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Visions & Reflections

A new view concerning an actomyosin motor

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Muscle contraction and cellular movements are achieved by the movement of myosin motors along protein tracks of actin filaments using the energy released from the hydrolysis of ATP. Recently, remarkable progress has been made toward understanding the mechanisms underlying myosin motors. In this short review, we present our view of the actomyosin motor rather than an overview of the field.

The development of three techniques – single-molecule detection, structural biology and molecular and cell biology – have contributed to the progress. Single-molecule detection techniques enable the visualization and manipulation of single molecules, allowing the behavior of the motors to be monitored [1–2] (fig. 1). The motile activities of a actomyosin have been ascribed to the operation of a unit machine that contains a single myosin molecule and an actin filament. Studying this unit machine offers many advantages, because individual processes can be measured and interpreted clearly.

The movement and force generated by single myosin molecules have been measured by manipulating actin filaments with a glass microneedle [3] and a laser trap [4, 5]. In the laser trap measurement, an actin filament is suspended taut between two beads, one at each end using a double trap and then brought into contact with a single myosin molecule immobilized on the glass surface. The displacement of the actin filament generated by single myosin heads was determined by measuring the change in the position of one of the beads with an accuracy of nanometers and milliseconds. Displacements with mean amplitudes of 4–15 nm during one ATPase cycle were observed (note that this method cannot determine individual displacements, only a mean value [6]). Individual ATPase cycles by single myosin heads were visualized using fluorescently labeled ATP and a total internal reflection fluorescence microscope [7]. Combining this technique with laser trapping nanometry, individual ATPase and mechanical events were simultaneously measured and thus the mechanochemical coupling was directly determined [8].

The atomic structure of the myosin head has been determined using X-ray crystallography [9]. The myosin head is responsible for the motile activity. Muscle contraction is thought to result from structural changes in the myosin molecules coupled with the hydrolysis of ATP. The structure of the myosin head is dependent on the chemical state of the ATP bound to it. Small changes in the structure of the binding site for ATP are transmitted and amplified to other parts of the molecule [10]. The angle of the long α helix at a neck domain extending from the motor changes between states before and after the power stroke. Based on this difference in structure, a model called the lever-arm model was proposed in which the neck domain rotates as a lever-arm when ATP is hydrolyzed, resulting in the sliding movement [11]. The consequence of this hypothesis is that the step size of the movement should depend on the length of the neck domain. In the case of muscle myosin (myosin II), the step size is expected to be at most 10 nm.

Advances in cell biology and protein engineering have allowed a variety of myosin molecules to be developed. Eighteen classes of the myosin superfamily have been discovered. Among them are myosins with neck domains of varying lengths. These myosins have been used to test the lever-arm model. Light chains or calmodulin wrap around the α helix at the neck domain. The length of the neck domain is classified by number of binding sites for light

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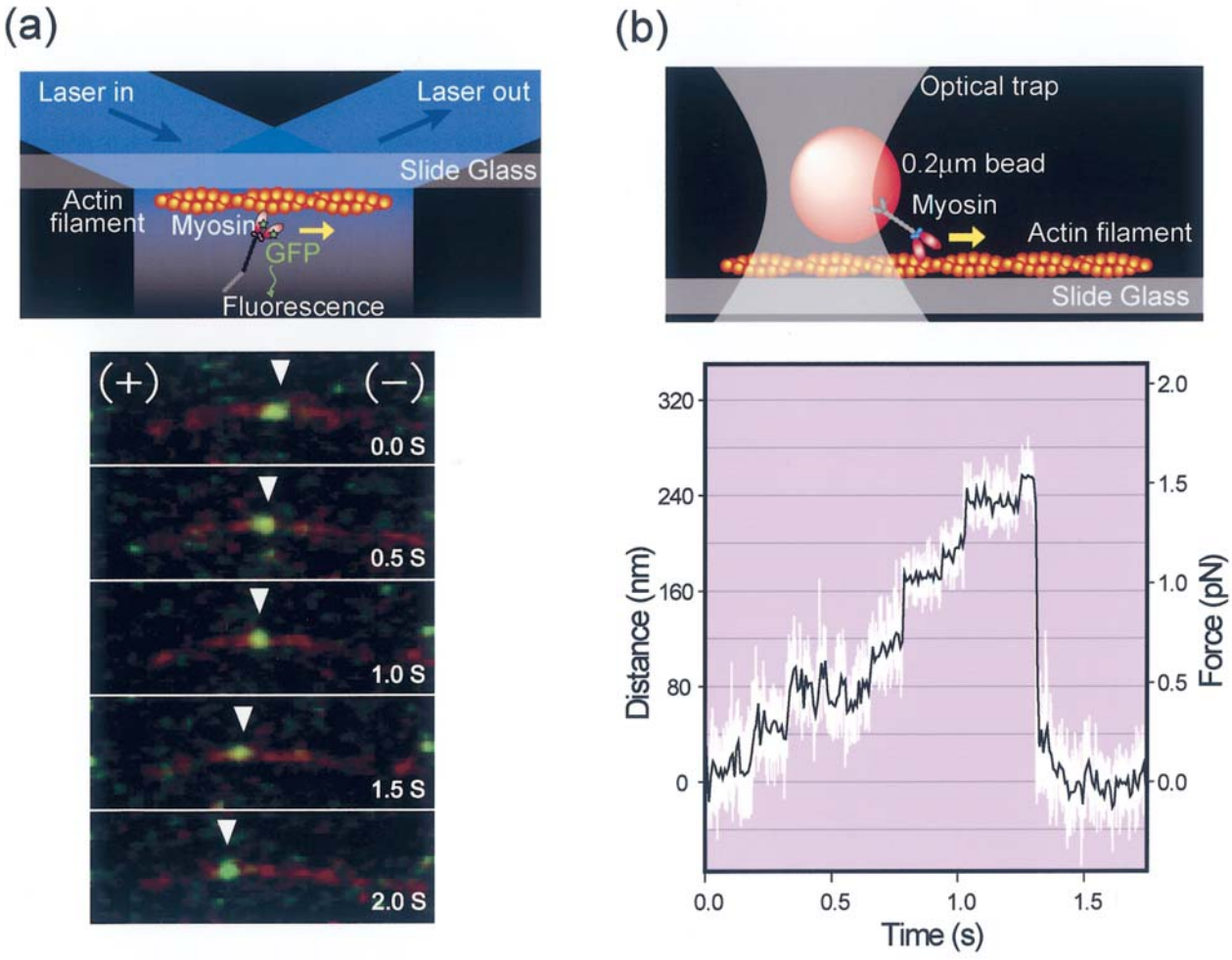

