the hydrolysis of ATP to conformational changes. The explosive rate of discovery of new molecular motors, combined with greatly improved imaging techniques and the development of *in vitro* assays have shown that motor proteins play a key role in intracellular trafficking and morphological changes in virtually all eukaryotic cells. More than one hundred different molecular motors are estimated to be involved in motile processes in any given eukaryotic cell and new functions of motor proteins continue to be discovered. Three classes of motor proteins regulate the highly complex movements in eukaryotic cells: the myosin family of actin-based motors, the microtubule-based kinesin and the dynein family.

The classic example of actin filaments and myosin at work is during skeletal muscle contraction. But the functions of actin and myosin extend to many other cellular events, motility, adhesion, endocytosis, cytoplasmic streaming, neuron growth, structural maintenance and polarization, to name some[1]. Like molecular cars on an actin track, myosins transport organelles and other cellular components, such as mRNA. Myosins can also aid in the formation or maintenance of an organized actin-based structures, as found in stereocilia. It is also becoming increasingly clear that myosins provide an intimate link between signal transduction pathways and cytoskeleton. The physiological importance of myosins is underscored by the discovery that mutations of these motors can lead to severe phenotypes such as cardiomyopathy, deafness, blind-ness, sterility, seizures, and death[1,2].

A combination of biochemical and molecular approaches has led to the identification of over 20 different myosin classes many of which were found to be expressed in a single cell[3]. Myosin superfamily members are grouped into different classes based on phylogenetic analysis of motor domains. Each class

is designated by a Roman numeral, largely in the order of their discovery. A total number of 18 classes have been officially designated, but there are at least six novel myosins that have yet to be classified. The first discovered and the most extensively explored myosin is the myosin II. Therefore it is referred to as "conventional" myosin. All other types of myosin are referred to as "unconventional".

Domain Structure of Myosins

All types of myosin that have been purified so far appear to consist of several polypeptide chains. Heavy chains (HC) and light chains (LC) form three functional domains, a globular head, a neck and an extended tail. In most of the cases the tail can self assemble to form two-headed homodimers (Figure 1). Myosin II can self-associate through the coiled-coil tail region into thick filaments which represent an array of heads capable of translocating actin filaments. In skeletal muscle, these thick filaments are bipolar and can move actin filaments towards their center.

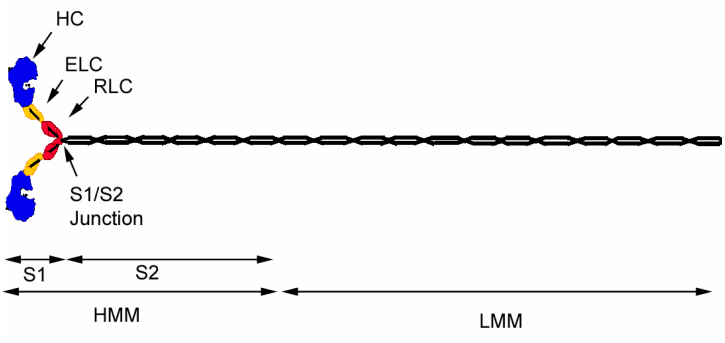

Figure 1: Schematic structure of the full-length myosin II. The Heavy Chain (HC) is shown in black, the N-Terminal head domain is highlighted in blue. The ELC is shown in yellow, and the RLC is shown in red. Heavy meromyosin (HMM) and light meromyosin (LMM) are functional fragments of limited proteolysis. HMM can be further proteolyzed to the enzymatically active single headed subfragment 1 (S1).

The head domains of myosins (also referred to as the catalytic domains) contain both actin and nucleotide binding sites. Comparative sequence analysis of the catalytic domain of most of the myosin classes suggest that basic structural elements which comprise the actin- and nucleotide-binding sites are largely conserved with the exception of several surface loops and the amino-terminus[4-6]. The second functional domain is the neck region which

consists of a long single α-helical strand from the heavy chain which is stabilized by binding of light chain subunits. Class II myosins have two different light chains associated with each neck, the regulatory light chain (RLC) and the essential light chain (ELC) (Figure 2).

In many of the other classes of myosin, varying numbers of calmodulin (CaM) molecules are associated with the neck region, possibly in addition to other types of light chains. The consensus sequence on the heavy chain that is involved in light chain binding is IQXXXRGXXRXXY (or W) and is referred to as "IQ motif". The number of IQ motifs present in the necks of different myosins can vary between zero and six.

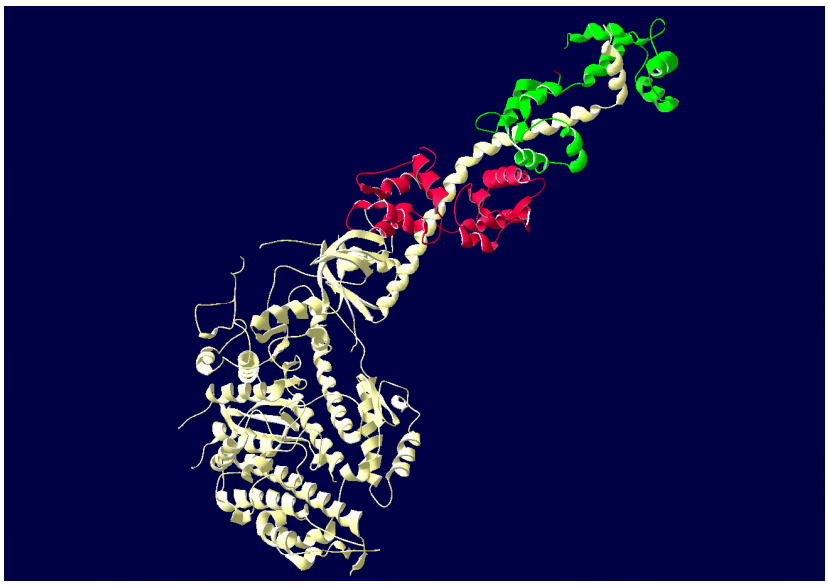

Figure 2: Structure of myosin II S1. Light yellow: heavy chain (HC), red: essential light chain (ELC), green: regulatory light chain (RLC).

