1P-197 膵臓α細胞と神経細胞は接着分子 CADM1 を介して相互作用する Communication between pancreatic islet cells and nerves is mediated by cell adhesion molecule 1 (CADM1)

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[Background and Aims] Cell adhesion molecule 1 (CADM1) has three immunoglobulin-like motifs in the extracellular domain and is expressed by a variety of cells. We previously showed that CADM1 mediated the attachment of mast cells to neurites and this attachment resulted in major enhancement of functional communication from the neurites to mast cells. To examine here whether CADM1 might mediate attachment and communication between islet α cells and peripheral nerves, we observed the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in superior cervical ganglia neurons and α cells after two-day coculture.

[Results] Immediately after the addition of scorpion venom, a depolarizer of neurons, the neurons became activated. Some α cells attendant to neurites appeared to respond to this nerve activation. Because α cells are apt to aggregate with each other, we sometimes observed the aggregates riding on neurites. Within the aggregates, the responses to nerve activation were detected not only in α cells directly or nearly attendant to neurites but also in those lacking direct contacts with neurites. In addition, the proportion of original α cells that responded to nerve activation was one-third out of all α cells attended to neurites, whereas the proportion of CADM1-deficient α cells responding was just one-sixth that of original α cells.

[Conclusions] These results suggested that CADM1 promoted communication from neurons to neurite-attendant α cells and between neurite-attendant and non-attendant α cells using an *in vitro* coculture model.

1P-198 移動性細胞のアクチン・ミオシン収縮機構の振動に対する細胞内力学的因子の変化 Quantification of Intracellular Mechanical Factors Involved in Rapid Cell Migration under Actomyosin Perturbation

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Cell migration involves a series of spatiotemporally coordinated processes in which coupling interactions among mechanical and biochemical factors play important regulatory roles. Although molecular pathways of cell migration are well investigated, mechanical factors and their roles are not yet fully understood. In this study, we have undertaken a detailed examination of the actin structure dynamics in the lamellipodia of rapidly locomoting fish keratocytes using a combination of fluorescent speckle microscopy and particle imaging velocimetry. Based on continuum mechanics, we have quantitatively determined retrograde flow field and the distribution of mechanical strain in the lamellipodial actin filament meshwork under different pharmacological interventions against actomyosin activity. We aim at understanding the role of actomyosin interactions on the deformation behavior of the meshwork, and how such deformation correlate with other motility processes such as retrograde flow and depolymerization. Our results highlight the presence of a dominant negative strain in the actin meshwork whose direction is parallel to that of cell locomotion. In addition, a systematic perturbation of actomyosin activity reveals that retrograde flow and meshwork deformation are a function of actomyosin contractility. Furthermore, by correlating strain and F-actin density distributions in the lamellipodia, we suggest that negative strain is involved in actin filament depolymerization.

1P-199 紡錘体の持つダイナミックな形状制御機構の解析 Probing dynamic shape regulation of the meiotic spindle

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The meiotic spindle equally segregates replicated genomes into two daughter cells during cell division. Miss-segregation during this process causes birth defects and canceration.

Spindles are mainly composed of microtubules and molecular motors. At metaphase, size and shape of the spindle are maintained constant. Despite the morphological stability of metaphase spindle, spindle microtubules spontaneously change the state between growth and shortening. Additionally, they are transported toward the spindle pole by molecular motors (poleward flux). The stability of the metaphase spindle therefore indicates that the microtubule dynamics and the interplay between microtubules and molecular motors are well coordinated.

In this study, we developed micromanipulation techniques for changing spindle shape. Spindles were spontaneously assembled in *Xenopus* egg extract and stretched along their pole-to-pole axis using two glass micro-needles. After a brief stretching they were able to recover their original size and shape. In contrast, when spindles were stretched and kept in this condition, they gradually recovered their original shape, but became larger. This result suggests that the meiotic spindle has an ability to adjust its size and shape to the externally applied force. To examine the mechanism of morphological adaptation, we studied the dynamics of each spindle microtubule using confocal microscopy. Our findings provide new insights into the mechanism of how metaphase spindle maintains its stable structure.

