TIMELINE

The myosin swinging cross-bridge model

James A. Spudich

No biological system has been studied by more diverse approaches than the actin-based molecular motor myosin. Biophysics, biochemistry, physiology, classical genetics and molecular genetics have all made their contributions, and myosin is now becoming one of the best-understood enzymes in biology.

Hugh E. Huxley's elegant combination of X-ray diffraction and electron microscopy studies on muscle (TIMELINE) led him to propose the swinging cross-bridge model of muscle contraction in 1969 (REF. 1) (FIG. 1). Since then, many important findings have shaped our understanding of molecular motors in general and myosin in particular. At a pivotal meeting in 1972 at Cold Spring Harbor, a coherent picture began to emerge about how the cross-bridge might work. Tension transient measurements using intact muscle[2], structural studies including those based on three-dimensional-reconstructions from electron micrographs[3], actin-activated myosin ATPase kinetics[4], the development of nucleotide analogues to probe the ATPase cycle[5–7], and other approaches began to come together in meaningful ways. The discovery of the first 'unconventional' myosin[8] pointed to the importance of non-muscle myosins in cells. By the 1980s, time-resolved X-ray diffraction on living fibres using synchrotron radiation revealed changes in cross-bridge order that were kinetically consistent with tension development[9]. Until the early 1980s, the field was limited by the lack of a quantitative *in vitro* assay to measure the essential aspect of the proposed swinging cross-bridge model — ATP-driven movement of myosin along actin. Since then, such assays have been developed, and have been essential in furthering our understanding of the crossbridge mechanism.

Quantitative *in vitro* motility assays, developed from 1983 to 1986, have not only been invaluable tools for the study of myosin, but also led to the discovery and characterization of the kinesin family of molecular motors[10–12]. The discovery of a large family of kinesin motors, together with studies that led to a compelling model of how kinesin works[13], have been pivotal in understanding the roles and mode of action of molecular motors *in vivo*[14,15].

For the actin-based myosin systems, quantitative *in vitro* motility assays immediately provided the evidence needed to rule out some models of contraction, such as

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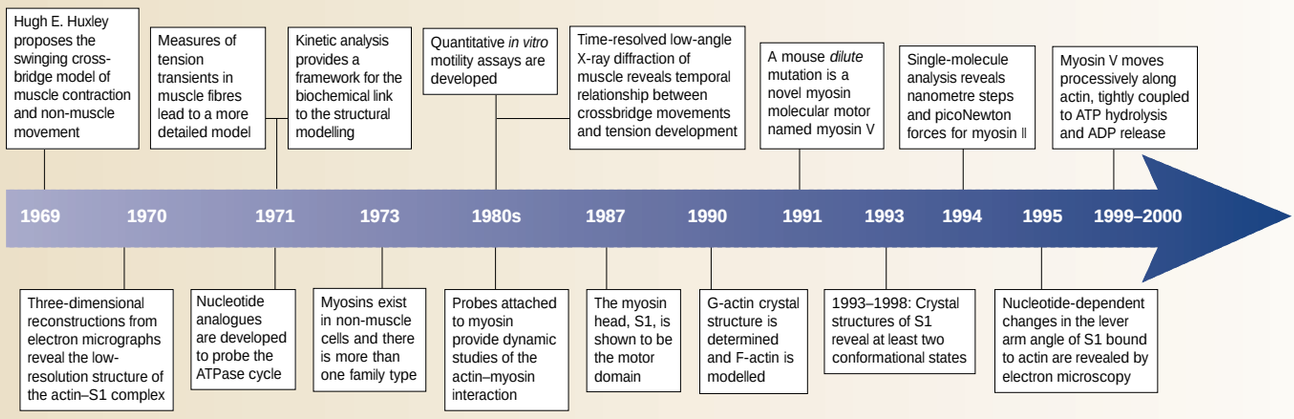

F-actin, filamentous actin; G-actin, monomeric actin; S1, subfragment 1.

