antisense probes are advantageous in monitoring of mRNAs due to their predominant kinetics in hybridizing with target mRNAs.

the tolerance against the restriction enzyme digestion by measuring the level of gene transcription.

1P-270 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 Zhang (1), Kohki Okabe (1), Takashi Funatsu (1) ((1) Laboratory of Bio-Analytical Chemistry, Graduate School of Pharmaceutical Sciences, University of Tokyo)

In response to stress, eukaryotic cells reprogram mRNA metabolism to repair stress-induced damage and adapt to changed conditions, which is associated with the assembly of stress granule (SG) and processing body (PB) in the cytoplasm. During stress, cytoplasmic mRNAs aggregate to these RNA granules, where they are sorted and remodeled for reinitiation of translation, degradation or storage. Till now, stress-induced assembly of SG and PB is mainly studied indirectly by using protein makers, and the actual behavior of endogenous mRNAs in living cells during stress has not been detected directly yet and remained uncertain.

In this study, we used a linear antisense probe to visualize endogenous cytoplasmic mRNAs in living mammalian cells in order to investigate the dynamics of mRNAs under stress in cytoplasm and obtain insight of the role and function of SG and PB. Poly(A)⁺ mRNAs were chosen as the target. A Cy3 labeled poly(U)₂₂ 2'-O-methyl RNA probe was used to detect poly(A)⁺ mRNAs. The probes combined with streptavidin were microinjected into the cytoplasm of COS-7 cells, followed by the induction of stress with 0.5 mM arsenite.

As a result, a remarkable enhance in both size and number of RNA granules was observed after arsenite stress, but such increase was not found in normal cells. The granules were rapidly induced within 20 min in response to the stress. A large amount of small granules were first aggregated, and then gathered to bigger ones. The detail of the assembly and the dynamics of mRNAs is under study.

1P-273 編むことで構成された3次元マイクロハイドロゲル構造
Three-dimensional Hydrogel Microstructures Fabricated by Knitting

Hiroaki Onoe (1), Shoji Takeuchi (1) ((1) Institute of Industrial Science, The University of Tokyo)

We propose a method to knit hydrogel fibers for fabricating 3D hydrogel microstructures. Hydrogel microfibers were generated with a coaxial laminar flow microfluidic device. We manually handled these microfibers in a liquid, and succeeded to knit them to form a complex woven structure.

Hydrogel-based micro-scale structures have recently garner attentions for their potential applications in biochemical science and biotechnology. Usually, hydrogel microstructures have been constructed using micro-molding techniques or microfluidic devices. However, building complex three-dimensional hydrogel structure is technically difficult and a challenging issue. On the other hand, handling of fibers is well established in fabric industries, and knitting of fibers can make various shapes in one to three dimensions such as wires, sheets and cylinders. Our concept is to use hydrogel fibers instead of hydrogel beads or molded hydrogel structures, and to knit them for constructing three-dimensional microstructures, as knitted products in our daily life.

We successfully knitted three alginate-based hydrogel microfibers to form braid structures. This demonstration indicates that various kinds of micro-scale hydrogel structures could be fabricated by our knitting approach.

1P-274 生物物理学のための細胞サイズ半透膜チャンバー
A cell-sized semipermeable chamber for biophysics

Masahiro Takinoue (1), Shoji Takeuchi (1) ((1) Institute of Industrial Science, The University of Tokyo)

Microchambers for biophysical research have actively developed in recent years. Since the chambers have uniform shape and size, they allow us to observe biophysical phenomena quantitatively and accurately, e.g., a single molecular analysis of a biomacromolecule, a network analysis of cells or tissue, etc. However, most microchambers are closed system, so that buffer and medium solutions cannot be exchanged easily. Thus, it has been difficult to observe environmental responses of a single biomacromolecule and to realize a long-term observation of a cell/tissue culture by addition of nutrient. Our group has given an eye to this point, and has developed a semipermeable microsphere with a diameter of several tens micrometers as a semipermeable microchamber. Now, we are developing a cell-sized semipermeable chamber with a diameter of several micrometers. The chambers are placed in a microfluidic channel, so that this microchamber system can achieve not only exchange of the solution inside the chambers but also construction of an interaction network between the chambers connected by the microfluidic channel. In this presentation, we will show the progress of our research and discuss the possibility of the cell-sized semipermeable chamber in biophysics and DNA/RNA nanobiotechnology.

