Dispatches

# Motor Proteins: Myosin Mechanosensors

**Mechanosensation is emerging as a general principle of myosin motors. As demonstrated in a recent study, the single-headed myosin I molecule is an exquisite mechanosensor, able to sense strain over a very small force range.**

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Mechanosensation, the sensing of mechanical forces, is a fundamental molecular and cellular capability that allows molecules and cells to sense their environments. At the cellular level, it seems highly likely that mechanosensation was one of the original sensory systems, along with nutrient, temperature, pH, and light sensation. As cells and organisms evolved, mechanosensation was refined to perform diverse functions, such as tissue formation, blood-pressure regulation, muscle contraction, hearing, bone remodeling and cell-shape control. At the molecular level, ion channels, myosin and kinesin motors, and polymerases are all tension sensors [1,2].

Single-headed myosin I motors are widely expressed in eukaryotic cells and are particularly concentrated at the stereocillia of inner ear hair cells and the brush border of intestinal epithelial cells [3]. In a new study by Laakso *et al.* [4], the myosin I isoform myosin Ib has now been shown to display an extraordinary ability to sense strain over a small force range (Figure 1). The authors used single-molecule methods to quantify the force-generating events mediated by individual myosin I molecules on an actin filament. Because myosin I is single headed, the authors used dual optical tweezers to control two beads, one attached to each end of an actin filament, in the context of immobilized myosin I. In this setup, the binding events are visualized by the reduction in the correlated thermal fluctuations of the beads of the actin filament upon myosin binding [4]. By analyzing the position of the trapped beads and their fluctuations, the motor step size, the duration of attachment, and the rate constants could be quantified under conditions of varying ATP concentration and resistive force (load). Under low load, the detachment rate increased with ATP concentration, whereas attachment rate was independent of ATP concentration. At a constant ATP concentration, the detachment rate decreased 75-fold within a small force range (0–2 pN). This led to a shift from 0.2 to 0.9 in the duty ratio, which is the fraction of time a motor spends bound to its actin track relative to the duration of its entire ATPase cycle. With applied load, the slow movement of myosin I revealed two distinct sub-steps (a 5.1 nm step followed by a 3.3 nm step). The smaller sub-step is due to an extra 32° swing of the lever arm and represents the force-sensitive transition. Overall, myosin I was sensitive to strain applied to the actin filament and converted into an actin-filament anchor in response to low loads.

The drastic change in duty ratio of myosin I seen in these experiments could be important for a number of cellular processes but is particularly striking in its possible role in hearing adaptation to different amplitudes of vibration in the stereocilia of inner ear hair cells, where the myosin Ic isoform might help to tune membrane stiffness [5]. However, this tension-sensing ability is not unique to myosin I as myosins II, V, and VI are also mechanosensitive [6–9]. Myosin V and VI are dimeric processive motors that transport cargos in cells [10–12], whereas myosin II assembles into a thick filament form that can produce contractile forces that act on the elastic cytoskeleton of the cell. All myosin motors hydrolyze ATP, converting the chemical energy in ATP into conformational changes of the motor, which, by sliding the actin filament relative to the motor, produces mechanical work. The different nucleotide-binding states lead to differential binding affinities of myosin to actin. Myosin in the ADP-bound or nucleotide-free (rigor) state has a high affinity for actin, while binding of ATP to the motor releases the myosin from the actin filament. Applied load results in the motor favoring this high actin affinity state, leading to an increased duty ratio. Most myosin motors achieve

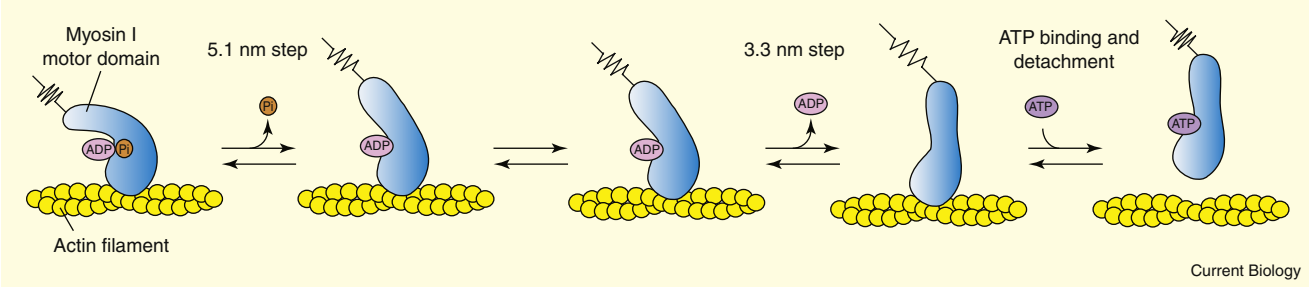


Figure 1. Model of mechanosensation by myosin Ib [4].

