decrease of attractant concentrations, and direct their swimming path toward eggs efficiently by the Ca^{2+} -mediated stereotype changes of flagellar waveforms.

2P-207 酢酸菌 *Gluconobacter oxydans* 621H 株の運動能

Motility of the acetic acid bacterium *Gluconobacter oxydans* 621H

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Acetic acid bacteria are obligate aerobes to produce acetic acid from ethanol via its ethanol oxidative respiratory chain. Ethanol is oxidized to acetic acid by two sequential reactions; the reducing equivalents reduce oxygen to produce water. Through this incomplete oxidation process, acetic acid bacteria can produce energy as the form of proton motive force. Flagella are locomotive apparatuses to consume proton motive force in the processes of both biogenesis and work. We hypothesize some relations between the incomplete oxidation and flagellar motility, because the proton motive force is a key for the both processes. Presumably, a balanced energy state is physiologically critical for the organism. The genome of *Gluconobacter oxydans* ATCC 621H strain, an acetic acid bacterium, includes one complete set of flagellar genes. Here we examined motility of *G. oxydans*.

G. oxydans spreaded on semi-solid agar via an unknown mechanism making trails as well as via swimming. Highest swimming motility of *G. oxydans* was observed at the exponentially growing phase on glycerol-glucose medium or ethanol-supplemented glycerol-glucose medium (YPGD-E). Using citrate-phosphate buffer, optimum pH for the swimming was pH 6.0; the cells rarely swam at pH 4.0. After grown on YPGD-E medium, pH of the medium dropped to approx. 3.5 by acetic acid production. Compared with citrate-phosphate buffer, acetate buffer kept the higher swimming motility even at pH 4.0.

2P-208 膜交通機構における植物特異的ミオシン VIII と XI の機能と制御

Function and regulation of plant specific myosin VIIs and XIs in membrane trafficking

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The motor protein is indispensable for not only the motive force but regulation and maintenance of the highly organized membrane trafficking in the cell. In contrast to the animal cell by which various microtubule based motors are taking part, the motor that takes part is only plant specific actin based class VIII and XI myosin in the plant cell. However, higher plant myosins have a lot of members, 4 members exist in myosin VIII and 13 members in myosin XI (*Arabidopsis thaliana*). The possibility is suggested that the function diversity of each member enables regulation of the plant membrane trafficking.

In order to identify function allotment and/or complementary among members, a detailed analysis of native full length myosin is indispensable. It succeeded from *Arabidopsis* in the cloning of the full length of 3 members (ATM1,2,VIIIA) for myosin VIII and 10 members (XI1,2,A,B,D,F,H,I,J,K) for myosin XI. Domain organizations such as the number of IQ motif and coiled-coil probability were different, suggesting the diversity in movement and regulation among members. GFP was fused into N terminal assumed for the influence not to exist in the motor function of myosin, then transiently expressed in the protoplast of *Arabidopsis* cultured cell. Live cell imaging revealed that each member's existing in the organelle with different shape and size, and doing a peculiar movement respectively. These results showed the wide function diversity among plant specific myosin members in membrane trafficking.

2P-209 細胞間相互作用の測定のための生細胞配列構造体の開発

Development of artificial cell crystal for studying cell-cell interactions

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Living cells interact each other, and thus it is basically important to elucidate cell-cell interactions. In the case of measuring the physical and chemical interactions between cells in a quantitative way, the control on the distance as well as the position among cells is one of important issues. In this study, we developed new culture systems, in which living cells were arranged in wells of microarray to control the physical connections among cells precisely. We firstly succeeded to place each of living cells in a well of the microarray with a spacing of ca. 20 μm and form a monolayer of living cell with a closed-pack structure, namely an artificial cell crystal. This sample is expected to be useful for measuring chemical interactions between cells. Moreover we found that the cell crystal was peeled off from the microarray in an appropriate condition, so that some type of cell sheet could be fabricated. Such a sheet with a periodic structure enables to investigate more quantitatively the mechanical interactions between cells rather than a single cell mechanical experiment. We will present the fabrication techniques of artificial cell crystal and the mechanical properties of cell sheet with a highly packed structure.

