1P-159 蛍光偏光顕微鏡を用いたミオシンヘッドダイナミクス解析システム

Dynamics of myosin head orientation during sliding as examined by polarized fluorescence microscopy

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Muscle contraction involves cyclic interaction of myosin cross-bridges with actin filaments coupled with ATP-hydrolysis on myosin. It is generally postulated that one ATP hydrolysis causes one unit of mechanical movement such as tilting of myosin head, independently from the external load. However, there is no definite reasoning to assume one-to-one correspondence between chemical reaction and the mechanical event. Oosawa and his colleagues proposed a loose chemo-mechanical coupling mechanism to explain experimentally found mysterious behavior of bio-molecular motors that slide past cytoskeletal polymers, such as actomyosin. A modular Feynman-type thermal ratchet model with two energy barriers, proposed by Vale and Oosawa well explains the force-velocity and heat-velocity profiles of the muscle. We previously observed freeze-replica images of sliding actomyosin in vitro and found that myosin heads were bound to actin filaments in several different modes including the attachment angles. Though the images might reflect actual sliding events, they are snapshots that captured the instantaneous structures of the targets and we cannot trace the time-course. To study dynamic behavior of myosin heads during sliding, we developed total internal reflection fluorescence microscope system to examine the polarization of fluorescent signals attached to myosin heads on the glass surface, during the movement of actin filaments. We will report on the orientational dynamics of actin-attached myosin heads, using several different fluorescent probes.

1P-160 細胞質ダイニンの細胞周期依存的な動態変化の解析

Cell cycle-dependent dynamic behavior of human cytoplasmic dynein

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Cytoplasmic dynein complex is a large multi-subunit microtubule-associated molecular motor involved in various cell functions. However, the mechanism of dynein-mediated vesicle transport is fully understood. In this study, we investigated cell cycle-dependent distribution and behavior of dynein complex in live cells. The dynein intermediate chain IC74 was labeled with a novel GFP-based fluorescent affinity tag and stably expressed in the HeLa cells to monitor dynein complex. Dynein complex was observed as fluorescent spots that move along with microtubules using a high-speed laser scanning confocal microscope. In interphase cells, these spots exhibited bi-directional movement, and the behavior was similar to that of EB1, showing a comet-like distribution on microtubule plus ends in the cell cortex. In prophase cells, microtubules around centrosome became clearly visible probably due to an increased binding of dynein to microtubules, and the average run length of fluorescent spots was larger than that in interphase cells. Moreover, long distance movements of fluorescent spots toward centrosome were frequently observed. The affinity-purified dyneins from the HeLa cells showed bi-directional movement on microtubules similar to that observed in live interphase cells. These results suggest that the cell cycle-dependent factors might participate in centrosome-directed long-distance movement of dynein as a regulator of dynein-microtubule interaction. Possible candidates for these factors will be discussed.

1P-161 アクチン G146 残基への変異はミオシンのクラス特異的な運動能欠損を引き起こす。

Mutation at Gly146 of actin causes class-specific motility defect of myosin.

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In the lever arm model for myosin motility, the only roles of actin filaments are to stimulate Pi release from myosin and to provide foothold for force generation. However, a number of studies have suggested that structural dynamics of actin filaments induced by myosin binding are also essential for force generation. To understand the roles that structural dynamics of actin filaments play in the motor functions, we have constructed and characterized mutations at the Gly146 residue of Dictyostelium actin, which is located between two domains of actin and acts as a hinge when actin is folded by chaperonin. In vitro motility assays using rabbit skeletal HMM, G146A actin moved normally, but with G146V and G146T actins, the fraction of moving filaments decreased dramatically and the speed of the moving filaments was reduced (0.54 $\mu\text{m/s}$ of G146V as compared with 3.1 $\mu\text{m/s}$ of wild type, at 23 deg). In contrast, actin activated S1 ATPase activity (Vmax) by G146V actin filaments was only modestly decreased to 60% of the wild type. Additionally, ATP sensitive binding of S1 to G146V actin filaments was normal by a cosedimentation assay. Thus, the motility defect caused by substitution from Gly146 to a residue with a bulky side chain was not caused by the loss of activity to stimulate Pi release or to bind strongly to myosin in an ATP-sensitive manner. Interestingly, G146V had no effect on the gliding velocity and force generation by myosin V. We are investigating why this mutant actin impairs motility of myosin in a class-specific manner.

