3P-117 アクチン変異体がミオシン 5 の運動性に与える影響の解析

The influence of mutated actin filament against processive myosin-V motility

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Myosin family is actin filament related ATP driven molecular motor. It is a widely accepted view that myosin interacts with an actin filament using ionic interaction. It is reported that 8 negative charged amino acids in actin relates to actomyosin interaction, while positive charged amino in myosin loop2 region relates to it. However, how the ionic interaction from these amino acids relate to the myosin stepping mechanism, in particular weakly actomyosin binding state, stall force or run length of processive motors, remain to be explained. To clarify these points, we constructed several actin mutants in which the number of negative charged amino acids were decreased to 0 - 6. According to results of actin gliding assay, some of mutants seem to have lower actomyosin interaction compared with that of wild type actin. Furthermore, we are planning to determine the properties of myosin-V on these actin mutants using single molecule measurement like TIRF imaging and optical trapping.

At this meeting, we will discuss how ionic interaction strength change affects the functional properties of processive myosin-V.

3P-118 GアクチンとFアクチンに存在する明確な 2 状態

Two Distinct Forms of Actin in Monomeric and Filamentous states

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One of the most abundant proteins in eukaryotes is actin, a ubiquitous protein that plays a role in the dynamic assembly and disassembly of structures such as cell migration, lamellipodia and filopodia. The dynamics of actin filament treadmilling is regulated by two actin structural states: globular actin (G-actin) and filamentous actin (F-actin). While G-actin's crystal structure has been solved by several groups, F-actin's has not. Although recently it was reported that the structure for the two actins differ (Oda et al), but there is still much to resolve on the matter of dynamic structures. Here we observed the dynamics of the actin structural states under various conditions by using single-molecule FRET in combination with total internal reflection fluorescence microscopy. To conduct these experiments, we first labeled actin residues 41 and 374 having substituted Gln 41 with Cys transgenically. The new Cys 41 site along with Cys 374 were used for site-directed labeling by SH-group reactive fluorescent dyes. We found that both G-actin and F-actin had at least two distinct states. In a low ionic solution, the main structural state of G-actin is described by high FRET structural states. Increasing the KCl concentration can, however, increase the proportion of the low FRET structural states. These results offer new insights on the structural dynamics of G-actin and F-actin and how they relate to function.

3P-119 モータータンパク質の 3 次元計測

3D tracking of motor proteins

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Molecular motor proteins play an important role in living organisms. They take nanometer scale steps, by hydrolyzing ATP, with a run-length in the order of micrometers. Myosin V and VI are one of the well studied molecular motors but even for them many aspects of their stepping are unclear despite an excellent resolution in two dimensions. To get a deeper understanding of their mechanics it is important to obtain information about the third dimension. 3D tracking utilizing optical astigmatism and two dimensional gaussian fitting, offers exactly that possibility. Applying 3D tracking to single molecule experiments, we achieved up to 1 nm resolution in all three dimensions. For imaging the third dimension it was important to use gold nano particles instead of fluorescent particles observed by a dark field microscope providing a higher signal to noise ratio coupled with a near perfect stability of the image, compared to conventional fluorescent probes. With this technique we are trying to clarify the motion of Myosin V and VI.

3P-120 ミオシン VI のストレインセンサーの定量解析

Quantitative analysis of strain-sensor for myosin-VI

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Recently, Brownian motion process in the unidirectional motion of myosin-V and -VI was directly observed (Shiroguchi et al., 2007; Arimoto et al., in preparation). In the process, myosin head randomly diffuses back and forth and then strongly binds to actin in the forward region. The forward strong attachment mechanism has unsolved yet. To clarify the mechanism, we examined transition from weak (Pi bound state) to strong binding (Pi released state) to actin using single molecule techniques and proposed a strain-sensor model, where weak-to-strong transition

was regulated by strain applied to the myosin head (Iwaki et al., 2009). Especially, backward strain applied to the myosin-VI head accelerated strong binding to actin, which means strong binding would be ensured in the forward binding site because backward intramolecular strain should be applied to myosin head in the forward binding site by spanning the actin helical pitch during walking motion. Thus, the strain-sensor mechanism would be utilized for directional sensing and rectification of Brownian motion, which leads to productive motion in the thermal noise.

In this meeting, we will report quantitative analysis of strain-dependency of weak-to-strong transition rate. To quantitatively analyze the sub-milli seconds attachments (weak binding) under constant load, we are now constructing an objective type dark-field observation system combined with an optical trapping, enabling a detection of small optically trapped particle and rapid manipulation.

3P-121 プロトン駆動型べん毛モーターのプロトン透過活性とトルク発生サイクルとの相関関係

Relationship between proton conductivity and the rate of torque-generation cycle of the proton-driven flagellar motor

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MotA and MotB are integral membrane proteins and form the stator complex of the proton-driven bacterial flagellar motor. The stator complex conducts protons and couples proton flow with motor rotation. A highly conserved aspartic acid residue, Asp-33 of *Salmonella enterica* serovar Typhimurium MotB, which is a putative proton-binding site, is critical for torque generation coupled with an inward proton translocation powered by proton motive force across the cytoplasmic membrane. To understand the energy coupling mechanism of proton translocation with torque generation, we have been studying the motor functions by bead rotation assay and pH imaging. In our previous study, we isolated pseudorevertants from the slow-motile *motB(D33E)* mutant and showed that the second-site mutations recover a torque generation step coupled with protonation/deprotonation of Glu-33 but not the maximum proton conductivity (Che et al., *J. Bacteriol.* 2008). In order to characterize the effects of these second-site mutations more clearly, we carried out careful measurements of near-stall torque produced by the *motB(D33E)* mutant and its pseudorevertants. We also measured proton conductivity of the MotA/B complexes with these mutations and deletion of a proton channel plug region at the external pH values of 5.5 and 7.0 using a pH-sensitive green fluorescent protein. Based on the obtained results, we will discuss relationship of proton conductivity of the MotA/B complex with the torque-speed curve of the motor.

3P-122 歩行運動中のミオシン V のモータードメイン一個の力学特性

The mechanical properties of a single myosin V motor domain during gait motion

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Myosin V takes multiple 36-nm steps along an actin filament by coordinating its two motor domains. The properties of myosin V have been addressed by means of several assays. In particular, optical tweezers techniques and FIONA have contributed widely to enlightening the motion mechanism. However, these two assays cannot be applied when studying the mechanism for coordinated force generation by a single motor domain during motion. For myosin V, optical tweezers assays are restricted to studying the mechanical properties of both motor domains when an optically-trapped bead is attached to the myosin V tail. On the other hand, although FIONA is capable of observing the motion of a single motor domain during gait motion, it is not capable of revealing the mechanical properties because these studies do not apply external force.

Here, we constructed a new optical tweezers system that incorporates a DNA linker to the myosin V. The DNA linker is used to connect a bead to one of the two motor domains. In this experimental setup, external force by the optical tweezers is applied to a single motor domain directly via the DNA linker during gait motion. By using this measurement system, we succeeded to observe single head behavior during coordinated motion.

3P-123 pH イメージングシステムを用いた細胞内 pH とべん毛モーター回転の同時計測

Simultaneous measurement of intracellular pH and bacterial flagellar motor rotation using a high-resolution pH imaging system

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The proton driven bacterial flagellar motor is powered by the electrochemical potential gradient of proton across the cytoplasmic membrane. Torque is generated by cyclic interactions between the MotA/B complex, which acts as the