In contrast to the conservation in the catalytic domain, the tail domains are quite divergent across the different myosin classes. The tail domains are the most diverse in their primary sequence and structure. Furthermore, their function is largely subject to speculation especially in the novel classes of myosin. The tails of many myosins contain coiled-coil sequences which allow the molecules to dimerize and produce two-headed molecules.

Myosin Crystal Structure

The three-dimensional crystal structure of myosin S1 including the ELC and the RLC has been resolved in atomic resolution only for chicken skeletal myosin[7]. The most important revelation of this remarkable structure is that the head contains a large subdomain that might act as a molecular lever. The domain consists of an 8 nm long α-helical light chain binding domain which is stabilized by a RLC and an ELC wrapped around it (Figure 2). This neck domain is ideally located to be the lever arm. It extends from near the nucleotide binding pocket to the distal end of the head where S1 joins S2 (Figure 1). The small conformational changes in the nucleotide binding site of the motor domain are thought to be translated into rotation of this putative lever arm, allowing its distal end to move through several nanometers. The subdomain at the end of the α-helix is pivotal, because it anchors the point on which the lever arm rotates. This converter domain translates small conformational changes in the core of the motor domain into a larger swing of the neck domain (Figure 3).

There is strong crystallographic evidence that the neck domain does indeed rotate during the ATP hydrolysis cycle. To date, the crystal structure of myosin II has been determined of four organisms in complex with a variety of nucleotides and nucleotide analogs[7-12]. Based on this data, the converter domain in the myosin motor domain exists mainly in two different conformations. This result strongly suggests that the position of the lever arm which immediately adjoins the converter domain varies significantly between the two major conformation states. These states are postulated to be the beginning (prestroke state) and the end (poststroke state) of the actomyosin power stroke (Figure 3).

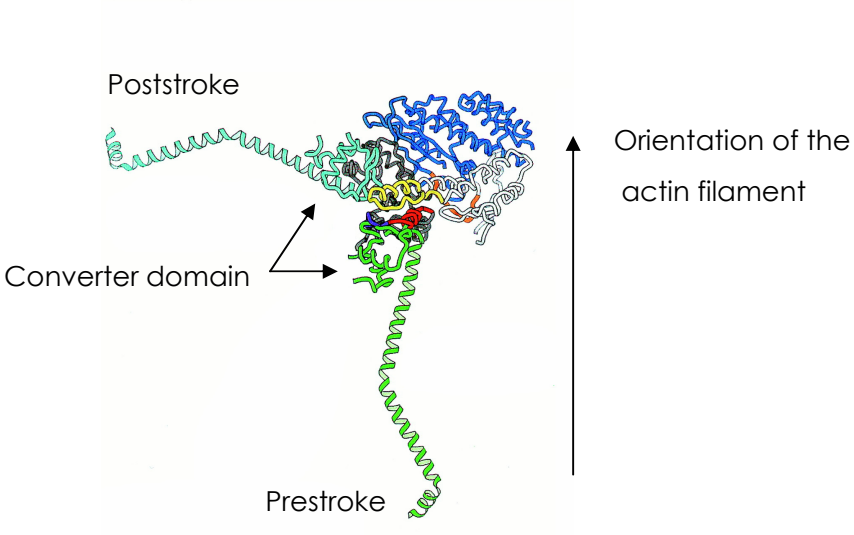

Figure 3: Myosin S1 in the putative "prestoke" and "poststroke" states.

Though the initial structural studies were performed on myosins lacking the neck domain to facilitate the crystallization (except the chicken skeletal S1 structure) modeling studies show that the changes in the converter domain are expected to lead to a rotation of the neck domain of 70-90 degrees[13-15]. This expectation was confirmed by solving the structure of the smooth muscle myosin head with the nucleotide analog ADPBeF_x in the ATP binding pocket that included one of the light chain binding domains[10]. The comparison of this structure to the chicken skeletal myosin S1 in the no nucleotide state revealed a 70 degree rotation of the converter domain with a corresponding rigid-body movement of the neck domain (Figure 4).

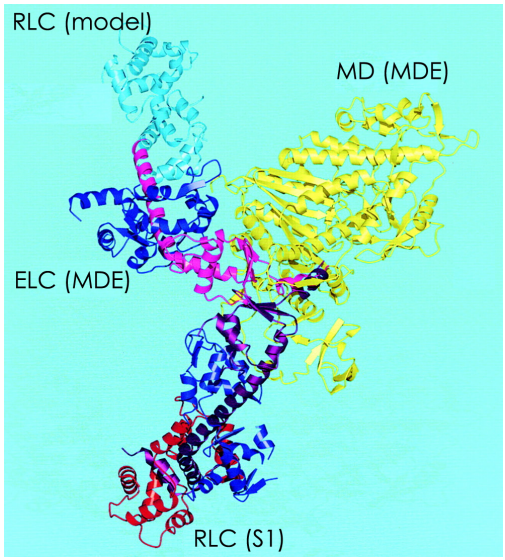

Figure 4: Ribbon diagrams of the smooth muscle myosin motor domain with the ELC (MDE) and the chicken skeletal myosin (S1) with the ELC and RLC. The superimposed motor domains (yellow) reveal a rotation of the converter domain coupled to the rotation of the lever arm.

Given the orientation of the head when it binds to the actin filament, the rotation associated with the dissociation of the nucleotide swings the distal tip of the neck domain about 10 nm towards the barbed end of the actin filament[7,13]. These structures give strong support to the hypothesis that muscle contraction is driven by a swinging lever arm. Further support for the lever arm model comes from single molecule experiments showing that the size of the working distance depend on the lever arm length[16].