1P-275 蛍光性 BODIPY ケージド化合物を用いた生体分子の時空間光制御
Spatial and temporal control of biomolecules by green light irradiation with use of fluorescent BODIPY-caged compounds

Nobuhiro Umeda (1), Yasuteru Urano (1), Tetsuo Nagano (1) ((1) Graduate School of Pharmaceutical Sciences, The University of Tokyo; (2) CREST, JST; (3) JSPS)

Cellular events and biological activities are controlled by spatial and temporal distribution of biomolecules in cell system. Therefore, spatiotemporal control of biomolecular activities is one of the most important technologies used in current biological researches. Caged compound is one of the most powerful tools for the spatiotemporal control of biomolecules in cell system, which can be activated by irradiation of light. However, since present caged compounds require UV light for its activation, cell damage and poor permeability of the irradiation light into the tissues are inevitable problem in the photo-control system. In addition, users could not know the distribution of caged compounds, since they are invisible in microscope.

In this meeting, we will describe the development of novel caged compounds which are fluorescently visible, and efficiently activatable with green light. We have developed caged histamine and caged glutamate based on novel photo-reaction of 4-phenoxy BODIPY derivatives. Since these caged compounds fluoresce, we can see if the caged compounds are present where we would like to activate them. In addition, because the light of the range of wavelength is much harmless and far more permeable into biological tissues, developed caged compounds can be applied to UV-vulnerable samples, or organs and tissues. In the future, BODIPY-caged compounds would offer dual uncaging system, in which two different biomolecules are activated with different wavelength of light.

1P-276 Rc-PYP と相互作用タンパク質を用いた酵素活性の光制御系の作製

1P-271 キネシン-カルモジュリン融合蛋白質を用いた分子シャトルの開発
Kinesin Calmodulin Fusion Protein as a Molecular Shuttle

Hideki Shishido (1), Kiyoshi Nakazato (1), Eisaku Katayama (2), Shigeru Chaen (3), Shinsaku Maruta (1) ((1) Div. of Bioinfo., Grad. Sch. of Eng., Soka Univ.; (2) Div. of Biomolecular Imaging, Inst. of Med. Sci., Univ. of Tokyo.; (3) Dept. of Int. Sci. in Phys. and Biol., Coll. of Humanity and Sci., Nihon Univ.)

Recently attention is focused on the application of molecular shuttles based on the motor protein kinesin and microtubule to the drug delivery system (DDS) and lab-on-a-chip. Previously biotin-avidin and antigen-antibody system have been used to attach target cargoes on kinesin. Although the systems are highly specific and tight, these are flawed as irreversible binding. In this study, we employed reversible cargo loading system using calmodulin (CaM) and M13 peptide for the molecular shuttle. We have designed kinesin K560 chimera protein fused with CaM at the C-terminal tail region of kinesin (K560-CaM). K560-CaM was expressed by *E. coli* expression system and purified. The ATPase activity and the microtubules gliding activity of K560-CaM were almost in the normal range of the kinesin wild type. The Ca²⁺ dependent reversible binding of K560-CaM and M13 peptide was monitored with HPLC using size-exclusion column (SEC-HPLC). Using rotary-shadowing electron microscopy, K560-CaM was observed as tetrameric configuration in the absence of Ca²⁺. In the presence of Ca²⁺, K560-CaM was observed as dimeric and tetrameric configuration. The Ca²⁺-dependent configurational change of K560-CaM was also monitored by SEC-HPLC and analytical ultracentrifugation. Finally, we successfully observed that K560-CaM transports M13 peptide coupled with quantum dot along the microtubule in the presence of Ca²⁺ using TIRF-Microscopy.

1P-272 プログラム死する大腸菌の開発
The development of *E.coli* programmed death system

Kumiko Arata (1), Kazuhiro Tabata (1), Hirohuki Noji (1) ((1) Department of Biomolecular Energetics, The Institute of Scientific and Industrial Research, Osaka University)

We developed a genetic system for an inducible programmed death of *E.coli* cells, with an aim for application to the genome engineering. We introduced, into *E.coli* cells, an expression vector of a restriction enzyme, SacI, of which expression level is strictly controlled by pET and pLysS system. Once expressed, SacI can digest the genomic DNA at ~150 positions, leading a fatal damage to the *E.coli* cell growth. When the SacI expression was induced, the cell growth which was monitored by O. D. of the culture medium actually stopped. However, unexpectedly, the cell growth again started after 5 hours. Then, we measured the population of viable cells from colony forming unit by plating the culture on an agar plate. After induction of SacI, the number of viable cells in 1ml medium decreased from 10⁸ to 10⁴ but not extinct. We carried out the same experiment on a ΔrecA strain. The percentage of survival cells decreased in RecA gene deficient strain, suggesting that the RecA-dependent recombination of genomic DNA conferred the tolerance against the SacI induction. However, extinction was still not achieved. We are analyzing what kinds of genetic alternations conferred

