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Monitoring functions and variations of expression profiles in a cellular system is a key to understanding the mechanisms of living organisms, especially to identifying those phenotypical characteristics. The *in situ* labeling for expressed biomolecules is one of useful methods to visualize amounts and spatial distributions of such molecules, and detailed measurement in single cell, electron microscopy (EM) is well used with gold nano-particle labels. At the simultaneous labeling of several molecules, different sizes of particles are used; that yielded derogation of quantitative capability because of the difference of labeling efficiency depended on the sizes of particles. Moreover, size variation of practicable particles is not so many; therefore, new labeling method should be developed for exhaustive study of expressed biomolecules in a cell.

We tried to establish a new labeling technology in the EM, which develops conventional labeling of the EM to "multi-color" one. We prepared nano-particles with different metals in the same size and used it as "multi-color" label. To evaluate labeling ability of these particles, probe DNAs were immobilized on the particles and target DNAs on a substrate were labeled. Kinds of the labeled particles were identified by measuring backscattered electron using a field emission scanning electron microscope. Our method would achieve simultaneous measurement of exhaustive molecular expression in a single cell.

2TA3-09 細胞内の局所的な温度変化の測定とその解析

2P-258 Measurement and analysis of local temperature change inside living cells.

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Activity of proteins such as Ca^{2+} -ATPase is thermosensitive and thermogenic. Feedback loop between their activity and local temperature change may play an important role in biological systems. But there have been no experimental evidence that a cell uses intracellular temperature change effectively for intracellular processes. Recently, a few techniques have been developed to measure temperature change inside single living cells. We and other groups, using fluorescent thermosensitive polymers, have shown the intracellular temperature elevation of about 1°C that was induced by chemical stimulations such as ionomycin (Suzuki, M. et al., 2007, *Biophys. J.*, **92**: L46-L48) and FCCP (Gota, C. et al., 2009, *JACS*, **131**: 2766-2767). However, single cells cannot produce enough amount of heat to explain these phenomena from the viewpoint of calorimetric studies with cell suspension.

In this study, we tried to explain this gap by measuring and changing local temperature inside HeLa cells. Aims of this study are (i) the detection of localized heat sources in a cell which could rise temperature heterogeneously during thermogenesis induced by ionomycin, and (ii) the determination of cellular thermophysical coefficients. Our results showed that intracellular temperature elevation induced by ionomycin was relatively uniform. We found that the thermal diffusivity of a living cell was less than that of water. The value, however, was insufficient to bridge the gap. Therefore it is suggested that there are other missing viewpoints to connect the data from single cells and those from cell suspensions.

2TA4-01 機能的細菌べん毛モーターにおけるモーター構成素子の交換割合の見積り

Turnover of subunit-exchange in functioning bacterial flagellar motor

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Bacterial flagellum is a supramolecular complex and consists of a basal body (rotary motor), a helical filament, and a hook. The flagellar motor is driven by the electrochemical potential of ions, and the interaction between stators and a rotor in flagellar motor is thought to generate the motor rotation. Recently, it was shown that stator parts are exchanged in a functioning motor and that the assembly of stator into the motor requires coupling ion for the motor rotation. In our study, we focused on the dynamics and the exchange of rotor components in a functioning motor. We constructed the expression systems of GFP-FlhN, FlhM-GFP, and GFP-FlhG as GFP-fusions of rotor component. In tethered cells producing each GFP-fusions, fluorescent spot was localized at the rotational center. Each GFP fusion was probably incorporated into flagellar motor as a rotor component. To observe the exchange of rotor components, we carried out Fluorescent Recovery After Photobleaching experiment using evanescent illumination to the tethered cell. In a tethered cell producing GFP-FlhN or FlhM-GFP, the recovery of fluorescence at the rotational center was observed after the complete-photobleaching. On the other hand, in a cell producing GFP-FlhG, the

recovery of fluorescence was not observed. From the complete- and partial-photobleaching procedures for the cells producing of GFP-FlhN, the rate constant of FlhN-dissociation was calculated as 0.0007 s⁻¹. Thus, FlhN and FlhM, at least, could be exchanged in a functioning flagellar motor, and FlhN subunits would be exchanged in tens of minutes. These results would mean that a bacterial flagellar motor is not a stable structure and exchanges their component flexibly even in functioning state.

