2P175 高圧負荷によって誘導されるクラミドモナス非運動性変異株鞭毛の屈曲運動

High pressure induces flagellar bending movements in *Chlamydomonas* paralyzed mutants

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Chlamydomonas mutants lacking flagellar central structures are paralyzed. Recently we found that the mutants display beating under high pressure (20-80 MPa). To further examine the effect of pressure, we analyzed pressure-induced beating in *Chlamydomonas* mutants by high-speed video microscopy. Previous studies showed that flagellar waveform changes from asymmetric to symmetric types over 10⁻³M Ca. Interestingly, pressure application increased the number of cells displaying symmetric waveform. In addition, similar waveform change was also observed in demembrated and reactivated flagella in the absence of Ca²⁺, indicating that pressure application induced symmetric beating without Ca²⁺. We suggest that pressure and Ca²⁺ have similar effects on flagellar structure.

2P176 バクテリアの集団運動による巨大渦パターン形成

Large-scale vortex pattern emerging from bacteria collective motion

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Swarming bacteria is an ideal example of collective motion because of their limited cognition between individuals and the experimental reproducibility. We found *Flavobacterium johnsoniae*, gliding bacteria with the rod shape of 5 µm in length and 0.5 µm in width, exhibits the large-scale vortex pattern during spreading on the non-nutrient agar plate. The size of the single vortex grew up to 3 mm in a 24-hour period, and the number of cells including the vortex was estimated to be 10⁷. Notably, all vortices rotated in a counter-clockwise manner at a speed of 0.4 µm/s, which is presumably attributable to the left-side biased movement of single cell revealed by the tracking method with a high accuracy.

2P177 シグナル変換のアダプター分子 LAT は細胞膜直下の小胞膜上で働く：1 分子追跡法による解明

Transmembrane signaling adaptor LAT works on the vesicles associated with the plasma membrane: a single-molecule tracking study

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Recently, Linker for Activation of T cells (LAT), a transmembrane adaptor protein vital for signaling in immune cells, is drawing extensive attention: it was reported that LAT constitutively forms clusters in the plasma membrane (PM) and that the LAT clusters are the key for signal transduction. However, our single-molecule tracking of LAT in mast cells revealed that ~10% of LAT exists in cytoplasmic vesicles located near the PM, and that, upon antigen stimulation, downstream signaling molecules are recruited to the LAT molecules on the vesicles rather than LAT monomers or clusters in the PM. These results suggest that LAT-containing vesicles serve as a signaling platform in immune cells. These vesicles were enriched in particular phosphoinositides and their kinases.

2P178 The bidirectional role of conserved charged residues in rotor-stator interaction in a rotary motor of bacterial flagella

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Bacterial flagellum is bidirectionally rotated by the flagellar motor located in the inner membrane. It is known that some conserved charged residues are crucial for rotor-stator interaction but this evidence is based on previous studies focused on the rotation in forward (counterclockwise) direction only. Here, using tethered-cell assay, we verified the function of the residues in the interaction even in the reverse (clockwise) direction. In WT and charge-neutralizing mutations of the stator, rotational speeds in both directions were almost the same. But in the case of some mutations in the rotor, they differed totally. These results support a current model that the rotor changes its conformation depending on rotational direction.

2P179 細胞-GUV 電気融合による µm サイズ人工物の導入

Introducing micro-meter size objects into live cells mediated by cell-GUV electrofusion

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Here, we report a method for introducing large objects, up to micrometer level, into living cells by electrofusion using an artificial giant unilamellar vesicle (GUV or Liposome). The mixture dispersion of GUVs and HeLa cells were exposed to an AC field to induce cell-GUV alignment and were loaded DC pulse to cause puncture of the contact area. We demonstrated that a plasmids, designed DNA nanostructure (origami), and magnetic beads could be introduced into live cells by using this approach. The treated cells were cultured to confluence without significant damage. By using external magnetic fields, we also demonstrated rotating HeLa cells that introduced magnetic beads. These bioengineered hybrid cells will be useful for elucidation of cell mechanisms in the future.

2P180 量子ドットを用いた脳がん幹細胞表面の膜タンパク質の運動解析

Membrane Protein Dynamics on Brain Tumor Stem Cell Evaluated by Using Quantum Dot

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Brain tumor stem cells (BTSCs) populate in a small portion of tumor and are responsible for the recurrence of the tumor after treatment. BTSCs have also been known to have unique characteristics, such as high resistance to radiotherapy and chemotherapy, invasion, metastasis, multilineage and self-renewal potential. Despite such well-known properties, the difference in membrane functions between BTSCs and non-BTSCs remains unclear. A potential factor is dynamics of the BTSC membrane protein marker, CD133. Thus, we evaluate dynamics of CD133 labeled with quantum dots using a confocal microscope. BTSCs showed higher motility and expression level of CD133 than that of non-BTSCs, suggesting that dynamics of CD133 might be a key property in membrane functions of BTSCs.