various pH conditions. Flickery gating was observed in the negative potentials. Kinetics and open channel noise analyses of the single-channel current were performed. Based on our present data, we will discuss how the KcsA channel regulates ion conduction using two separate gates.

1P-194 サブミリ秒時間分解能での KcsA カリウムイオンチャンネル
開閉構造変化計測
Conformational Changes of Single-Molecular KcsA Potassium
Channels during Gating Recorded in a Sub-Millisecond Time
Resolution

Hirofumi Shimizu (1), Masayuki Iwamoto (1), Takashi Konno (1), Reo Sakakura (2), Yoshimitsu Aoki (2), Shigetoshi Oiki (1) ((1) *Department of Molecular Physiology and Biophysics, University of Fukui Faculty of Medical Sciences*; (2) *Department of Electronics and Electrical Engineering, Faculty of Science and Technology, Keio University*)

We had succeeded in recording global twisting motions of KcsA potassium channels during gating in a single molecule with Diffracted X-ray Tracking (DXT) method (1). The channels were fixed on a glass plate and a gold nanocrystal was attached to the other side as a probe. Synchrotron white X-ray was irradiated and a diffraction spot from the attached nanocrystal was recorded at video rate, which can be translated into the motions of channel. While this method was sensitive to detect motions of the channels (~0.1 degree), its time resolution was shortened to reveal the whole gating motion (30 frames per second). To overcome this problem we installed a new detecting system which was composed of x-ray image intensifier and high-speed camera. With this system the twisting motions during gating were successfully recorded at 5000 frames per second (200 μ s/frame) which is equivalent to the sampling rate in single channel current recordings. In this presentation we will discuss about the contribution of this development to revealing the gating motions in detail.

(1) H. Shimizu, M. Iwamoto, T. Konno, A. Nihei, Y.C. Sasaki, S. Oiki, *Cell* 137, 67-78, 2008

1P-195 T細胞認識における PUFAs の影響
Effects of disruption of lipid rafts by PUFAs on T cell recognition

Keiko Udaka (1), Satoshi Komaniwa (1), Arisa Yano (1), Toshihiro Komatsu (1), Akifumi Hagi (2) ((1) *Department of Immunology, Kochi Medical School*; (2) *Otsuka Pharmaceutical Factory, Inc.*)

see ITA3-08

1P-196 FPGA を用いた 1 分子生体シミュレーター
Single Molecule Bio-Simulator on FPGA

Michihiro Hori (1), Masahiro Ueda (1) ((1) *Osaka University*)

Progressing in single molecule imaging technology, stochastic nature of signaling molecules has been revealed in living cells. This leads to a fundamental question on the intracellular signaling processes: how does the signaling system in living cells reliably operate under thermal and stochastic fluctuations for flexible and robust responses in fluctuating environments? For understanding the design principle and computational principle of the system, it is essential that the relationships between cell's behaviors on the macroscopic level and stochastic properties of single molecules on the microscopic level are clarified. To carry out the simulation of the information processing in a cell including stochastic behaviors of single molecules, we have developed a calculation method by utilizing field programmable gate arrays (FPGAs). FPGAs are the LSI devices which can change digital circuits easily by computer programming. FPGAs are more flexible than application-specific integrated circuits (ASICs), and FPGAs can process information faster than software-based calculation. In this study, we propose a method for FPGA implementation, in which stochastic nature of biochemical reactions and diffusion of single molecules can be included in the simulation of the information processing in a cell. We applied this method to the stochastic simulation of chemotactic signaling system.

1P-197 細胞内 1 分子解析に基づいた走化性応答における受容体-G 蛋白
質間相互作用のモデル
Two state model of G protein-coupled chemoattractant receptors for
directional sensing in chemotaxis

Yukihiro Miyana (1), Michihiro Hori (1), Tatsuo Shibata (2), Mashahiro Ueda (1) ((1) *Graduate School of Frontier Biosciences, Osaka University*; (2) *Department of mathematical and life science, Hiroshima university*)

