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HOW TWO-FOOT MOLECULAR MOTORS MAY WALK

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1. INTRODUCTION

Myosins and kinesins each constitute a large family of linear molecular motors that track along a filamentous rail, myosins along an actin filament and kinesins along a microtubule. These motors are powered by free energy derived from ATP hydrolysis, and the mechanisms of chemo-mechanical conversion in these motors have been under intensive study (Kinosita et al., 1998; Vale and Milligan, 2000; Mehta, 2001; Vale, 2003; Schliwa and Woehlke, 2003; Endow and Barker, 2003). Most of myosins and kinesins have two globular domains that bind to the filamentous rail and that hydrolyze ATP in a rail-dependent manner. The two domains are usually called "heads" or "motor domains" and are connected via a neck-like structure to a common stalk. Some of the two-headed motors are processive, in that a single molecule moves along a rail for many ATPase cycles without detaching from the rail. These processive motors appear to "walk," using the two heads alternately in a hand-over-hand fashion, as has recently been demonstrated for myosin V (Yildiz et al., 2003) and conventional kinesin (Asbury et al., 2003; Yildiz et al., 2004). Unlike walking of a human being, molecular motors cannot rely on inertia, which is negligible for biological molecules that work in water or membranes. A human has a right and a left foot, but the two heads of a molecular motor are identical (Fig. 1*a*). How, then, do they walk? In this article, we focus on the mechanism(s) that warrant forward, not backward, stepping. Because we discuss walking mechanisms in this article, we call the heads "feet" and necks "legs."

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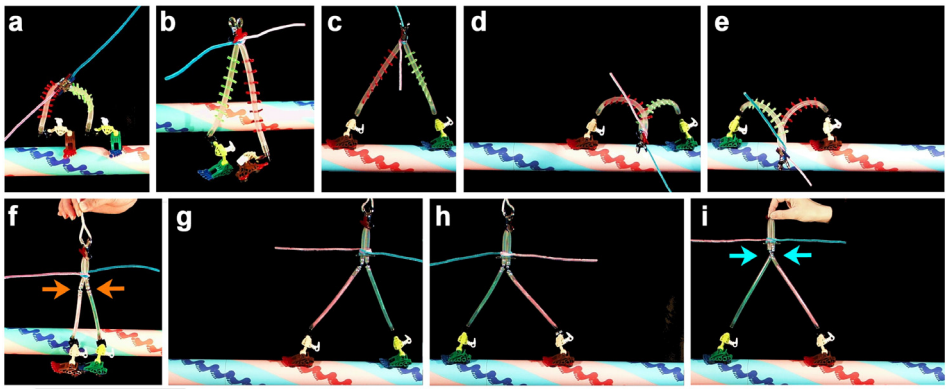

Figure 1. Walking with two identical feet. The legs are made of elastic vinyl pipes, which can accommodate both torsional and flexural strains. (**a**) Two feet of a molecular motor are identical, because they are coded by the same gene. (**b**) The two legs are joined in twofold symmetry. Thus the two feet would be oriented in opposite directions, one toe facing east if the other faces west, unless the legs are flexible. (**c**) When two feet land simultaneously during walking, both toes must be oriented forward, because the landing sites on the rail are all identical. Elastic legs would thus be severely distorted. In the configuration shown in this image (*b*), most of the strain is in torsion of the legs (note the directions of the small bars on the legs) whereas bending is relatively small. (**d**) By turning one of the feet by 360°, much of the torsional strain is relieved, resulting in severe bending. The total strain is the same as in *c*. (**e**) When the motor moves forward by one step and attains the same posture as in *d*, a cargo (pink and blue long bars) attached to the body rotates by 180°. A 180° rotation also accompanies every step if the motor steps with the posture in *c*. (**f**) Flexible joints in the legs (arrows) allow the two feet to adopt the same orientation. (**g**) With flexible joints, landing with two feet does not introduce strain. (**h**) But, because of the basic twofold symmetry, the body still tends to rotate 180° every time the motor steps. (**i**) With flexible joints, though, the 180° rotations can be prohibited by an external force without introducing too much strain in the legs. Note that the two legs are now crossed near the junction (arrows); a small torque suffices to bring about this configuration.

2. HINTS ON WALKING MECHANISMS

2.1. Walking with Two Identical Feet

The two legs of a myosin or kinesin molecule emerge from a coiled coil of α-helices (the stalk, or "body"), each helix extending into a leg (see Figs. 2*c* and *d*). Because the coiled coil is twofold symmetric, as confirmed for kinesin (Kozielski et al., 1997) and myosin (Li et al., 2003), the two identical legs are also arranged, basically, in the same twofold symmetry: if there were no flexibility, the two feet would be oriented in opposite directions, making it extremely difficult to walk on landing spots that are unidirectionally oriented on a rail (Figs. 1*b*-*e*). In fact, each leg of kinesin is a single polypeptide chain and is considered flexible over the entire length, unless it is "docked" onto the foot (Fig. 2*d*); in a crystal structure of kinesin dimer where both legs were docked (Kozielski et al., 1997), the two feet were not arranged in twofold symmetry, indicating that the short undocked portion is already flexible. Myosin's legs are reinforced with light chains (Figs. 2*a* and *b*), and thus are probably semi-rigid. Flexibility likely resides at the leg-body junction. Indeed, analyses of rotational Brownian motion of myosin II (conventional myosin) indicated the presence of a flexible joint near the leg-body junction (Kinosita et al., 1984; Ishiwata et al., 1987), and electron micrographs of myosin

