measure the dynamics of the interaction between the AFM probe and the live cell surface, we designed a new cantilever with a scooping function which can seamlessly manipulate bead probes adhered on cells one after another. There is no need for the time consuming process of replacing probes in each experiment. The newly designed cantilever has a semicircular cutout for the scooping function at its arrow head edge, thus, a “micro scoop cantilever”. The micro scoop cantilever allowed us to perform various experiments such as recovery of proteins interacting with ligands immobilized on a bead, detection of the bead adhesion force up to micronewtons, and simultaneous measurements of input and output force.

1J1448 蛍光ナノ温度計を用いた細胞小器官内の温度測定 P78 Temperature measurement inside organelles with fluorescent nanothermometers

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Fluorescence thermometry of single living cells has recently succeeded in measuring not only the temperature changes externally induced by local heating techniques, but also the self-active temperature increase related to the thermogenesis which is induced by chemical stimulations. On the other hand, living cells respond to the external temperature changes in the form of Ca^{2+} burst, pH adjustment, and so on. Therefore, the local temperature changes induced by chemical reactions in living cells may have potential to affect the activities of biological systems nearby. This viewpoint of “Thermo-Chemical Signalling” will be essential for understanding how the living cells effectively use local temperature changes. The conventional fluorescence thermosensors, however, are generally sensitive to physical and chemical parameters in addition to temperature. Here, we fabricated fluorescent nanoparticles with no sensitivity to, at least, pH and ionic strength. Due to their high fluorescence intensity, the temperature change could be determined by the changes in fluorescence intensity of each nanothermometer with 115.79 Hz temporal resolution. We found that the nanothermometers, which were captured into living cells by endocytosis, were encapsulated into acidic endosome and transported along microtubules. We demonstrate that both the temperature and the position of the moving organelles can be measured simultaneously inside living cells; a unique application of this probe as a “walking nanothermometer”.

1J1512 サルモネラ菌 L 型直線形べん毛繊維のラマン光学活性に対するべん毛繊維の長さによる影響

Effect of the flagellar filament length on Raman optical activity of L-type straight filament of *Salmonella*

Ai Suga¹, Masashi Sonoyama¹, Yoshiaki Hamada², Hideyuki Yoshimura³, Masashi Asahina⁴, Fumio Hayashi¹, Kenji Oosawa¹ (¹*Grad. Sch. Chem and Chem Bio., Gunma Univ.*, ²*The Open University of Japan*, ³*Biophysics, Meiji Univ.*, ⁴*Biosciences, Teikyo Univ.*)

The flagellar filament of *Salmonella* is an assembly of a single protein, flagellin. There are different helical and straight shape filaments from wild type and mutants. We previously reported that the Raman spectra of normal and two straight filaments were almost same, but in the Raman optical activity (ROA) spectra, some intensive ROA peaks were observed from only one of two straight filaments, L-type. When the L-type filaments were depolymerized or fragmented, the intense ROA bands disappeared. It was noted that the ordered structure formed by the main chain of the protein in the L-type filament seems to be one of the essential factors for producing the intensive ROA signals. If the ordered structure in the L-type filament contributing to the intense ROA signals is derived from a specific alignment of monomers along the filament and the filament length is one of important factors, there would be the minimum length to arise the ROA intensities. To investigate of effects of the filament length on ROA intensities, it is necessary to control filament length. We prepared filament samples with the protocols reported by I. Yamashita et al. (*J. Mol. Biol.* (1991) **217**, 293-302) and measured of these filaments. In this meeting, we will discuss effects of filament length on ROA.

1J1524 マイクロウェルと蛍光相関分光法を用いた単一細胞内タンパク質定量測定

Quantification of total number of the protein in single cell by using Microwell and Fluorescence Correlation Spectroscopy

Sho Oasa, Akira Sasaki, Masataka Kinjo (*Grad. Life Sci., Hokkaido Univ.*)