Myosin Regulation

Cells display numerous motile activities such as moving organelles towards their cell center and away, activating and repressing organelle movements or moving chromosomes towards the equator. All of these events require specific molecular motors which in turn must be regulated precisely. Molecular motors and/or the associated proteins are subjected to reversible phosphorylation and dephosphorylation. In some cases changes of the phosphorylation state have been correlated with changes in localization or in the transport of cargo by the motor, in other cases with a change in motor activity as described below.

Another widely used mechanism for regulation of molecular motors is calcium binding to their light chains.

Myosin Regulation via Phosphorylation

The most extensively explored phosphorylation mediated regulation is in class II myosins. Depending on the myosin, phosphorylation might occur on the light chains or heavy chains or both. In some cases, phosphorylation is regulatory by profoundly affecting the actin-activated MgATPase activity of the myosin, in others it is rather modulatory and has only a small effect on the ATPase activity.

The actin activated ATPase activities of *Dictyostelium*, vertebrate smooth and nonmuscle myosin II are regulated by reversible phosphorylation of their regulatory light chains. The RLC phosphorylation increases their actin activated ATPase activity[17-20]. The mechanism of regulation is a mystery since the RLC is topographically far removed from the catalytic domain in the available crystal structures (Figure 62).

However, no double headed myosin has been crystallized so far and there is only one myosin S1 structure containing both light chains[7]. The importance of a crystal structure of an intact double headed myosin becomes evident if it is considered that the RLC is located at the S1/S2 junction and its binding properties might be altered in the S1, missing the S1/S2 junction (Figure 1). In addition, there are a number of data supporting mechanisms that predict formation or breakage of direct interactions between the two heads in myosin II depending on the phosphorylation state of the RLC[21-23]. No structural data at atomic resolution of a double headed myosin is available to date to validate these models. However, a three-dimensional image reconstruction of electron microscopy data taken on unphosphorylated and thiophosphorylated smooth muscle HMM revealed long inferred asymmetric interactions between two S1 heads in the unphosphorylated state in which one of the motor domains is bent towards the other head. This kink was straightened in the phosphorylated structure (Figure 5)[24,25].

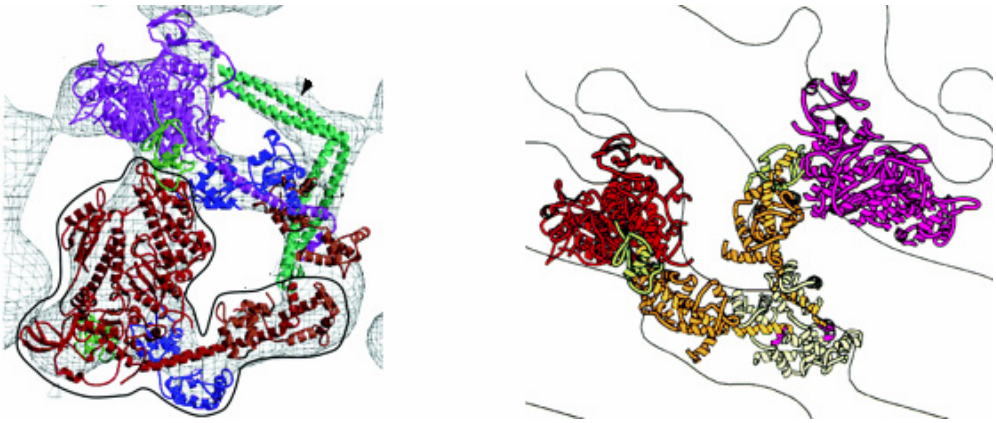

Figure 5: (Left) Arrangement of the unphosphorylated myosin heads, suggesting direct interactions between the heads of one HMM molecule in the inhibited, unphosphorylated state. The interacting heads are shown in red and purple. **(Right)** Arrangement of the thiophosphorylated myosin heads. No interactions between the two heads are observed in the phosphorylated state. The head domains are shown in red and magenta[25].

Based on this data, two models that are not mutually exclusive models for inhibition of the myosin ATPase activity in the unphosphorylated state are possible: either individual rate constants of the ATPase reaction are modified by the head-head interaction or the head orientations would not favor actin binding, resulting in a great barrier for actomyosin interactions[24,25].

Similar structural studies on double headed myosin have not yet been carried out with *Dictyostelium* myosin II. However, the catalytic domains of *Dictyostelium* myosin, chicken skeletal myosin and smooth muscle myosin are structurally highly conserved[7,9,10]. Also, although there are primary differences in the sequences, the structures of the regulatory domains of chicken skeletal muscle[7], chicken smooth muscle[10], and scallop striated muscle[26,27] are similar. This suggests that while the biochemical events leading to activation or inhibition may vary for different myosins, the structural role of the RLC can be a more fundamental feature of myosin II motors. Another important finding is that the sequences around the phosphorylation sites of smooth muscle and nonmuscle myosin II are highly conserved[17]. Furthermore, the enzymatic activities of the smooth muscle myosin are regulated essentially in the same way as the *Dictyostelium* myosin II: both enzymes display a highly actin

activated ATPase activity upon phosphorylation. In contrast, the single headed S1 is active regardless of the phosphorylation state[23,28].

The phosphorylation in the myosin tail region can also regulate the activity of myosins not by affecting the enzymatic activity, but by changing the affinity of the myosin to its cargo. The calcium/calmodulin dependent CaMKII kinase phosphorylates a serine in the C-terminal organelle-binding domain in *Xenopus* myosin V in a cell-cycle dependent manner[29,30]. The onset of mitosis results in an increase in myosin V tail phosphorylation and a concomitant decrease in the level of myosin V associated with melanosomes in melanophores. The increased phosphorylation is predicted to cause cargo (melanosome) release from myosin V. Therefore, HC phosphorylation in the myosin V tail region may be one mechanism for regulating myosins involved in organelle transport.

