lineage. These obvious cases of cross-over mediated recombinational exchange are a hallmark of meiotic sex. Indeed, a number of different meiotic recombination events can be readily ascertained by direct inspection of the SNP variation. So not only are *Giardia* apparently experiencing meiotic recombination, but when they are assessed in the right conditions, they may be doing it frequently enough to detect multiple, separate events of genetic exchange.

The presence of meiotic genes [9] and the new evidence of recombination from population genetics [12] together hold the first keys to unlocking *Giardia*'s sexual secrets. How often and under what conditions does *Giardia* have sex? Such questions are important beyond academic interest, because the answers also have strong implications for disease epidemiology and treatment [15]. To finally unlock sex in *Giardia*, we need to catch them in the act. Investigations of genetic exchange in *Giardia* at the cellular level need to be done [16] — experiments that would have been considered pointless under the previous assumption of asexuality. With a completed genome in hand in which multiple meiotic genes are already identified, and molecular cell biology methods being developed and applied to *Giardia* [17,18], we might not have to wait long until details of its sex life are exposed for all to see.

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Molecular Motors: A Surprising Twist in Myosin VI Translocation

A recent study has revealed an unexpected change in conformation of the myosin VI converter domain, essential for twisting the lever arm through a ~180° rotation to achieve a large step along actin.

James A. Spudich

A eukaryotic cell has nearly 100 distinct molecular motors moving along cytoskeletal tracks, providing dynamic organization of cellular components. While myosins overall are the best understood family of molecular motors, myosin VI has represented the biggest challenge to conventional views of how this family of motors works. In a seminal paper in *Cell* [1], Anne Houdusse and her colleagues provide the answer to a key mystery of this provocative motor.

The conventional view of how myosins function [2] is illustrated by myosin II, the motor used for muscle contraction and cytokinesis in nonmuscle cells. The head of myosin II comprises the motor domain [3] and can be subdivided into a globular amino-terminal catalytic region, ending with a converter, followed by an α helix composed of two IQ motifs [4], each of which binds a calmodulin-like light chain (Figure 1). According to the swinging lever arm model, myosins exist in a prestroke state when ADP and Pi occupy the active site. Upon binding to actin, conformational changes near the active site associated with Pi release cause the converter to move as a rigid body toward the plus end of the actin filament. The converter rectifies and amplifies the structural changes near the active site. The converter movement is further amplified by a swinging of the light-chain-binding region (the 'lever arm') through ~60–70°, providing a stroke of ~10 nm (Figure 1A).

Myosin V is a dimeric, processive plus-end-directed motor, involved in transport of a wide variety of cargos. Each head domain has a long light-chain-binding region with six IQ motifs, primarily binding calmodulin (Figure 1B). These longer arms allow for this myosin to take 36 nm steps [5] with a lever arm swing through an angle similar to myosin II.

Myosin VI exists as both a monomer and a dimer, its oligomerization state being regulated *in vivo* [6,7]. Processive as a dimer, it has a wide variety of cellular roles, including vesicular transport and a dual translocation and anchoring role in the function of sensory hair cells [8]. Myosin VI has a single calmodulin-binding IQ motif. It is most distinct from myosin II and myosin V by the presence of a unique

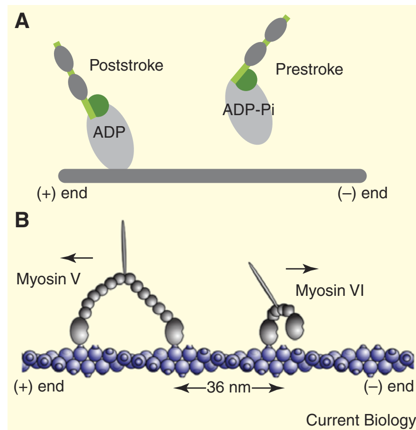

Figure 1. Movement by the myosin family of molecular motors involves transitions between prestroke and poststroke states.