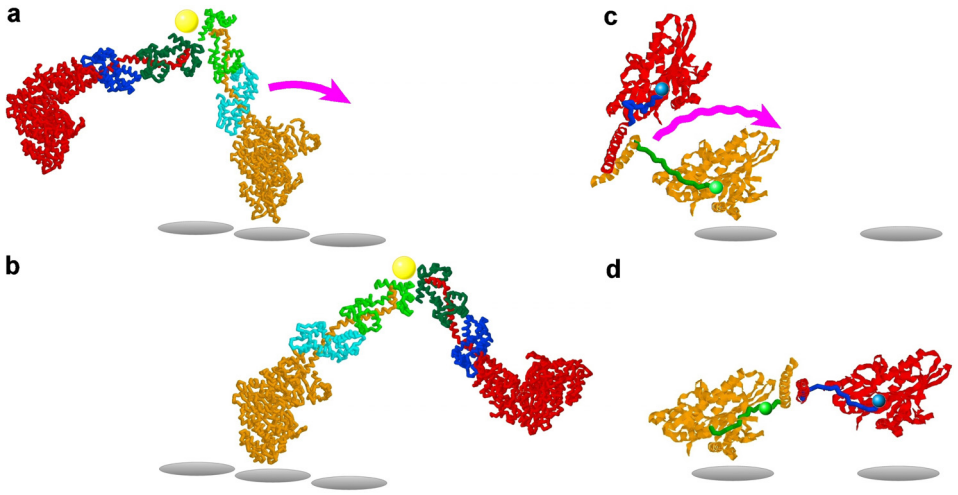

Figure 2. Postulated lever action in two-foot motors. The motors in this figure move toward right. (**a, b**) Scallop myosin. This myosin is non-processive and thus does not "walk." Nevertheless, we show possible walking postures in order to indicate lever action (arrow) in myosin: the orange leg (the long α-helix) rotates between *a* and *b*. Flexible joints likely exist around the location indicated by the yellow ball, beyond which the two α-helices form a coiled coil (the "stalk"; not shown in the figure). Thus, the red leg presumably undergoes rotational diffusion around the yellow ball. The lever action of the orange leg biases the Brownian motion of the red foot forward. Each figure is composed of two structures of scallop myosin subfragment 1 (S1), arranged arbitrarily. The orange foot/leg in *a* represents the structure of S1 binding MgADP·VO_4, and that in *b* is S1 without a nucleotide (Houdusse et al., 2000). The red foot/leg is S1 binding MgADP, which mimics a structure after detachment from actin (Houdusse et al., 1999). Cyan/blue, essential light chains; green/dark green, regulatory light chains. The gray disks with a diameter of 5.5 nm represent binding sites on an actin filament. (**c, d**) Conventional kinesin. The blue and green legs ("neck linkers") between the balls are presumably flexible, and thus the red foot undergoes Brownian motion. The flexible green leg in *c* "docks" onto the orange foot, in an ATP-dependent process, through Brownian motion (wavy arrow). After docking (*d*), the Brownian motion of the red foot occurs around the green ball, and thus is biased toward the forward binding site. The docking is equivalent with the lever action in myosin. The gray disks with a diameter of 4 nm represent binding sites on a microtubule. The figures are constructed from a structure of a dimeric kinesin (Kozielski et al., 1997) by assigning arbitrary structures to the neck linkers (except the green one in *d*) and orienting the central coiled coil (stalk) arbitrarily.

V revealed the angle between the two legs to be quite variable (Walker et al., 2000). In a crystal of the coiled coil portion of myosin II, the end next to the legs was disordered (Li et al., 2003).

The flexibility in the undocked legs of kinesin and that at the body-leg junction of myosin allows the two feet to adopt the same orientation, which is a required posture when the two feet simultaneously land during walking. Because the two feet and legs are identical, the landed posture with least strain must be the same whether one or the other foot leads, although the flexibility may allow many other postures without imposing much strain. If the motor reaches the unique, most stable posture after every step, then symmetry dictates that the body must turn around its axis by 180° as the leading and trailing feet swap (Howard, 1996; also see Figs. 1*g* and *h*). Hua et al. (2002) sought for, but did not observe, the 180° reorientations that would accompany every step in kinesin. They thus suggested inchworm walking in which one foot always

leads. We also failed to observe the 180° reorientations in myosin V (Ali et al., 2002) and myosin VI (Ali et al., 2004). Recent studies, however, have established that myosin V and kinesin walks in a hand-over-hand fashion in which the two feet alternate in the lead. A single fluorophore attached to one of the two feet of myosin V (Yildiz et al., 2003) or kinesin (Yildiz et al., 2004) advanced, for every two ATP hydrolysis cycles, over a distance twice the motor's step size which is the distance traveled by the body of the motor in one ATP cycle; inchworm walking would advance the fluorophore by a distance equaling the step size. Also, in some mutant kinesins, dwells between steps were alternately long and short, indicating an asymmetric hand-over-hand walking with two different conformations for two-foot landing (Asbury et al., 2003). Why, then, have the 180° reorientations not been detected so far? Previous observations were made by attaching a large probe, a microtubule or micron-sized bead, to the motor. The attached probe may well have broken the symmetry of the motor, as in the mutations, and impeded the reorientation. With the assumed flexibility, the torque needed to break the symmetry is small (Fig. 1*i*). Genuine motion of these motors, without a cargo, might still involve 180° reorientations, although the flexible joints likely obscure the rotation.

