dynamics of IMA is important to develop and maintain cell function through regulation of morphology and stiffness of cell surfaces. Researchers have emphasized a focus on filamentous components of IMA (i.e. cytoskeletons), especially actin cytoskeleton, and its dynamics has been well studied. Previously, by using high-speed live-cell scanning probe microscopy (HsLc-SPM), we found microgranular components of IMA (MGs) in leading lamella and revealed that MGs exhibited superdiffusion and moved collectively toward cell body. However, the origin of such dynamics of MGs has been unclear. In this study, by hybridizing HsLc-SPM with fluorescence microscopy, we found that the motion of MGs was partly correlated with the motion of fluorescence-labeled actin. Pharmacological cessation of retrograde flow of actin cytoskeleton disordered direction of the motion of MGs, while a part of MGs remained moving. These results indicated that the retrograde actin flow forced MGs back toward cell body, suggesting that actin cytoskeleton constrained dynamics of MGs. Further observations showed that actin stress fibers developed toward MGs and that vinculin, a focal adhesion protein, was localized at a part of MGs. MGs may be involved in remodeling of actin cytoskeleton to assemble actin stress fibers.

3P-159 デジタル画像相関法により得られた 局所伸展刺激下における細胞骨格ネットワークの不均一な変形分布

Heterogeneous deformation of cytoskeletal network under external stretch processed by digital image correlation method

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Physical force plays a critical role in regulating cell proliferation, growth and death. However, the mechanisms how cells sense the force have been unclear yet. A clue to solve the problem is to simultaneously measure map of mechanical information and the following biochemical signals. As a first step, we started to observe intracellular displacement under external stretch. The purpose of this study is to observe the manner of which the displacement propagates in a cell.

In order to measure intracellular displacement under stretch, a pair of fluorescent image for a cell, i.e. two images before and after stretch, was processed by digital correlation method. The procedure of this method is as follows: First, Identical pixel within the pair is searched by going on their intensity. Next, Coordinates of identified pixels are decided. Last, subtracting the coordinates for every pixels points makes displacement map in a cell. Intracellular displacement was impressed via the movement of glass needle.

We examined whether the applied deformation isotropically propagates into cell body. Actin filaments around the pocked area were moved to the direction of movement of the needle. In addition, some actin filaments away from the pocked area were moved. These results indicate that applied deformation propagated heterogeneously into cell.

We plan to report how the heterogeneous deformation propagates into cell body after local force is added to GFP fusion protein expressing cells by performing time laps observation.

3P-160 伸展刺激に対する細胞の硬化応答は細胞が2サイクル以上の周期伸展を記憶することで消失する

Effect on cellular stiffness response to external stretch by memorizing more than 2 cycles of stretch in a myoblast

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Organisms have an ability to memorize external stimuli. In most case, corporation of some cells enables to perform the ability. However, it has been hardly ever reported that a single cell does. We found memory effect of a single cell. Stiffness of a myoblast cell increases in response to 8% of step-like stretch. However, it remains constant under the condition that 30 cycles of cyclic stretch are added prior to the step-like stretch. This finding implies that cellular stiffness response to step-like stretch is changed by memorizing cyclic stretch. The purpose of this study is to clear the mechanism how the cyclic stretch inhibits the cellular stiffness response. First, we checked if the cyclic stretch destroys actin fiber network because cellular stiffness mainly originates in actin fiber. Fluorescent micrograph for filamentous actin showed that actin fiber network after the cyclic stretch didn't significantly differ from that under static condition. This result suggests that the change in stiffness response by the addition of cyclic stretch is not due to disruption of actin fibers. Second, to examine the minimal effective cycle on the stiffness response, we monitored stiffness response with decrease of the number of cycle. The stiffness response shifted between 1 and 2 cycles. This result implies that 2 cycles are minimum cyclic numbers to inhibit cellular stiffness response. In this meeting, we will also report results when pattern of the 2 cycles stretch is varied.

