ployed YFP bimolecular fluorescence complementation (BiFC). In BiFC, two potentially interacting proteins are fused to N- and C-terminal half-molecules of YFP, respectively. If these fusion proteins interact, YFP may be reconstituted. Thus, if BiFC occurs, it would lend strong support for FPR dimer formation. Here, we performed single-molecule imaging of BiFC pairs to directly detect FPR dimers. The two-dimensional dimer dissociation constant was determined to be 3.6 copies/square micron, with a dimer lifetime of 91 ms (dissociation rate constant of 11.0/s) and a 2D association rate constant of 3.1/ [copies/square micron]/s. Under a physiological expression level of ~2.1 receptor copies/square micron (~6,000 copies/cell), monomers continually convert into dimers every 150 ms, dimers dissociate into monomers in 91 ms, and at any moment, 2,500 receptor molecules participate in transient dimers and 3,500 molecules exist in transient monomers.

2P229 Independent response of Shc and PI3K to ErbB signals suggested by FCS and FCCS in living cells

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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 factors in the ErbB pathway. We focused on lateral diffusions and interaction of Shc52 and PI3K (p85a) which are major factors for EGF (ErbB1 ligand) and HRG (ErbB3, 4 ligand) signaling process, respectively. Analysis using MCF-7 and its mutant cell lines overexpressing wild-type or mutant ErbB1 indicates that diffusional responses of the two factors were differently affected depending on the cell types and ligands. Interestingly, direct interactions between Shc52 and PI3K are not found in FCCS measurements, even though diffusional response was clearly activated for both factors. These results suggest that the diffusional responses and interaction of these factors with ErbB receptors are independently regulated in living cells, even details of the response have not been described until now. Analysis of other factors including Grb2, cAbl, cCbl, Cdc25A, Crk, PLCg and PTPN1 is also in progress.

2P230 FCS 解析で明らかとなったビブリオ菌 Na⁺ 駆動型モーターにおける FliF の C 末端と FliG の相互作用 C-terminal tail of FliF is responsible for interaction with FliG in the Na⁺-driven motor of *Vibrio alginolyticus* revealed by FCS analysis

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Bacterial flagellar motor is composed of the rotor and the stator, and torque is generated by their interaction that couples to translocation of the specific ion through the stator. The rotor is composed of several rings and a rod, and among them C ring is involved in torque generation, most closely by FliG. The C ring is mounted on the cytoplasmic face of the membrane-embedded MS ring composed of FliF, and association of FliF with FliG is the crucial step to initiate flagellar basal body formation. Previously, we reported our initial application to detect interactions between FliF and FliG of *Vibrio alginolyticus* by Fluorescence Correlation Spectroscopy (FCS). This time, we applied FCS measurements to mutant FliF proteins and explored regions of FliF responsible for FliG interaction. First, we examined the C-terminal region of FliF by constructing a series of C-terminally truncated FliF variants. We found that truncation of eight residues at C-terminal end abolished flagellar assembly, and FCS measurement of this mutant showed that FliF-FliG interaction was greatly reduced. This C-terminal tail contains a well-conserved tryptophan, and replacement of this residue to alanine (W575A) severely impaired both motility and interaction with FliG. These results suggest that C-terminal tail residues of FliF are responsible for FliG interaction that is critical to flagellar assembly. Role of the C-terminal portion of FliF will be discussed in the poster.

2P231 Mutational analyses of PIP3/F-actin wave nucleation and propagation

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Amoeboid cell movement is essential for a large number of biological processes including phagocytosis, metastasis, and chemotaxis. The major driving

force for amoeboid cell shape changes is actin polymerization at the protruding edge. Elongations of filamentous actin meshwork push out membrane. The regulation of this structure is thought to be complex and involves change in the activity of WASP and SCAR/WAVE complex by Ras, PIP3 and TORC2. Recently, self-organizing reaction-diffusion waves of actin have been observed. These waves vary in shape, change directions, and are capable of pushing membrane forward when they reach the cell perimeter. However, the functions of this self-organization remain unclear. It is necessary to characterize the functions of the waves in the cell movement such as migration, phagocytosis, and chemotaxis. Here, we studied the dynamics of the PIP3, F-actin and other components in Dictyostelium by live cell imaging. In wild-type cells, their dynamics on the substrate-attached area is similar to excitable chemical reaction field, for example spiral wave, target pattern, and propagating wave, starting at stochastic nucleations. In contrast, we observed that in mutants of PIR121 - a negative regulatory component of SCAR/WAVE complex, PIP3 waves fail to propagate after nucleation. When actin polymerization is inhibited by treatment with Latrunculin A, we find that propagation of PIP3 waves is recovered in a dose-dependent manner. We will discuss the function of the wave on chemotaxis.

2P232 フェムト秒レーザー誘起衝撃力による単一細胞の操作と刺激 1C1520 Single cell manipulation and stimulation by femtosecond laser-induced impulsive force

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When an infrared femtosecond laser pulse is focused into water through an objective lens, a stress wave is generated at the laser focal point. The stress wave localizes in micron scale at the threshold of the generation, it is possible to manipulate and stimulate a single animal cell by the impulsive force due to the stress wave. First we quantified the impulsive force by utilizing atomic force microscopy (AFM). From the bending movement of the AFM cantilever, the magnitude was estimated to be 10^{-13} ~ 10^{-11} [Ns], corresponding to the average force of about μ N in μ s timescale. By the force loading, we can detach single cell from a cell culture substrate. For example, the impulsive force required to dissociate leukocyte-endothelial adhesions was estimated as being on the order of at least 10^{-13} Ns, which was reliable in comparison with the force estimated previously by cell force spectroscopy. Furthermore, activity of a single cell was modulated by the impulsive force and monitored it by fluorescence imaging using a calcium indicator (fluo-8). When the laser pulse was focused at a position 10 μ m far from a mouse myoblast (C2C12) cell, fluorescence of the fluo-8 in the cell was increased, suggesting the cell was stimulated by the impulsive force. From these results, we present a potential of the impulsive force as a research tool for cell biology.

2P233 Stochastic cell fate decision in PC12 cells

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Population of cells inherently becomes heterogeneous because of every kind of intrinsic or extrinsic noise. Such heterogeneity leads to apparently stochastic decisions of cell fates including proliferation, differentiation, and death, and affects sensitivity of each cell against external stimuli. To understand the effects of those inherently generating heterogeneity on stochastic cell fate decisions, we have performed the following studies.

We chose PC12 cells to study stochastic cell fate decisions, because we can easily determine those cell states from the morphology of each cell. For instance, those cells are usually spherical in shape during the proliferative phase in the presence of EGF or under the serum-rich environment. In the presence of NGF or serum starved environment, some cells gradually undertake neurite outgrowth and differentiate into neuron-like. Under any circumstance, some cells die of apoptosis (blebbing and shrinkage) and necrosis (expansion and burst).

We counted the time evolution of the number of these three patterns of cell fates in different conditions and made a 3-state cell fate decision model using differential equations to determine rate constants for each cells to decide three different fates. Comparing these parameters, we found that stochasticity of cells in a low serum condition is higher than that in a high serum, and observed higher sensitivity to stimuli from both EGF and NGF in low serum. Those results imply that heterogeneity of a population of cells augments sensitivity of cells.

2P234 マクロファージの細胞固有の貪食特性の娘細胞への伝承現象 Transmission of the intrinsic properties of each macrophage cell in phagocytosis to its daughter cells