Myosin Regulation via Calcium Binding

Myosins have one to six IQ motifs per HC and most light chains bind in the absence of calcium. Calcium binding to LC can result in a weakening of affinity and may cause its dissociation. More importantly, calcium binding also influences actin-activated MgATPase activity, actin binding, and alters the translocation velocity of actin filaments in *in vitro* motility assays[31]. The effects of calcium binding are different for each myosin.

In case of myosin V, micromolar calcium concentration results in higher actin-activated MgATPase activity and in increased affinity for actin, but decreased motility[31,32]. In contrast higher calcium levels result in both decreased actin-activated MgATPase activity and motility[33].

ATPase Activity of Myosins

To generate motility motor proteins convert chemical energy into mechanical energy by coupling the hydrolysis of ATP to conformational changes. Myosin II has served as a model system for understanding motility for decades. Unfortunately, an intact myosin II molecule is not well suited for kinetic studies of

the MgATPase activity, particularly in presence of actin as it polymerizes into thick filaments at physiological and lower ionic strengths. These filaments do not mix well and thus contribute to large amount of light scattering. Furthermore, thick filaments interact heterogeneously with actin filaments, complicating the kinetic analysis. Therefore, most of the data obtained on the kinetic cycle of myosins came from soluble proteolytic fragments, S1 or HMM (Figure 1). The same is also true for the unconventional myosins.

According to the lever arm model, a cycle of actin–myosin interaction is thought to occur as follows: the S1 domain binds to ATP, and releases its attached actin filament. Next, the S1 domain hydrolyzes the ATP causing a large conformational change in the molecule. Actin rebinding triggers phosphate release, which in turn prompts the S1 domain to return to its starting conformation, in a motion termed the powerstroke. The net result is that the attached actin filament is translocated about 10 nm in the direction of its pointed end[4].

Processivity and Duty Ratio of Myosins

The time a myosin motor spends bound to actin filaments is an important consideration when evaluating its function. Duty ratio 'r' refers to fraction of the ATPase cycle that myosin spends strongly bound to actin.
The duty ratio 'r' is defined as:

$r = T_{on} / T_{on} + T_{off}$

Myosins that spend a significant proportion of the cycle strongly bound to actin have a high duty ratio such as myosin V (Figure 6), while those that spend a small fraction of the cycle strongly bound to actin are considered to have low duty ratio such as myosin II.

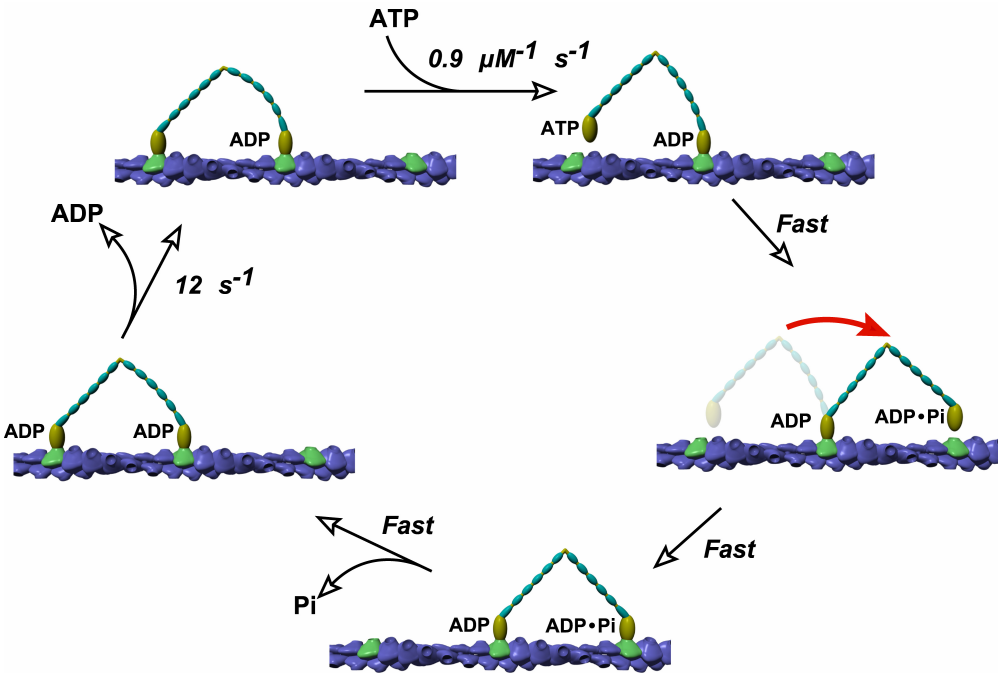

Figure 6: Nucleotide depended stepping of myosin V along an actin filament. The slow ADP release of the trailing head (12 s^{-1}) ensures that the motor stays attached to its filaments with both heads during one ATPase cycle.

Conventional muscle myosin II is a low duty motor that detaches after each ATPase cycle from its track[14]. This property is reflected in the fact that large coordinated assemblies of motors are needed to produce high speed motility as in muscle contraction. A low duty ratio motor would not be suitable for a persistent transport of cargo, as it would detach after a single step on actin. In contrast, motors with a high duty ratio are good candidates to serve as long range transport motors. Such single myosin molecules can translocate along an individual actin filament for long distances.

Myosin V was the first discovered processive motor in the myosin superfamily. In single molecule experiments myosin V was shown to take multiple steps per encounter with actin[34]. A single myosin V molecule attached to surface can promote continuous movement of an actin filament. Therefore the actin velocity is independent of the myosin V density.

The next discovered processive motor in the myosin superfamily is myosin VI[35]. The rate-limiting ADP release (5.6 s^{-1}) and the slow rate of ATP binding rate

(0.018 $\mu M^{-1} s^{-1}$) in the presence of actin ensure that myosin VI has a high duty ratio. Thus myosin VI spends significant proportion of its ATPase cycle strongly bound to actin and can act as a processive motor *in vivo*[36,37]. High duty ratio motors can be identified by *in vitro* motility assays of single myosin molecules. Myosins from at least three classes are shown to be processive: myosin V[34], myosin VI[35,36] and myosin IXb[38].