2.2. Lever Action and Biased Diffusion

Until recently, researchers working on myosin and kinesin had somewhat different views. Because myosin's legs are likely stiff, prevailing theory states that a landed leg acts as a lever (Huxley, 1969; Holmes and Geeves, 2000): when a landed ankle is bent forward, the leg leans forward, carrying the body forward (Figs. 2*a* and *b*). The tilting is best evidenced for myosin V (Walker et al., 2000; Moore et al., 2001; Veigel et al., 2001; Burgess et al., 2002; Forkey et al., 2003). The lifted leg thus easily finds a forward landing site. Kinesin's legs, in contrast, are flexible and unlikely to serve as a stiff lever. Instead, a lower part of a landed leg docks onto the landed foot such that the upper leg emerges from a forward part of the foot (Figs. 2*c* and *d*). This biases the Brownian motion of the lifted foot forward, and the foot lands on a forward site. The relatively small bias could be efficient, because kinesin's legs are short and must be fully extended to reach a forward or backward site: of the two sites that are available, only the forward site can be reached after the docking of the landed foot. The docking in kinesin may be regarded as the equivalent of the lever action in myosin, and then the two schemes are apparently quite similar. Myosin researchers, however, tend to stress the lever action whereas kinesin researchers diffusional search. The distinction seems to be coupled to the question of which process produces force: lever action itself in myosin, and landing of the diffusing foot in kinesin. Is this really so?

3. SPIRAL MOTION OF MYOSIN AROUND AN ACTIN FILAMENT

3.1. Observation of Spiral Motion

Myosin V is now one of the best characterized molecular motors. It moves toward the barbed, or plus (fast-growing) end of an actin filament using two long (~23 nm) legs each reinforced with six light chains (Cheney et al., 1993; see Fig. 3). The movement is processive (Mehta et al., 1999; Rief et al., 2000; Sakamoto et al., 2000). The step size

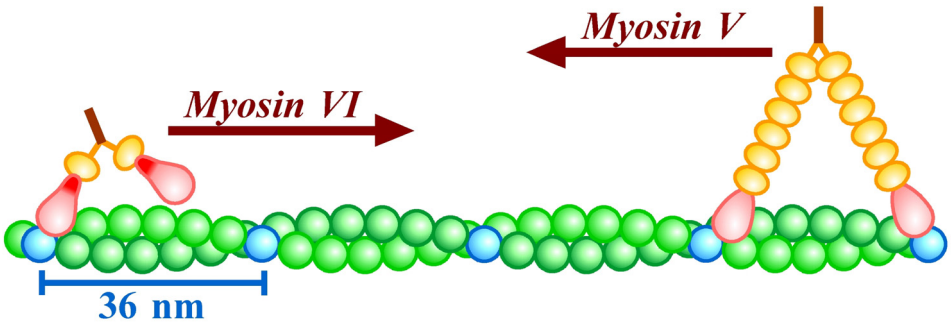

Figure 3. Myosin V and myosin VI. Myosin V has two legs each reinforced with six light chains (orange) and walks on an actin filament (green) toward the barbed, or plus, end (left in the figure). Myosin VI, on the other hand, moves toward the pointed, or minus, end. Previously, myosin VI was thought to bind one light chain per leg, and thus myosin VI was considered short-legged as shown in this figure. The red portion in the feet of myosin VI indicates an extra insert not seen in other myosins. Every 13th monomer of the actin filament is shown in blue. Walking along one side of an actin filament implies an average step size of ~36 nm. Adapted from Ali et al. (2004).

of myosin V has been estimated to be about 36 nm (Mehta et al., 1999; Rief et al., 2000; Rock et al., 2001; Tanaka et al., 2002), which coincides with the helical repeat of an actin filament of 36 nm (Holmes et al., 1990; see Fig. 3). Electron micrographs of myosin V (Walker et al., 2000) have shown that bound feet are separated by ~36 nm on an actin filament, corroborating the step size measurements. In most of these previous studies, however, myosin V could approach an actin filament from only one side, and thus myosin V may have been forced to step on the actin sites that are 36-nm apart (Fig. 3).

To address this possibility, we designed an experiment in Fig. 4*a*, where myosin V could freely rotate around an actin filament that was suspended between two large (4.5 μm) beads. We attached a duplex of 1-μm beads to myosin V to facilitate visualization of rotation. The microscope focus was set such that the bead closer to the observer appeared white and the bead farther from the observer black. As seen in Fig. 4*b*, the bead duplex moved in a left-handed spiral around the right-handed double helix of actin. The rotational pitch was 2.2±0.3 μm per turn, compared to the pitch of the actin double helix of 72 nm. The slight left-handed spiral can be explained if the step size of myosin V is slightly less than the actin helical repeat (half pitch) of 36 nm: in Fig. 4*a*, stepping on blue subunits that are 36-nm apart produces straight motion without spiraling, whereas slightly smaller strides, e.g. on the purple subunits, would lead to a left-handed spiral.