The single-cell level measurement is required when the heterogeneous amounts of target protein could be estimated in each cell. However, protein quantification in living cell has been performed by biochemical methods. The only blunted value is obtained in this case, because a total amount of target proteins and a number of cells can be estimated. In order to achieve single-cell level analysis, the small space (Microwell) constructed on the PDMS (polydimethylsiloxane) chip was studied in our laboratory. The single cell was isolated in Microwell and the number of EGFP in single cells was measured in Microwell. However, the quantitative measurement was difficult due to background fluorescent signal from cell lysate. In this study, we have measured the various concentrations of EGFP solution in Microwells by using FCS (Fluorescence Correlation Spectroscopy). The background fluorescence was corrected by using the numerical equation and the lower limit for quantitative analysis of EGFP concentration was determined. The cell volume was estimated in order to confirm whether EGFP is completely extracted from the cell in the Microwell. These verifications revealed that the Microwell system and FCS determine the amounts of target protein quantitatively. Moreover, another fluorescent protein is expressed as internal standard so that the target proteins can be estimated the absolute number of molecules in the cell. We will try to apply Microwell system to aggregate proteins.

1J1536 miRNA の高感度な定量を目指した DNA マイクロアレイの開発 Development of DNA microarray for high-sensitive quantification of miRNA

Taro Ueno, Takashi Funatsu (*Grad. Sch. Pharm. Sci., Univ. Tokyo*)

MicroRNAs (miRNAs) are 18-25 nucleotides in length, which bind to target mRNAs and suppress their expression levels in all eukaryotes. Because the amounts of some miRNAs are downregulated or upregulated in blood serum of cancer patients, miRNAs in blood serum can be potential standard biomarkers for cancer diagnosis. Aiming at the high throughput-quantification, we constructed and evaluated the DNA microarray for miRNA quantification. First, we immobilized the fluorescent ssDNA probes, which are complementary sequences to the miRNA-141, on the glass surface using an inkjet spotting machine. In an area of ~4000 m in diameter, 121 fluorescent spots of ~200 m in diameter were arrayed. When polyethylene glycol (PEG) was used as a long linker between ssDNA and glass surface, the HY rose up ~15 times, which implies the probes immobilized via PEG are more mobile and acceptable for miRNA than the probes bound to solid surface directly. Next, synthesized Cy5-labeled miRNA was introduced onto the glass and incubated for overnight. After stringent washing, hybridized Cy5-miRNAs were observed on the probes. The normalized fluorescence intensities of Cy5 were proportional to the input amount of Cy5-miRNA, although its hybridization yield (HY) was not sufficient (~0.007%). Actually, the polarization (P) of the fluorescent dye attached to probes with PEG is twice higher than P of ssDNA probes without PEG. We concluded long PEG-linker has an advantage on bio-molecules interaction on glass surface, leading to high sensitive quantification of miRNA.

1J1548 ナノポーラスメンブレンを用いた単一細胞遺伝子発現解析のための基礎技術

New tool for analyzing gene expression of single cells using nanoporous membrane

Maiko Tanabe¹, Masataka Shirai¹, Tomohiro Konno², Kazuhiko Ishihara², Hideki Kambara^{1,2} (¹*Central Research Laboratory, Hitachi, Ltd.*, ²*Dept. Bioeng., Univ. Tokyo*)

Cells are the basic structural and functional units of living organisms. Individual cells vary in behavior during the formation of tissues. The molecular science data obtained to now are the averages over ensemble of cells which may mask the variations. The analysis of single-cells is very important to understand morphogenesis of tissues, such as developmental biology, cancer biology and regenerative medicines. A small number of cells may play an essential role in the function of a tissue. For clarify these, we need to develop new tools for analyzing precise and high-throughput gene expressions of single-cells. Here, we report a novel quantification method for analyzing multiple gene expressions in single-cells. We use thin nanoporous membranes with penetrating pores of 100-200nm in diameter to construct cDNA libraries in the membrane from cells that keep the positional information of mRNA. The cDNA in the membrane is quantified by fluorescent counting in 3 dimensional images of the membrane using a laser scanning confocal microscope. We discuss the counting accuracy by comparing the counted data with those obtained with qPCR for a controlled

mRNA sample. This research was supported by the Strategic International Cooperative Program of Japan Science and Technology.