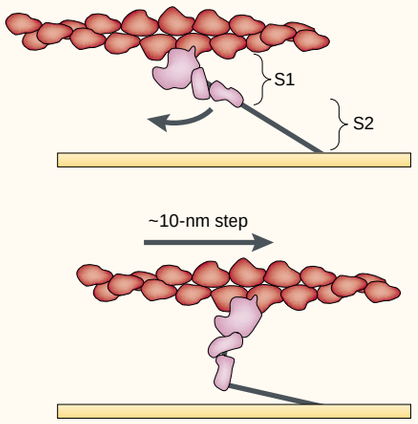

Figure 1 | **The swinging cross-bridge model.** The model shown is that of H.E. Huxley[1], modified to indicate bending near the middle of the elongated cross-bridge (subfragment 1, or S1) to provide the working stroke. The S1 is tethered to the bipolar thick filament (horizontal yellow line) through the S2 domain of the myosin molecule. The actin filament structure is based on the model proposed by Holmes *et al.*[44], and the S1 structures are based on various crystal structures[18–21]. S1 and filamentous actin (F-actin) are drawn to scale.

those involving conformational changes in the tail region of the molecule. The cross-bridge itself (subfragment 1 of myosin, or S1; FIG. 1) was shown directly and unequivocally to be the motor domain of myosin[16]. The tail of myosin II has the crucial function of forming bipolar thick filaments that anchor the S1 motor domains to a macromolecular assembly used in muscle contraction and in contractile processes such as cytokinesis in non-muscle cells. To understand the motor activity, attention focused on S1.

Insights from the structure of S1

As we entered the 1990s, the main limitation was the lack of a high-resolution crystal structure of the S1 motor and the actin track along which it moves. One of the most important breakthroughs of the last decade was the determination of the high-resolution crystal structures of monomeric actin (G-actin)[17] and of S1 (REFS 18,19). S1 consists of a catalytic domain and a light-chain-binding domain (FIG. 2). The catalytic domain contains a nucleotide-binding site of the P-loop variety that is closely associated with switch-I and switch-II helices. The nucleotide site is ~4 nm away from the actin-binding site, and these two sites communicate with one another through the switch-I and switch-II helices, which move in response to the state of the nucleotide, especially the presence or absence of P_i in the active site. Extending from the edge of the catalytic domain furthest from the actin-binding site is an α-helix that is surrounded by two calmodulin-like light chains. It has been suggested that this light-chain-binding region acts like a lever arm, which amplifies smaller conformational changes near the nucleotide-binding site by swinging through a large angle from a prestroke state to a poststroke state (FIG. 2). Strikingly, X-ray crystallography has revealed more than two distinct states of the lever arm, one a putative prestroke state and another a putative post-stroke state[18–21].

The asymmetric shape of S1 allowed the docking of the X-ray crystal structure of S1 into low-resolution three-dimensional images of the actin–S1 complex generated by electron microscopy[22,23]. Three-dimensional reconstructions from electron micrographs in the presence and absence of ADP revealed different conformations of the lever arm, indicating that, at least for some myosins, there might be a small additional stroke that derives from the release of ADP from the active site[24,25].

Although the static electron-microscope and X-ray crystal structures are essential for understanding how S1 works, important contributions have come from dynamic studies of nucleotide-dependent S1 conformational changes. Probes placed at the most reactive cysteine near the active site showed very little change in orientation during contractile events[26]; this led to the suggestion that the banana-shaped S1, as observed by electron microscopy, might actually swing not at the actin-binding site, but rather in the middle of the S1 (REF. 27). The subsequent crystal structures drove home this modified swinging cross-bridge model: the main change in