The average step size of myosin V is calculated from the spiral pitch in the following way. If the step size is $(36 - x)$ nm, then myosin V will rotate toward left by $180° \times (x/36)$ per step. To accumulate one turn, myosin V has to make $360° / [180° \times (x/36)] = (72/x)$ steps, or to proceed over a distance of $(72/x) \times (36 - x)$ nm. This latter distance is equal to the spiral pitch of 2200 nm. Thus, $(72/x) \times (36 - x) = 2200$. Solving for x yields $x = 1.1$ nm, and thus the step size is 34.9 nm. (This calculation is slightly different from the one in Ali et al. (2002), but the answer is essentially the same.) With this average step size, the predominant landing site from the position in Fig. 4*a* is on the blue subunit, or the 13th subunit counting both strands. Occasionally myosin V lands on the 11th subunit (purple), and less frequently on the 15th or 9th.

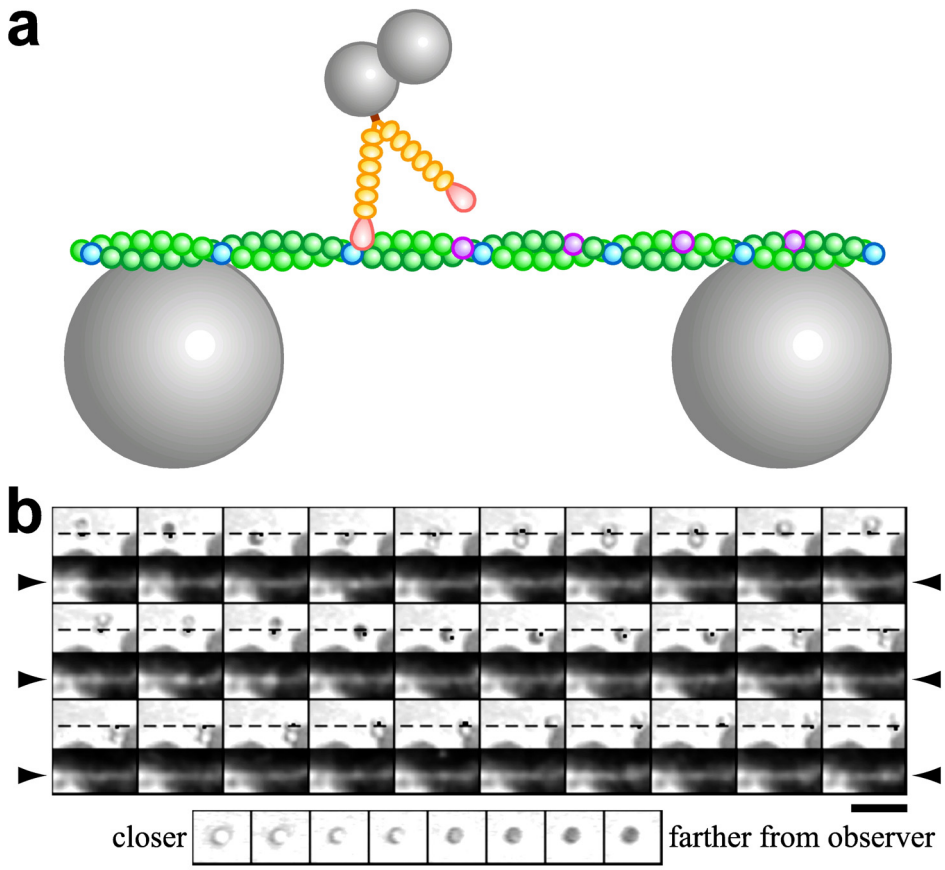

Figure 4. Spiral motion of myosin V around an actin filament. (**a**) Experimental setup. An actin filament, fluorescently labeled, was suspended between beads of diameter 4.5 μm placed on a glass surface. To myosin V, a duplex of 1-μm beads was attached. (**b**) Sequential images, at 1-s intervals, of spiral motion of myosin V at 400 μM ATP. Upper frames in a row show transmitted images of the bead duplex, where a bead closer to the observer appears white and a bead farther away black (see control images at bottom). Lower frames show fluorescence images of the actin filament (between arrow heads). The dashed line in the upper frames show the position of the actin filament deduced from the lower frames. The dot in the upper frames indicates the center of the bead which we judged to be attached to actin. Bar, 5 μm. Adapted from Ali et al. (2002).

The step size we obtained under no load (viscous load is negligibly small for the slow translational motion) is not so different from the previous estimates. Previous steps of ~36 nm were not imposed by experimental geometry. Myosin V naturally walks almost straight, on one side of an actin filament. The implication is that the lever action in myosin V occurs, on the average, almost precisely forward, or in a plane including the actin filament, to let the lifted foot aim from above, not obliquely from side, at the landing sites on the filament. The slight tendency to spiral to the left is explained if forward bias by the lever action is not sufficient to comfortably place the lifted foot on the 13th subunit. Or, the ankles of myosin V may have an intrinsic tendency to bend slightly to the left. When many molecules of myosin II carry an actin filament forward,

the filament rotates as a right-handed screw (Nishizaka et al., 1993) with a pitch of ~1 μm (Sase et al., 1997). Although myosin II is not processive and thus does not walk, the right-handed spiral can be explained if myosin II "kicks" actin by bending a landed ankle slightly obliquely to the right. The structure of the foot (motor domain) of myosin V is similar to that of myosin II (Coureux et al., 2003; Holmes et al., 2003), but a small difference in the direction of ankle action is not inconceivable.