2P-210 細胞-細胞外マトリックス結合力の AFM を用いた力学解析

Mechanical analysis of cell-matrix binding force as studied by AFM

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The cell-matrix adhesion plays an important role in various cellular activities, such as cell division, and transmembrane signal transduction, where integrin receptors for extracellular matrix have crucial roles in the signal traffic through reorganization of the actin-containing cytoskeleton. In this study, we focus on the mechanical linkage between the integrin-ligand affinity and the reconstructed actin-containing cytoskeleton. For this purpose, the unbinding force of integrin-fibronectin complex was measured by atomic force spectroscopy (AFM) using a fibronectin coated-bead which was attached the AFM cantilever while the reconstructed actin-containing cytoskeleton was visualized as GFP-fused actin filaments expressed in rat fibroblasts. We will report the the magnitude of the force required for receptor-fibronectin unbinding on the live cell surface together with the cell stiffness.

2P-211 極低温電子顕微鏡によるべん毛フックの 7 Å 分解能での構造解析

High resolution structure of the flagellar hook by cryoEM image analysis

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The bacterial flagellum is a motile organelle made of the basal body as a rotary motor and the filament as a helical propeller connected by the hook as a universal joint. A partial atomic model of the hook has been built by fitting the X-ray crystal structure of a FlgE fragment made of domains D1 and D2 into a cryoEM density map (Samatey et al., 2004, Shaikh et al., 2005). However, the model lacked domain D0 made of the terminal chains due to their removal for crystallization, and therefore the central core structure was still missing. Although it was predicted that the terminal chains form an α -helical coiled coil, the previous cryoEM work did not resolve the coiled coil structure due to the low resolution around 15 Å. The fitting of the crystal structure was not so accurate, either. So, we have carried out cryoEM image analysis of the straight hook and obtained a density map at 7.7 Å resolution. Now, we can clearly see the α -helical coiled-coil in the core domain D0 and most of the loops and hairpin structures in outer domains D1 and D2. Domain D1 was over-tilted from the hook axis by 20° in the previous model, although this correction did not change the mechanistic explanation for the bending flexibility of the hook. We found another feature in the axial packing interactions of the α -helical coiled coils in the core domain, which also explains the flexibility of the hook. We compared the structural differences between the rod and the hook to see how domains of almost identical fold can produce distinct mechanical functions.

2P-212 細胞性粘菌の走化性シグナル伝達系における自己組織化現象の蛍光イメージ解析と理論的解析

Fluorescent image analysis and theoretical study of self-organization in chemotaxis signal processing of *Dictyostelium*

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In *Dictyostelium* cells, phosphoinositide-3-kinase (PI3K) and tumor suppressor phosphatase and tensin homolog (PTEN) have been identified as signaling molecules essential for chemotaxis in response to concentration gradient of cAMP. PI3K and PTEN are enzymes that produces and degrades phosphatidylinositol 3,4,5-trisphosphates (PtdIns(3,4,5)P3), respectively. Along the cAMP gradient, PI3K is localized in the anterior region along plasma membrane while PTEN is localized on the lateral and posterior region of plasma membrane. Furthermore, it has been known that cellular motile activities are not necessary for the formation of such reciprocal distribution of PI3K and PTEN. In this study, we studied the distribution of these molecules in the situation of chemoattractant gradient being absent. We have found that these signaling molecules formed localized domains on membrane, which were not stationary in time but traveling along membrane. From the noisy time lapse data of fluorescent images, we reconstructed the temporal dynamics of reaction among molecules such as PTEN, PI3K and PtdIns(3,4,5)P3 using principal component analysis. The reconstructed dynamics indicates a reciprocal localization of PTEN and PtdIns(3,4,5)P3 and its oscillatory nature. Based on the reconstructed dynamics, we next developed a theoretical model, which can successfully reproduce several patterns observed experimentally. Our theoretical model predicts that PtdIns(3,4,5)P3 negatively regulates the localization of PTEN on the plasma membrane.

2P-213 1 分子イメージングによる三量体 G タンパク質の動態解析

Heterotrimeric G protein dynamics in early signaling events of chemotactic responses revealed by single-molecule imaging analysis.

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Heterotrimeric G proteins constitute a diverse superfamily of transmembrane signaling molecules in eukaryotic cells for various physiological responses. According to the prevailing scheme, G proteins dissociate into the activated α and $\beta\gamma$ subunits via complex formation with agonist-bound receptors. However, the relevance of these early activation events of G proteins to the intracellular dynamics on plasma membrane has not been revealed. To reveal the intracellular dynamics of G proteins, we used single-molecule imaging techniques in living *Dictyostelium* cells, which provided direct measure of mobility in the plasma membrane and revealed a dynamic shuttling of G proteins between the membrane