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# Propagation of a signal coordinating force generation along an actin filament in actomyosin complexes

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**Abstract**

Force generation due to ATP hydrolysis by myosin molecules contacting an actin filament was found to be coordinated along the filament. We marked several points along the actin filament with the aid of a fluorescent material, and measured the temporal development of the acceleration of each marked point in the stationary coordinate space. Variations in the acceleration of the sliding movement of the actin filament propagated backward along the filament. Variations in the acceleration propagating in the direction opposite to the direction of the sliding movement show that force generation at each active site of an actomyosin complex could be regulated in a communicative manner along the actin filament. 

*Keywords:* Actin filament; Force generation; Myosin; Sliding movement

## 1. Introduction

ATP hydrolysis due to myosin molecules contacting an actin filament asks the intriguing question of how each myosin molecule would start hydrolyzing an ATP molecule in relation to the similar hydrolysis proceeding in its neighborhood [1–11]. If each myosin molecule contacting the actin filament hydrolyzes ATP molecules in a manner totally independent of other neighboring myosin molecules contacting the filament, it would be hard to actualize a seemingly uniform sliding movement of the filament on myosin molecules. The absence of any coordination among the myosin molecules contacting the actin filament with regard to their ATP hydrolysis would disturb a proper functioning of the sliding movement. In the absence of such a coordination, the phase of each actin-activated ATP hydrolysis cycle of a myosin molecule could be random among themselves, with disturbing consequences upon the coordination of force generations along the filament. Unless proper coordination is available for the force generations along the filament, there would be no coherent supra-molecular movement

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of an actin filament relative to the contacting myosin molecules. At issue should be to see how such a coordination could be established in reality.

We examined how an actin filament would develop its sliding movement longitudinally as interacting with myosin molecules in the presence of ATP molecules, and measured variations in the accelerations of several marked points along the filament. Each marker along the filament denoted as a speckle was a fluorescent material attached to the filament. We prepared a speckled actin filament for the purpose. Although variations in the acceleration of a marked point put on the actin filament are only indirectly related to the force generation proceeding there, the observed variations revealed how the actin filament comes to establish a coordination of the acceleration along the filament.

## 2. Materials and methods

### *2.1. How to prepare a speckled actin filament?*

Actin and myosin molecules were prepared from rabbit skeletal muscle [12–14]. Speckled actin filaments were made from a mixture of actin filaments, both unlabeled and labeled by rhodamine–phalloidine. Unlabeled and labeled actin filaments [25 mM KCl, 25 mM imidazole-HCl (pH = 7.4), 4 mM $MgCl_2$, 1 mM DTT] were made and treated with equal molar phalloidin, and their concentrations were maintained at 33 $\mu$g/ml. The mixture of the suspensions of labeled and unlabeled actin filaments with a volume ratio of 1:4 were subjected to supersonic dissecting (Yamato, Branson 3200) for 4 min. The mixture of dissected actin filaments, both labeled and unlabeled mixed together, was placed at a temperature of 4°C for 24 h for reformation of the filaments that could be speckled ones.

### *2.2. Measurement apparatus*

We prepared a standardized in vitro motility assay [15]. The slide glass to fix a concentration of 0.2 mg/ml HMM was hydrophobically treated with butyltrimethoxysilane (Shinetsu Chem. Co.). The solution condition for observing the sliding movement of an actin filament was 25 mM KCl, 25 mM imidazole-HCl (pH = 7.4), 4 mM $MgCl_2$, 2 mM ATP, 1 mM DTT at a temperature of 25°C. The microscope (Olympus, IX70) attached with the object lens (Olympus, Uplan Apo 100 ×, oil) was used with the aid of the fluorescent equipment (Olympus, IX-FLA) and the fluorescent filter (Olympus, rhodamine filter). The image of the microscope was taken by a video cassette recorder (Sony, EVO-9500A) carrying the camera ICCD-Camera (DHT Co., DX1700). The images were processed by the image-processing software NIH Image 1.6 (Wayne Rasband, National Institute of Health, Research Services). Each image was taken at every 1/30 s through a video grabber board (Scion Co., LG-3 PCI) into a computer. The spacing of neighboring pixels was 100 nm, and the spatial resolution of identifying the end of each speckled segment along the filament was 100 nm. Observation of the displacement of an actin filament was done at every 1/15 s interval.

