at an interval of 3 degrees. The image reconstruction of neural network was performed by cone-beam filtered back-projection algorithm. The 3-D network of dendrites and axis cylinder were revealed in the reconstructed image.

2P325 高速原子間力顕微鏡によるプロセス糖質加水分解酵素の一分子観察
Single molecular observations of processive glycosidases by high-speed atomic force microscopy

Kiyohiko Igarashi¹, Takayuki Uchihashi², Masahisa Wada¹, Toshio Ando², Masahiro Samejima¹,
 (¹ Graduate School of Agricultural and Life Sciences, The University of Tokyo; ² Department of Physics, Kanazawa University)

In nature, filamentous fungi are the most potent degrader of cellulose using various cellulose-hydrolyzing extracellular enzymes, generally called cellulases. Although extensive studies have been done, the mechanisms of hydrolysis of celluloses by cellulases are quite ambiguous, because the reaction occurs at a solid/liquid interface. Fungal cellobiohydrolases have the ability to hydrolyze cellulose chains of a crystalline substrate due to their two-domain structure, i.e., cellulose-binding domain (CBD) and catalytic domain (CD), and unique active site architecture. However, the details of the action of the two domains on crystalline cellulose are still unclear. Here we present real-time observations of *Trichoderma reesei* (Tr) cellobiohydrolase I (Cel7A) molecules sliding on crystalline cellulose, obtained with a high-speed atomic force microscope. The average velocity of the sliding movement on crystalline cellulose was 3.5 nm/sec, and the catalytic domain without the CBD moved with a velocity similar to that of the intact TrCel7A enzyme. However, no sliding of a catalytically inactive enzyme (mutant E212Q) or a variant lacking tryptophan at the entrance of the active site tunnel (mutant W40A) could be detected. This indicates that, besides the hydrolysis of glycosidic bonds, the loading of a cellulose chain into the active site tunnel is also essential for the enzyme movement.

2P326 Toward ultimate size down of Aequorea fluorescent protein Venus

Kimura Taro (1), Matsuda Tomoki (1), Nagai Takeharu (1), (Laboratory for Nanosystems Physiology, Research Institute for Electronic Science, Hokkaido University)

Application of green fluorescent protein (GFP) from the jellyfish *Aequorea victoria* and its color variants, as well as GFP-like proteins from other organisms, has revolutionized our ability to analyze a wide range of biological issues such as gene expression, protein localization, and cell motility in living specimen. However, when fused with a smaller protein of interests, the relatively large size (Mr = approx.30,000) of GFP, which is composed of 238 amino acids, would have a possibility to hamper function of the fused protein. To overcome this problem, size reduction of GFP has been desired. So far, Li et al successfully made deletion mutant of GFP. However, more than 16 amino acids deletion from GFP completely diminished the fluorescent property. To further reduce the size of fluorescent protein, we performed iteration of amino acids deletion, identification of deletion mutant with weak fluorescence, and introduction of random mutation to restore the fluorescence intensity. Among the fluorescent protein variants, we used the brightest version of yellow fluorescent protein, Venus, since the folding and chromophore forming ability is superior than those of other fluorescent proteins. So far, we have already succeeded in obtaining the smallest fluorescent protein reported to date. Despite 29 amino acids deletion, the Venus mutant showed comparable fluorescence intensity with that of native Venus. In the meeting, we will present our current study toward development of a miniature Venus comprised of less than 200 amino acids.

2P327 蛍光標識 DNA アプタマーと分解酵素を用いた可逆的な細胞ラベリング技術の開発
Labelling of live cells using fluorescent aptamers: binding reversal with DNA nucleases

Hideyuki TERAZONO¹, Yu ANZAI¹, Kenji YASUDA^{1,2}, (¹ Yasuda "On-chip Molecular cell" Project, Kanagawa Academy of Science and Technology, KSP East 310, 3-2-1 Sakado, Takatsu-Ku, Kawasaki, Kanagawa, 213-0012, Japan; ² Department of Biomedical Information, Division of Biosystems, Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University, 2-3-10 Kanda-Surugadai, Chiyoda-ku, Tokyo 101-0062 Japan)

Separation of uniform phenotype cells maintaining its native states is important both for post genome cell based studies and practical applications of those cells for regenerative medicine. For non-destructive and non-invasive cell purification, reversible cell labeling method has been developed exploiting high affinity single strand DNA (ssDNA) against target molecule on cells, aptamer, and following DNase digestion procedure. Human acute lymphoblastic leukemia cells were labeled with Qdot ssDNA aptamer, and were restored into the label-free condition by nuclease digestion. The conjugation between the

fluorescent-aptamer and the cells was evaluated on the fluorescence intensity by flow cytometer, and confirmed the decoration of aptamers. After 10 min DNase nuclease digestion, the intensity of the fluorescence of labeled cells returned to that of the non-labeled intact cells by the removal of aptamers. For the confirmation of damage on cells by aptamer or by nuclease, the growth rates of the nuclease-treated cells were evaluated and were equivalent to that of the non-treated intact cells, and therefore the digestion process had no significant influence on the growth of the cells in following the cultivation. The results indicated the potential of non-invasive reversible labeling and cell separation with maintaining the native cell activity and further applications of those cells instead of conventional antibody technology.

