3P-185 アクチンネットワークの流れ及びミオシンがラメリポディア上の後方輸送運動へ及ぼす影響

Dependence of retrograde flow on actin network flow and myosin activity.

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In the front of the cell, there is thin membrane structure called lamellipodium, where motion of surface-bound object is observed toward the cell center. This flow is thought to be coupled to the retrograde flow, a flow of actin network toward the cell center in lamellipodium. By analyzing the motion of 1 micrometer bead bound to the surface of lamellipodium of Swiss 3T3 cell, our group has demonstrated that cytochalasin D, latrunculin A and jasplakinolide significantly slowed the bead movement, whereas myosin-II inhibitors (blebbistatin and Y-27632) affected the motion to much less extent. Hence, actin polymerization seems to play the major role in retrograde flow. However, another group has shown that myosin-II-based contractile force plays the major role. Thus, the identity of the driving force for the retrograde flow remains elusive. One possibility is that drugs affecting the actin cytoskeleton disrupted the cytoskeletal structure, thereby inhibiting the effective transmission of the myosin-II-based contractile force. We will thus analyze the state of actin cytoskeleton and myosin-II distribution in the cell treated with the above inhibitors and will relate it to the mechanism of retrograde flow.

3P-186 位相差電子顕微鏡による氷包埋シアノバクテリア細胞内 DNA 高次構造の観察

Observation of in vivo DNA conformation in ice embedded cyanobacterial cells by Hilbert differential contrast TEM

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The Hilbert differential contrast transmission electron microscope (HDC-TEM), one of several phase contrast electron microscopes developed recently, makes it possible to visualize the ultrastructure of ice embedded whole cyanobacterial cells. Surrounded by smooth cell walls, thylakoid membranes, carboxysomes, and polyphosphate bodies can be clearly identified. Various particles and filamentous structures are also discernable in the cells. We attempted to identify in vivo DNA in cyanobacterial cells by incorporation of BrdU (bromodeoxyuridine) into newly synthesized DNA, a technique widely used to investigate in vivo DNA synthesis in cell biology research. Rapidly growing *Synechococcus* sp. PCC 7942 cells were cultured in BrdU containing liquid media for 24 h. Incorporation of BrdU into cyanobacterial cells was confirmed by fluorescent labeled anti-BrdU antibodies and EDX analysis of resin embedded ultrathin sections. HDC-TEM images of the BrdU treated cells exhibited electron dense areas similar in shape and size to the fluorescent images of BrdU as incorporated in the cells. Interestingly, incorporation of BrdU tended to be restricted to the distal poles of the cells although DAPI stained DNA was evenly distributed throughout the whole cells. The apparent in vivo conformation of HDC-TEM visualized DNA was further compared with compiled images of DNA reconstructed from conventional TEM images of serial sections of resin embedded cells.

3P-187 弾性体基板上における心筋細胞の拍動周期ゆらぎと牽引力との相関解析

ANALYSIS OF FLUCTUATION OF INTERBEAT INTERVALS AND TRACTIONAL FORCE OF CARDIOMYOCYTES ON THE ELASTIC SUBSTRATE

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Fibrotic cardiomyopathy, which caused by scarring after cardiac infarction, may induce serious affection of beating behavior as cardiac disease. In fibrotic tissues, physical aspects of the microenvironment, such as stiffness or mechanical compliance of the extracellular matrices quite varied as compared from normal tissue, which has been suggested that these different mechanical environments affect tractional force of cardiomyocytes and causes irregular beating process. However, relation between the beating process of cardiomyocyte and mechanical difference of cellular environment is remaining unclear. In this study, we have tested polyacrylamide (AA) gel as model of elastic substrate. Also, polystyrene latex beads were embedded in the acrylamide gel to measure tractional forces of the cell. Using this typical traction forces microscopy, it was possible to visualize and analyze mechanical force of cardiomyocyte as real time imaging (32frame/sec). Primary cultures of cardiomyocytes were prepared from 1-day-old ICR mice and isolated by treatment with collagenase and trypsin. Using this method we investigated how traction force and interbeat intervals of cardiomyocyte are affected by different mechanical environment. Interestingly, strain of the gels generated by beating cardiomyocytes was proportional to stiffness of the gels, that is, tractional force of the cells did not depend of the elasticity in the range of 3.1kPa to 23.5kPa.