In vitro Motility Assays

The study of motor proteins was revolutionized by the development of *in vitro* motility assays in which the motility of purified motor proteins along purified cytoskeletal filaments is reconstituted in cell free conditions. Today the most commonly used assay for myosin motors is the gliding filament assay (Figure 7). In this assay, the motor is attached to a surface and the fluorescent filaments are imaged onto an intensified charge-coupled device (CCD) camera. The fluorescent filaments are observed to diffuse in solution, eventually attach to and glide along the motor coated surface. The motions can be acquired on a computer and the speed can be measured by tracking the leading edge of the fluorescent filaments from frame to frame using appropriate imaging software[39].

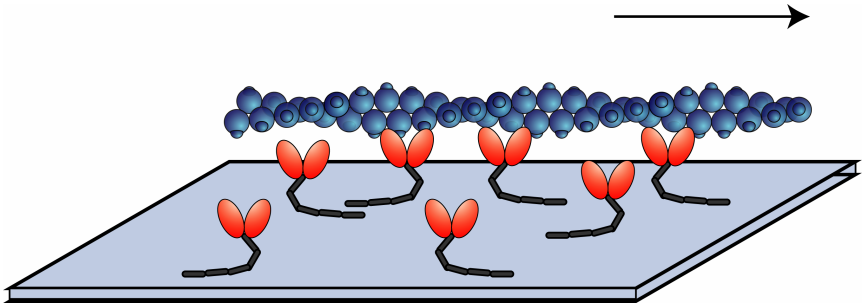

Figure 7: Gliding filament assay. The surface attached motors drive the unidirectional motility of single actin filaments in the presence of ATP. The arrow indicates the direction of the filament movement.

Single Molecule *in vitro* Assays

Recent developments in the fluorescence microscopy allow the observation of fluorescently labeled single molecules *in vitro* in aqueous solutions[40]. Since then a whole arsenal of single molecule techniques have been developed for

studying proteins and other macromolecules. Single molecule spectroscopy in general provides unique information on distribution functions of relevant observable events and resolves sub-populations in a heterogeneous sample. In addition, single molecule spectroscopy circumvents synchronization problems and allows distinction between multiple kinetic pathways as well as detecting individual transient intermediates. Recently developed assays for detection of single biomolecules such as total internal reflection fluorescence (TIRF) microscopy[40,41] or single molecule fluorescence polarization anisotropy (smFPA) play an important role in single molecule studies[42]. TIR makes use of the evanescent field formed at the glass-water interface (Figure 8). Its amplitude decays exponentially away from the interface, with the 1/e decay constant about 200 nm[43]. Under these conditions only fluorophores attached to the surface or near the surface are excited. Thus, sample molecules in the bulk medium are not excited, reducing the background several orders of magnitude compared to conventional epi-fluorescence microscopy. Using the TIR fluorescence microscopy, the processive movement of fluorescently labeled single myosin V molecules along actin filaments has directly been observed[42,44,45].

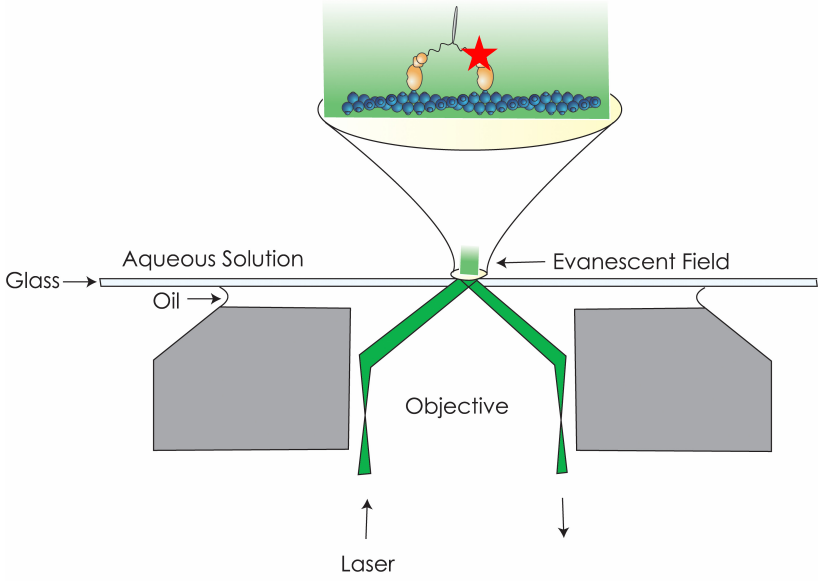

Figure 8: Schematic drawing of the formation of an evanescent field. The laser beam is focused to propagate along the objective edge. The beam is then totally internally reflected back into the slide. The electrical field however cannot vanish abruptly and produces an oscillating electromagnetic field, termed an evanescent wave, penetrating some hundred nanometers into the aqueous solution and excites fluorophores, shown as a red star, close to the surface[43].

Another widely used single molecule technique for motor proteins is the optical trapping technique. Optical trapping is based on the principle that small dielectric particles or objects in a size range from nanometer to micrometer can be trapped in the waist of a strongly focused continuous wave laser beam. The optical trap results from the fact that the objects trapped in the focus of the laser beam experience a restoring force if they try to leave the high intensity volume. The force from the optical trap is in general weak (in the order of pN) although fully sufficient for manipulation of individual objects on a cellular level it has any physical influence. In the so called dual bead assay the actin filament is stretched to tension between two beads to form a dumbbell (Figure 9). This dumbbell is then lowered onto the surface which is sparsely coated with motors. Binding and motion events are apparent from the bead displacement. These displacements are measured using photodiode detectors capable of sensing nanometer displacements with millisecond temporal resolution.