13:00~17:00 K会場/ Room K

細胞生物の課題 1

Cell biology 1

1K1312 細菌べん毛蛋白質輸送ゲート複合体は H^+ でも Na^+ でも駆動されるデュアルフューエルシステムである

Bacterial flagellar type III protein export gate complex is a dual fuel system that utilizes both proton and sodium ion for protein export

Tohru Minamino^{1,2}, Yusuke Morimoto¹, Keiichi Namba¹ (¹Grad., Sch., Frontier Biosci., Osaka Univ., ²PRESTO, JST)

For self-assembly of the bacterial flagellum, which is responsible for motility, most of the flagellar component proteins are translocated into the central channel of the growing flagellar structure by the flagellar type III protein export apparatus. Flil is the ATPase of the export apparatus and forms a complex with FliH and FliJ to escort export substrates and chaperone-substrate complex from the cytoplasm to the export gate complex made of six integral membrane components of the export apparatus. The export gate complex is known to utilize proton motive force (PMF) across the cytoplasmic membrane as an energy source for protein unfolding and translocation. A specific interaction of FliJ with an export gate protein, FlhA, brought about by the docking of the FliH₂FliI₆ ring complex to the gate is required for efficient PMF-driven protein export. To clarify whether only proton is the coupling ion for the export gate complex to drive flagellar protein export, we analyzed the effects of various cations on flagellar protein export at an external pH value of 7.5. Sodium ion significantly facilitated protein export while other cations did not. Phenamil, a blocker of sodium ion channels, markedly reduced the secretion rate of export substrates. These results suggest an interesting mechanism in which the export gate complex is a dual fuel system that can utilize not only PMF but also sodium motive force across the membrane for flagellar protein export.

1K1324 細菌べん毛タンパク質輸送装置近傍の局所 pH の測定

Direct measurement of local pH around the bacterial flagellar protein export apparatus

Yusuke V. Morimoto¹, Nobunori Kami-ike¹, Tomoko Miyata¹, Keiichi Namba¹, Tohru Minamino^{1,2} (¹Grad. Sch. of Frontier Biosci., Osaka Univ., ²PRESTO, JST)

The bacterial flagellum, which is responsible for motility, is a biological nanomachine consisting of a rotary motor and a filamentous helical propeller. Most of the flagellar proteins are translocated into the central channel of the growing structure for self-assembly at the distal end by the flagellar protein export apparatus. The export gate complex utilizes proton motive force across the cytoplasmic membrane for protein unfolding and translocation. Recently, it has been shown that the proton flows through the gate is coupled to protein translocation. To understand the energy coupling mechanism, proton movements coupled with flagellar protein export must be directly measured. So far we have developed a high-resolution pH imaging system that can measure the intracellular pH distribution at a pH resolution of 0.02 using a pH-sensitive GFP, pHluorin. In this study, we fused pHluorin to the N-terminus of FliG, which is a C-ring component of the rotor, to measure local pH near the export apparatus. Electron microscopic observation revealed that the pHluorin part of the fusion protein is located inside of the C-ring and hence faces the export apparatus. We measured local pH around the export apparatus of wild-type cells and a Δ fliH-flil bypass mutant that forms flagella even in the absence of two soluble export components, FliH and FliI. We show that the local pH near the export gate of the Δ fliH-flil bypass mutant was significantly higher than that of wild-type cell. We will discuss this local pH difference.

1K1336 PomA 変異体を用いた Na^+ 駆動型べん毛固定子のモーターへの集合能の解析

The stator assembly around the rotor in the Na^+ -driven flagellar motor with mutations of the stator component PomA

Norihiro Takekawa¹, Natsumi Nonoyama¹, Na Li¹, Takashi Terauchi¹, Hajime Fukuoka², Seiji Kojima¹, Michio Homma¹ (¹Div. of Biol. Sci., Grad. Sch. of Sci., Nagoya Univ., ²IMRAM, Tohoku Univ.)

