mechanochemical energy translation, but not the actual torque generation step within the cycle by the stator-rotor interactions.

3P154 大気中及び液中における軟体動物ミオシン分子の構造変化 : AFM 計測と脆弱部位の予測 Conformational changes of single oyster myosin molecules in air and in solution: Atomic force microscopy and prediction of fragile points

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Myosin is a mechano-chemical motor that converts the chemical energy of ATP hydrolysis into mechanical energy. Myosin molecule has two heads and a long tail. Muscle contraction is regulated by calcium ion. In invertebrates, notably molluscan muscles, the regulation is associated with myosin. Therefore it is important to know whether the coiled-coil structure of the rod is regular or not. The purpose of this research is to explore the conformational flexibility of single oyster myosin molecules in salt solutions directly using AFM. We successfully imaged single myosin molecules in solution and in air, respectively. In the absence of CaCl₂, these molecules are mainly in the extended form. In the presence of CaCl₂ the rods bend sharply at specific positions. We determined some flexible regions of bending in a single myosin rod in solution and in air by AFM imaging analysis. The results showed four flexible regions in a single myosin rod, but the angular dependency to each bending position seems slightly different in solution and in air, respectively. These bending positions of oyster myosin rods were compared to those of rabbit skeletal myosin rods (Tanizawa et al., 2009). We also determined the fragile points and heptad breaks in a scallop myosin rod using our prediction system (SOSUicoil). All of these values predicted were located in the bending positions of scallop myosin rods using EM Images. We discuss that the conformational specificity of myosin around the fragile points may be tightly coupled to muscle contraction.

3P155 ベンモーターにおける温度依存トルクステップの解析 Analysis of torque steps of the bacterial flagellar motor induced by heating

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We developed a simple method of temperature control for single molecule measurements and reported an application for Na⁺-driven chimeric flagellar motor in *E. coli* (Annual meeting, 2009). We found that the transient heating over 40°C can induce the stepwise change of torque in a reversible fashion. To understand the mechanism of the torque change, we examined the torque steps of the chimeric motor and the temperature-sensitive mutants using back-focal-plane interferometry.

Long heating of the chimeric motor at ~40°C for 10-20 min induced the slower change in torque than that measured in the transient heating for 1-2 min. Analysis with an automatic step-finding algorithm showed that the step size of the torque change decreased with increasing the heating time. However, maximum number of the torque steps, 10-13, did not depend on the heating time up to 20 min. Point mutation in a stator protein at a possible interface to a rotor protein showed similar results at 5-10°C lower temperature: reduced step size of torque and comparable maximum number of the steps. Since it is reported that maximum number of the torque generation in a motor is > 11 (Reid et al., 2006), each step likely reflects dissociation or incorporation of the stator.

Thermally induced stator dynamics and the change in the unitary torque found in this study may be a key to understand the mechanism of torque generation. Further test of the local heating with a new system would be discussed in the meeting.

3P156 蛍光顕微鏡を用いた F-actin と cofilin の協同的結合の観察 Cooperative binding of cofilin to actin filaments observed by fluorescence microscopy

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Cofilin binds to actin filaments and severs them at neutral pH, but at acidic pH, binds to actin filaments without severing. EM observation showed that binding under the latter condition is cooperative, in a sense that cofilin forms clusters along the filaments, while other parts of the filaments are bare (Galkin et al., 2001), which presumably depends on the shortening of helical pitch of the filaments induced by cofilin binding (McGough et al., 1999). In this study, we observed this binding using fluorescence microscopy in order to observe the binding events along large number of filaments in a semi-quantitative manner.

We mixed 5 μ M of Alexa 488-labeled skeletal actin filaments with various concentrations of cofilin-mCherry at pH6.5, fixed them with EDC, and diluted for observation. In the presence of 10 μ M cofilin-mCherry, most of the filaments were very short with bound cofilin-mCherry. In 2.5 μ M cofilin-mCherry, we observed both short actin filaments with bound cofilin-mCherry and longer filaments without detectable fluorescence of cofilin-mCherry. Additionally, some filaments had both bound and unbound regions along the lengths. The fluorescence intensity of cofilin-mCherry is more or less uniform in the bound regions, suggesting that there is only one mode of cooperative binding under this condition. These results are qualitatively consistent with the earlier EM observation. We plan to investigate the effects of bound nucleotides and Pi, as well as of ageing, on this cooperative binding.

3P157 DIFFUSIVE MOVEMENT OF A SINGLE-MOLECULE MAMMALIAN CYTOPLASMIC DYNEIN

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Cytoplasmic dynein performs almost all the minus-end-directed microtubule-based movements in most eukaryotic cells. Unlike kinesin and myosin, the motile mechanism of dynein is not sufficiently understood. An in vitro motility analysis showed that a single molecule of porcine cytoplasmic dynein didn't move along a microtubule unidirectionally nor processively (Miura et al. 2010). To further investigate the behavior of mammalian cytoplasmic dynein, we isolated functional human dynein complex from HEK 293 cells expressing multifunctional GFP-tagged dynein intermediate chain (Kobayashi and Murayama, 2009) and conducted single-molecule analysis. The human dynein showed biased diffusive movements on microtubules with the drift velocity of 64±8 nm/s and the diffusion coefficient of 2.5±0.1×10⁴ nm²/s. To estimate the contribution of thermal fluctuation to these diffusive movements, we executed the single molecule analysis without ATP to eliminate the effect of the energy from ATP hydrolysis. We measured dwell times and diffusion coefficients in the presence of ADP, ADP with Vi, AMP-PNP, or no nucleotide. The human cytoplasmic dynein was most diffusive in ADP-Vi condition and least in ADP condition. In these diffusive movements, dynein doesn't consume any energy released from ATP hydrolysis, suggesting that thermal energy is the primary source of movements. These one-dimensional diffusive movements may relate to the working mechanism of mammalian cytoplasmic dynein.

3P158 低温におけるアクトミオシンモーターの一分子計測 Single molecule measurement of actomyosin motors at low temperature

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Myosin generates force and displacement in a timescale of sub-millisecond using ATP hydrolysis (Tanaka et al., 1998). Unitary 5.3 nm step of skeletal myosin in a single turnover of ATP was detected by scanning probe nanometry (Kitamura et al., 1999). However, the multiple steps in a single ATP turnover is still controversial, since the steps have not been reproduced by optical trapping nanometry, which is widely used in the studies of motor proteins with a time resolution of a few millisecond. We previously constructed a new cooling chip to slow down the myosin kinetics (Takeda, master's thesis). In the present study, we tested the cooling chip for detecting the unitary steps using optical trapping nanometry. Force and displacement of actomyosin was measured on skeletal myosin molecules fixed on a coverslip by manipulating single actin filament with an attached bead (single trapping) or two beads (dual trapping) using optical tweezers. In single trapping, rising speed of the actin filament drastically decreased at low temperature, however maximum forces were constant at wide range of temperature. From these results, our system is useful for measuring the interaction between actin and myosin by controlling of temperature. To detect the step of single myosin molecule, experiments in dual trapping with decreasing number of active motors at low temperature is now in progress. In the meeting, we hope to show an evidence for the multiple unitary steps of skeletal myosin and to discuss about mechanism of myosin motors.

3P159 Impact of the off-axis loads on the processivity of myosin VI

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Myosin VI is perhaps the most unconventional actin-based motor, which puzzled the researchers over the world with several unique features of its structure