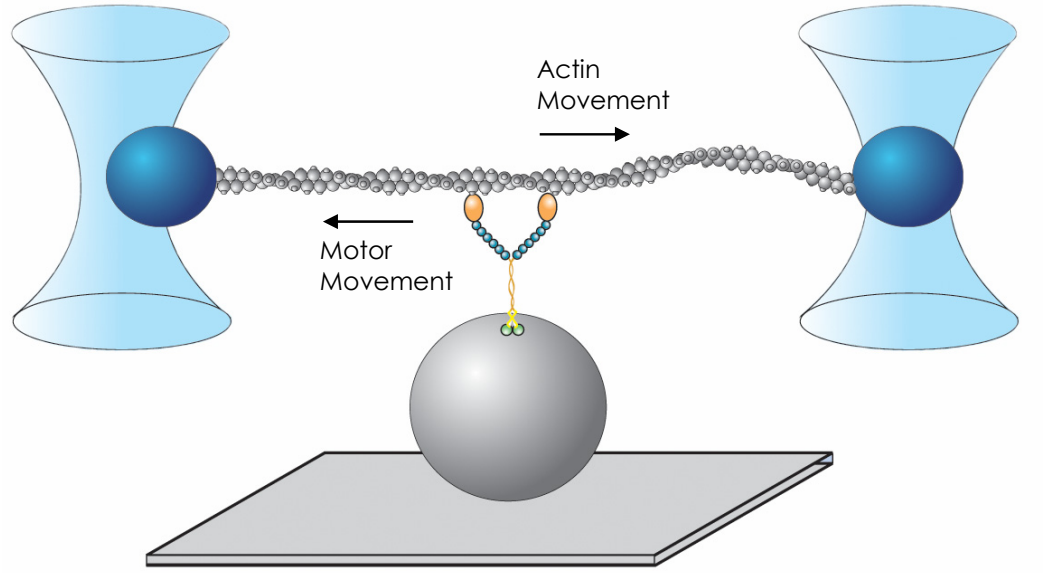

Figure 9: In a dual-bead assay the beads (shown in blue) are attached to the either end of the actin filament (shown in grey). In this geometry the filament is stretched to tension and lowered down to the surface that is sparsely coated with motor protein. As the motor takes discrete steps the bead is moved out of the light trap (illustrated in light blue). The position of the bead can then be tracked with nanometer resolution on quadrant photodiode detectors. A force feedback control maintains the distance between the bead center and trap center constant.

Myosin V and Myosin VI as Processive Motors

Myosin V is a double headed molecular motor that binds six light chains per heavy chain. As a result, myosin V has neck domain which is three times longer than that of myosin II. Thus, according to the lever arm model myosin V should be able to take 30 nm steps. Mehta *et al.* demonstrated that myosin V takes 36 nm steps after each diffusional encounter with the actin filament in optical trapping experiments[34]. The pseudorepeat of the coiled coil F-actin is 36 nm. Thus the measured step size of myosin V may allow the molecule to step along the 36 nm pseudorepeat of the F-actin helix without having to spiral around it. To investigate the lever arm length depended stepping of myosin V Purcell *et al*. created mutants with lever arms of varying lengths by deleting their IQ domains. The stroke size was found to be depended on the lever arm length (Figure 10). Myosin V molecules with four light chains is still processive but takes only 24 nm steps. A further reduction to one light chain per head results in a non processive motor with a 6 nm step size[16].

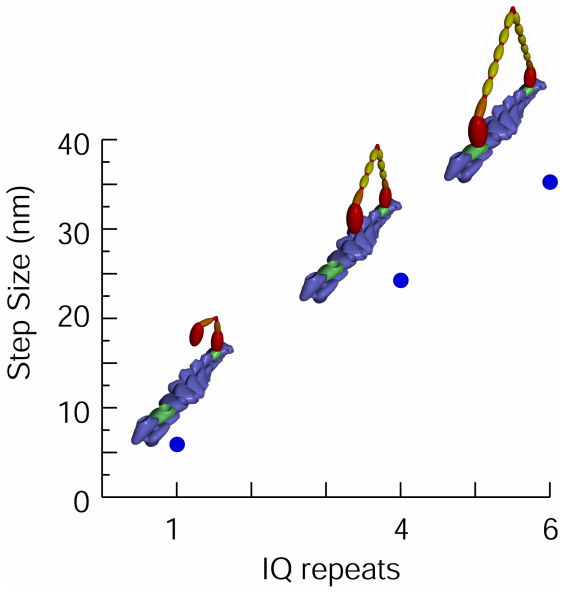

Figure 10: Dependence of the step size on the number of the IQ domains in myosin V. The step size decreases in a proportionally with decreasing number of the IQ domains.

Rief *et al*. determined the stepping kinetics of myosin V and proposed a hand-over-hand model for myosin V processivity[46]. In this model, myosin V spends the majority of its lifetime with both head domains bound simultaneously to actin. For a processive motor that operates in such a hand-over-hand manner, some

form of communication between the two heads must exist to prevent both from detaching at the same time. The nucleotide dependent processive stepping is illustrated in Figure 6. According to the hand-over-hand model, the heads swap orientation during the stepping cycles resulting in alternation of the leading position. After each ATP hydrolysis the trailing head becomes the new leading head. An alternative to the hand-over-hand model is the inchworm model, which involves one head always serving as the leading head with the trailing head pulled up from behind at each step.

As myosin V, myosin VI is a double headed motor but has only one IQ repeat and therefore a significantly shorter lever arm. The second calmodulin binds to a 53 amino acid unique insert that is not present in other characterized myosins[47,48]. In a dual bead assay Rock *et al.* found that myosin VI is a processive motor, which can take 30 nm steps along an actin filament under 1.7 pN load (Figure 11)[35].