On binding to actin, the myosin I motor domain releases the bound phosphate and takes a 5.1 nm sub-step, which stretches a compliant element (spring) in the motor. This mechanical strain slows a second 3.3 nm sub-step that occurs through an additional 32° rotation of the lever arm. Low mechanical loads inhibit this second sub-step and reduce the rate of ADP release up to 75-fold. Once the motor completes this slow transition, the ADP is released, allowing ATP to rebind and the motor to release from the actin filament.

higher duty ratios by limiting the release of ADP. Myosin VI, on the other hand, responds to load by increasing the rate of ADP re-binding, which in turn prevents ATP binding, leading to anchoring of the motor on the actin track [6]. Regardless of the mechanism (reduced ADP off-rate or increased ADP on-rate), mechanical strain leads to the maintenance of the ADP-bound state, locking the myosin motor on the actin filament. Thermodynamically, each myosin is limited by the free energy of ATP hydrolysis (~100 pN●nm). If the work done by the motor (W = applied load●step size) exceeds this, then the motor must either release from or lock onto the track.

In addition to the impact of applied load, single-molecule studies have shown that the directionality of the applied load impacts how the myosin molecule interacts with the actin track [7]. Without load, myosin II molecules have a low duty ratio (typically on the order of 0.05), meaning that most of the motors are unbound to actin at any point in time. An assistive forward push (towards the plus end of actin) can accelerate the release of ADP, lowering the duty ratio and speeding up the motor [8,9]. However, myosin II locks tightly to actin when a resistive force pulls against its direction of movement, which slows the release of ADP up to 10-fold, depending on the myosin II isoform. Processive dimeric myosin V moves in a 'hand-over-hand' fashion, taking a step size roughly equivalent to the length of the pseudo-repeat of the actin filament (36 nm) [13,14]. When low to moderate forces are applied to recombinant monomeric forms of myosin V, this motor also responds differentially to applied load, depending on the direction of the load [7]. Backward resistive force inhibits the head from releasing from the track while assistive forward force helps release the head from the actin filament. However, super-stall loads (loads greater than the maximum force the motor can generate, i.e. >3 pN) cause dimeric myosin V to step backwards in a force-dependent but ATP-independent manner [15]. This behavior may allow myosin V to adjust its position along the actin filament until it finds its isometric position. In native myosin V, the front and rear heads probably also take advantage of these directional differences to generate intramolecular strain, allowing the coordination of their binding and conformational changes to achieve processive movement as they pull cargos along an actin filament.

Myosin mechanosensitivity is emerging as a general feature of the motor domain as it has now been observed in four classes of myosin. Because the myosins are ATPases, generating forces actively, Newton's Third Law implies that the connectors of the actin filament and the motor will also prove to be critical players in this mechanosensing system (Figure 2). These connections must be able to withstand at least as much force as the motor can generate in order for the motor to feel a large enough load to lock into the ADP-bound state. In the case of the single-headed myosin I molecule, the mechanism of anchorage of myosin I to the plasma membrane or to other anchoring factors takes on special importance because, unless the motor is anchored, the one-headed motor cannot generate enough force to trigger the shift in duty ratio. Myosin I has a phosphatidylinositol-4,5-bisphosphate ($PIP_2$)-binding site in the tail domain that mediates high-affinity interactions with phospholipids, and myosin I's neck (IQ repeat) region can bind to myristoylated proteins that have homology to calmodulin, a common myosin light chain [16,17]. It would be interesting to determine whether any of these associations are similarly modulated by mechanical strain. One strategy for a motor protein to accumulate locally in the cell might be for it to have two strain-dependent associations — one through the motor domain and a second through the tail domain with a receptor. Local mechanical strain could slow both dissociation rates, allowing the motor and perhaps the receptor to lock into place. This could then strongly influence where both proteins accumulate, explaining their subcellular colocalizations.

