binding of D-cofilin was also observed for F-R-actin, suggesting that the cooperative binding of cofilin was not origin-dependent phenomenon. We are now examining the cooperative binding of D-HMM to F-D-actin. We will present detailed data of the cooperative interactions containing mutually exclusive binding at this meeting.

2PS040 棘皮動物コラーゲン性のキャッチ結合組織における可逆的な硬さ変化の分子機構

Molecular mechanisms of reversible changes in stiffness of echinoderm collagenous 'catch' connective tissues

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'Catch' connective tissues shown in echinoderm animals such as sea urchins, sea stars, and sea cucumbers can extensively and reversibly change their stiffness in a few minutes under the regulation of the nervous system. Neurotransmitters such as acetylcholine, adrenaline, and some oligopeptides cause changes in the stiffness of the living pieces dissected from these tissues. They are no more effective, however, after the treatment with detergent solubilizing cell membranes. There are some granule-containing cells in the tissues, which are assumed to secrete some factors under the regulation of nerves causing changes in the tissue stiffness. The tissues contain a large amount of the extracellular matrix mainly consisting of collagen fibrils. It seems that cross-linking between the fibrils are formed or broken during the change of the stiffness of the tissues. Its molecular mechanisms are, however, not yet fully understood. We isolated a protein factor called 'tensilin' from an extract of sea cucumber body wall dermis, one of the known 'catch' connective tissues. It stiffens the detergent-treated dermal pieces and induces aggregation of collagen fibrils isolated from the tissue. We also isolated a crude extract solution which softens the detergent-treated dermal pieces. It is possible that there are other factors affecting on interactions among dermal fibrils and the stiffness of the tissues. Molecular mechanisms of the stiffness changes of the 'catch' connective tissues should be clarified by purifying and characterizing these factors.

2PS041 Cooperative function of raft domains and actin skeleton utilized for anthrax toxin assembly and internalization: a single-molecule study

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How raft domains and cortical actin filaments (actin-based membrane skeleton) collaboratively work in the plasma membrane (PM) is one of the key issues in understanding the functions of the raft domains and internalization of raft-associable receptors. To address this question, we examined the mechanism by which a component of anthrax toxin, called protective antigen (PA), bound to the cell surface receptor (PA-receptor complex, called PARC) form heptamers in the PM and internalized. The lethal and edema protein components of the toxin are known to only bind to PARC heptamers, and after internalization, cross the endosomal membrane to enter the cytoplasm. The process for the PARC heptamer formation has not been totally understood, because, previously, only PARC monomers and heptamers were detectable due to methodological limitations. Here, by using CHO cells stably expressing the PA receptor, CMG2, and the PA specifically labeled with a fluorescent dye precisely at a 1:1 mole ratio, we succeeded in observing intermediate PARC clusters and the recruitment of a specific lipid to those clusters by simultaneous dual-color single-molecule tracking. With an increase of the cluster size, the residency time of the raft lipid at the clusters was prolonged. Furthermore, the PARC intermediate clusters induced actin polymerization, which is enhanced with an increase of the cluster size. How these events are correlated and lead to heptamer formation and the subsequent internalization is under investigation.

2PS042 核内を運動する粒子の分子同定と、可視化、トラッキング解析 Imaging, tracking and analysis on the intranuclear moving particles

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Cell nucleus is a site of gene expression. The expression process should require dynamic change in architectural framework of nuclear structures and active transport of many kinds of molecules and subcellular components. Many researchers microinjected fluorescently labeled molecules into the nucleus and showed high mobility of the molecules in nucleoplasm. Moreover, advanced in fluorescently tagged protein leads to more precise analysis on dynamics of nucleoplasm. However not so many studies were reported in the living unstained nucleus. We, therefore, used pupil projection apodized phase contrast (PPAPC) microscopy, (external phase contrast with special frequency filtering). The PPAPC revealed many moving particles in living cell nucleus, which were unable to observe with conventional phase contrast microscopy. We reported cell cycle dependent movement of the intranuclear particles. The particles were identified by interactive observation of optical (PPAPC) and electron microscopy. Here, we identified the molecules involved in the moving particles, with immunofluorescence method. Moreover, we tracked the moving particles and analyzed the trajectory. Motile mechanisms of the particle and biological meaning of the movements were discussed in the presentation.

2PS043 クライオトモグラフィーによる動的生細胞内イメージングの開発

Development for dynamic Live Cell imaging by cryo-electron tomography

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Cryo-electron tomography of intact cells is an emerging technology that compliments crystallography, NMR and single molecule imaging techniques. Its strength is in that it reveals the spatial arrangements of key proteins and complexes during intracellular signaling and mechanical events like motility and division. In this meeting, we describe dynamical Live Cell imaging by cryo-electron tomography using the Titan Krios microscope. Observed living cells were grown directly on Holey Carbon Support Film, 200 mesh molybdenum EM specimen grids (Quantifoil R2/2). We analysed normal cell movement and cell division on Quantifoil using Image J and compared these properties with those of cells grown on a normal cell culture plastic plate. After phosphate-buffered saline washing and rapid freezing, the specimen grids were transferred onto the microscope immediately while keeping the environment under liquid N₂. Cells were imaged over an angular range from -70 degrees to 70 degrees at 2 degrees x cosθ tilt increments automatically and analyzed with Amira software to provide 3D images. We will present several 3D images in several types of Live Cell and would like to discuss them with everyone.

2PS044 Live cell imaging of the growth factor-induced nuclear translocation of ERK in PC12 cells

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ERK (extracellular signal-regulated kinase) is one of the most major MAPKs and its dynamic activation regulates cell fate such as the growth and differentiation in PC12 cells. Specifically, nuclear translocation of ERK after the activation of Raf/MEK/ERK cascade leads to further activation of proteins in the nucleus such as the transcription factor, resulting in cell fate decisions. However dynamics of the ERK translocation within individual cells have not been well characterized. For further understanding and quantitative modeling of this signal transduction system, dynamical behavior of ERK in response to extracellular signals in single cells should be addressed. EGFP-tagged ERK was transfected into PC12 cells, and visualized in living cells using confocal laser microscopy, which allows quantitative measurement of the dynamics of ERK nuclear translocation induced by growth factor stimuli. The results revealed that the amount and time course of the translocation were varied among individual cells. To investigate the mechanism to induce such heterogeneity, translocation of Raf, the MAPKKK in the ERK cascade, to the cell membrane upon growth factor stimuli are being investigated, using TagRFP-tagged Raf. Thus, we will discuss the relation between dynamics of ERK and Raf, and further perspectives toward single molecule measurement and modeling.