Light dependent enzyme activity control by use of interaction of the Rc-PYP

Harumasa Kubo (1), Yoichi Yamazaki (1), Hironari Kamikubo (1), Mikio Kataoka (1) ((1) *NARA INSTITUTE of SCIENCE and TECHNOLOGY*)

To introduce light dependent activity regulation to various enzymes, we applied light dependent complex formation system discovered in photosynthetic bacterium. Rc-PYP is one of the Photoactive Yellow Protein isolated from *Rhodobacter capsulatus*. It absorbs UV-A and blue light by use of a covalently attached chromophore, p-coumaric acid. Rc-PYP interacts with its binding protein by exposure to UV-A light. This complex is dissociated with blue light irradiation. Light dependent complex formation and dissociation are achieved in this protein-protein interaction system, so two divided enzyme fusing with the Rc-PYP and binding protein are expected to reconstruct in the complex form light dependent manner. We verified whether this system was available for light dependent enzyme activity control by use of divided luciferase as a fusion enzyme firstly. Luciferase was divided into two sub domains, and these were fused to N or C terminal of Rc-PYP and the binding protein via glycine-serin linker. Among possible 8 patterns of fusion, two patterns were firstly constructed and examined their light dependent lusiferase activities. One of them was successfully introduced light dependent luciferase activity, but the other was not. It is revealed that the latter shows less binding affinity of fused Rc-PYP and its binding protein. Thus, this light dependent complex formation system is hopeful for light regulation attachment to various enzymes, but the fusion design requires more improvement.

2P-001 電子線結晶構造解析による水チャネル AQP1 の高分解能構造解析
High-resolution structural analysis of a water channel, AQP1, by electron crystallography

Kaoru Mitsuoka (1), Hiroko Endou (1), Daisuke Kasuya (2), Nobuhiko Gyobu (2) ((1) *BIRC, AIST*; (2) *BIRC, JBIC*)

Water is the most abundant molecule in all living cells and therefore an effective water transport function of the cell membrane is required. For this purpose, a family of membrane proteins called aquaporin exists from bacteria to animals and controls the entry and exit of water from cells. For example, the high water permeability of red blood cells is explained by the large amount of expressed aquaporin-1 (AQP1), a specialized water pore, in the plasma membrane.

Previously, we created two-dimensional crystals of human AQP1 from erythrocytes and determined the structure at 3.8 Å resolution by electron crystallography (Murata et al. 2000). However, to fully understand the molecular basis of the water transport, the higher resolution structures of aquaporins are of great interest. Here we report a large-amount expression of AQP1 from Sf9 cells using a baculovirus system and better 2D crystals using the expressed proteins. Required amount of protein for 2D crystallization is usually less than those for 3D crystallization and it enables us a successful crystallization using an expressed protein. The crystals diffracted beyond 2.5 Å resolution and were found to be suitable for determination of an atomic model including water molecules.

Ref. Murata, K., Mitsuoka, K., Hirai, T., Walz, T., Agre, P., Heymann, J. B., Engel, A., Fujiyoshi, Y. (2000) *Nature* **407**, 599-605.

2P-002 Folding simulations of chignolin by self-learning multiscale method

Wenfei Li (1), Shoji Takada (1) ((1) *Kyoto University, Department of Biophysics*; (2) *CREST, Japan Science and Technology Inc.*)

see 2TP5-02

2P-003 アミロイドーシス原因タンパク質トランスサイレチンの中性子結晶構造解析に向けて
Towards the Neutron Crystallographic Analysis of Amyloid Protein, Transthyretin

Takeshi Yokoyama (1), Yuko Nabeshima (2), Mineyuki Mizuguchi (2), Ichiro Tanaka (1), Nobuo Niimura (1) ((1) *Frontier Research Center for Applied Atomic Sciences, University of Ibaraki*; (2) *Graduate School of Medicine and Pharmaceutical Science, University of Toyama*)