2TA4-02 高温におけるキメラべん毛モーターの回転トルク応答

2P-132 Torque response of bacterial flagellar motor induced by transient heating.

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We have demonstrated the torque-speed curve of Na⁺-driven chimeric flagellar motor in *E. coli* (Inoue et al., 2008). The chimeric motor produced almost constant torque at constant temperature, as reported for WT motor in *E. coli*. We found that transient heating over 40°C can induce torque change in a reversible fashion. To understand the mechanism of the thermal response of torque, we examined effect of high temperature on the chimeric motor using back-focal-plane interferometry.

Heating at 40-50°C for 2-5 min induced reduction of motor torque (reducing phase) and cooling to room temperature induced resurrection of torque (resurrection phase) mostly in several minutes. In the resurrection phase, torque recovered in a stepwise manner. Histograms of resurrection phases measured with 0.35-1.0 μm bead showed several peaks up to 11 with unitary torque of 190-210 pN nm. The results are consistent with the report showing that the motor can be equipped with ~11 torque generating units (Reid et al., 2006). Reducing phase of torque also showed similar peaks in the histograms with unitary torque of 180-200 pN nm.

These results suggest that active number of torque generating units in a motor can be controlled by manipulating temperature. Thermal response of torque may be a key to understand the dynamics of motor components and the mechanism of torque generation.

2TA4-03 F₁-ATPase のストールトルク Stall torque of F₁-ATPase

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F₁-ATPase is a rotary molecular motor; hydrolyzing ATP to ADP and phosphate, the central γ subunit rotates unidirectionally. On the other hand in cells, it works as a part of F₀F₁-ATP synthase and rather synthesizes ATP from ADP and phosphate with rotating in the opposite direction of hydrolytic rotations. Thus, it is crucial to study the rotations in the synthetic direction to reveal the nature of F₁-ATPase. Here, we applied an external torque to F₁-ATPase in the synthetic direction using an electrorotation method. We found that F₁-ATPase shows stepwise rotations in the synthetic direction at a sufficiently strong torque in the presence of ATP, ADP, and P_i. The torque necessary to rotate the wild type F₁-ATPase in the synthetic direction times 120° was almost equal to the free energy change of a single ATP hydrolysis under various conditions. On the other hand, smaller torque was enough to rotate mutated F₁-ATPase (βG181A, T165S, Y341W) in the synthetic direction.

2TA4-04 チューブリンC末端の伸長した構造が弱結合に果たす役割

2P-134 The role of extended C-terminal tail of tubulin on weak-binding interaction between motor proteins and microtubules

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The mechanism of weak binding between motor proteins and microtubules (MTs) is not fully understood in spite of previous kinetic, structural and motility studies. To get a comprehensive picture of the weak-binding interaction, we combined two methods: simulation of the weak-binding interaction using positively charged nanoparticles (made of polyacrylamide gel, ~60 nm in diameter) and MTs, and in vitro motility assay using mutant MTs. The charged particles show one-dimensional (1D) Brownian motion on MTs in a charge-dependent manner. Analysis of this interaction using mutant MTs revealed that the C-terminal tail (CTT) of tubulin is not essential for 1D Brownian motion of the charged particles, but the duration of Brownian motion was significantly reduced by the deletion of the CTTs. There are two possible interpretations for this observation: 1) 1D Brownian motion of the charged particles was prolonged merely due to the negative charges on the CTTs, and 2) prolonged interaction of the charged particles with MTs is a result of both charge effect and the extended structure of the CTTs. According to the latter interpretation, the structure of CTTs extending into the solution may prevent the charged particles from firmly attaching to the surface of the MTs. To confirm which of these interpretations is true, we are studying the motility of the particle/kinesin along those mutant MTs having the

same number of negative charges but varying CTT lengths. The results of this analysis will be presented in the poster.