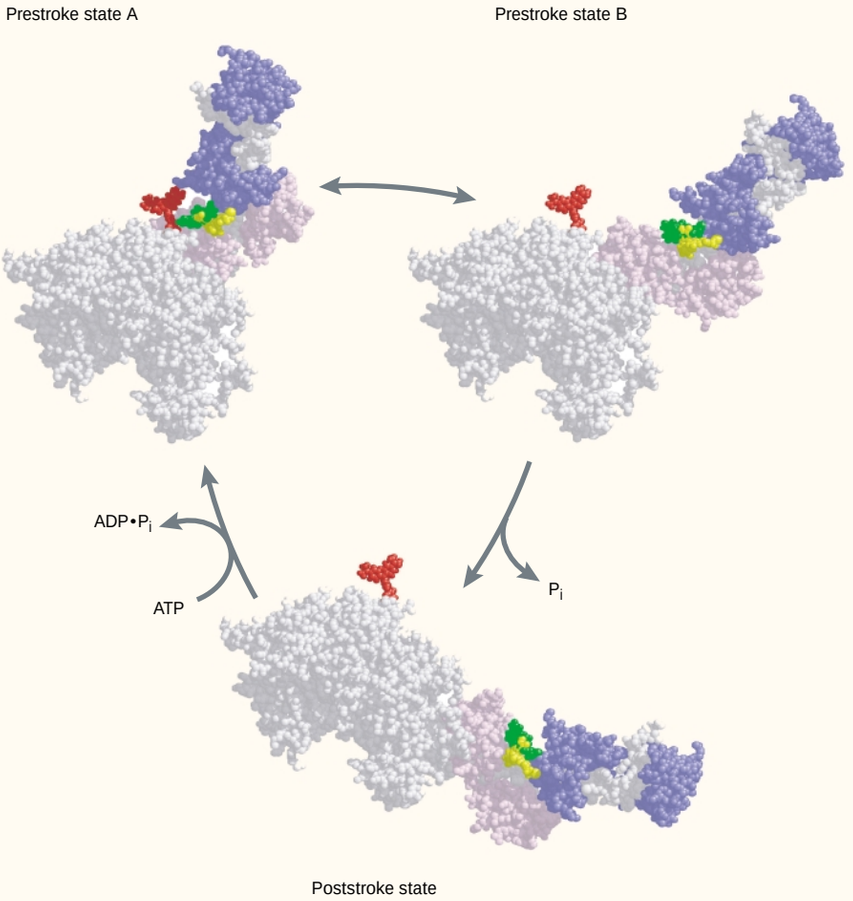

Figure 2 | **Prestroke and poststroke states of subfragment 1.** Structures modelled from the prestroke and poststroke subfragment 1 (S1) crystal structures[18–21] are shown, with the catalytic domain of S1 kept in a fixed orientation. The lever arm moves through an angle of ~70°, going from prestroke state A to the poststroke state. Prestroke state B was predicted from fluorescence resonance energy transfer (FRET) measurements[28]. The red colour shows the position of an acceptor dye for the FRET measurements. The green and yellow colours show two independent positions for placing a donor dye on the regulatory light chain. Adapted from REF. 28. © (2000), with permission from Elsevier Science.

orientation was suggested to occur between the catalytic domain and the light-chain-bound lever arm[22] (FIG. 3). To visualize the suggested change using a dynamic approach, molecular genetic manipulation of the S1, removing existing reactive cysteines and placing new ones at sites that should reveal large changes in lever arm orientation was necessary. Using this approach, an ~70-degree rotation was revealed with at least three distinct states of the lever arm position[28] (FIG. 2).

How big is the myosin step?

The swinging cross-bridge model is only consistent with a small, ~10-nm, step in motion when myosin interacts with actin. Using single muscle sarcomeres from which the Z lines — structures that anchor the thin filaments in the sarcomere — had been removed by protease treatment, a much larger step in motion for each ATP hydrolysis step was reported[29]. Debate ensued for a decade about the size of the step taken when myosin interacts with actin, with some experiments indicating a possible step size of more than 100 nm (REF. 30). A refinement of the *in vitro* motility assay permitted measurement of myosin steps, one molecule at a time[31]. This simplified system established that there is a unitary step in motion for conventional myosin II of ~5–15 nm, each step probably linked to the turnover of a single ATP (although see REF. 32 for an alternative view).