(A) Schematic of the myosin II head, in its prestroke and poststroke states. The prestroke state (right) is bound to ADP–Pi and has weak affinity for actin. Upon binding to actin, the converter (green) and associated lever arm swing toward the actin plus end, associated with Pi release and strong binding to actin. (B) Myosin V and myosin VI are processive motors that move in opposite directions along actin.

insert of ~40 residues [9] between the catalytic region and IQ motif that binds a second calmodulin molecule (Figure 2). Myosin VI translocates along actin toward the minus end (Figure 1) [10].

Myosin VI has seriously challenged the lever arm hypothesis [11,12] since it takes large 36 nm steps in the absence of a long lever arm [13–15]. Single-molecule analyses reveal stroke sizes of monomeric constructs of up to ~20 nm [16–18]. How the calmodulin-bound regions could serve as a lever arm to amplify such a large stroke has been difficult to imagine. Since the structure of the converter in the myosin VI poststroke state [9] is the same as that in all other myosins in all states, the dogma has been that the converter always rotates as a rigid body. Modeling a lever arm swing for myosin VI with a conventional converter movement [9] fails to give a stroke size anywhere near that measured. Thus, a less conventional model was favored by Menetrey *et al.* [9], and Yanagida and colleagues have suggested a radical departure from the lever arm hypothesis, proposing that the myosin head moves along an actin filament one actin monomer at a time by biased Brownian motion [11,12].

The power of *in vitro* motility assays and single-molecule analyses to provide detailed structural information about functionally important transitions became most apparent in two studies using monomeric constructs of myosin VI terminated at different positions after the converter [18,19]. Importantly, the converter itself was shown to move toward the plus end of the actin filament, as for myosin II and myosin V. The carboxy-terminal ends of the calmodulin-binding regions, however, were shown to move toward the minus end. When step sizes, velocities and direction of movement of four different myosin VI constructs were mapped onto the known poststroke structure of the motor [9], a low-resolution picture of the prestroke structure emerged with a ~180° rotation of its light chain binding region [18]. The recent crystallographic study by Menetrey *et al.* [1] is in full agreement with this low-resolution prestroke structure. Most importantly, this seminal crystallographic study shows how this remarkable ~180° lever arm swing is achieved.

Surprisingly, the converter itself rearranges to achieve a new conformation. Comparison of the converter structure in the prestroke and poststroke states shows that the β sheet of the converter is found in a similar orientation and that its interactions with the relay and SH1 helices, two pivotal communication elements in all myosins, are mostly conserved (Figure 3). The α helices of the converter are, however, dramatically reoriented by changes in the hinges connecting them. These reorientations result in a surprising twist of the converter that allows for the ~180° swing of the lever arm.

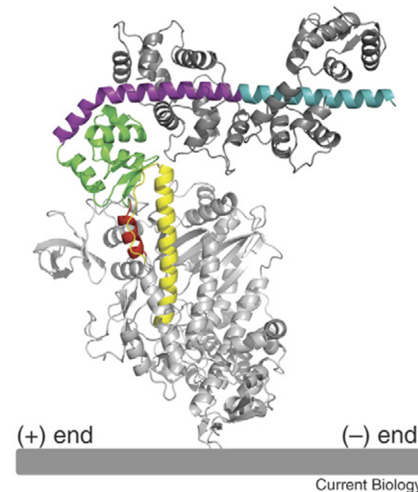

Figure 2. The myosin VI head has a unique insert that directs its lever arm in a different orientation from that found in myosin II and myosin V.

The head of myosin VI is shown in its putative poststroke state with its unique insert (purple) redirecting its lever arm in the minus end direction of the actin filament. The converter (green) associates with the unique insert. The IQ helix is shown in blue. The complete IQ helix with its bound calmodulin is shown modeled into the structure of Menetrey *et al.* [9].

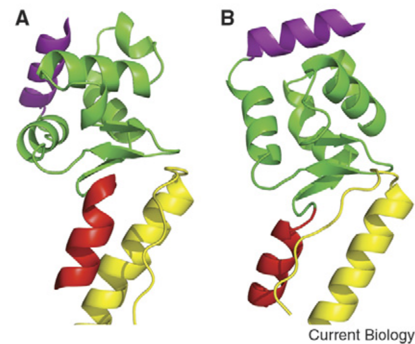

Figure 3. A new conformation of the converter reorients the lever arm in the prestroke state.