Figure 1. Processive movement of a single molecule of myosin VI with a very short neck domain. (*a*) The fluorescence from single molecules was visualized using an evanescent field in which only fluorescent molecules in the vicinity of the glass surface were excited. Myosin VI was labeled with green fluorescent protein (GFP). A fluorescent spot from GFP-myosin (arrow) moved along an actin filament fixed on the glass surface. (*b*) Optical-trapping nanometry of bead-tagged myosin VI. A myosin VI molecule moved processively along an actin filament with large (~36 nm) steps [14].

chains or calmodulin. Myosin V contains six repeats of the calmodulin-binding sites whereas myosin II (muscle myosin) has only two binding sites and myosin VI only one. According to the lever-arm model, the step size is expected to be large for myosin V and small for myosin VI. As expected, myosin V exhibited a large step (36 nm compared with 4~15 nm for muscle myosin) [12]. In contrast to myosin II, myosin V can move long distances along an actin filament without dissociating. Such long-distance or processive movement has been observed using single-molecule imaging [13, 14]. The fluorescent spots from single-molecules of green fluorescent protein attached to myosin V have been observed moving along actin filaments. Thus, it was tempting to conclude that for double-headed myosin V, one of the heads moves with a large step while the other head stays at the same position and then, after the step has been completed, the role of the two heads changes [15]. However, a deletion mutant of myosin V which contains only one calmodulin-binding site also showed the same large step [16]. Thus, the length of the neck domain is not essential in determining the processivity of the movement or the step size. A similar conclusion was obtained using natural proteins. Myosin VI, which has one calmodulin-binding site, i.e., a very short neck domain, also showed a processive movement and a large step size [14, 17] (fig. 1). Thus, the lever-arm model does not explain these data [18, 19]. The rotation of the neck domain may be utilized for other functions such as the regulation of kinetic processes, which may act as a strain sensor.

What then causes the movement of myosin, if the structural change does not? A hint to answer this question was obtained when techniques that enabled the manipulation of single myosin head molecules instead of an actin filament were established. In the laser trap measurement, the actin filaments are manipulated. The actual displacement

of myosin molecules is damped and disturbed by thermal noise because of the compliant linkages between myosin and the bead. To overcome this problem, myosin molecules instead of actin filaments were manipulated and the displacements measured, although these were very difficult experiments to perform. Measuring the displacement of a myosin head itself provided better resolution [20]. For this measurement, single myosin heads were captured and manipulated using a scanning probe, while being visualized. The measuring system of the scanning probe was less compliant than the laser trap, thus the details of a single displacement could be explored. Within a single displacement there were one to five regular steps, giving rise to a total displacement of ~5–30 nm. The total stepping process of the single displacement was coupled to the hydrolysis of a single ATP molecule. The average number of steps in a single displacement was three, giving a total displacement of 13 nm. The size of a single step was 5.5 nm, coinciding with the interval between adjacent actin monomers on one strand of an actin filament. The dwell time between steps was independent of the ATP concentration and increased when the temperature was lowered. In the measurement system, the load applied could be increased by increasing the spring constant of the microneedle. At large loads, the number of steps in a single displacement was smaller and the dwell time between the steps was greater, while the size of the step remained the same. The steps took place stochastically. Some of the steps (<~10% of the total) were in the backward direction. The probability that steps occurred in the backward direction increased with an increase in the applied load.