Another important development is the use of molecular genetics to dissect the roles of various domains and specific residues of S1 (for review, see REF. 33). The data assembled from multifaceted approaches applied to this research area best fits the following model (FIG. 3). The myosin cross-bridge binds to ATP, and then releases its attached actin filament. The cross-bridge then hydrolyses the ATP, and primes itself in preparation for a productive working stroke. Actin rebinding triggers phosphate release, which in turn prompts the myosin cross-bridge to return to its starting conformation, in a motion termed the 'powerstroke.' This powerstroke involves a relatively fixed catalytic domain bound to actin and swinging of the light-chain-binding region through a considerable angle, providing a working stroke of 5–15 nm. The net result is that the attached actin filament is translocated in the direction of its minus (pointed) end. Although FIG. 3a is simplified to show only forward directions around the cycle, reversibility of the steps is established and important (FIG. 3b). For example, rebinding of myosin–ATP and myosin–ADP to actin is rapid, and the actin–myosin–ATP and actin–myosin–ADP complexes are important intermediates. Although this model is widely accepted, interpretation of data has been at times difficult and controversial. This is partly due to the relatively small step size of the muscle-type myosin II and the fact that the molecule spends a very short time strongly bound to actin.

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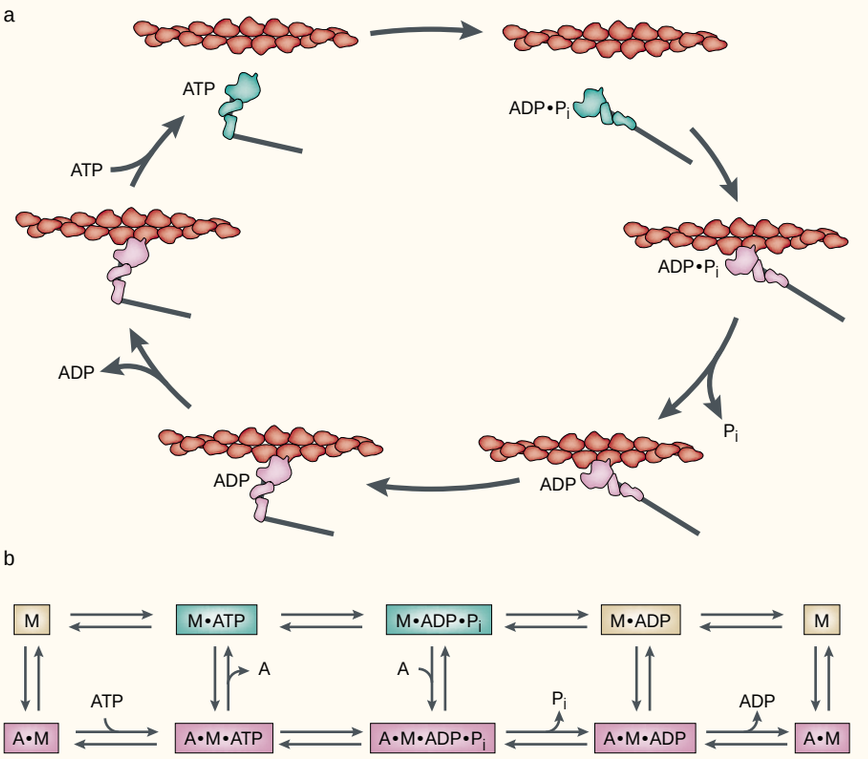

Figure 3 | **The actin-activated myosin-II ATPase cycle. a** | The states of the myosin subfragment 1 (S1) domain that are strongly attached to actin are shown in pink. Myosin II is only strongly attached ~5% of the total time of the cycle. That is, the myosin spends most of its time off the actin or only weakly associated (green states). The structures shown were modelled as described in FIG. 1. **b** | All of the myosin and actin–myosin nucleotide states are shown. All steps are reversible. A, actin; M, myosin. A primary path corresponding to the structures shown in **a** is A•M to AM•ATP to M•ATP to M•ADP•P_i to AM•ADP•P_i to AM•ADP to AM.