Transmembrane signaling for directional sensing in chemotaxis of eukaryotic amoeboid cells is mediated by heterotrimeric G protein-coupled receptors. Early activation events of G proteins by the receptors have been explained by a two state model, in which the receptors have both high and low affinity for

chemoattractants, and the receptor with high affinity promotes activation of heterotrimeric G protein. We used single-molecule imaging techniques to reveal the intracellular dynamics of G proteins in living *Dictyostelium* cells, which provided direct measurement of the mobility on membrane. We found that receptor stimulation induced a slow down of G α subunits in their lateral mobility, attaining a half-maximum response at about 320 nM of a chemoattractant. The mobility shift was probably due to a complex formation between the receptor and G α subunit. This dose-dependence of the response corresponds to low affinity of receptor but is different from that of G protein activation that was obtained previously to be a half-maximum at about 10 nM, roughly high affinity of the receptor. To explain these observations, we extended the two state model, in which the receptor with high affinity serves as an activator for heterotrimeric G protein in an enzymatic manner while the receptor with low affinity serves as a binding site for the activated G protein, which have important implications in the mechanism underlying the generation of a spatiotemporal pattern of active G proteins on membrane for directional sensing in chemotaxis.

1P-198 トロンボポエチンシグナルの初期段階は脂質ラフトによって制御
されている
Initial thrombopoietin signaling is regulated by lipid rafts

Akihiko Sakamoto (1), Takashi Kato (2), Takashi Funatsu (3) ((1) *Graduate School of Pharmaceutical Sciences, The University of Tokyo*; *Center for Medical System Innovation, The University of Tokyo*; (2) *Faculty of Education and Integrated Arts and Sciences, Waseda University*; (3) *Graduate School of Pharmaceutical Sciences, The University of Tokyo*; *Center for NanoBio Integration, The University of Tokyo*; CREST, Japan Science and Technology Agency)

Thrombopoietin (TPO) is a cytokine that primarily regulates megakaryocyte maturation and platelet production. Binding of TPO to its receptor, MPL, causes a conformational change of MPL, which allows cross-phosphorylation of Janus kinase 2 (JAK2). Phosphorylated JAK2 then phosphorylates MPL and several downstream effectors including signal transducers and activators of transcription, mitogen-activated protein kinase, and phosphoinositide 3-kinase. Activation of these pathways promotes proliferation and differentiation of megakaryocytes. However, how TPO signaling is regulated has not been clarified. Here, we show that initial TPO signaling is regulated by the distribution of MPL on the cell surface. Fluorescently labeled TPO gradually clustered on the cell surface of MPL-expressing cells, followed by internalization. Cholesterol depletion by methyl- β -cyclodextrin inhibited both clustering and internalization of TPO. Furthermore, phosphorylation of JAK2 and MPL were dependent on clustering of TPO rather than its internalization. Cell fractionation by sucrose density gradients revealed that TPO induced a translocation of MPL and JAK2 from lipid raft fractions to nonraft fractions. These data suggest that the distribution of MPL on the cell surface is a key regulator of initial TPO signaling. We are now studying (i) a molecular mechanism for TPO-dependent translocation of MPL; and (ii) the behaviors of MPL on the cell surface by single molecule imaging.

1P-199 コントラスト変調と異常分散を用いた膜蛋白質結晶中の脂質二重
膜の可視化
Visualization of lipid bilayer in the crystal of the membrane protein
using x-ray contrast variation and anomalous scattering

Yoshiyuki Norimatsu (1), Junko Tsueda (1), Ayami Hirata (1), Shioh Iwasawa (1), Chikashi Toyoshima (1) ((1) *Institute of Molecular and Cellular Biosciences*)

see ITA3-07

1P-200 小脳核グルタミン酸作動性シナプスにおけるモノアミンを介した
修飾作用
Monoaminergic cross-talk action at the mossy fiber-deep cerebellar
nuclei synapses

Fumihito Saitow (1), Hidenori Suzuki (1) ((1) *Dept. Pharmacology, Nippon Medical School*)

Activity of the deep cerebellar nuclei (DCN) takes an important role in outputting processed information from the cerebellum. Modulatory effects of synaptic transmission are important for regulating the information flow. As reported previously, one of monoamine, 5-HT shows the inhibitory effects on both glutamatergic and GABAergic transmissions through the presynaptic mechanisms. In this study, we examined a role of other monoamines, noradrenaline (NA) and dopamine (DA) on the synaptic transmission in the DCN using rat cerebellar slices. We recorded stimulation-evoked EPSCs from DCN neurons using whole-cell patch-clamp recording. Bath application of 10 μ M NA or DA decreased the amplitude of glutamatergic EPSCs but not GABAergic IPSCs. Biophysical and pharmacological studies revealed that presynaptic α_2 -adrenergic (α_2 -AR) and D2-like receptors (D2R) were responsible for the inhibitory effects on the EPSC amplitude, respectively. Although ~40 % of synapses did not show the inhibitory effects by the application of a D2R agonist quinpirole, application of DA