2P200 PI3K-PTEN システムにおける自己組織化現象
○新井 由之¹、松岡 里実¹、柳田 敏雄¹、上田 昌宏¹
¹阪大院・生命機能
Traveling waves in PI3K-PTEN system for spontaneous cell migration and tactic responses.
Yoshiyuki Arai(1), Satomi Matsuoka(1), Toshio Yanagida(1) and Masahiro Ueda(1). (1:Graduate school of Frontier Biosciences, Osaka University)

Spontaneous cell migration is the basis to realize the tactic response to environmental cues. In Dictyostelium cells, the spontaneous migration and shape changes are not random, but driven in an oscillatory manner. How spontaneous cell migration is generated remains to be unsolved. To investigate the mechanisms of spontaneous cell migration, we have focused on intracellular dynamics of both phosphoinositide-3 kinase (PI3K) and phosphatase tensin homolog deleted on chromosome 10 (PTEN) molecules. PI3K and PTEN are localized exclusively on membrane, where catalyze the production and degradation of PIP3 respectively. Such complementary localization of PI3K and PTEN is a molecular basis of PIP3 localization at the PI3K-dominant side, which leads to pseudopod formation. Thus, the PI3K-PTEN system can be regarded as a compass for cell migration. Here we report spontaneous generation of traveling waves in the PI3K-PTEN system on membrane of Dictyostelium cells. Fluorescent-labeled PI3K2 and PTEN were observed simultaneously in the cells, which revealed self-organizing oscillatory changes on membrane without external signals. This means that spontaneous pseudopod formation was driven by oscillatory activities of PI3K-PTEN system. External signals may bias the direction of cell via modulation of PI3K-PTEN system for tactic responses. We will discuss the possible mechanisms for the traveling waves in the inositol phospholipids signaling pathway based on reaction-diffusion dynamics of PI3K and PTEN.

2P202 バクテリア走化性受容体の共有結合修飾によるシグナル増幅への影響
○黒岩 延裕¹、柴田 達夫¹
¹広大院・理・数理分子生命科学
2aC07 Effect of Covalent Modification on Signal Amplification in Bacterial Chemotactic Receptors
Nobuhiro Kuroiwa (1), Tatsuo Shitaba (1). (1: Dept Mathematical & Life Sci, Graduate School of Sciences, Hiroshima Univ)

Bacterial chemosensory signaling pathway is known for ultrasensitive amplification of chemical input stimuli. In the pathway covalent modifications of the receptor, methylation and demethylation by CheR and CheB, are essential to the adaptation, kinase activity of the receptor complex transiently changed by ligand binding in an ultrasensitive manner then relaxed to basal level. A mechanism of the adaptation is explained, however, amplification mechanism remains poorly understood although the structures of molecules and components of the system are well studied. Here we analyze contribution of methylation/demethylation dynamics onto the ultrasensitive signal amplification. The model suggests that methylation and demethylation play an important role in the signal amplification, and capability of ultrasensitive amplification even in the absence of cooperative ligand binding and receptor-receptor interactions.

2P201 細胞膜におけるPI(3,4,5)P₃脱リン酸化酵素PTENの局在形成の分子メカニズム
○松岡 里実¹、柳田 敏雄¹、Devreotes Peter N.²、上田 昌宏¹
¹阪大院・生命機能・ナノ生体科学、²Dept. of Cell Biology, Johns Hopkins Univ.
2aC06 Dynamic regulation of PI(3,4,5)P₃ phosphatase activity of PTEN on membranes revealed by single-molecule imaging
Satomi Matsuoka (1), Toshio Yanagida (1), Peter N. Devreotes (2) and Masahiro Ueda (1). (1: Laboratories for Nanobiology, Graduate School of Frontier Biosciences, Osaka Univ.;2: Dept. of Cell Biology, Johns Hopkins Univ. School of Medicine)

Chemotaxis of eukaryotic cells involves an establishment of cellular polarity along the moving direction, and one hallmark is a localization of PI(3,4,5)P₃ on membranes at the leading edge. By acting at lateral and posterior membranes, the PI 3-phosphatase, PTEN, plays an indispensable role in restricting PI(3,4,5)P₃ to the front. We have revealed the mechanisms of regulation of PTEN by observing single molecules of Halo-tagged PTEN in living *Dictyostelium* cells. Single PTEN molecules showed transient association and lateral diffusion on membranes. From an analysis of the life-times of membrane interaction, we revealed two populations of membrane-bound PTEN. These two populations distributed on membranes in a spatially regulated manner along cell polarity. The population with the slower dissociation rate was detected exclusively at posterior membrane, while the faster one was observed at whole membrane. From an analysis of the lateral diffusion, we found membrane-bound PTEN undergoes a transition between two states with different diffusion coefficients. The difference in the dissociation rate could be attributed to the difference in the transition rate to the state with the slower diffusion. The transition likely involves the interaction with some membrane-associated molecule. It is possible that this unidentified molecule enriched on posterior membrane controls the membrane-binding stability of PTEN molecules on membrane, which is a novel mechanism how PTEN is locally activated on the posterior membrane.

2P203 細胞への針挿入の成功率と細胞構造
中村 史^{1,2}、○上石 英希²、中村 徳幸^{1,2}、三宅 淳^{1,2}
¹産総研・セルエンジニアリング研究部門、²東京農工大院・工・生命工
Insertion probability of needle into a cell and structure of the cell
Chikashi Nakamura(1,2), Hideki Kamiishi(2), Noriyuki Nakamura(1,2) and Jun Miyake(1,2). (1: Res. Inst. Cell Eng., AIST, 2: Dept. Biotechnol., Tokyo Univ. Agric. & Technol.)

We have been developing a novel cell manipulation technology by using atomic force microscopy (AFM) and an AFM tip etched to several hundred nanometers in diameter by focused ion beam. We call the etched tip nanoneedle. The nanoneedle is approached to and is withdrawn from a living cell using an AFM apparatus. Mechanical force monitoring using AFM is useful to sense the nanoneedle position toward the cell. In approach process, the nanoneedle apex contacts with the cell surface and it indents the cell, hereby a repulsive force exerted on the nanoneedle increases, and then a steep repulsive force drop appears. The force drop indicates penetration event of the nanoneedle through the cell membrane (1). Insertion probabilities of nanoneedle to cells vary in cell species. To decompose stress fiber or actin filament, Y27632 or cytochalasin D were used to several kinds of cells. Insertion probabilities decreased with cell stiffness. It was suggested that insertion of nanoneedle into cell depended on cellular stiffness. (1)I.Obtaya et al., Nano Letters,5,27-30 (2005)