Following the myosin-V trail

A captivating aspect of basic research is that pivotal information about a particular biological problem comes from unexpected places. The clearest evidence for nucleotide-dependent coupling of conformational changes in myosin leading to stepping of myosin along an actin filament has resulted from initial genetic experiments on mutant mice with reduced hair colouring. It must have been a surprise to the authors of that work when they determined that the defective gene leading to the *dilute* phenotype coded for a new member of the myosin family, myosin V[34]. This myosin has a typical catalytic domain but the putative lever arm is three times longer than that of myosin II[35] (FIG. 4). The tail of myosin V is also considerably different from that of myosin II, presumably because its role is to bind to vesicular cargo rather than to form bipolar thick filaments. The unique feature of the myosin-V S1, the long lever arm, should, according to the swinging cross-bridge model, allow for a much longer step size than that observed for myosin II. In a relatively short time, studies of myosin V have revealed its probable mechanism of action, and further clarified how the myosin family of molecular motors works. The

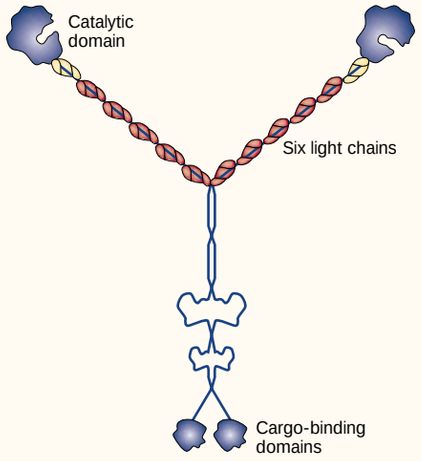

Figure 4 | **Predicted structure of myosin V.** The putative lever arm of myosin V is roughly threefold longer than that of myosin II. The tail domain is also different between the two myosin types because the tail of myosin V functions to bind vesicular cargo in non-muscle cells as opposed to myosin II, which functions to form bipolar thick filaments for contractile events.

advantages of studying the myosin-V motor are that it is built to take an exceedingly large step along actin and it remains tightly bound to actin for a large part of its ATPase cycle. These characteristics allow myosin V to move processively along an actin filament. These properties are essential when one considers the task that this molecule fulfils *in vivo*. In melanocytes, melanin-containing vesicles are presumably carried along actin filaments anchored in the cell cortex. Not all faces of the actin filaments are available to the myosin but, nevertheless, it walks processively along the actin filament to move the melanin-containing vesicles into the dendritic spines of the melanocytes, from where they are taken up by keratinocytes. It is not surprising, then, that the step size of myosin V is ~36 nm (FIG. 5)[36,37], the helical repeat of the actin filament. Furthermore, myosin V moves processively along actin filaments *in vitro* even when those filaments are tightly adhering to a surface along one face of the filament[36–38]. In this configuration, it is impossible for myosin V to follow the long-pitch actin helix as it moves along the filament; it clearly must step from one helical segment of the filament to the other.

The conformation of myosin V when strongly bound to actin has been revealed by electron microscopy[39]. The authors point out that, in low concentrations of ATP, when the myosin heads both remain bound to the actin filament, the molecule often seems to be straining forward, in the form of a 'telemark skier' (FIG. 6a).

A combination of biochemical studies and single-molecule analyses on myosin V provide compelling evidence that the dwell time between successive 36-nm steps is limited by ATP binding at subsaturating ATP concentrations and by ADP release in the presence of saturating ATP[37,40,41]. Affinity constants and on- and off-rate constants for ATP and ADP calculated from traditional biochemical approaches are remarkably consistent with those revealed by single-molecule analysis[40,42,43]. By examining the myosin-V molecules one at a time, it is possible to distinguish mechanistically between slowing the rate of stepping owing to sub-saturating ATP concentrations versus owing to competition of binding of ADP and ATP for the active site[37]. An unusually low myosin-V ATPase activity reported by Ando and co-workers[38] led them to suggest that myosin V might move 400 nm for each ATP hydrolysed. Myosin-V ATPase has subsequently been shown to be much higher, 13 s^{-1} (REF. 40). This value is consistent with one ~36-nm step per hydrolysed ATP and the observed velocity of ~0.5 μm s^{-1}. So, the combination of biochemical, structural and single-molecule analyses of the past few years provides strong evidence for a mechanism of movement of this myosin along an actin filament that involves arm-over-arm 36-nm steps that are limited by the release of ADP from the rearward head (FIG. 6b).