## 3. Results and discussion

We first observed how each speckle on the actin filament moved in the stationary coordinate space as demonstrated in Fig. 1, in which we fabricated five speckles on a single actin filament. The spacing between the neighboring speckles was greater than 1 $\mu$m. The traces of the movements of two marked points, speckle *p1* situated near at the pointed-end of the actin filament and speckle *p5* near at the barbed-end, recorded on the stationary coordinate space, are displayed in Fig. 2. The observation that the portion of the filament near the barbed-end did not faithfully follow the trace of that portion near the pointed-end reveals that the actin filament is flexible enough with regard to both its bending and elastic displacement. In order to further examine how each portion of the filament would move in relation to its neighboring portions, we measured variations in the acceleration of each speckled point. Variations of the magnitude of the acceleration at each speckled point of *p1* and *p2* are

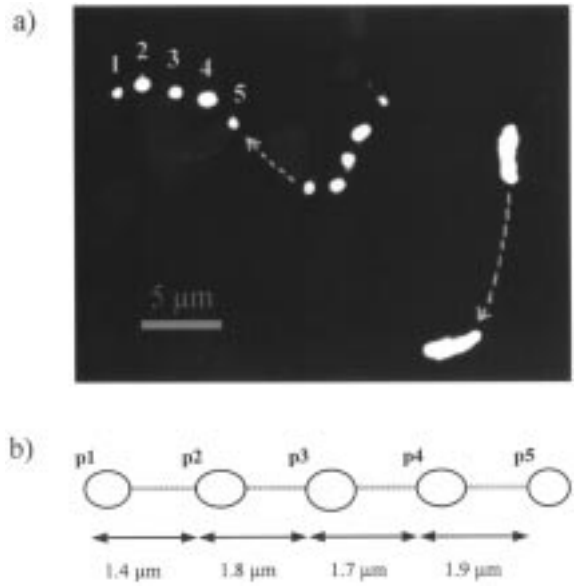


Fig. 1. (a) Fluorescence image of a speckled actin filament (left), and of a normal actin filament (right). Two images separated over 2 s were superimposed for the speckled actin filament. Broken arrows indicate the direction of the sliding movement of the actin filament. Each image of the speckled actin filament consisted of five fluorescent regions. The total length of the filament was approx. 6.8 $\mu$m. (b) A schematic representation of a speckled actin filament. Elliptic circles correspond to fluorescent regions. Elliptic circles are denoted as *p1* through *p5*. Distance between the neighboring elliptic circles is measured as referring to the left-end points of the circles.

found in Fig. 3. One could see those variations of the portion ( *p2*) situated at the downstream were slightly retarded compared to those ( *p1*) situated at the upstream. This suggests that variations in the acceleration of the filamental sliding movement propagated backward, being opposite to the direction of the sliding movement itself. The propagation of variations in the acceleration backward could be further confirmed by measuring how the correlation of those variations was propagated and delayed toward the downstream direction, as depicted in Fig. 4.

Variations in the acceleration of a supramolecular portion of an actin filament exhibit only part of an indirect effect of the force generation from actin-activated ATP hydrolysis of myosin. Nonetheless, the variations in the accel-

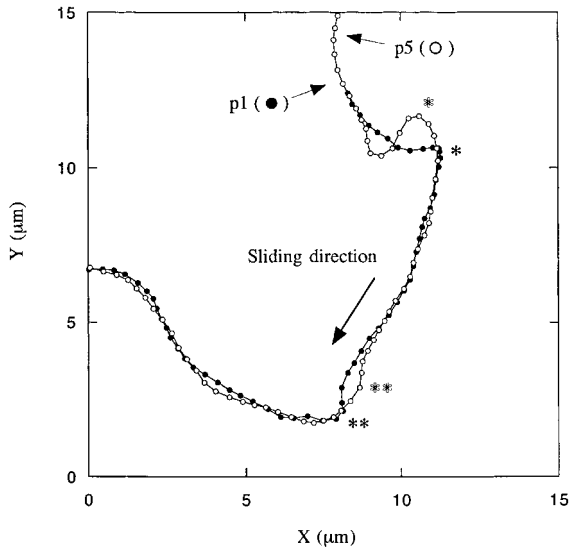


Fig. 2. Loci of the speckled actin filament sliding on myosin molecules in the presence of 2 mM ATP. The loci of *p1* (open circle) and *p5* (filled circle) were measured at every 1/15-s interval. The asterisks denote the points in the neighborhood of which the movement of *p5* did not faithfully follow that of *p1*. The average sliding velocity of the actin filament was 6.3 $\mu$m/s.