2TA4-05 KIF1A の 1 次元ブラウン運動にチューブリン C 末端は必要か？

2P-135 Is the tubulin C-terminus tail necessary for the 1D Brownian motion of KIF1A?

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A single-headed kinesin superfamily protein, KIF1A, can move processively along the microtubules for more than 1 micrometer. In contrast to the unidirectional motion of two-headed kinesin, KIF1A displays a biased Brownian motion along microtubules. Previous study using KIF1A mutants demonstrated that the key mechanism for the single-headed processivity lies in the weak, electrostatic interaction between the positively charged, lysine-rich loop of KIF1A (K-loop) and the negatively charged, glutamate-rich C-terminal region of tubulin (E-hook). Two different explanations are possible for the mechanism of this electrostatic anchoring; (1) KIF1A moves along the microtubule by hopping through the fluctuating E-hook, like Tarzan swinging on the vine. (2) The raw of negatively charged C-terminal tails produces a uniform electrostatic track on the microtubule protofilament and KIF1A moves along this track like a monorail, with K-loop as the flange on the wheel. To examine which is actually the case, in this study, we prepared a series of mutant microtubules with their C-terminals tails varied in their lengths and/or number of net negative charges and tested the interaction of KIF1A. Our preliminary result showed that, on the contrary to the previous report using subtilisin-treated microtubules, KIF1A motor can move along the microtubules without C-terminal tails. Our result challenges the Tarzan model and rather supports the latter mechanism.

2TA4-06 細胞質ダイニンのステップ機構

Walking mechanism of cytoplasmic dynein

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Cytoplasmic dynein is a two-headed molecular motor, which takes hundreds steps along a microtubule (MT) without dissociation. To elucidate the mechanism of this processive motion, we expressed the hetero-dimeric dynein constructs, in which one domain completely lost its ATP-binding activity due to the mutation in the Walker A motif in its AAA1 module (P1T mutation). Our single-molecule motility assays showed that the heterodimeric dynein of the wild type and the P1T mutant (Wild/P1T) moved processively on MT with its velocity approximately 40% of that of the wild-type homo-dimer. Because P1T mutant head in the heterodimer cannot dissociate from MT by itself, this processive motion suggests that the wild-type head pull the P1T head off from MT. Therefore, intramolecular tension between two heads is predicted to play an essential role in the processive movement of dynein. We then examined the effect of intramolecular tension through the tail domain linking the two heads. We inserted a Gly-rich flexible linker with 20 or 30 residues between the tail domain and the hetero-dimerizer to reduce the tension. Unexpectedly, the wild-type homodimer and the Wild/P1T hetero-dimer with the flexible linker moved processively; their run length and velocity were similar to those of the homo- and hetero-dimer without the flexible linker, respectively. These results suggest that the tension through the tail domain does not play a critical role in the processive motion. The direct interaction of the two AAA rings in the dynein heads may be responsible for sustaining alternative steps of the two heads on MT.

2TA4-07 ダイニンの力発生に伴う構造変化

Structural changes in dynein molecules accompanying force generation

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Oscillatory bending of cilia and flagella is driven by dynein molecules, which move along a doublet microtubule (MT) to cause relative sliding of the MTs. Because the neighboring doublet MTs are interconnected by the nexin link structures and also fixed at their bases on a basal body, dynein has to move against the mechanical stress provided by these connections. In order to understand the roles of dynein molecules in cilia and flagella, it is important to know the structures of dynein during movement against the mechanical stress. One of the models that explain movement of dynein is the stalk-rotating model, in which the stalk of dynein changes its angle to push a MT. However, we found that the dynein stalk did not change its angles relative to the MT between the ADP/Vi state (in the presence of ATP and vanadate) and the no nucleotide state (with apyrase), which are thought to correspond to the pre-powerstroke and post-power stroke states, respectively. Instead, we observed shift of the head and stalk with

respect to the tail-MT attachment. Based on these results, we proposed a model in which dynein pulls the MT by shortening the distance between the head and the tail-MT attachment. Then, how does dynein change the head position and the stalk orientation during movement against mechanical stress? To investigate the structures of dynein under mechanical stress, we are developing a new complex that comprises dynein, MTs, and double-stranded DNA. Preliminary in vitro motility assays showed extension of DNA upon addition of ATP.