3P-161 IgE 受容体会合体への Lyn キナーゼの動的リクルート : 2 色同時蛍光 1 分子追跡法による研究

Dynamic recruitment of Lyn kinase to IgE receptor cluster: Dual color single-molecule tracking study

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Recruitment of cytoplasmic signaling molecules to the engaged receptor is a key step for the signal transduction. However, very often, recruitment is detectable only biochemically, and not by imaging the receptor and the signaling molecule. A typical example is given by the recruitment of Lyn kinase, located on the cytoplasmic surface of the plasma membrane to the engaged IgE receptor clusters (Fc ϵ RI). Immediately (~ 30 s) after crosslinking Fc ϵ RI by the multivalent antigen, Fc ϵ RI phosphorylation by Lyn was found, but not Lyn concentration at Fc ϵ RI clusters by confocal imaging. To reconcile these apparently contradictory observations, we explored the possibility that Lyn recruitment occurs very transiently, by simultaneously observing single individual Fc ϵ RI clusters, induced by the multivalent antigen, and each individual Halo-tagged Lyn molecule, expressed in RBL-2H3 cell (real-time overlaying at video rate, with an accuracy of ~ 20 nm). Within 30 s after stimulation, single molecules of Lyn were frequently recruited to Fc ϵ RI clusters very transiently with an exponential residency time of ≈ 160 ms, suggesting that high levels of Lyn accumulation at the receptor cluster detectable by conventional fluorescence microscopy do not occur. Further investigations on the involvement of SH3 and SH2 domains of Lyn are in progress.

3P-162 Na⁺駆動型べん毛モーターのCリング付き基部体精製の試み ATTEMPT TO PURIFY THE HOOK-BASAL BODY WITH C-RING FROM THE Na⁺-DRIVEN FLAGELLAR MOTOR

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The flagellum is the biggest architecture in the bacterial organ. At the base of each flagellum, there is the ion-driven rotary motor embedded in the membrane. *E. coli* and *Salmonella* have the H⁺ driven motor, and *Vibrio alginolyticus* has Na⁺ driven type. The motor is composed of the rotor and the stator, and torque is generated by the interactions between them. The hook-basal body (HBB) is the rotor part of the motor composed of rod, LP-, MS-ring and hook. C-ring is mounted on the cytoplasmic side of the MS-ring of HBB, and believed to involve in torque generation of the rotor side. C-ring is composed of three proteins; FliG, FliM, and FliN. FliG participates most directly in the torque generation among them. To elucidate the mechanism of torque generation, we undertake the isolation of C-ring from the Na⁺-driven polar flagellum of *V. alginolyticus* to investigate rotor-stator interactions. We applied the C-ring isolation method using Triton X-100, established for *Salmonella*, but that resulted in the HBB without C-ring. Consequently, many detergents were tested and we found that CHAPS could solubilize HBB with FliG. Electron microscopic observation showed significant difference between FliG-associated HBB and FliG-dissociated HBB. Currently we are going to analyze more detail structure by cryo-EM. Since FliM and FliN seem to be very fragile and dissociated during the C-ring preparation, we need to improve current protocol to isolate the complete rotor. Updates will be discussed in the meeting.

3P-163 試験管再構成モデルを用いた力刺激による接着関連タンパク質の集積

Force-dependent assembly of adhesion-related proteins in an *in vitro* model of adhesive structures

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Cells adhere to extracellular matrices (ECM) and neighboring cells, and the resulting adhesive interactions are crucial for morphogenesis, migration, proliferation and differentiation of the cells. Cell adhesion receptor proteins, integrin and cadherin are clustered into the adhesive structures, focal adhesion (FA) and adherence junction (AJ), respectively. At these adhesive structures, integrin and cadherin are linked to actin cytoskeleton *via* a plaque of the cytoplasmic proteins, enabling actin cytoskeleton to form and to strengthen mechanical linkage with ECM. Mechanical stresses generated mainly in an actin cytoskeleton are known to enlarge the size of FA and AJ. However, the molecular mechanisms underlying the force-dependent protein assemblies are largely unknown mainly due to the complex structures of the FA and AJ. In this study, we developed an *in vitro* model for the force-dependent molecular assembly at adhesive structures. The model composed of 5 μ m NEM-myosin-coated glass beads linked to a cover slip coated with NEM-myosin *via* actin filaments, which were embedded in HeLa cell extract. By centrifuging the model system, mechanical forces (0.18-30 nN for 5 min) were applied to the bead. After the centrifugation, samples were fixed, immunostained against FA and AJ related proteins. The staining showed that the force facilitated the accumulation of FA and AJ related proteins between the beads and the glass surface. Accumulation of