Backwards load from the drag of a vesicle attached to myosin V or VI implies that cargo attachments for these myosins must withstand at least the forces that the motors generate. In this case, because many of these cargos have multiple families of motors (including microtubule-based motors), the myosins may have to directly pull against other active motors [18]. For myosin II, the functional unit is the bipolar thick filament, which is a large, rigid coiled-coil bundle of tens to hundreds of myosin II dimers,

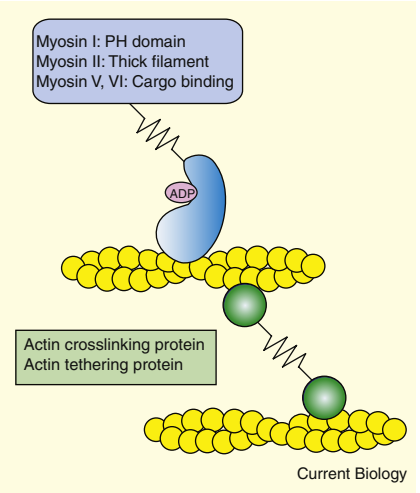


Figure 2. The actin–myosin mechanical circuit.

Myosin motor domains are mechanosensitive and undergo force-dependent shifts in duty ratio. However, the complete circuit must include the anchoring of the motor and the filament. Through different carboxy-terminal tails, the motor is linked to cargoes, thick filaments or lipids. The actin filament is also anchored through actin-associated proteins to the membrane or other actin filaments. PH, pleckstrin homology.

depending on the isoform. Myosin II associates with a variety of actin structures that range from meshworks to bundles. For myosin II to generate contractile stress in the cell, the actin filaments must be anchored to the greater actin network, which is further linked to the plasma membrane. The actin crosslinkers that hold these filaments together are then likely to be critical in generating the resistive load that triggers the mechanosensory capability of the myosin molecule [19,20]. Importantly, these crosslinkers would also impact the behavior of myosin I as it pulls against large crosslinked assemblies of actin filaments, and the crosslinkers help set the viscous drag of the cytoplasm and cortex through which the processive myosin V and VI molecules must pull their cargos.

Overall, the recent work on myosin I has revealed that this motor is extremely sensitive to force and that mechanosensation is emerging as a common feature among myosins. Myosin motors can move freely over actin filaments without load, but then readily convert into molecular-scale tension generators in response to mechanical load. This core principle

provides the basis for diverse processes, ranging from hearing to vesicle trafficking and cell division.

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# Behavioural Genetics: The Social Fly

**Two studies report the presence of a social influence on pheromonal communication in fruit flies, demonstrating that the production of pheromone blends and mating behaviour are profoundly influenced by social context.**

**Michael G. Ritchie**

One reason why *Drosophila melanogaster* has become one of the favourite model organisms for behavioural genetics is the apparent simplicity of its courtship behaviour: A male fly reared in isolation can display the full repertoire of co-ordinated mating behaviours when it encounters a female — including orientation, chasing, singing, genital licking and copulation. In classical ethology, such behaviours are called 'fixed action patterns', emphasising their apparent lack of flexibility [1]. Critical stimuli for the male courtship sequence are the appearance and smell of female flies, with the smell being a cocktail of pheromones within a mix of waxy cuticular hydrocarbons [2]. Males will even court females of other *Drosophila* species who have been 'perfumed' with the cuticular cologne of their own females [3]. That these mating behaviours are to a large extent genetically determined seems clear from the wealth of genetic mutations and manipulations that are known to affect courtship — including the recent development of impressive techniques for eliciting male behaviour in females by genetic manipulation [4] or photoactivation of appropriate neural circuitry [5]. However, it is also becoming clear that courtship and mating is perhaps not strictly 'fixed' and can be influenced by environmental factors, not the least of which is the presence of other flies (Figure 1). Now, two papers [6,7] from the laboratory of Joel Levine published in this issue of *Current Biology* start to shed some light onto how social experience affects courtship signals and mating behaviours.

In the first paper, Krupp *et al.* [6] examine the temporal regulation of pheromone production in male *Drosophila.* They find that pheromone production varies diurnally, under the control of a peripheral clock which is regulated at the site of cuticular hydrocarbon synthesis — the so-called 'oenocytes' located in the dorsal abdomen. However, patterns of pheromone expression vary with the social context in which flies find themselves, depending on whether the group is genetically uniform or comprises individuals that are genetically different. In the accompanying paper, Kent *et al.* [7] examine the variation in pheromone production in a quantitative genetic context and quantify the influence of various parameters — such as diurnal conditions, male genotype and social environment — and their interactions. Remarkably, phenotypic variation in important mating pheromones is largely influenced by interaction of these parameters with social context. Therefore, a male flies' courtship signal is not just a product of his genotype, development and the time of day, but also of whom he is interacting with socially.

Diurnal variation in fly behaviour is influenced, as in other animals, by an interaction between external cues, the day–night and light–dark cycles, and the internal clock machinery whose molecular components determine circadian cycles of activity [8]. Krupp *et al.* [6] demonstrate that the oenocytes — the sites of pheromone production lying just underneath the