property of GR is direct indicator of transcriptional activity. To clarify an intranuclear whole landscape of transcriptional activity of GR, raster imaging correlation spectroscopy (RICS) and FCS measurement were performed in nucleus in which the intranuclear structure was distinguished as hetero or eu-chromatin. Our results indicate that the diffusion property of GR inhomogeneously distributed in the nucleus. Moreover, it is possible that this distribution correlates the intranuclear structures.

3P204 隔壁に局在を示すヒスチジンキナーゼ (BaeS & HydH) の局在変化と機構の解析 Characterization of histidine kinases that localize to the septum in *Escherichia coli*

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Escherichia coli has many two-component systems to respond to various stimuli. In general, a two-component system consists of a histidine kinase (HK) and a response regulator (RR). In this study, we constructed the fusion proteins consisting of each HK and green fluorescent protein (GFP). Each fusion was expressed in the wild-type or HK deletion strain. As a result, localization patterns of HK-GFP were classified into six groups. As expected, NtrB, a cytoplasmic protein, was diffused throughout the cytoplasm, and CheA was localized at the pole. The other HKs, all of which are transmembrane proteins, were diffused throughout the membrane, formed several foci in the membrane, localized to the polar region of the membrane, or localized to the septum. We then focused on HKs localized to the septum (*i.e.* BaeS and HydH). Because the septum is a structure that appears only at the time of cell division, it was not clear whether they actually localize to the septum. To address this issue, we constructed plasmids, with which the expression of BaeS-GFP or HydH-GFP can be tightly controlled with the inducer arabinose. Cells carrying each plasmid were incubated with arabinose and then the expression of HK-GFP was turned off by adding glucose just before and during observation. When single cells were observed, either HK-GFP was diffused throughout the membrane but when cells underwent division, either HK-GFP appeared to cluster at the septum. We therefore conclude that these HKs localize to the septum during cell division.

3P205 小腸絨毛における上皮下線維芽細胞を介した SubstanceP による情報伝達 Signaling roles of substance-P in intestinal villi via subepithelial fibroblasts network

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Subepithelial fibroblasts of intestinal villi form contractile network under the epithelium. They are in close contact with neural and capillary networks, smooth muscles and immune cells. They have unique characteristics such as: cAMP-dependent cell-shape changes, cell-shape dependent mechanosensitivity that induces ATP release as auto/paracrine mediator, and expression of receptors for endothelins (ETA, ETB), ATP (P2Y1), substance-P etc. From these features obtained by primary culture, we proposed that subepithelial fibroblasts work as a mechanosensor and signal transduction machinery in the intestinal villi. This time, we have focused on substance-P (SP) and its tachykinin receptor, NK1. SP is a potent activator of intracellular calcium in subepithelial fibroblasts. Pharmacological measurements showed that NK1 is the major in subepithelial fibroblasts. Immunohistochemistry of rat duodenum villi by light and electron microscopy showed that NK1R immunoreactivity localized on the plasma membrane of subepithelial fibroblasts. We also found that these subepithelial fibroblasts formed synapse-like structure with SP immunopositive neural varicosities. From these findings we propose that ATP released from subepithelial fibroblasts by mechanical stress activates SP neurons via P2X receptor and then SP released from the neurons activates subepithelial fibroblasts again via NK1R. This ATP and SP mutual signaling system may regulate physiological functions of intestinal villi, such as villous movement, nutrients uptake and peristalsis.

3P206 嫌気呼吸調節に関するヒスチジンキナーゼ TorS の局在メカニズムの解析 Localization mechanism of the histidine kinase TorS of *Escherichia coli* controlling anaerobic respiration

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Escherichia coli has 30 histidine kinases (HKs) that sense various stimuli. We have constructed GFP-fusions to all of them and classified their localization patterns into six groups. In particular, some transmembrane HKs localize to cell poles like methyl- accepting chemotaxis proteins (MCPs). To understand the mechanism of polar localization, we focused one of this class of HKs, TorS that is involved in regulation of anaerobic respiration. We constructed and observed five deletion derivatives of TorS-GFP with deletions in the cytoplasmic domain of TorS. All mutants localized at cell poles like full-length TorS-GFP. We constructed chimeric proteins between TorS and the osmosensor HK EnvZ. TorZ-GFP, which has the periplasmic domain and the transmembrane (TM) regions of TorS, localized to the cell poles like TorS-GFP. EnrS-GFP, which has the cytoplasmic domain of TorS, distributed throughout the cell membrane like EnvZ-GFP. These results suggested that polar localization of TorS requires the periplasmic domain and/or the TM regions. It has been reported that DcuS and CitA alter their localization in response to the stimuli they sense. We examined whether localization of TorS-GFP is also affected by signal input. The TorS ligand, trimethylamine *N*-oxide (TMAO), had slight effects on the localization of TorS-GFP. Incubation under microaerobic or anaerobic conditions had greater effects on the localization of TorS-GFP, implying some link between localization and function of TorS.

3P207 空間的な硬さ分布に依存した繊維芽細胞群のパターン形成 Spatio-temporal Pattern of Fibroblasts on Multi-Rigid Surfaces

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Collective cell behavior plays the critical roles in many physiological and pathological processes, including embryonic development, morphogenesis, and wound healing etc. It is known recently that cell motility changes substantially depending on the substrate stiffness of the external environment, which is known as mechanotaxis, *e.g.* cells prefer to locomote toward stiffer regions on the culture substrate. Most of previous works on mechanotaxis focused on the analysis of individual cell motility, regarding the cell as unicellular object. On the contrary, interaction between cells is an essential aspect in multi-cellular organisms.

Here, we focus on the understanding of the collective behavior of 3T3 fibroblasts under geometrically arrangement of different stiffness substrates. We adapted a gel with micro elasticity gradient (MEG) surface via photo curable styrenated gelatin being able to modify the extracellular matrix. We cultured the cells on the prepared patterned MEG gels consisting of elasticity jumps and different elasticity regions. We measured movement and orientation of cells along with their proliferation rate, and observed that cells grow to form various spatio-temporal patterns under different extracellular conditions. This study provides new insights in the collective behavior of mechanosensing-cells under different environmental conditions.

3P208 Importance of Vein Creation at Front for Network Designing in Plasmodium of *Physarum polycephalum*

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The plasmodium of true slime mold *Physarum polycephalum*, a single cellular organism with a naked protoplasm, forms a vein network with amoeboid locomotion. It has been researched that this network is optimized to an environment with a time-dependent rule. Considering the formation of vein network, we have to take not only these time development but also the creation of new vein structures at frontal tips into account.

In this study, the latter is focused. First, we observed several morphological mutants migrating directionally on plain agar gel. One showed sheet-like flat pattern at its advancing front. The frontal tip was spreading broadly in this case. The anterior vein structure formed a network consisting of many cycles, *i.e.*, it can be regarded as a closed graph. Another showed finger-like pattern. The tubes are spreading dendritically without overlapping each other. This vein can be regarded as an opened graph. Second, we simulated these different pattern by cellular automata with a simple rule and could reproduce the similar results.

Fixing the amount of the protoplasm, a plasmodium forming the dendrite structure without overlapping can cover wider effective area than that forming the sheet structure. Therefore, it can be considered that both the structure of rear vein network and the ability to search for a new place is determined by creation and annihilation process of advancing fronts. We also would like to report about some experiments on how the difference comes from.