The stochastic stepwise movement of the myosin head is most likely driven by thermal motion (fig. 2). Brownian movement along protein tracks has been observed for several types of linear motors but not myosin. KIF1A, a single-headed motor in the kinesin superfamily, and single-headed truncated kinesin were found to undergo Brownian movement along microtubules [21, 22]. There are extra interactions with the track protein that help retain the motor proteins on the protein track while they move thermally. Such long-distant Brownian movement has not yet been observed for myosin, most likely because myosin, readily dissociates from the actin filaments. Only in some states during the hydrolysis of ATP does the myosin head step from one actin monomer to the next, while remaining attached to the actin filament.

Given that the Brownian motion is random by nature, the movement of myosin must be biased to one direction, because the observed motion of myosin is oriented to one direction. This must be accomplished using the energy of the ATP hydrolysis. If this were not the case, the second law of thermodynamics would not hold. The directional movement is expected to occur when the interaction potential is tilted in one direction. Brownian particles move preferentially toward low energy levels. The binding of myosin molecules may possibly cause structural changes in several neighboring actin monomers in a direction-dependent manner, so that the myosin molecule is attracted more to one direction than the other. Analysis of the data of the stochastic processes of myosin II shows the energy difference between two directions is 2~3 k_BT [K. Kitamura, private communication]. The other way to bias Brownian movement is by dynamic fluctuations of interaction potential between myosin and actin. Simulations of the motion of Brownian particles shows that non-random fluctuation in the interaction between myosin and actin may bias Brownian movement.

Actin may play an active role in the motor function of myosin in addition to the structural changes in myosin. It is important to study the effects of the interaction with active myosin on the structure and state of actin and the mode of interaction of actin with myosin. Recently,

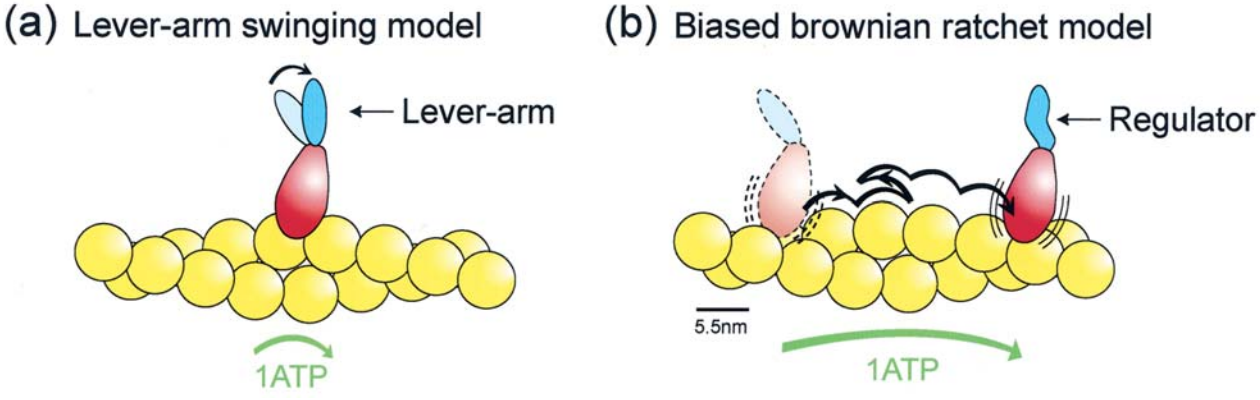

Figure 2. Two models for the sliding movement of myosin along actin filaments. (*a*) The movement of the myosin heads is driven by the conformational changes in the neck domain of myosin. (*b*) The myosin heads move stochastically and the directionality of movement is biased by the energy released from the hydrolysis of ATP. The biased Brownian model predicts that myosin can move long distances with several steps during the hydrolysis of a single ATP molecule [6].

myosin has been shown to bind to an actin filament with ~36-nm intervals in the presence of ATP [14]. These results suggest that the binding of active myosin heads evokes 'hot spots' on the actin filaments that attract other myosin heads. This may explain the large step sizes observed for myosin V and VI.

Myosin and actin are not special proteins. Many of their characteristics are shared by many other proteins. Myosin can generate and convert energy. Myosin and actin self-assemble and must be able to recognize each other in order to interact. The knowledge we have acquired as well as the techniques we have developed for the system of myosin and actin will be useful for future studies in the diverse field of bioscience.

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