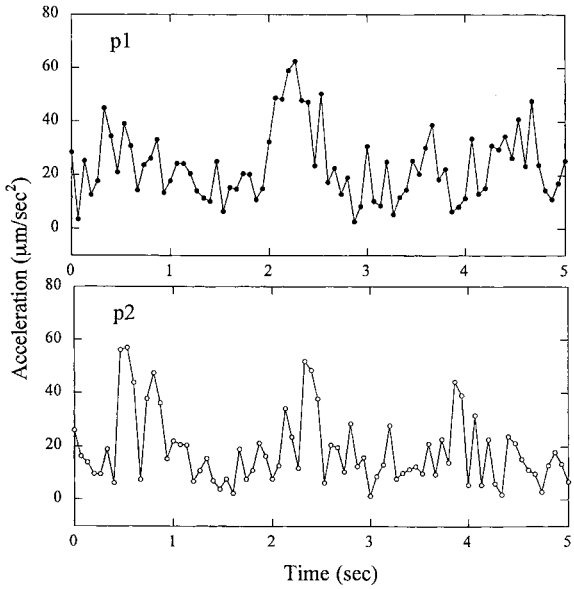


Fig. 3. Time developments of the accelerations of the two points *p1* and *p2* on the actin filament. The acceleration at each point on the actin filament was measured by consulting the difference of the sliding velocities at two time points separated over 1/15 s.

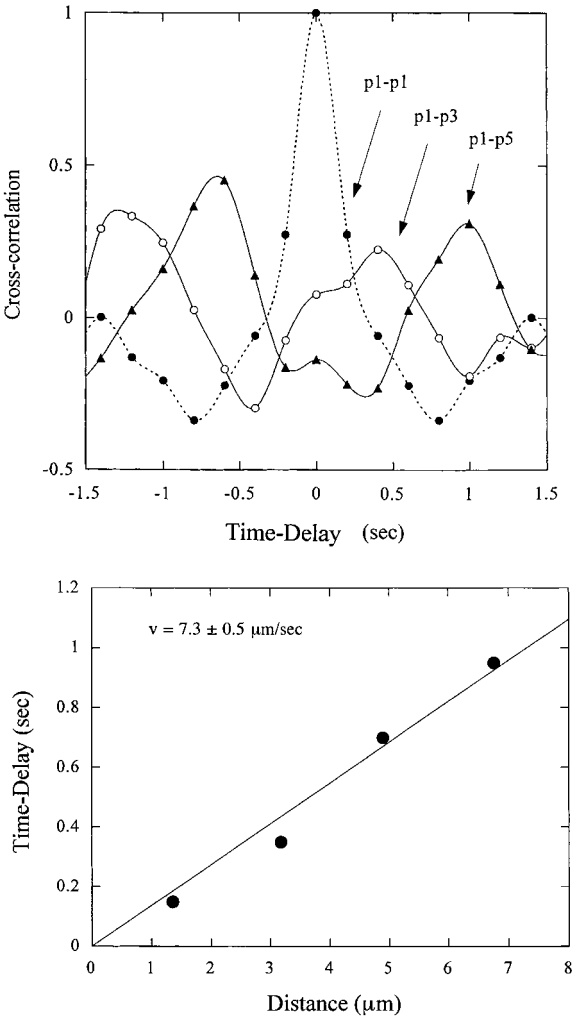


Fig. 4. (a) The cross-correlation functions of the accelerations between two points, *p1* and *p3*, and *p1* and *p5*, along the actin filament, with the designated time-delay between the two. For reference, the auto-correlation function between *p1* and *p1* is also presented. (b) The distance between two arbitrary points on the filament vs. the time-delay exhibiting the largest cross-correlation. The linearity between the distance and the delay-time gives the propagation velocity of changes in the acceleration, 7.3 $\mu$m/s.

eration were found to be coordinated in a propagating or communicative manner along the actin filament.

## 4. Concluding remarks

Needless to say, each actomyosin complex along an actin filament is instrumental for the generation of the force responsible for the sliding movement. In this regard, there are at least two processes proceeding in a mutually coordinated manner. One is the unit process of the hydrolysis of an ATP molecule at each actomyosin complex, and the other is the communication or coordination of the unit processes between the neighboring actomyosin complexes along the filament [3,16]. We have concerned ourselves mainly with the latter process of coordination. The propagation of variations in the acceleration of each supra-molecular segment of the filament in the direction opposite to that of the sliding movement implies that it could be related to a signaling process that may coordinate and regulate the force generations over the entire filament. Each actomyosin complex acts upon and is acted upon by the similar complexes in the neighborhood along the filament. Interaction between neighboring actomyosin complexes could be mediated by a signal propagating in between. Although we have not yet identified the nature of such a signal that could propagate along the actin filament, the propagation of variations in the acceleration along the filament suggests that the internal coordination of actomyosin complexes could be due to the unidirectional propagation of a signal of a local character.

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