3.2. Myosin VI May Move Like Kinesin

Myosin VI moves, unlike most other myosins, toward the pointed, or minus, end of an actin filament (Wells et al., 1999; Homma et al., 2001). It is a processive motor (Rock et al., 2001; Nishikawa et al., 2002), and thus is considered to "walk" using its two feet. Myosin VI has only one typical light-chain binding site per leg (Wells et al., 1999) and was initially considered short-legged (Fig. 3). Nevertheless, its step size was long, ~30 nm (Rock et al., 2001) or ~36 nm (Nishikawa et al., 2002), apparently incompatible with the expected physical size of the legs. Again, these measurements were made in a configuration that allowed myosin VI to approach from one side of an actin filament, raising the possibility that this constraint somehow forced the short-legged myosin VI to stride on the 36-nm spaced landing sites on one side of the filament. We thought that, if we allowed myosin VI to rotate freely around an actin filament, it would spiral more extensively toward left than myosin V. It might even spiral to the right with a pitch of 72 nm, if its natural step size is smaller than 18 nm such that the motor tracks along one strand of the actin double helix. So we tested this idea, again using the actin-bridge assay (Fig. 4*a*).

A result is shown in Fig. 5*a*, where the bead duplex spiraled as a right-handed screw with a pitch of ~1.4 μm. Many more right-handed spirals were observed, the pitch averaging 2.4 μm. The shallow right-handed spiral indicates an average step size slightly greater than 36 nm (e.g., walking on purple subunits in Fig. 5*b*); the average step size calculated as above is 37.1 nm. There were, however, many more bead duplexes (>80%) that failed to show clear rotation (>0.5 revolution) during a processive run of a few μm. If these represent straight motion, the step size would be 36 nm (on blue actin subunits in Fig. 5*b*). Or, these may also be a part of long-pitch right-handed spiral. In any event, the average step size of myosin VI somewhat exceeds 36 nm, being the longest among known molecular motors. Other long steppers include myosin V, and myosin XI with the average step size of 35 nm (Tominaga et al., 2003).

The long strides of myosin VI are obviously incompatible with walking with short legs. In an electron micrograph (Nishikawa et al., 2002), legs of myosin VI were somehow extended, possibly by unzipping of the stalk coiled coil. Another possibility that the insert unique to myosin VI (red part in Fig. 3) may adopt an extended conformation (Rock et al., 2001) has been denied by a recent study (Bahloul et al., 2004) which shows that the insert is actually a second calmodulin (light chain) binding domain. More recent work (B. R. Rami and J. A. Spudich, Stanford University, and H. L. Sweeney and C. Franzini-Armstrong, Pennsylvania University, personal communication) shows that the coiled coil is indeed unzipped over a considerable length and the unzipped portion is flexible (Fig. 5*b*). The long and flexible legs can account for the unconstrained step size of 36-37 nm. Thus, myosin VI seems to walk almost like kinesin, relying on biased diffusion. A lower part of the legs of myosin VI is

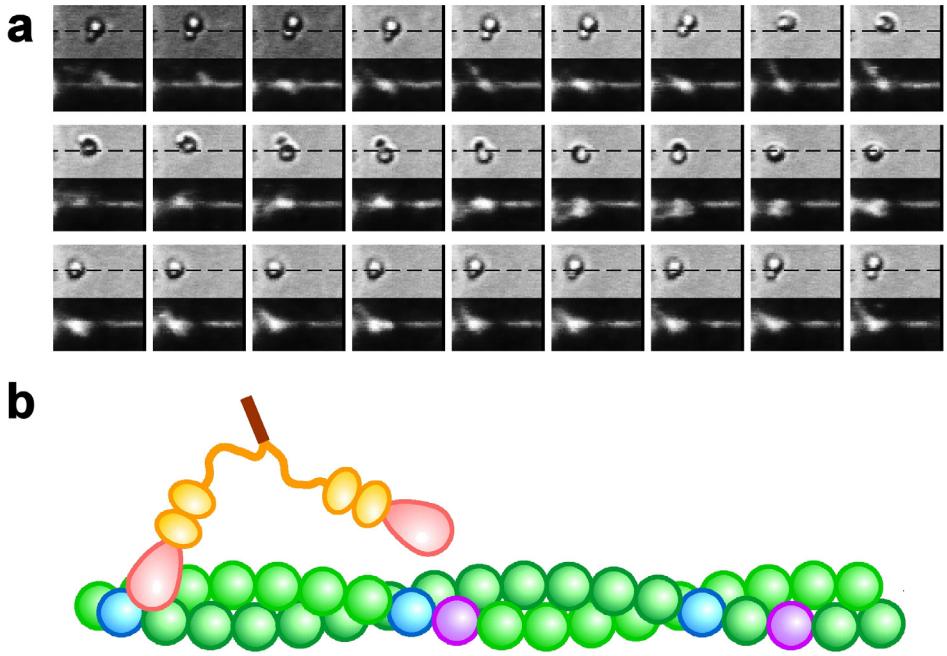

Figure 5. Spiral motion of myosin VI around an actin filament. (**a**) Sequential images, at 0.4-s intervals, of spiral motion of myosin VI at 400 μM ATP. See Fig. 4 for details. This bead duplex (1 and 0.45-μm) carried a short actin filament, which also rotated as seen in the fluorescence images in the lower frames. Of the bead duplexes examined, only ~15% showed right-handed spiral, as seen in these images. Others moved straight without spiraling. (**b**) A recent study (Bahloul et al., 2004) shows that the extra insert in the leg sequence (red part in Fig. 3) actually binds a calmodulin light chain. Also, the coiled coil beyond the light-chain binding region was found to be unzipped. Myosin VI can thus span >36 nm, as required by the right-handed spiraling. Adapted from Ali et al. (2004).

presumably semi-rigid, and its lever action would serve the purpose of biasing the diffusion of a lifted foot forward.