2TA4-08 in vitro 滑り運動中のミオシン頭部の構造と SH1-SH2 架橋ミオシン頭部の 3 次元再構成像との比較

Reconstructed 3-D structure of SH1-SH2 crosslinked myosin head and its comparison with intermediate crossbridge configuration during in vitro motility

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Quick-freeze deep-etch replica electron microscopy showed that a majority of myosin crossbridges during in vitro motility take a unique configuration, i.e. kinked oppositely to ADP/Vi-form, holding actin filaments inside the curvature. Since its appearance was similar to that of SH1-SH2-crosslinked myosin whose atomic coordinates are not yet solved, we attempted to reconstruct its 3-D structure from replica images by "single-particle analysis", which is usually applied to transmission images like electron cryo-microscopy, of the particles much larger than myosin heads. Utilizing the high contrast by metal-shadowing, we classified the images of SH-crosslinked heads and made the average for each class. Each averaged image was subdivided into the motor-domain and the leverarm, and their Euler angles were separately determined using "differential structure detection method" (Kimori et al., 2007). Since the motor-domain of Cys-Lys-crosslinked myosin (1L2O.pdb) best-resembled the new one, among myosin conformers, it was used as a template for the later analyses. Relative orientation of two modules in each class-average matched nicely to each other, assuring the validity of our estimation. We made a tentative model of a new structure by relocating two modules according to their relative angles. After reconstruction of the 3-D shell from class averages and their angles, by a new procedure, we compared the images of actin-attached myosin heads during motility, with the above structure as a reference. Among three class-averages with different appearance, two of them showed the arrangement of two modules similar to the reconstructed shell, but the torsion angle of the leverarm in the third image was substantially deviated from the standard, possibly corresponding to new "tension-bearing" configuration.

2TA4-09 細胞内の特定のアクチンフィラメントは、ミオシン II の S1 と結合しやすい

Specific subsets of actin filaments in cells have a high affinity for S1 of myosin II.

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Filaments of myosin II are localized to the posterior of migrating cells and to cleavage furrows in dividing cells. However, myosin II molecules are unable to localize to specific sites if filament assembly is inhibited, leading to the widely accepted hypothesis that those specific localizations of myosin II within cells are primarily determined by localized filament assembly/disassembly. We propose an alternative interpretation that the weak affinity between single myosin II molecules and actin filaments make the potential of individual myosin II molecules to localize indiscernible, and reasoned that S1 portion of myosin II may show appropriate localization if the affinity is increased. We thus expressed GFP-fused S1 with mutations to enhance the affinity for actin [i.e., G680A (Patterson, 1998; Batra et al., 1999) and L596S (Uyeda et al., 2002)] in *Dictyostelium* cells. In live imaging, both GFP-mutant S1s indeed localized along specific portions of cell cortex, while GFP-wild type S1 was only diffusely distributed in the cytoplasm. Comparison with localization of actin filaments, after fixation and staining with rhodamine-phalloidin, demonstrated that GFP-mutant S1s preferentially bind to filaments along the cortex in the posterior in polarized cells and the furrow region in dividing cells. They were also intensively localized along thin cytoplasmic strands connecting two cells during cytokinesis C (traction-mediated cytotfission) of myosin II-null cells. Actin filaments with abundantly bound S1 are in general either relatively stable or mechanically stretched, suggesting the possibility that stabilization and/or stretching of actin filaments increase the affinity for S1. We plan to co-express GFP-mutant S1 and mCherry-actin for simultaneous localization of both proteins in live cells.

2TA4-10 アメーバ様細胞の走化性運動の戦略

Strategies for chemotaxis of amoeboid cells

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