lipid dependence, using water-in-oil (W/O) droplets lined by a lipid monolayer. To monitor the gene-expression in real time, we encapsulated the cell-free transcription/translation systems with plasmids encoding GFP in the droplets, and observed the gene expression of GFP using confocal fluorescence microscope. The GFP intensity per unit volume was increased linearly with time, and the change rate was markedly dependent on the droplet size. By analyzing them for ~30 droplets with different sizes from 20 to 100 micrometer, the intensity is found to be proportional to the reciprocal of droplet size. This result indicates that the gene-expression on the lipid membrane is more dominant than that inside the droplet. In addition, we used three types of lipids (DOPG, DOPE, and DOPC) to examine the lipid dependence on their acceleration effects. The degree of the enhancement of the expression reaction follows the order of PG > PC >> PE. These observations are discussed in relation to the significant effects of cell-sized space on the activity of genetic DNA.

3L1534 ErbB signaling - Quantification of protein diffusion and interaction networks in MCF7 cells using FCS and FCCS

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The signaling pathway of ErbB receptors including ErbB1 - 4 is involved in diverse physiological processes such as cell proliferation, differentiation, apoptosis, and carcinogenesis. Systems-level analyses have been recently carried out for this pathway, combining conventional biochemical methods in vitro with computational simulation. However, information about spatiotemporal dynamics and reaction-kinetics of related proteins are largely lacking. It will be worthwhile to directly quantify localization, diffusion, and interaction of related proteins in living cells. We established a live cell FCS and FCCS analysis of the proteins in the ErbB networks. We focused on the quantification of diffusions and interaction of Shc52, Grb, Cbl, and PI3Ks (p85alpha,beta and p110alpha,beta) which are major cofactors for EGF (ErbB1 ligand) and HRG (ErbB3, 4 ligand) signaling process. Analysis using MCF-7 and its two mutant cell lines overexpressing wild-type or mutant ErbB1 indicates that diffusional movements of these cofactors were differently affected depending on the cell types and ligands. Interestingly, direct interactions among them were not found in FCCS measurements, even though interactions with ErbBs were detected for all the factors. These results suggest that spatiotemporal response of these factors with ErbB receptors are independently regulated in living cells, even details of the response have not been described until now. Our study will pave the way toward a new insight for ErbB signaling mechanism.

9:00~12:00 M会場 / Room M

分子モーター 4

Molecular motor 4

3M0912 ミオシン V エネルギー変換素過程の1分子計測

P48 Single molecule measurement of the myosin V energy transduction process

Keisuke Fujita¹, Mitsuhiro Iwaki², Atsuko Iwane¹, Toshio Yanagida¹ (¹Graduate School of Frontier Bioscience, Osaka Univ., ²Graduate School of Medicine, Osaka Univ.)

Myosin V is an ATPase molecular motor that takes 36 nm processive steps along actin filaments in a hand-over-hand fashion. Experiments using gold nano particles have revealed that this movement consists of two processes: a deterministic lever arm swing and diffusional Brownian search. Although both are thought to contribute to the ATP chemo-mechanical energy transduction, details of their contributions remain poorly understood.

Here, we constructed a new optical tweezers system that incorporates a DNA linker to the myosin V to investigate this matter. The optical tweezers exert an external force onto one of the motor domains via the DNA linker, allowing us to observe conformational changes by the lever arm during gait motion. Furthermore, by analyzing the load dependency of the conformational change, we estimated the energy difference between the pre- and post-state of the lever arm to be 3.2 k_BT (that is, maximum work of the lever arm). This was far less than the 14 k_BT work we observed during the Brownian search-and-catch. We further discuss theories on how the Brownian search-and-catch produces mechanical work.