4. TOE UP-DOWN MECHANISM

4.1. Moving Forward against a Backward Force

Myosin and kinesin both appear to walk forward by biasing the diffusion of the lifted foot by an action of the landed ankle, either lever action or docking. Does this mechanism really warrant that the lifted foot lands on a forward, not backward, site? Such a mechanism, alone, would not work properly when the body is pulled backward, particularly when the legs are flexible as in kinesin and myosin VI. If the legs are flexible over a large part, diffusion will even be biased backward (Fig. 6*a*). In kinesin, the free energy difference between the docked and undocked states is small (Rice et al., 2003), implying that docking would fail when the body is pulled back by a load. Then, there will be little bias for diffusion, or backward diffusion may be preferred (Fig. 2*c*).

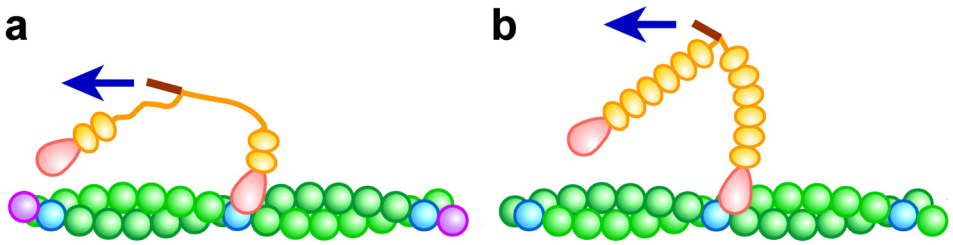

Figure 6. How to move forward against a backward force (arrows). (**a**) If legs are flexible over a considerable length, diffusion would move the lifted foot toward a backward site. (**b**) Under a high backward force, the presumably semi-rigid leg of myosin V may also be bent backward, such that the diffusion is biased backward.

Myosin V might appear to be free from these problems, but its legs cannot be perfectly rigid. Veigel et al. (2002) have estimated its leg stiffness to be 0.2 pN/nm, implying that the body would be pulled back by 10 nm under 2 pN of backward force (Fig. 6*b*). Also, the ankle of the landed foot may yield under the external force, because sustaining 2 pN at the end of the 23-nm leg requires a torque of 46 pN·nm, comparable to the torque of the powerful rotary motor F_1-ATPase (Yasuda et al., 1998; Kinosita et al., 2004). In spite of these problems, all these motors are known to move forward under a few pN of backward load. An additional mechanism, other than biasing the translational diffusion of the lifted foot by moving the effective pivot forward, must be involved.

4.2. Toe Up-Down Mechanism

The mechanism we propose (Ali et al., 2004) is the ankle action in the lifted foot: proper up-down motion of the lifted toe will orient the sole correctly such that landing on a forward site is favored over a backward site even if the body is pulled backward. The toe up-down mechanism warrants forward motion even if legs are completely flexible.

To illustrate the mechanism, let us again use the toy model introduced in Fig. 1. First we deal with the case of semi-rigid legs as in myosin V (Fig. 7). We assume the presence of a flexible joint near the leg-body junction (Fig. 1*f*), and thus there will be little torsional strain in the two legs. Bending strain, however, cannot be relieved by the flexible joint. In Fig. 7, a posture with a bent leg is attained only at the expense of a high energetic cost, or is rarely attained (unless coupled to a reaction accompanying a large drop in free energy).

Figure 7*a*1 shows a posture of the motor after the trailing foot (green) is lifted from the rail. The ankle of the leading foot (red) is already bent forward (lever action), such that the pivot for rotary diffusion of the lifted leg is forward of the landed foot (red) and is ready to bring the green foot forward. However, if the lifted toe is up, as in Fig. 7*a*1, the sole will be misoriented after a forward swing (Fig. 7*a*2), making forward landing difficult. Forced landing would bend the leg severely (Fig. 7*a*3). Natural landing sites for the toe-up foot will be next to the landed foot (Fig. 7*a*4), front or back, and thus the motor will hardly walk forward.