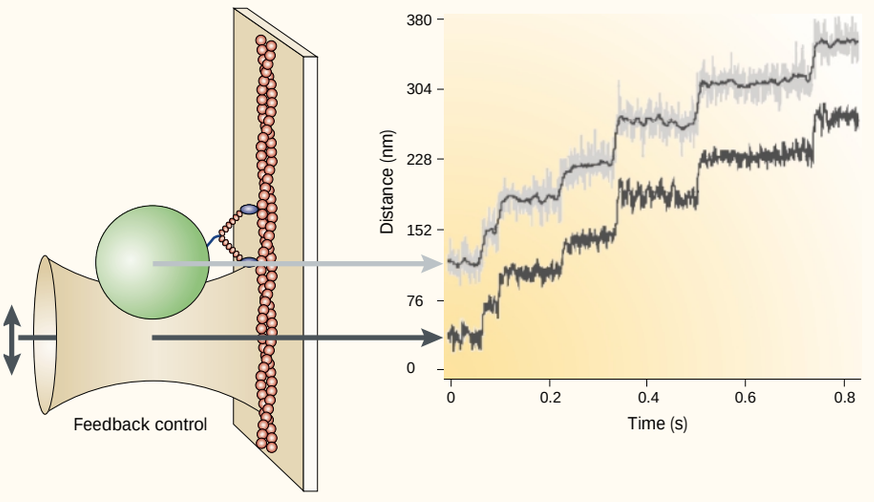

Figure 5 | **Experimental scheme of the force-feedback-enhanced laser trap.** A feedback loop keeps the distance between the centre of the polystyrene bead (grey curve) and the centre of the laser trap (lower black curve) constant as the myosin-V molecule steps along the actin filament. With this set-up, the myosin-V molecule is kept under constant load as it moves[47]. Adapted from REF. 37.

What remains to be done

A diverse array of biophysical and biochemical approaches have lent strong support for the swinging cross-bridge model of muscle contraction proposed in 1969. Although muscle researchers will be quick to point out that there is much to be done to understand how the complex muscle system works, there is little doubt that the globular head domain of myosin II, seen as a cross-bridge between actin and myosin filaments in muscle, acts as the motor domain of the molecule and pulls actin filaments along by repetitive conformational changes coupled to ATP hydrolysis. But to understand fully even this fundamental aspect of muscle and non-muscle contractile processes, several key issues still need to be resolved.

Although a crystal structure of filamentous actin (F-actin) has not been obtained, the modelled structure[44,45] is likely to be correct for the most part. A crystal structure of the F-actin–S1 complex, however, is essential. The high-resolution structure of the actin-bound state of S1 will reveal important aspects of the actin–myosin interface and clarify how the communication between the actin-binding site, the nucleotide site and the lever arm occurs.

Myosin V might be the best myosin type for sorting out other fundamental details of how the myosin motors work. For example, one should be able to show directly that the stepping of the myosin V along actin is associated with the release of ADP from the rearward post-stroke head but not the forward pre-stroke head, followed by binding of ATP to the rearward head to release it

