3P176 ケラトサイトと好中球と粘菌の遊走のための異なるメカノセンシング機構

Mechanical responses of keratocytes, neutrophils and *Dictyostelium* cells for their optimal migrations

Chika Okimura¹, Takafumi Mizuno², Yoshiaki Iwade¹ (¹*Faculty of Science, Yamaguchi University*, ²*Biomedical Research Institute, National Institute of Advanced Industrial Science and Technology (AIST)*)

Crawling migration is achieved even under no external attractants. How do cells generate their migrating polarity? Cells adhering to substrata must receive and respond to mechanical stimuli from the substrata. The mechanical force is a potent candidate which regulates migrating polarity. Cyclic stretching-recovery of substratum (CSS) is one of the most appropriate techniques to estimate the relationship between the force from the substratum and the cell function.

Fish keratocytes, neutrophil-like HL-60 cells and *Dictyostelium* cells are commonly used as materials for investigation of fast crawling cell migration. In response to the CSS, they showed different migrations. We will discuss the difference dependent on cell types and molecular dynamics in each cell type.

3P177 マイコプラズマは左回りに進むのか？

Do Mycoplasmas glide to the left?

Hanako Morio, Taishi Kasai, Makoto Miyata (*Grad. Sch. Sci., Univ. Osaka City*)

Mycoplasma mobile, a fish pathogenic bacterium, forms a membrane protrusion at one pole and glides in its direction. The gliding does not occur along a straight line. We checked the gliding direction and found that 77% cells glide with curving to the left. The units of gliding machinery may align helically around the cell neck and generate the propelling force biased from the cell axis. The gliding machinery pulls the other part of cell, and generates the drag force at other cell parts, resulting in the bias in gliding direction. We traced a 200 nm bead artificially bound to the cell, and found that the cell does not rotate around the axis. This may be explained by assuming that the legs always pull the cell body rather than row.

3P178 微小管 X 線繊維回折：チューブリンピッチの動的変化 Dynamic changes of the axial pitch of tubulin repeat in live microtubules revealed by x-ray fiber diffraction

Shinji Kamimura¹, Yosuke Fujita¹, Yuuko Wada¹, Hiroyuki Iwamoto² (¹*Dept. Biol. Sci., Chuo Univ.*, ²*JASRI, SPring-8*)

Microtubule (MT) is one of the key components of eukaryote cytoskeleton. Our question is how MT assembly/disassembly dynamics is correlated with the conformation of tubulin dimers inside MT. The technique we developed for the quick shear-flow alignment of biological filaments enabled us to analyze the x-ray fiber diffractions of MTs under various physiological conditions. We found that MTs were classified into 3 groups of distinct structural properties, which varied depending on GTP-hydrolysis and taxol content. Interestingly, the axial pitch of tubulin repeat of GMPCPP-MTs became longer by lowering temperatures. It is strongly suggested that even in assembled MTs, both GTP- and GDP-tubulin dimers can undergo dynamic conversion between short- and long- configurations.

3P179 生細胞内における厳密な PI3K ヘテロダイマー複合体のシグナル応答

Dynamic Signal Response of Rigorous PI3K Heterodimer in Living Cells

Chan-Gi Pack¹, Yuko Saeki², Mariko Okada², Yasushi Sako¹ (¹*Cellular Informatics Laboratory, RIKEN*, ²*Laboratory for Integrated Cellular Systems, RIKEN IMS-RCAT*)

In mammals, class IA phosphoinositide 3-kinases (PI3Ks) consist of a p110 catalytic subunit (p110a or p110b) bound to any of five regulatory subunits including p85a and p85b. It has been suggested previously that free p85a negatively regulates PI3K signaling by competition with p85/p110 heterodimers for recruitment to phosphotyrosine residues of ErbB. In contrast, it was recently shown by quantitative mass spectroscopy that p85 and p110 subunits are present in equimolar amount in mammalian cell lines suggesting the nonredundant complex formation between PI3K subunits. However, compositions of PI3K subunits have never been studied in vivo conditions. Here, we for the first time detect spatio-temporal dynamics of the interactions between PI3K subunits in living cells.

3P180 Cell signaling occurs by a specific mobility and clustering state of epidermal growth factor receptor

Michio Hiroshima^{1,2}, Yasushi Sako² (¹*RIKEN QBiC*, ²*RIKEN Cellular Informatics Lab.*)

An information-theory based analysis combining with fluorescence single-molecule imaging showed that epidermal growth factor receptor (EGFR) moves across the plasma membrane with rapid transitions among several mobility and clustering states. EGF stimulation induced an equilibrium shift to the state of slowest diffusion and larger cluster where interactions between EGFR and Grb2 adaptor protein occurred significantly more frequent compared to other states. Receptor molecules in the state undergo confined diffusion and the diffusion characteristics were affected by membrane cholesterol depletion. Therefore, EGF signaling arises through dynamic interactions between the receptor molecules in a specific state susceptible to membrane components.

3P181 Detection of Cellular Responses to a Differentiation Factor Using Raman Microspectroscopy

Sota Takanezawa^{1,2}, Shin-ichi Morita¹, Yasushi Sako¹, Yukihiro Ozaki² (¹*Cellular Informatics Lab., RIKEN*, ²*School. Sci. Tech., Kwansei-Gakuin Univ.*)

Raman microspectroscopy is one of powerful methods to obtain comprehensive information about molecular compositions in living cells. We measured Raman spectra in the cytoplasm and nucleus of human breast cancer derived MCF-7 cells at 0-2 hours of heregulin (HRG) stimulation. HRG induces differentiation of MCF-7 into mammalian grand-like cells. Average and standard deviation of the spectra from HRG-stimulated cells were compared with those from control cells. In the HRG-stimulated cells, decreases of lipid and protein fractions were observed with an increase of water fraction. This result suggests that cell morphological changes were induced, and/or concentration of intracellular molecules was diluted by HRG stimulation.