2P-160 ミオシンは単分子より多分子の方がエネルギー消費を節約できるのはなぜか

Why the energy consumption of myosin can be reduced when they work together?

Takeshi Nakagawa (1), Hiroto Tanaka (2), and Kazuo Sasaki (1). (1: Dept. of Applied Physics, Tohoku Univ.; 2: PRESTO, JST)

It is curious that the distance one myosin interacting with the actin filament moves by the consumption of 1 ATP (the interaction-length, IL) is found to be 5-15nm and 60-200nm in the experiment of single and multiple myosins, respectively. At the last annual meeting one of us (HT) suggested a model of multiple myosin molecules which are connected with springs and move on a two-dimensional chemo-mechanical potential surface (BSJ 2007). It was demonstrated that IL of multiple myosins is longer than that of single myosin. Due to the complexity of the potential, we cannot intuitively understand the mechanism underlying such behavior of multiple myosins.

To understand the mechanism it would be worth constructing a simpler model that captures the essence of the above model. We have succeeded to construct a ratchet model which shows longer IL for multiple myosins than that for a single myosin. This model will allow us to gain insight into why the energy consumption per unit distance of a myosin in multiple myosin system can be lower than that of one myosin.

2P-161 キネシンの協調的な二足歩行運動に対するネックリンカーの役割

Role of the neck linker on the coordinated processive movement of kinesin dimer

Hiroshi Isojima(1), Yuji Toyokita(1), Teppei Mori(1) and Michio Tomishige(1). (1: Department of Applied Physics, The University of Tokyo)

Kinesin is a motor protein that is involved in intracellular transport, and has recently been shown to walk along microtubules by alternately moving two motor 'heads'. Such processive movement requires a mechanism for coordination between two heads, and to explain this mechanism a model has been proposed that internal tension built up through the neck linker is essential for the head-head communication. To test this idea, we reduced the tension posed to the neck linker by extending the neck linker using poly-Gly insertions and observed conformational states of these mutants using single-molecule fluorescence resonance energy transfer. Under low nucleotide (ADP) conditions, where wild-type kinesin is known to take one-head-bound state, the neck linker extended mutants stably took two-head-bound state. We also found that in this state the neck linkers in the two heads extended rearward and took similar conformations. These neck linker conformations are distinct from those observed for wild-type kinesin in two-head-bound state whose neck linkers in front and rear heads point rearward and forward respectively. These results suggest that the optimal size of the neck linker is important for maintaining the opposite geometry of the two neck linkers, which might be involved in the head-head coordination.

2P-162 走査プローブと光ピンセットの実験結果の相違を説明するミオシンの運動モデル

Construction of myosin model explaining difference between experimental results observed with scanning probe and optical tweezers.

Hiroto Tanaka. (PRESTO, JST)

Single molecule measurement (SMM) techniques have been applied to myosin. Then, SMMs' results show that, during single ATP hydrolysis cycle, myosins II & V repeat several cycles of association with- and dissociation from an actin filament to generate sliding motion, suggesting that myosin can convert ATP energy by multi-step processes (MSPs). This MSPs cannot be explained by conventional "lever-arm model", then "Biased Brownian motion (BBM) model" has been proposed for a mechanism of myosin. However, the MSPs have been observed only by SMM with scanning probe (SP), and not observed with optical tweezers (OT) widely used for SMM. Because MSPs have been observed clearly with myosin II & V, it is strongly suggested that BBM is movement mechanism of myosin. Then, why have MSPs not been observed with optical tweezers? In order to answer this question, here, we construct model including characteristics of SP & OT, and simulate movement of myosin attached to measurement probes (SP or OT). Taking into account the effects of measurement probes, we construct 2-dimensional potential along an actin filament, and simulate movement of myosin on the 2D potential by Monte Carlo method. For simulation, spring constants of probes parallel and perpendicular to an actin filament are set according to characteristics of each probe. As a result, sliding velocity with SP (~0.5 $\mu\text{m/s}$) becomes slower than that with OT (~3 $\mu\text{m/s}$), then MSPs are clearly observed with SP. This result explains well the experimental results with SP and OT.

2P-163 一分子 FRET を利用した F_1 -ATPase の触媒サブユニット β の構造変化の検出

Detection of conformational changes of the β subunits in F_1 -ATPase by FRET

Masaru KOBAYASHI(1), Takayuki NISHIZAKA(1), Tomoko MASAIKE(1). (1: Department of Physics, Gakushuin University)

F_1 -ATPase is a rotary molecular motor in which the central γ subunit rotates against hexagonally arranged $\alpha_3\beta_3$ subunits. Rotation of the γ is assumed to be driven by conformational changes of the catalytic β subunits during ATPase turnover. Changes in orientation of the C-terminal domain of β against the N-terminal domain during rotation of γ have been detected by the polarization modulation system (Masaike *et al.*, 45th Biophysical Society Meeting of Japan,

2007). To reveal the mechanism of driving rotation from the viewpoint of inter-subunit distances of domains, we have applied fluorescence resonance energy transfer (FRET) method to measure changes in absolute distance between fluorescent molecules attached to C-terminal residues of two different β . Cysteines were introduced into C-terminal domain of the β and labeled with either Cy3- or Cy5-maleimide.