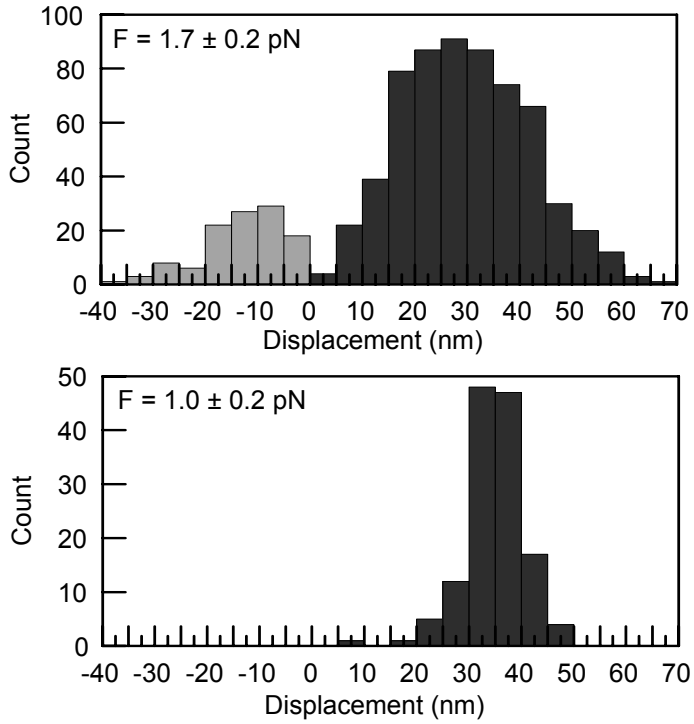

Figure 11: Step size distribution of myosin V and myosin VI in a dual bead assay. Compared to myosin V, myosin VI has a highly broad step size distribution as a direct result of accessibility of binding sites on the left side of the actin filament to the unbound myosin head.

Myosin VI also displays a significant number of back steps compared to myosin V (Figure 11). The colored actin subunits shown in Figure 12 represent the preferred binding sites for the leading head toward both the pointed end resulting in a mean step size distribution of 27 nm and the barbed end resulting in a mean step size distribution of 11 nm (Figure 12).

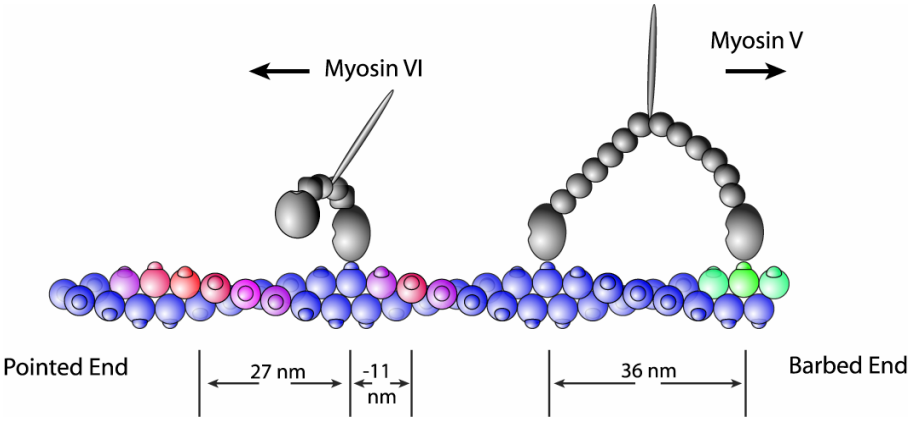

Figure 12: Myosin V with its long lever arm can span the 36 nm actin pseudorepeat within +/- 1 binding site (shown in green and turquoise). Myosin VI however can not reach the speudoactin repeat and has to search for the next available binding site, which are colored pink to red.

Given the short size of the lever arm of myosin VI, a canonical lever arm theory cannot fully account for its observed step size and processive movement (Figure 12). The discovery of a myosin with a short lever arm but a large step size led to controversial proposals for the working mechanisms of myosin VI[35,49].

Rock *et al.* proposed a modification of the lever arm model that predicts a step consisting of a working stroke of the short lever arm followed by a diffusive search for an available binding site on actin[35]. The working stroke provides part of the step size and imparts directionality while the diffusive search allows the leading head to find further actin binding sites. This proposal predicts mechanically extensible elements in the myosin VI protein, which extends the reach of the myosin heads and allows larger step sizes. The proposed mechanically extensible element could be in the tail domain, C-terminal to the light chain binding region (Figure 13, A). The proximal tail domain is an attractive candidate because of its low propensity to form a coiled-coil[50]. The coiled-coil region is likely to be involved in creating a functional dimeric motor.

Interestingly, Lister *et al*. have found that the full-length myosin VI from chicken intestinal brush border is a monomer[51]. As the authors point out, it seems likely that the dimerization state of myosin VI might be regulated *in vivo* in a manner that remains to be elucidated.

A mechanically extensible element could also derive, however, from some part of the structure N-terminal to the IQ domain that binds calmodulin. The unique insert could be involved in the required flexibility (Figure 13, B). This putative diffusive search of the leading head would also explain the observed broad step size distribution for myosin VI (Figure 11)[35].

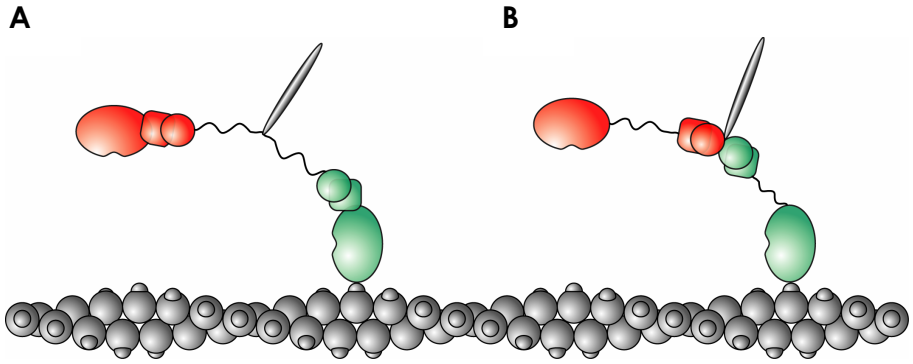

Figure 13: Proximal tail domain unfolding C-terminal to the IQ bound light chain (**A**) and catalytic domain unfolding N-terminal to the IQ bound light chain (**B**). The leading heads are colored red and the trailing heads are colored green. Small squares represent the unique insert bound calmodulin light chains and the small spheres represent the IQ bound calmodulin light chains.