1P-162 強い結合状態へ遷移する前のミオシン II に負荷を加えるための

高速スキャン実験

High-speed scanning experiment to apply a strain to myosin II before the transition to the strong binding state

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Muscle contraction is caused by the directional movement of myosin II along actin filaments. It has been proposed that the attachment rate of myosin VI to actin is accelerated by strain, which increases the efficiency of the directional movement (Iwaki et al., in preparation). If attachment rate of a muscle myosin is also accelerated by internal or external strain, we can propose a molecular mechanism of A. F. Huxley's 1957 model in which attachment rate is accelerated in the forward actin target. Therefore, we examine the effect of strain on the attachment kinetics of myosin II.

Myosin is strongly bound to actin via short-lived weak binding state. In order to apply a strain to myosin head before the transition to the strong binding state, a myosin II-coated bead (200 nm diameter) was optically trapped and scanned rapidly (400 nm/100 μsec) along an actin filament using an acousto-optical deflector. In this experimental system, we prepared a bridged actin filament, or "actin-bridge", by using etched glass to prevent myosin from interacting with the glass surface. To measure the rigidity of the actin-bridge, displacement of the bead bound to actin during the rigor state was measured showing the actin-bridge was rigid enough to apply the strain to myosin during High-speed scanning. Currently, we are observing interactions between several myosin molecules and actin-bridges and analyzing the movement. We will also discuss the results of high-speed scanning of single myosin molecule along an actin-bridge.

1P-163 α サブユニット変異体を導入したハイブリッドF₁の非対称な回転

Asymmetric rotation of the hybrid F₁ containing one mutant α subunit.

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F₁-ATPase hydrolyzes ATP by rotating central γ subunit anti-clockwise in 120° steps within the $\alpha_3\beta_3$ cylinder, each step consuming one ATP molecule. To elucidate the mechanism how the catalytic activity of one of three β subunits coordinates with the other two β subunits to drive γ subunit rotation, we previously showed that hybrid F₁ containing one or two mutant β subunits with altered catalytic activity rotates in an asymmetric stepwise fashion and that all three β subunits participate in driving each 120° rotation with a 120° phase difference [1]. Here, to investigate the role of α subunit in the rotation, we constructed hybrid F₁ with another mutant of α R364K, whose side chain contributes to the catalysis as "Arg finger" and observed γ subunit rotation. The hybrid F₁ containing one mutant α subunit showed similar asymmetric rotation to that containing one mutant β subunit. This result indicates that α subunit also contributes the "sequential three site mechanism" of F₁-ATPase. Furthermore, the angle interval between two catalytic pauses in each revolution appeared to be over 130°, suggesting an interesting possibility that about 10° sub-substep rotation exists between two catalytic dwells. We will report further angle analysis of hybrid F₁ with various mutants and discuss candidate models for each elementary event while considering other recent reports.

[1] Ariga, T., Muneyuki, E. & Yoshida, M. *Nat Struct. Mol. Biol.* **14**, 841-846 (2007)

1P-164 高感度 pH イメージングシステムを用いた細菌の細胞内局所 pH の測定

Local pH measurement within *Salmonella* single cells using a high-resolution pH imaging system

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The bacterial flagellar motor is powered by the electrochemical potential gradient of proton or sodium ion across the cytoplasmic membrane. Torque is postulated to be generated by cyclic interactions between the MotA/B complex, which acts as a stator that conducts protons, and the C ring, which functions as a rotor. The mechanism of energy coupling between proton influx and torque generation remains unknown.

To understand the energy coupling mechanism, the proton influx coupled with flagellar motor rotation should be measured with high spatial and temporal resolution. We have developed an intracellular pH imaging system, which can measure the local pH near the motor using a pH-sensitive green fluorescent protein, pHluorin, as a ratiometric fluorescent probe with excitation at 410 and 470 nm and emission at 508 nm (Miesenbock et al., 1998). This system can measure the intracellular pH distribution at a pH resolution of 0.03 and a spatial resolution of 0.2 μm at every 3 ms. We measured intracellular pH of *Salmonella* cells expressing pHluorin in the cytoplasm and found that intracellular pH was 7.3 - 7.4 when the external pH was 7.0. We fused pHluorin to a bacterial chemoreceptor protein, Tar, at its C-terminus to measure the pH near the cytoplasmic surface of the cell membrane, and found that the local pH value there was 7.2 - 7.3, which is lower than that of the cytoplasm by 0.1. We will discuss these pH differences. Local pH measurements by fusing pHluorin to motor proteins are under way.