Inter-subunit FRET between Cy3 and Cy5 attached to different β in the $\alpha_3\beta_3\gamma$ was detected by spectrofluorometer. To reveal the relationship between stepwise changes in FRET efficiency and rotation of the γ , we developed an optical system for simultaneous detection of Cy3 and Cy5. Alternate-stepwise intensity changes of Cy3 and Cy5 were observed under a fluorescence microscope. Additionally, in the present system, the micro beads attached to the γ subunit is simultaneously monitored in another wavelength. New mutants will be designed and tested if larger changes in FRET efficiencies are observed using a spectrophotometer and also under the microscope at the single molecular level.

2P-164 負荷存在下でのキネシンの構造状態の一分子 FRET 観察

Single molecule FRET observation of the conformational state of kinesin under load

Shota Kobashigawa(1), Michiko Nakajima(1), and Michio Tomishige(1). (1: Dept. of Applied Physics, School of Engineering, Univ. of Tokyo)

Kinesin is a motor protein that converts chemical energy derived from ATP hydrolysis to the mechanical work to move processively along microtubules. Optical trapping studies showed that the velocity is reduced as the external load applied to kinesin increases. However, it is still unknown which conformational transition in ATP hydrolysis cycle is the load-dependent step. Last year, we reported that using rapamycin-FK506 binding protein (FKBP) crosslinking system we crosslinked two kinesin dimers and observed the movement of this kinesin complex along microtubules. In this study, we replaced one of the two crosslinking kinesins by a mutant kinesin that tightly binds and cannot detach from the microtubule. Then we used this complex to apply load to the other wild-type kinesin dimer and observed its conformational state on the microtubule using single molecule fluorescence resonance energy transfer technique. We found that at saturating ATP concentration, the wild-type kinesin spent most of the time in one-head-bound state, which is clearly distinct from the configuration of wild-type kinesin before crosslinking that spends most of the time in two-head-bound state. These results suggest that the transition from one- to two-head-bound state might be prohibited under high load.

2P-165 分子モータの逐次性に関する理論的研究

Theoretical study of processivity of double-headed molecular motors

Noriko Tsuchiyama and Takahiro Harada. (Department of Human and Artificial Intelligent Systems, University of Fukui)

For linear processive motors such as kinesin, processivity is one of the most important functions. By experimental studies on various motors, the relations of processivity to a molecular structure and to environmental parameters have been reported. In order to clarify the factors affecting processivity of a molecular motor, construction of a simple mathematical model is highly important, because we can survey wide range of parameters with a relative ease. This enables us to study the general properties of linear processive motors on the basis of the mathematical model. However, a satisfactory model to explain existing experimental data has not been presented. Here, we studied processivity of linear processive motors by constructing a simple mathematical model. In order to take a detachment of a motor into consideration, we constructed a two dimensional model by extending a flashing ratchet model. Moreover, the motor was made double-headed by elastically coupling two heads. As a result, we confirmed that this model can make a sliding motion in a hand-over-hand fashion. Furthermore, it was found that the dependence of the run length on ligand concentrations in this model is consistent with the previous observations for kinesin. As a next step, we will examine whether this model can successfully explain the experimentally observed dependence of processivity on other parameters including the temperature and the magnitude of a load. In the presentation, we report the detail of our numerical investigation of this mathematical models.

2P-166 II 型トポイソメラーゼが手品のように DNA の絡まりを解くところを一分子操作・観察する

Single-molecule observation of unlinking magic by type-II topoisomerases with real-time control of DNA substrates

Katsunori Yogo(1), Taisaku Ogawa(1), Saki Obata(1) and Kazuhiko Kinoshita Jr(1). (1: Dept. of Phys., Grad. School of Science and Engineering, Waseda Univ.)

Type-II DNA topoisomerases (topoII) are molecular machines that play key roles in the maintenance of DNA topology in cells. They control the degree of supercoiling of DNA and untangle the catenanes that arise during replication or recombination. Lack of their activities during cell division ultimately causes cell death. TopoII untangles DNA catenanes in an ATP-dependent manner, by catalyzing the transport of one DNA segment to the other side of a second DNA segment through a transient double-stranded break in the second segment. The work of topoII would seem like that of a magician who fascinates the audience. We developed the system that enables us to observe directly this unlinking magic under an optical microscope. Single-molecules of DNA are fixed on chamber surface and mechanically braided with dual optical tweezers. One of the advantages of this method against bulk assay is that we can control geometry and tension in the DNA braid in real time and observe unlinking reaction repeatedly