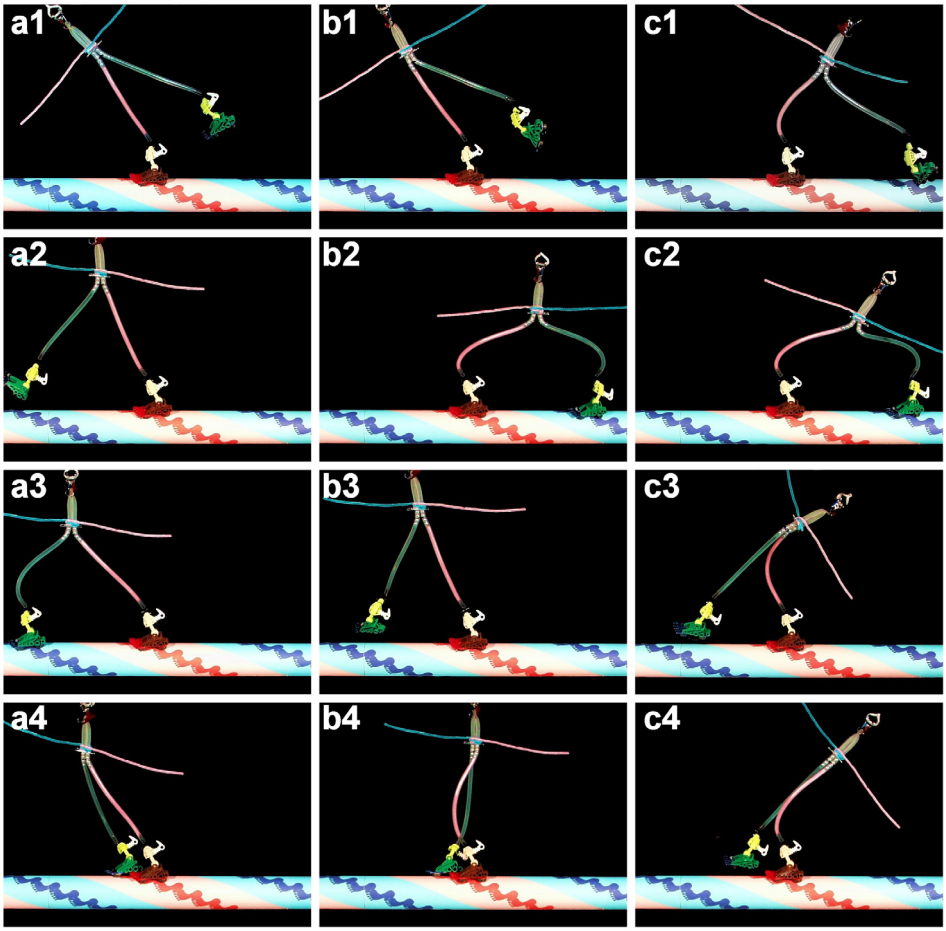

Figure 7. Toe up-down mechanism for the case of semi-rigid legs. (**a**) If the lifted toe is up, landing on a forward site is difficult, and natural landing would be on a nearby site (*a*4), if one is available. (**b**) If the toe goes down upon lifting of the foot, landing with the least strain will be on a distant forward site. (**c**) A backward force will pull the pivot backward (*c*1), but, as long as the lifted toe is down, landing on a distant forward site is favored. Under a high force, landing on a nearby site is equally probable (*c*4). Note that, in *c*4, bending of the red leg is caused by the external force. Adapted from Ali et al. (2004).

If, on the other hand, the lifted toe goes down, as in Fig. 7*b*1, re-landing on the backward site will be prohibited (Fig. 7*b*2), whereas landing on a distant forward site will be smooth and natural (Fig. 7*b*3). Landing on a nearby site would result in a strain (Fig. 7*b*4) and thus is much less likely.

A nice feature of the toe up-down mechanism is that it operates properly even in the presence of a backward force that tends to pull back the pivot beyond the position of the landed foot (Fig. 7*c*1). Thanks to the sole orientation, backward landing is still prohibited (Fig. 7*c*2), whereas a small thermal agitation will allow landing on a distant forward site (Fig. 7*c*3). Landing on a nearby site is also allowed under a high backward force (Fig. 7*c*4), but this is the condition where the motor begins to stall.

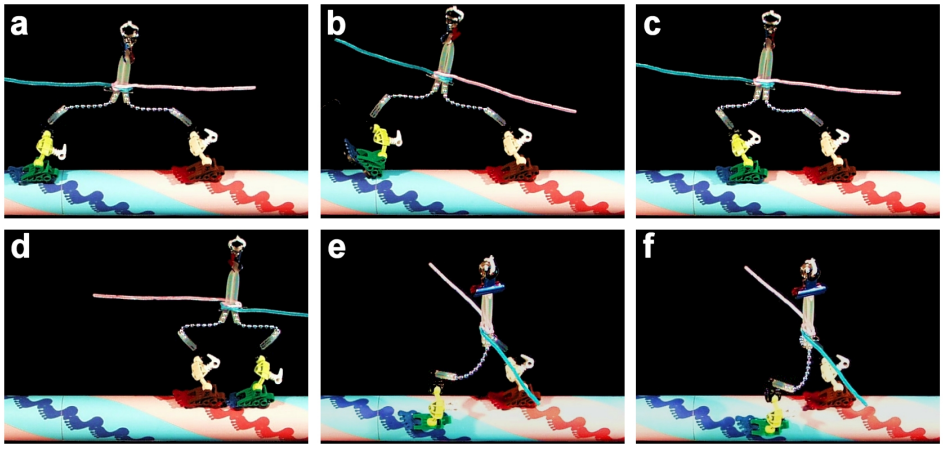

Figure 8. Toe up-down mechanism for the case of completely flexible legs. The legs in the model are made of metal chains. (**a**) The size of the legs are such that landing on a distant forward site is allowed only when the legs are fully extended and the leading toe is down and trailing toe up. (**b, c**) With the green toe up, forward landing is impossible. (**d**) As long as the green toe is down, there is no hope in backward landing. (**e, f**) If a lower part of legs is stiff, oblique landing on a side of the cylindrical rail is also prohibited. The postures here represent the lowest possible position for the green foot, which fails to reach a blue foot mark below.

