3P170 揺らぎの定理による V-ATPase のトルク測定：F サブユニットの働き

F-subunit reinforces torque generation in V-ATPase

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V₁, a water soluble domain of V-ATPase (rotary ATPase) is a rotary motor protein and the minimal rotary unit of V₁ is A₃B₃D. The rotor D-subunit is likely considered to be a structural analog of γ-subunit of F₁ although it lacks a globular domain unlike γ-subunit. To investigate how the lack of a globular domain affects on the rotary motion of V₁, we performed a single molecule experiment and measured the torque of A₃B₃D. We found that A₃B₃D produced significantly low torque compared with V₁, and recovered the high torque value only when globular shaped F-subunit was fused with D-subunit. The torque was measured precisely by using the fluctuation theorem that is the recently proposed method in the field of non-equilibrium statistical mechanics.

3P171 Binding interface between rotor subunits with low binding affinity in V_oV₁

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The central rotor complex of V_oV₁ of *Thermus thermophilus* is composed of V₁-DF shaft and V_o-CL₁₂ rotor ring. ATP synthesis/hydrolysis in V₁ is coupled with proton translocation in V_o by rotation of central rotor complex relative to a surrounding stator apparatus.

In this study, we directly demonstrate that low binding affinity of V₁-DF for V_o-CL₁₂ by both FRET and reconstitution experiments. Our results conflict with an idea that the binding interface between DF and C subunit should be tight sufficient for energy coupling between V₁ and V_o. A unique mode for the binding interface between rotor subunits should be necessary to explain the torque transmission mechanism in V_oV₁.

3P172 マイクロピラーアレイ上で成長したフィブロブラスト細胞の大きさと形状

Size and Shape of Fibroblast Cells Growing on a Micro Pillar

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We investigated human fibroblast cells (ATCC CRL-2097) growing on silicon micro pillars to study mechanical properties of the cells, such as locomotion, adhesion, and elasticity. Using a silicon-on-insulator wafer, we fabricated micro pillars (20 μm in width), which were approximately equal in size to a fibroblast cell before its adhering to the substrate. The cells were able to bridge geological gaps until 15 μm between the pillars. For larger gaps, the cells sank down to the bottom of the gap. Once the cell clings to the top of the pillar, it could grow over a gap onto the next pillar. The height of the pillars (35 μm) enabled us to know the vertical location of the cells. Our results will contribute to ways to know mechanical properties of cells on microstructures.

3P173 細胞および接着分子の極性の人為的制御

Artificial control of the polarity of cells and molecular assemblies

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The subcellular localization of individual focal adhesions, a molecular assembly necessary for cell adhesion, determines the cell morphology; however, the molecular and biophysical mechanisms remain unclear regarding how the localization is determined. Here we observed that cells exhibited marked Rac1-dependnet membrane ruffles when the polarity of focal adhesions was artificially controlled, using our novel micro-patterning technique, not to be consistent with that of the cells. Thus, our results suggested that focal adhesions align preferentially along the cellular long axis because of the high efficiency for inactivating Rac1, which in turn activates RhoA and then promotes cell adhesion maturation.

3P174 Study on membrane microfluidity of living cells using Muller Matrix microscopy

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It is widely accepted that membranes of living cells have inhomogeneous fluidity but direct measurements on such heterogeneity of membrane fluidity are scarce. Recently we developed a MuellerMatrix imaging system which can measure any polarization changes by the sample at optical microscopic resolution. It operates two measurement modes; one is the transmission mode which can visualize optical anisotropy, e.g. retardance and its direction, and the other is the epi-fluorescence mode which can visualize local orientation and rotational diffusion rate of fluorophores. We applied this apparatus to Swiss 3T3 fibroblast cells. The results will be discussed on poster presentation.

3P175 負荷をかけた状態での単離マウス気管上皮シリアの三次元運動

Three-dimensional motion of an isolated murine tracheal cilium under load

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Beating of airway epithelial cilia generates fluid flow to protect the mammalian respiratory system from harmful inhaled materials. Thus their response to the external force is crucial to understanding of the mechanism of regulated strokes. To address this point, we here combined 3-D tracking microscopy with optical tweezers. A fluorescent bead was attached to the tip of the isolated tracheal cilium, and trapped with various power of the infrared laser in the presence of ATP. The bead escaped from the trap when the low laser power, < 20 mW, was applied, whereas it showed irregular oscillation within the range of 350 nm under the higher power of laser. The apparent maximum force is estimated as 15 pN, and the periodic beating was randomized under that load.