(A) The prestroke conformation of the myosin VI converter is altered compared with (B) the conventional poststroke conformation. The major dramatic changes are in the orientations of the three converter helices (green). These reorientations redirect the unique insert helix (purple). SH1 helices are shown in red and relay helices in yellow.

This change in converter conformation was totally unexpected since, as already indicated above, the converter had been assumed to always rotate as a rigid body. Since the structure of the myosin VI converter in the poststroke conformation had been shown to be very similar to those of all other myosins [9], until now it was reasonable to assume that the structure in the prestroke conformation would be the same.

Given these results, one might assume that the converter must be forced out of its conventional conformation into the unusual prestroke conformation. But no! Structural considerations that derive from the 1.75 Å crystal structure indicate that the unusual prestroke conformation may be the favored conformation in the absence of constraints [1]. Apparently, it has to be forced out of this conformation and into the more conventional converter conformation. To hold the converter in the poststroke conformation, it must be stabilized by specific interactions with the amino-terminal subdomain.

In summary, the pivotal myosin VI prestroke structure reported by Menetrey *et al.* [1] resolves the mystery of the large stroke size by a short lever arm, and the swinging lever arm hypothesis as a general mechanism for myosin-mediated motility is on very firm ground.

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Non-Neural Reflexes: Sponges and the Origins of Behaviour

Sponges ‘sneeze’ without the benefit of nerves or muscles. While genomic analysis has uncovered a surprisingly complex set of molecular components in these ancient metazoans, physiological studies have revealed equally sophisticated cellular coordination.

Robert W. Meech

In 1825 Robert Grant beheld, for the first time, sponges “vomiting forth, from a circular cavity, an impetuous torrent of liquid matter” [1]. He argued that such excretions meant that sponges must be animals and not, as Aristotle believed, vegetables. Now Elliott and Leys [2], working on the freshwater sponge *Ephydatia muelleri* (Figure 1), have reported a cellular basis for what they call sponge ‘sneezing’. The surprising thing is that sponges have neither nerves nor muscles with which to produce a ‘sneeze’. Apparently the non-neural origins of reflex behaviour are already represented in this ancient phylum.

Sponges are multicellular animals. They sit near the base of Darwin’s evolutionary tree, at the interface between the single-celled Protozoa and metazoans with nervous systems, like those highly coordinated hunters and trappers — the jellyfish. At some stage in evolution, colonies of single-cells developed a competitive edge by moving to true muticellularity with individual units becoming specialized for different tasks. Cell communication is a prerequisite for multi-tasking, however, for without coordination the advantages of specialization disappear. Now, with the publication of the genome of the demosponge *Amphimedon queenslandica* (Joint Genome Institute 2005–7), the molecules of communication are being identified at last. Links with higher animals are reported monthly, such as the signals that specify the first embryonic axis in *Amphimedon* [3] and the scaffold-like synaptic proteins encoded in its genome [4]. Do proto-synapses have proto-transmitters also? And do proto-synapses lead to proto-behaviour? Perhaps they do.

A sponge lives by filtering water for food. Water is pumped through a system of canals by beating flagella in internal choanocyte chambers. The main problem arises with damaging sediment in the incoming water. The three major classes of sponge deal with the problem in different ways. The glass sponges (Hexactinellida), which apparently lack contractile cells, stop the flow of water by directly arresting the beating flagella with calcium from action potentials that propagate to them by syncytial transmission pathways [5,6]. The cellular sponges (Calcarea and Demospongiae), on the other hand, have no such pathways but regulate the incoming flow by compressing the flagellated chambers or by contracting the canal system (see [7]). It is this process of compression and contraction that Elliott and Leys [2] have examined in detail.

It is difficult to follow the course of contraction in a large opaque animal like a sponge and so Elliott and Leys [2] turned to transparent juveniles hatched from over-wintering cysts. They found that, by 7–10 days, the juvenile sponge was fully formed and filtering water. It was tent-shaped with an osculum,