The toe up-down mechanism also works with flexible legs, as long as landing sites are widely separated such that landing requires almost full extension of the legs. This is because, on a fully extended leg, the orientation of the sole is restricted, in the same way as in a rigid leg. In Fig. 8, the legs of the toy model are made of flexible chains. Suppose, for the moment, that only those landing sites (foot marks) that are at the top of the rail cylinder are available. This situation applies to kinesin on a microtubule. The green foot can land on the forward site if its toe is down (Fig. 8*a*), but it cannot land if the toe is up (Figs. 8*b* and *c*). When the green toe is down, forward movement is warranted because landing on the backward site is impossible (Fig. 8*d*). The preferential forward landing also operates in the presence of backward force. In Fig. 8*d*, even if the red ankle yields to a backward force and bends backward (kinesin "undocks" by backward pull), the green foot cannot reach the backward site as long as its toe remains down. Forward landing (Fig. 8*a*) would still be possible, when the body happens to move forward by Brownian motion (a rare event when the backward force is high).

On an actin filament along which myosin VI moves, all foot marks in Fig. 8 are basically available for landing. Unless the legs are too long, however, landing on a distant backward site is prohibited when the green toe is down (Fig. 8*d*). In addition, the presumably stiff lower portion of the legs of myosin VI opposes oblique landing on a side of the filament (Figs. 8*e* and *f*). Thus, toe-down landing will be made primarily on a distant forward site (Fig. 8*a*), and occasionally on sites next to the landed foot. The frequency of landing on a nearby site depends critically on the make of the legs and ankles (less easy if shanks are long and ankles are bent acutely), and also on the backward load. The large variation in the size of individual steps of myosin VI (Rock

et al., 2001; Nishikawa et al., 2002) may result from mixing of ~36-nm strides with short ones (±5.5 nm for the adjacent landing sites).

4.3. How to Walk Forward

In our view, at least four mechanisms are needed to let a two-foot motor walk forward: (1) the lever action or docking in the landed foot, (2) diffusion of the lifted foot, (3) toe up-down in the lifted foot, and (4) preferential detachment of the rear foot. (1) The lever action has been considered, by many myosin researchers, to play the central role in throwing the lifted leg forward. Its true significance, however, might be in preventing the pivot from pulled back too much by a backward force (Fig. 6). (2) Diffusion around the pivot is the only way by which the lifted foot move, whether forward or backward. It is quite unlikely that ATP-dependent conformational changes alone, in the landed and/or lifted feet, carry the lifted foot all the way onto a landing site. Passive diffusion must be involved, which assists forward and backward movements equally. (3) Toe up-down in the lifted foot, we propose, plays the major role in selecting a forward landing site over backward ones. (4) When both feet land, it must be the rear foot that is lifted first. This is accomplished by a strain dependence of the nucleotide kinetics in the feet. For kinesin, for example, pulling a landed foot forward has been shown to increase the affinity of that foot for ADP (Uemura and Ishiwata, 2003). This would take place in the rear foot, and the affinity for a microtubule binding site is weakened when kinesin's foot binds ADP.

In somewhat different contexts, a human being also uses the four mechanisms above when he/she walks. The major differences, apart from the distinction between the right and left feet, are that a human relies on inertia, particularly in (2) and (4), and that gravity helps in (2) and (3).

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DISCUSSION

Pollack: Could you comment on how single-headed kinesins could advance along a microtubule?

Kinosita: Some part of kinesin would stick non-specifically to the microtubule, as has been shown for KIF1A, and another part would pull the molecule forward, by a lever action or by extending itself forward by diffusion followed by binding to a microtubule site.

Gonzalez: Without strain, on which direction would myosin V move?

Kinosita: If there were no strain-dependent regulation of nucleotide kinetics, either foot, front or rear, could be lifted from actin. The motor would still move forward, but the efficiency would be low, because lifting the front foot would not contribute to forward motion while ATP is expended.

Pollack: (comment) With single filaments sliding past one another, you do see backward steps of $n \times 2.7$ nm.

Sugi: One single question is the biased diffusion you mentioned in your talk is the same as so-called Brownian ratchet mechanism?

Kinosita: The Brownian ratchet, in my opinion, is a broad term that applies to almost

any mechanisms of molecular machines. Moving the pivot forward, by a lever action, to help forward landing is a kind of ratchet. Assuring forward landing by bringing the toe up, without necessarily the pivot motion, is another way of realizing a ratchet.

Kushmerick: You stated the ATP synthesis by F_1-ATPase may be nearly 100% efficient. What is the efficiency of the H^+ proton-driven component of F_oF_1 ATP synthase? Is the overall F_oF_1 rotary motor reversible?

Kinosita: For ATP synthesis, I expect the number efficiency (the number of ATP molecules synthesized per 120-degree rotation) to be close to one, under favorable nucleotide concentrations and at low rotary speeds. The energetic efficiency (free energy for ATP synthesis divided by mechanical work done on the gamma subunit) is critically dependent on the nucleotide concentrations and applied torque, and would be high only near reversal (low torque). Peter Gräber's group, among others, has shown that F_oF_1 ATP synthase is reversible, and that synthesis/hydrolysis in F_1 is balanced by the proton flow through F_o. When the proton motive force across F_o is greater than the free energy needed for ATP synthesis, ATP is synthesized. ATP is hydrolyzed when the proton motive force is lower. They have shown that synthesis of one ATP molecule requires translocation of about four protons, but, in addition, some protons may leak through the membrane or the F_o motor. Thus, experimental determination of the efficiency of synthesis is very difficult.