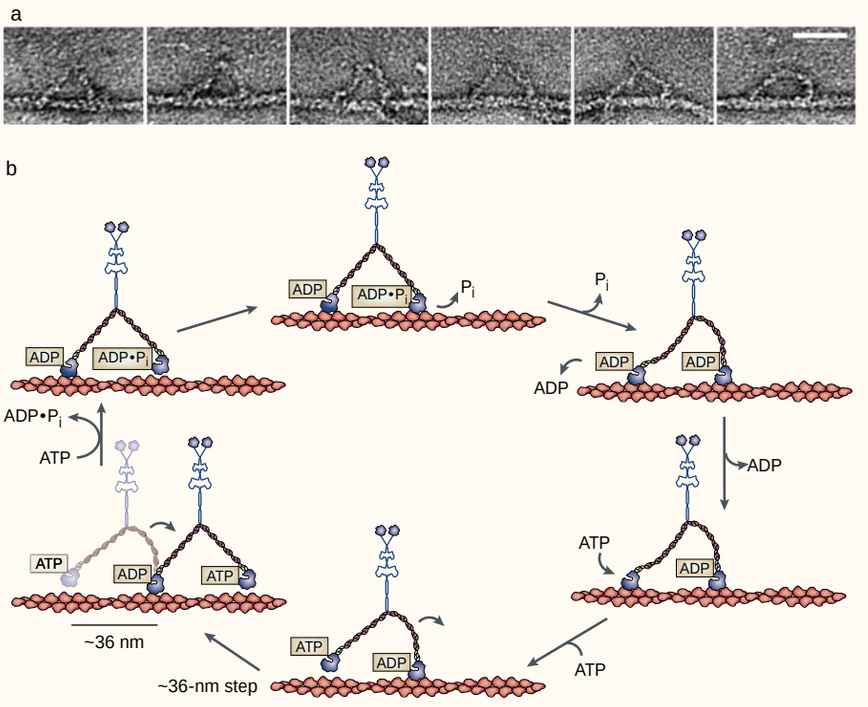

Figure 6 | **Nucleotide-dependent processive stepping of myosin V along an actin filament. a** | The electron micrograph images reveal the two heads of myosin V bound to the actin filament with a separation of ~30 nm, or straddling 11 actin monomers, in the three panels on the left. The three panels on the right show binding of the two heads with a separation of ~36 nm, or straddling 13 actin monomers. Some images show a configuration reminiscent of a 'telemark skier.' Bar, 36 nm. Adapted with permission from REF. 39 © (2000) Macmillan Magazines Ltd. **b** | A model for myosin-V stepping. As illustrated in the lower right panel, myosin V dwells with both heads attached to the actin filament, the leading head with ADP and the trailing head in rigor. ATP binding to the trailing head promotes its dissociation from actin, and forward movement of the released head then discharges intramolecular strain. The previous leading head then becomes the trailing head (lower left panel). Note that the trailing head moves 72 nm to reach its new site of attachment, but this results in only a 36-nm step of the body of myosin V or of any cargo attached to the cargo attachment domain. The new, detached leading head quickly hydrolyses ATP and then binds actin. Force generation follows either actin binding or phosphate release, which itself occurs either concomitant with or immediately following actin binding. These steps are fast relative to ADP release. At this point, the molecule is in its kinetically dominant state: both heads bound to actin and ADP, the leading head in a prestroke configuration and the trailing head in a poststroke configuration (upper right). The leading head is stressed against the direction of motion and the trailing head is stressed along this direction, an asymmetry that should bias the following ADP release to occur at the trailing head and not the leading head.

from the actin filament. Single-molecule analysis of myosin V (REF. 37) coupled to total internal reflection microscopy will allow observation of single ADP and ATP molecules as they bind and let go of the myosin[46]. Experiments that reveal whether myosin V takes fairly rigid 36-nm steps or, more likely, takes large steps with a good deal of diffusive capability, where the 36-nm pseudo-repeat of the actin filament dictates the step size, will be important for understanding fundamental aspects of the movement. Details of strain-dependent changes in nucleotide affinities, so crucial in models of myosin function, also need to be understood. Extensive mutational analysis will help answer these and other remaining questions.