1P-200 Rhoキナーゼによって Ca^{2+} -非依存性に直接リン酸化された平滑筋ミオシンは、アクチンとの滑り運動を最大速度で引き起こすことが出来る Direct and Ca^{2+} -independent RLC phosphorylation by Rho-kinase is alone sufficient to stimulate actomyosin sliding at the maximum velocity

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In contrast with the Ca^{2+} -dependent physiological contraction of vascular smooth muscle (VSM), the Rho-kinase (ROK)-mediated Ca^{2+} -sensitization contributes to abnormal VSM contraction such as vasospasm. As the downstream mechanism of ROK leading to elevation of myosin light chain (MLC) phosphorylation, inhibition of MLC phosphatase by ROK is regarded as major or sole pathway, although direct and Ca^{2+} -independent phosphorylation of MLC by ROK was also reported. The latter possibility has been ignored without any strong evidence. In order to test the latter possibility, in this study we used *in vitro* motility assay system with highly purified contractile proteins of smooth muscle, but in the complete absence of the MLC kinase and phosphatase, both of which are essential for the former mechanism. Smooth muscle myosins were purified as recombinant proteins in a baculovirus expression system and were confirmed by MALDI-TOF MS. The MLC was phosphorylated by either ROK or MLCK. The *in vitro* motility assay of the recombinant myosin revealed that average velocity of actin sliding on unphosphorylated and ROK-phosphorylated myosin was 0.0 and 0.3 $\mu\text{m}/\text{sec}$, respectively, the latter value of which is comparable to the maximum value of MLCK-phosphorylated myosin (0.27 $\mu\text{m}/\text{sec}$). Finally, in the permeabilized VSM constitutively active ROK2 induced large Ca^{2+} -sensitization and MLC phosphorylation in the absence of cytosolic Ca^{2+} and MLCK activity. These results indicate that direct phosphorylation of MLC by ROK can alone lead to Ca^{2+} -sensitization.

1P-201 AFM による細胞骨格変形の側方力測定 Lateral force measurement of cytoskeletal deformation by AFM

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The force measurement technique of Atomic Force Microscopy (AFM) has been widely used in biophysics and cellular biology. The technique used in commercial instruments mainly detects vertical deflections of a cantilever. Recently, however, with development of AFM related technology, it is now applied for measuring a lateral force by detecting a torsional deflection of the AFM cantilever. Our aim is to measure the lateral force on the cell surface by using AFM while its cantilever was moved sideways keeping it in touch with the cell surface at a certain positive contact force. In this way, we measured mechanical rigidity of the cytoskeletal structure maintained by actin filaments and other proteins. Actin filaments maintain the cell structure and adjust its shape to various internal and external signals. We prepared transfected cells with a plasmid which had DNA of fusion protein of green fluorescent protein (GFP) and β -actin. We found actin filaments in the cell expressing the fusion protein by examining fluorescence of GFP. Based on this experimental setup, we captured fluorescence images of the deformation of actin filaments together with the force response data. We, thus, were able to compare the fluorescent image of actin filaments while they were pushed and pulled by an AFM tip.

1P-202 蛍光イメージング法を用いたサルモネラ菌べん毛特異的 ATPase の基体部への離合集散機構の解析 Fluorescence imaging of assembly and disassembly of the bacterial flagellar protein export ATPase FliI to the flagellar basal body

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For construction of the bacterial flagellum, most of the flagellar proteins are translocated into the central channel of the growing structure by the flagellar protein export apparatus. FliI ATPase forms a complex with its regulator FliH and facilitates the initial entry of export substrates into the export gate made of six membrane proteins. The FliH/FliI complex also binds to a C ring protein, FliN, through the FliH-FliN interaction for efficient export. However, it remains unclear how these reactions proceed within the cell.

In this study, we constructed FliI-CFP and FliI-YFP fusion proteins and analyzed their localization by fluorescence microscopy. A few bright spots within each cell suggested that many of them are bound to the C ring, and breaching experiments showed their rapid assembly and disassembly. We confirmed that both FliH and the C ring are required for the localization, but faint spots observed even in the absence of the C ring suggested binding of the FliI hexamer to the gate. FliI-YFP formed a complex with FliH δ 1 missing residues 2-10 but the complex did not show the localization. FliH δ 1 interacted with neither FliN nor the gate-forming proteins. Alanine-scanning mutagenesis of FliH revealed that only two residues, Trp-7 and Trp-10, are responsible for these interactions. Taken all together, hydrophobic interactions of two Trp residues of FliH with other export components seem to drive the cycling reaction of assembly/disassembly of FliI for efficient export.