3M0924 クライオ電子顕微鏡法による車軸藻ミオシンの高速移動分子メカニズムの解明

Elucidation of the molecule mechanism of Chara myosin's fast movement by electron cryomicroscopy

Hiroaki Hidaka¹, Hiroshi Mine¹, Kohji Ito², Keiichi Yamamoto², Takuo Yasunaga¹ (¹Department of Bioscience and Bioinformatics, Faculty of Computer Science and Systems Engineering, Kyushu Institute of Technology, ²Department of Biology, Chiba University)

Chara myosin is a motor protein, which is involved in cytoplasmic streaming of *Chara corallina*. The myosin slides along actin cables the fastest among all known myosin classes (~70 μm/s). To unravel the mechanism how Chara myosin moves so fast, the kinetic properties of the myosin have been analysed and showed that Chara myosin has high ATPase activity. In addition, in its ATPase cycle, the ATP binding rate is extremely fast and the ADP release rate is dramatically accelerated by binding to actin (1000-fold). The sequence analysis and electron microscopy showed that Chara myosin consists of a motor domain (motor domain : MD) that belongs to class XI, a neck region with six tandem IQ motifs that serve as binding sites for six light chains, and α-helical coiled-coil domain for dimerization, and a globular tail domain. Nonetheless, its detailed structural features are not clear enough to understand the molecular mechanism of its kinetics.

Thus, we have analyzed the structure of Chara myosin by electron cryomicroscopy and 3D reconstruction and obtained preliminary 3D maps of single myosin head and actin-bound one, which showed that nucleotide-binding pocket should be opened by actin binding. We continue to acquire the refined 3D-structure of Chara myosin MD with/without nucleotides by cluster analysis, which may identify the structural differences in each 2D/3D-particles and enable us to obtain the highly resolved 3D-structure. We will compare them with actin-bound one, which should be also obtained at the nanometer resolutions.

3M0936 シロイヌナズナミオシン 11 は多分子による高速移動に有利である *Arabidopsis thaliana* Myosin XI properties make it ideal for fast transport by multiple myosins

Takamitsu Morikawa¹, Kohji Ito², Atsushi Kimura², Keigo Ikezaki¹, Tomotaka Komori¹, Keisuke Fujita¹, Mitsuhiro Iwaki³, Toshio Yanagida¹ (¹Graduate School of Frontier Biosciences, Osaka University, ²Department of Biology, Faculty of Science, Chiba University, ³Graduate School of Medicine, Osaka University)

Myosin XI is an actin-based dimeric motor protein expressed exclusively in plant cells and involved in cytoplasmic streaming. Like myosin V, myosin XI has six light-chain (calmodulin) binding sites in its neck domain, yet its velocity is ten times faster. Further unlike myosin V, myosin XI works in cooperation with multiple to conduct transport by binding to a single organelle. Here, to clarify how myosin V and XI achieve distinct functions despite similar structures, we studied myosin XI by single molecule techniques.

Arabidopsis thaliana myosin XI was expressed using a baculovirus expression system. To visualize its motility at nanometer precision, we labeled the motor domains with a fluorescent quantum-dot and measured the step size by combining TIRF microscopy and the FIONA method, finding step sizes of ~29 nm, 29 nm and 59 nm. This result indicates myosin XI walks using both hand-over-hand and inch-worm like steps. We also measured the force generated by these steps using optical trapping, finding a stall force of 0.85 pN, less than half that of myosin V (2-3 pN).

The weak force generation and multiple step sizes indicate myosin XI has a relatively flexible structure. We speculate that such flexibility may enable multiple myosin XI molecules to transport organelle quickly compared to other myosins.

3M0948 尿素及び尿素誘導体のミオシン ATP 加水分解活性に及ぼす効果

Effects of urea and its derivatives on myosin ATPase activity

Shin-ichiro Yasui, Altansukh Batnasan, Tetsuichi Wazawa, Noriyoshi Ishida, Takuya Nakagawa, Makoto Suzuki (*Grad. Sch. Eng., Tohoku Univ*)

Myosin heads translocate along an F-actin during cycles of the hydrolysis reaction of ATP involving association and dissociation with F-actin. The effect of cosolvents of aqueous solutions, such as urea, on actomyosin interaction has provided a wealth of experimental information to understand the energetics of ATP hydrolysis. Although the concentration range of urea as a denaturant are in