Myosin VI is a Point-end Directed Motor

Unique among characterized myosin motors, myosin VI moves toward the pointed end of actin filaments (Figure 12)[47]. It is assumed that the mechanistic coupling in the converter domain will differ between the barbed end directed and pointed end directed motors. A simple mechanical model illustrated in Figure 14 may explain the polarity of movement. Minor rearrangements in the converter domain may produce a swing of the lever arm in the opposite direction. As seen in the recent three-dimensional structure at atomic resolution, the unique insert, a 53 amino acid insertion between the converter

domain and the lever arm plays an essential role in repositioning the lever arm to reverse directionality[52].

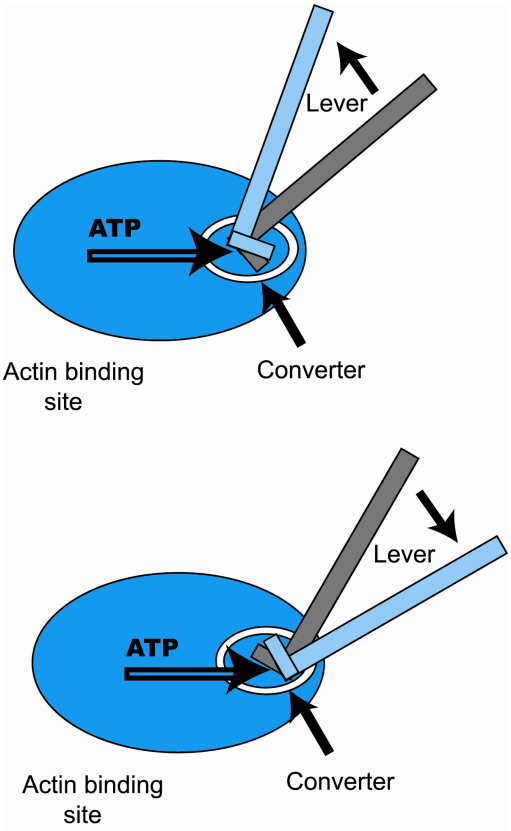

Figure 14: A slight difference in the topology of the rigid elements in the converter region shown in the white circle can respond to the same small conformational changes in the core with a reversed swing of the lever arm.

Support for this theory comes from another family of molecular motors, the kinesins. Kinesins family of motors has members that can move in both directions along the microtubules[53]. Atomic structures, in conjunction with functional studies, have given insights into the molecular basis of the directional bias[54]. While the core structures of plus- and minus-end directed kinesins are virtually identical, the topological relationships between the head and the neck domain differ significantly[55,56]. There is strong evidence that the neck is crucial in determining directionality[56-59]. Thus, in both myosin and kinesin, the regions between the catalytic domain and the adjacent neck including the converter domain seem to be important for specifying the direction of movement. Although both regions in these different types of molecular motors

are considerably divergent in their structures, they seem to share functional equivalence.

Roles of Myosin VI as a Point-end Directed Motor in Cells

Myosin function can be inferred in part from the direction of motor movement on actin filaments. At the cell cortex, where endocytosis occurs, polarized actin filaments are anchored with their barbed end at the plasma membrane with their pointed ends facing inwards. This allows oriented transportation of cargo into or out of the cell. Conventional myosins as well as unconventional myosins travel towards the barbed end of the actin filament. Thus, a barbed end directed myosin will transport attached cargo outwards towards the cell surface, such as a vesicle on the exocytic pathway. Conversely, a pointed end directed myosin will transport attached cargo inwards, away from the cell surface, such as a vesicle on the endocytic pathway. Recent studies on myosin VI, the only pointed end directed myosin characterized to date, provide compelling evidence that myosin VI and therefore also actin is involved in distinct steps in endocytosis. Endocytosis is an essential function in all cells and is required for nutrient uptake, receptor internalization and synaptic transmission. Immediately under the plasma membrane where the endocytosis occurs is a cytoskeletal layer consisting of primarily actin filaments and associated proteins. In polarized cells myosin VI is associated with endocytic domains. In kidney proximal tubule cells and intestinal enterocytes, myosin VI is enriched at specialized clathrin-coated invaginations at the base of the brush-border microvilli[60-62]. A role for myosin VI in the endocytosis could also relate to various biological processes involving membrane restructuring. As a high duty ratio motor[36], it is ideally adapted for maintaining membrane tension and providing force. A very different polarized cell type, the inner ear sensory hair cells in mice have actin rich extensions (modified microvilli) called stereocilia at their apical surface. These structures are organized into units or bundles that move together when stimulated. These bundles are deflected in response to sound vibrations and gravity, resulting in the opening of ion channels and eventually transduction of electrical signals to the brain[63]. The Snell's waltzer (*sv*) mouse mutant carries an intragenetic deletion in the gene encoding Myo6[64]. These animals are deaf and also display hyperactivity and circling behavior. Myosin

VI is localized to the base of stereocilia and is enriched in the cuticular plate, a region of densely packed actin filaments that the stereocilia is embedded into, as well as being diffusely distributed throughout the cytosol[64,65]. Molecular analysis of the inner ear cells in the *sv* mice supports a role for myosin VI in maintaining the stereocilia structure. Stereocilia bundles appear to be normal at birth, but after three days after the birth the bundles become fused together and disorganized[66]. One model for this fusion is that myosin VI provides the required force to anchor the membrane at the base of each individual stereocilium. The organization of actin filaments in the stereocilia and the minus end directionality of myosin VI are such that this motor may pull down the membrane around each stereocilium. In the absence of myosin VI, stereocilia form, but these structures are not preserved as the mice age resulting in stereocilia fusion.