2P328 Gene expression in growing cells

Mikihiro Hashimoto, Yuichi Wakamoto, (Department of Basic Science, Graduate School of Arts and Sciences, The University of Tokyo)

Gene expression levels measured by the amount of proteins or mRNA are not static but dynamically fluctuating at the single-cell level, even if cells are endowed with the same genotype and under the same environmental conditions. The fluctuation of gene expression can potentially generate the epigenetic diversity. Although the importance of the fluctuation is being recognized little by little, its biological meanings and functions still remain unclear. In addition, it is an important and difficult problem to evaluate the factor of the container size changing according to growth. To understand the role of the fluctuation for biological activity, we developed a simple new experimental system which can obtain a large amount of data at one time utilizing microfabrication and live-cell imaging techniques and made software to analyze time series of fluorescence intensity at the single cell level focusing on *Escherichia coli* cells as a simple model. As a result, it has been revealed that growth rate and time-scale of the correlation decay of the gene-expression fluctuation in single cells is closely related in a manner that could enhance plastic adaptation to stress environments. The characterized relation may underlie the universality of cellular phenotypic adaptation such as persistence.

2P329 ストレス環境における生きた細胞の内在性 mRNA のリアルタイムイメージング
Real-time Imaging of Endogenous mRNAs in Living Cells during Stress

Junwei Zhang¹, Kohki Okabe¹, Tokio Tani², Takashi Funatsu¹, (¹ Laboratory of Bio-Analytical Chemistry, Graduate School of Pharmaceutical Sciences, The University of Tokyo; ² Department of Biological Science, Graduate School of Science and Technology, Kumamoto University)

During stress, cytoplasmic mRNAs aggregate to RNA granules called stress granule (SG), where they are sorted and remodeled for reinitiation of translation, degradation or storage. Till now, SG is mainly studied indirectly by using protein markers, and the real time behavior of endogenous mRNAs in living cells remained uncertain. Here we used a linear antisense probe to visualize endogenous cytoplasmic mRNAs in living cells in order to investigate the dynamics of mRNAs under stress. A Cy3 and biotin labeled poly(U)₂₂ 2'-O-methyl RNA probe combined with streptavidin was microinjected into the cytoplasm of COS7 cells, detecting poly(A)⁺ mRNAs. After that, 0.5 mM arsenite stress was induced. We also transfected TIA-1-GFP plasmid into the cells to visualize SG.

Endogenous poly(A)⁺ mRNAs visualized by the antisense probe aggregated to granules during stress, which colocalized with SG marked by TIA-1-GFP. Furthermore, release of the stress caused the disassembly of the aggregated mRNAs in SG. Next, the mobility of mRNAs in SG was analyzed by fluorescence recovery after photobleaching (FRAP). As a result, 35 % of the mRNAs in SG was immobile. Time constants of the fluorescence recovery of the mobile mRNAs were 35 s and 265 s. This result is independent of neither the continuous time of stress nor the kind of mRNA. Moreover, dynamics of cytoplasmic mRNAs analyzed by fluorescence correlation spectroscopy (FCS) agreed with the results of imaging. Physiological significance of immobility of the mRNAs in SG is under study.

2P330 HaloTag を用いた大腸菌定着性受容体の局在観察
HaloTag works in *Escherichia coli* for observation of the chemoreceptor localization

Takehiko INABA¹, Tomoko Saito², Ikuro Kawagishi^{1,3}, (¹ Research Center for Micro-Nano Technology, Hosei Univ.; ² Faculty of Engineering, Dept. Frontier Bioscience, Hosei Univ.; ³ Faculty of Bioscience and Applied Chemistry, Dept. Frontier Bioscience, Hosei Univ.)

In *Escherichia coli*, the chemoreceptors form clusters with the histidine kinase CheA and the adaptor CheW that localize to the cell pole. This clustering is thought to be important for effective sensing of and responses to a wide range of stimulation. However, the mechanisms underlying receptor localization