3P-188 EGF (上皮増殖因子) 固定化マイクロビーズを用いた局所シグナル伝達機構の解析

The Analysis of Local Signal Transduction Systems By EGF (Epidermal Growth Factor)-Immobilized Micro-Sphere

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Intracellular signal transduction takes place in the context of subcellular compartments, including the highly organized meshwork of the cytoskeleton. Scaffolding proteins also contribute to the organization of the signal transduction machinery. Series of local signal transductions to regulate various cellular functions are observable in focal adhesion to extracellular matrix, focal complex by fluorescence microscopy. However, in comparison with adherent ligand-receptor signaling, it is difficult to monitor signaling of soluble factor. In order to quantitatively analyze local effect of liquid factor, visualization of liquid factor and technology to measure are necessary. And also, method that could control spatially local stimulation of growth factor is applicable for tissue engineering. Stimulating growth factor in desired way could possibly regulate cellular function such as inducing chemotaxis and morphogenesis. In this study, we established micro-sphere with epidermal growth factor (EGF) immobilized by genetic engineered method. Using our novel EGF micro-sphere, we found that the micro-sphere could induce stimulation of EGF locally as motility and morphology of the cells were drastically varied. In stimulation experiments of EGF-micro-sphere to Madin Darby canine kidney (MDCK) cells, phosphorylation of EGF receptor was detected by western blotting, and a drastic change of migration was observed.

3P-189 分子モーターの軌道の効率的なベイズ推定法

Effective method of Bayesian estimation for a trajectory of a single molecular motor

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In single-molecule experiments of molecular motors, it is a widely adopted strategy to visualize the motion of a molecule by attaching a large probe particle. In such a situation, only the motion of the probe can be monitored while the motion of the molecule is hidden. Therefore, estimation of the trajectory of the molecule based on the observed trajectory of the probe is an important but non-trivial task for the study of single molecule dynamics. In this presentation, we present a novel approach to this problem, which is based on Bayesian statistics and statistical mechanics. As a working model, we consider a relatively simple system that consists of two Brownian particles (a molecule and a probe) interacting with each other. As a first step, we assume that we already know the system parameters. However, it is still non-trivial to find out a single trajectory of the molecule using the trajectory of the probe. Here, we formulated this problem in the Bayesian framework. This simplifies the problem into a minimization of an "action" functional. We further developed an effective approximation technique which reduces a computational cost drastically. This technique enabled us to obtain a correct estimation of the trajectory of the molecule in an efficient manner. It is a next step to estimate the system parameters by extending above-mentioned method, and we expect that the method of empirical Bayesian estimation can be utilized for this problem. We will report to what extend our strategy is effective.

3P-189 脂肪細胞への分化過程で見られる高密度カベオラ構造形成：フリーズエッチ電子顕微鏡法によるアプローチ

High-density caveolar formation just beneath the plasma membrane during adipogenesis, as revealed by freeze-etch electron microscopy

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Caveola, a kind of membrane invagination, was found on the cytoplasmic surface of the cell membrane of the blood capillary (Palade et al., 1953) or the gall bladder epithelium (Yamada, 1955), which diameter was 60-80 nm. Caveolae represent an impressed design on the cytoplasmic surface as the flask shape in the ultra-cut section and the striated coat in the deeply etched image (Rothberg et al., 1992). Previously, we have reconstructed three-dimensional images of the undercoat structure on the cytoplasmic surface of the upper cell membrane by freeze-etch electron tomography, with 0.85-nm-thick consecutive sections (Morone et al., 2006). It found that most of caveolae or clathrin-coated pits might be bound with the actin-based membrane skeleton (MSK). Here we could visualize the entire cytoplasmic surface of the plasma membrane of the adipocytes fully differentiated from the murine-derived embryonic fibroblast by the freeze-etch replica electron microscopy. The entire surface of the cytoplasmic cell membrane could be occupied with caveolae, with extremely higher number density, and separated with the actin-based MSK. These data almost agree well with the previously reports (Scherer et al., 1994; Kanzaki et al., 2002; Parton et al., 2002). The high-density caveolae might play a role in the platform to function a glucose uptake on the plasma membrane. This electron microscopic technique could be applied for neuron as well as these fibroblasts.

3P-190 流れ刺激により誘導される P2X4 チャネルの細胞膜への移行

Up-regulation of cell surface P2X4 receptor by flow shear stress

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P2X4 receptor is a non-selective ATP-operated cation channel distributing in various tissues including vascular endothelial cells (ECs). Previous studies have demonstrated that P2X4 in ECs mediates flow-induced Ca^{2+} influx and