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Links

DATABASE LINKS myosin | kinesin family | myosin II | myosin V

ENCYCLOPEDIA OF LIFE SCIENCES Motor proteins | Actin and actin filaments

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OPINION

Cell control by membrane–cytoskeleton adhesion

Michael P. Sheetz

The rates of mechanochemical processes, such as endocytosis, membrane extension and membrane resealing after cell wounding, are known to be controlled biochemically, through interaction with regulatory proteins. Here, I propose that these rates are also controlled physically, through an apparently continuous adhesion between plasma membrane lipids and cytoskeletal proteins.

Many cellular processes have important mechanochemical components. These include some obvious mechanical activities such as endocytosis, cell motility and membrane resealing, but also others, such as replication and transcription. Taking the motor proteins as an example of mechanochemical enzymes, it is clear that both physical and biochemical controls can modify the rate of motor movement. For all motors, an inverse relationship exists between the rate of movement and the force resisting movement, called 'load'. Above a certain load, the motor force cannot overcome the resisting force, and the motor stops. An analogous situation probably exists for membrane mechanochemical activities. For example, the endocytic machinery has to generate sufficient force to bend the membrane bilayer and form an endocytic vesicle. If the resistance of the plasma membrane — the load — is stronger than the force exerted by the endocytic machinery, no vesicles can bud, and endocytosis is inhibited.

If the load of the membrane controlled the rates of endocytosis, exocytosis or of lamellipodial retraction and extension, the cell would 'automatically' adjust the membrane area to compensate for changes in morphology, growth or secretory activity. For example, if the membrane area was too small, the increased resistance to movement of membrane into an endocytic vesicle would decrease the rate of endocytosis but would allow exocytosis to proceed normally, the result being a net increase in the membrane area. So, the physical load involved in moving membranes will naturally set the balance of membrane transport in the endocytic and exocytic pathways, and the balance of process extension and retraction in a physical feedback control system[1].

What determines the load of the plasma membrane? In almost all cells, the plasma membrane bilayer is an inelastic, two-dimensional fluid; therefore, for membrane to flow laterally into new areas, bonds to old areas must be broken. Resistance to the movement of membrane would come from tension in the membrane if the cell was round and had no cytoskeletal support. However, most animal cells have complex morphologies because the membrane adheres strongly to the cytoskeleton. In animal cells, movement of membrane into an endocytic vesicle or lamellipodium requires movement of bilayer away from membrane–cytoskeleton bonds. Thus, much of the load of forming a vesicle or a lamellipodium should be the energy of membrane–cytoskeleton adhesion.

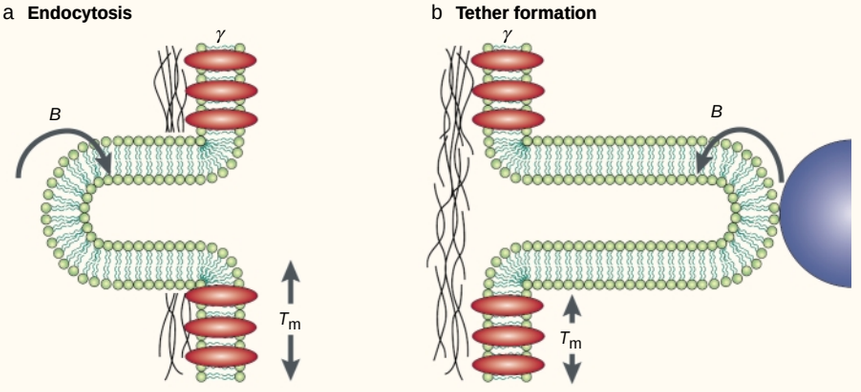

Figure 1 | **The energetics of endocytic vesicle formation are similar to those of tether formation.** This diagram illustrates that the factors that contribute to the load of forming a vesicle are the same as those that contribute to forming a tether. The major factor is the membrane–cytoskeleton adhesion energy, γ, which must be overcome to separate membrane from cytoskeleton. A secondary term is the tension in the bilayer plane, T_m, which resists deformation of the membrane. Because the curvature of vesicles and tethers are similar, the force needed to bend the membrane should be similar (membrane bending stiffness, B, defines the force for a given radius of curvature[39]). See Box 1 for details.