The polar flagellar motor of *Vibrio alginolyticus* rotates using Na^+ influx through the stator which is composed of PomA and PomB. About a dozen stators dynamically assemble around the rotor dependent on the Na^+ concentration of the environment. It is believed that the motor torque is generated by the interaction between cytoplasmic region of PomA and C-terminal region of FliG, a component of the rotor. In a previous study, we showed that mutations of FliG affect the stator assembly around the rotor, suggesting that the PomA-FliG interaction is required for the assembly. Now, we examined the effect of the PomA mutations on the assembly. All the examined mutant stators, which lost the motor function, assembled at lower level than the wild-type PomA. His-tag pull-down assay showed that some mutations in PomA reduced the PomA-PomB interaction, but the other mutations did not. Next, we tried to examine the ion conductivity of the mutants using the plug-deleted stator PomA/PomB(Δ 41-120) that impairs cell growth presumably because high amount of Na^+ is conducted into the cells. Some of the PomA mutations suppressed this growth inhibition, suggesting that such the mutant stators reduced the Na^+ conducting activity thereby they could not assemble. The mutations H136Y and D220K are not belonging to these two categories. We speculate that these particular mutations may affect the PomA-FliG interaction and prevent the activation of the stator assembly around rotor. Based on these results, possible mechanism of stator assembly will be discussed.

1K1348 Effect of the plug region deletion of PomB on function of the Na^+ -driven flagellar stator in *Vibrio alginolyticus*

Na Li^{1,2}, Seiji Kojima¹, Michio Homma¹ (¹Division of Biological Science, Graduate School of Science, Nagoya University, ²Division of Microbiology, college of life science, Northwest A&F University)

The stator proteins PomA and PomB form a complex that couples Na^+ influx to torque generation in the polar flagellar motor of *Vibrio alginolyticus*. This stator complex is anchored to an appropriate place around the rotor through a putative peptidoglycan-binding (PGB) domain in the periplasmic region of PomB (PomBC). To investigate the function of the periplasmic region of PomB, a series of in-frame deletion derivatives in the linker region between the transmembrane (TM) segment and the PGB domain of PomB were constructed. In-frame deletion (PomBAL) of up to 80 residues retained function and its over-expression with PomA impaired cell growth. This inhibitory effect was suppressed by a mutation at the functionally critical Asp (D24N) in the TM segment of PomB, suggesting that high Na^+ influx through the mutant stator causes growth impairment. Overproduction of the functional PomA/PomBAL stators also reduced motile fractions of the cells, and such effect is slightly relieved by a mutation (L168P) in the putative N-terminal α -helix connecting to the PGB domain without affecting the growth inhibition, suggesting that the conformational change of the region including the PGB domain affects stator assembly. Our results reveal the common features of the periplasmic region of PomB/MotB, and that flexible linker that contains 'plug' segment is important for the control of Na^+ influx through the stator complex as well as the stator assembly.

1K1412 ビブリオ菌 Na^+ 駆動型べん毛モーター固定子タンパク質 PomB のペリプラズム領域の結晶構造

Crystal structure of the periplasmic region of PomB, the stator protein of the Na^+ -driven flagellar motor of *Vibrio alginolyticus*

Seiji Kojima¹, Masato Takao², Mayuko Sakuma¹, Tatsuya Ibuki³, Michio Homma¹, Katsumi Imada² (¹Div. Biol. Sci., Grad. Sch. Sci., Nagoya Univ., ²Dept. Macromol. Sci., Grad. Sch. Sci., Osaka Univ., ³Grad. Sch. Front. Biosci., Osaka Univ.)

Stator protein PomA and PomB form a complex that functions as a Na^+ channel and couples Na^+ influx to torque generation in the polar flagellar motor of *Vibrio alginolyticus*. This stator complex must be anchored to an appropriate place around the rotor through a putative peptidoglycan-binding (PGB) domain in the periplasmic region of PomB (PomBC). Biochemical evidence suggests that PomBC interacts with MotX, a component of the T-ring structure of the flagellar basal body, to be stably incorporated into the motor. To understand the role of PomBC, we constructed a periplasmic fragment of PomB consisting of residues 135 through 315 (PomBC5) and determined its crystal structure at 2.1 Å resolution. PomBC5 forms a dimer in the crystal, which is consistent with the data obtained from size-exclusion column chromatography. PomBC5 appears as a single-domain structure, and its overall fold is almost identical to that of *Salmonella* MotBC, an ortholog of PomB. One distinct structural difference is the shorter N-terminal α -helix 2 (α 2) of PomBC5. Interestingly, this α 2 and connecting α 1, whose conformational changes in *Salmonella* MotB are speculated