2PS012 アクトミオシン活性に対する尿素とトリメチル N オキシドの中和効果
Counteractive effect of urea and trimethylamine N-oxide on the activity of the actomyosin motor

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It is known that urea induces unfolding of proteins with solvation whereas trimethylamine N-oxide (TMAO) stabilizes their structure due to peripheral hydration. In addition, deleterious effect of urea on the structure of certain proteins is completely counteracted by the presence of TMAO. We examined whether their counteraction is effective in actomyosin functions. CD spectroscopy showed that urea decreased unfolding transition temperature (T_m) of heavy meromyosin (HMM), on the other hand, TMAO increased its T_m . The decrease of T_m by 0.6 M urea was recovered by 0.3 M TMAO. Both urea and TMAO in the concentration range of 0-1.0 M considerably decreased the sliding velocity of actin filaments on HMM molecules under in vitro motility assay. Similarly, actin-activated ATPase activity was decreased by urea and TMAO although the ATPase activity of HMM alone was not suppressed. The urea-induced decrease of ATPase activation was further reduced by the addition of TMAO. Meanwhile, urea and TMAO were found to have counteractive effects on motility at concentrations of 0.6 M and 0.2 M, respectively. The difference of counteractive effects on HMM structure and on the motility suggest that water-related action in addition to the structural dynamics of HMM structure contributes to actomyosin activity.

2PS013 アクチン線維が滑走中のミオシン線維トラックからの ADP 遊離速度計測

Measurement of ADP release from bipolar myosin filament track along which actin filament slides

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The motility assays have shown that along myosin thick filament, actin filaments moved at a fast speed towards the central bare zone and at a slower speed away from the bare zone. The force and displacement of myosin crossbridges interacting with actin filament in the backward direction was smaller than that in the forward direction. Sellers and Kachar speculated that myosin heads in the backward movement might rotate 180 degrees so as to face the actin in the right configuration, and might be constrained so that the detachment of the rotated heads from actin would occur at slower rate. Recently, we have found that the thermal activation energy of the backward movement was significantly higher than that of the forward movement, suggesting that the backward movement causes the myosin heads to be constrained, and increases the energy required for the ADP release step. However, there is no direct evidence for the slow ADP release rate in the backward movement. Here, in order to examine whether rate of ADP release step depends on the direction of the actin movement, we measured nucleotide turnover rates of bipolar myosin thick filament along which actin filament slides by monitoring the displacement of prebound fluorescent ATP analog, Cy3-EDA-ATP on flash photolysis of caged ATP.

2PS014 細胞性粘菌ミオシン II の SH1 ヘリックス領域の変異がその運動特性に与える影響

Effect of mutation of the SH1 helix region of Dictyostereum myosin II on the motile characteristics

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A human myopathy has been reported to be caused by a point mutation in a fast myosin heavy chain gene. The mutation, glutamic acid to lysine at the 706th amino acid, is located at the SH1 helix region of the myosin II motor domain. The SH1 helix region acts as a linker for transmitting the structural changes of ATP-binding site in the catalysis domain to the lever arm. To investigate the effect of the mutation on the actin-myosin motility, we have introduced a corresponding mutation into the SH1 helix of Dictyostereum myosin II (E683K). The mutation resulted in a decrease in the actin-myosin sliding velocity (about 1/8 of the wild type), a decrease in thermal stability and the thermal aggregation of the myosin, which might be implicated in the disease process. This suggests that SH1 helix has an important function in the motor activity.

2PS015 ミオシン首振り運動の分子動力学シミュレーション
Molecular dynamics simulation for the swinging lever-arm motion of a myosin molecular motor

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I have proposed a theory called "Driven by Detachment (DbD)" mechanism for the working principle of myosin molecular motors. In this theory, the power stroke of myosin is not directly caused by the hydrolysis energy of ATP, but is produced by distortion in a myosin molecule, which is generated during the docking of myosin against an actin filament.

To verify the validity of this theory, a series of molecular dynamics (MD) simulation have been conducted. However, it was found that very long calculation time is necessary to generate the distortion in a myosin molecule during the docking process. Therefore, instead of directly producing the pre-power stroke configuration, external force was applied to the end of neck domain of a myosin molecule.

The MD software used was Gromacs code with its default Gromos96-43a1 force field and SPC water model. The initial structure of myosin was taken from PDB file 1M8Q. The AFM pull function of Gromacs was used to apply external force to the end of the neck domain toward the minus-end direction of an actin filament. The residues assumed to be engaged in the docking were fixed to the space.

When the applied force was 17 pN (10 kJ/(mol*nm²)), the neck domain bent about 60 degrees around the junction between the head and neck domains after 48 nm. If the applied force was removed, the neck domain returned to the initial position. As shown in these simulations, the junction between the head and neck domains works as an elastic element that can produce the power stroke of myosin molecular motors.

2PS016 Movement of cortical actin cables is driven by type V myosins in fission yeast cells during interphase

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Actin cytoskeleton is involved in various cellular activities such as cell morphogenesis, membrane traffic, polarized growth and cytokinesis. In the fission yeast *Schizosaccharomyces pombe*, actin filaments form three major structures; patches, cables and contractile rings. During interphase, actin cables extend from the growing ends toward the cell equator. Although actin cables have been shown to be important for establishment of cell polarity, their detailed behavior has still not been clear. We found that thick actin bundles were formed 15 min after addition of the actin stabilizer, Jasplakinolide (Jasp), in interphase fission yeast cells. Interestingly, these actin bundles showed an oscillatory movement along the long axis of the cell. We aimed to elucidate the mechanism of this movement. Most of the actin bundles moved along cell cortex at a maximum speed of $0.14 \pm 0.04 \mu\text{m}/\text{sec}$. To identify factors which are involved in the movement of the actin bundles, we treated various mutant cells with Jasp. We found that type V myosins and the formin For3 are required for the rapid movement of actin bundles. Especially, Myo51/Myo5 (Win et al., 2001; Motegi et al., 2001), which is one of the two type V myosins in fission yeast, colocalized with actin cables at the cell cortex. These results suggest that actin cables elongating from ends of interphase cells are guided toward the mid-region by cortical type V myosins.

2PS017 Contribution of the conformational change of myosin II in the coupled sliding and binding process of the force-generation

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The mechanism of the actomyosin motor is still under debate. In the most widely believed lever-arm model, it has been hypothesized that the mechanical force is generated by the conformational change of the myosin head which is strongly bound to the actin filament. In contrast, a single molecule observation has shown that the unidirectional Brownian motion of the myosin head, while keeping weakly bound to the actin filament (Kitamura et al., Nature (1999)). Furthermore, a molecular dynamics simulation study has suggested that the unidirectional Brownian motion is indeed possible due to the asymmetric slope of the energy landscape arising from the electrostatic interaction between actin and myosin (Takano et al., PNAS (2010)). In that simulation, however, the