Transthyretin (TTR) is one of more than 30 nonhomologous proteins linked to human amyloid disease. TTR is found in the blood and cerebrospinal fluid, and is composed of four identical 127-aminoacid β-sheet-rich subunits. Dissociation of TTR appears to be the rate-determining step that allows subsequent partial misfolding and misassembly, leading to the formation of cross β-sheet amyloid fibrils, as well as several other aggregate morphologies. Deposition of TTR amyloid fibrils is linked to four different amyloid diseases: senile systemic amyloidogenesis, familial amyloid cardiomyopathy, familial amyloid polyneuropathy, and central-nervous-system-selective amyloidosis. In spite of the

many high-resolution crystal structures of wild-type and amyloidogenic mutant TTR, comparisons of mutant amyloidogenic TTR structures to native TTR structures do not reveal any significant structural diversity that could account for amyloidogenesis.

In order to investigate molecular property including hydorogen atoms and hydrogen ions of native TTR, neutron crystallographic analysis have been under an investigation. Current status is that the crystals are grown in the presence of 1.4M ammonium sulfate and 0.1M sodium acetate buffer at pH4.0, and crystal size are approximately 0.4mm³ (1.2mmx1.0mmx0.4mm). The crystallization phase diagram which is helpful to grow large crystals were prepared, because single crystals larger than at least 1 mm³ are necessary for neutron diffraction experiments.

On the other hand, several x-ray crystal structures were determined at various pH(4.0 5.5, 7.5). Comparison among these structures suggests that Asp99 and water molecules around FG-helix are partially responsible to undergo conformational changes, likely stabilizing/destabilizing the tetramer.

2P-004 ×線小角散乱による DNA 相同組換えのメディエータである分裂酵母 Swi5 と Sfr1 の溶液構造解析
Solution structures of DNA recombination mediators Swi5 and Sfr1 in fission yeast studied by small-angle x-ray scattering

Yuichi Kokabu (1), Tomotaka Oroguchi (1), Yasuto Murayama (1), Naoyuki Kuwabara (1), Tsutomu Yamane (1), Hiroshi Hashimoto (1), Satoru Unzai (1), Toshiyuki Shimizu (1), Hiroshi Iwasaki (1), Mitsunori Ikeguchi (1) ((1) *Yokohama City University*)

The Swi5 and Sfr1 proteins in the fission yeast *Schizosaccharomyces pombe* are mediators of DNA homologous recombination. The Swi5 – Sfr1 complex simulates Rhp51 (a homologue of Rad51)–dependent DNA strand exchange that is a central reaction of homologous recombination. Despite its importance, a three – dimensional structure of the Swi5 – Sfr1 complex has not been determined yet. Therefore, we investigated solution structures of Swi5 and Sfr1 by using small – angle X – ray scattering (SAXS).

The SAXS results indicate that Swi5 forms a tetramer in solution, and has an elongated shape. In the Swi5 – Sfr1 complex, two Swi5 molecules are bound to one Sfr1 molecule, and the length of the Swi5 – Sfr1 complex is longer than that of a Swi5 tetramer. However, due to low resolution of SAXS structures, it is unable to determine locations of the N – and C – terminal domains of Sfr1 in the complex. Thus, by using specific binding of the IgG antibody to the N – and C – terminal domains of Sfr1, we attempt to determine the locations of these domains in the complex. Currently, we identified the epitope of Swi5 and Sfr1 by a Western blot and measured SAXS for the IgG antibody and Swi5 – Sfr1 – IgG complex.

2P-005 ニンジンレグインスリン結合タンパク質の結晶構造
Crystal structure of leginsulin binding protein from carrot

Takuya Yoshizawa (1), Hiroshi Hashimoto (1), Toshiyuki Shimizu (1), Satoru Unzai (1), Yuji Nishiuchi (2), Hisashi Hirano (1), Mamoru Sato (1) ((1) *Yokohama City University*; (2) *PEPTIDE INSTITUTE, INC.*)

Leginsulin binding protein (LBP) is expressed in response to biotic or abiotic stress, and localizes in the plasma membrane and middle lamellae of cell wall. Homologous proteins of LBP are widely present in plants, such as legume, wheat, and cotton.

LBP shows kinase activity and the activity is stimulated by the biding of 4-kDa hormone-like peptide from soybean called leginsulin. Carrot callus tissue cultured containing the peptide grows more rapidly than in medium lacking the peptide, suggesting that leginsulin may regulate growth, differentiation, and cell proliferation. The kinase activity of LBP is inferred to involve in an early step of signal transduction cascade triggered by the hormone peptide. On the other hand, LBP has binding activity of endo- β -1,4-glucanase secreted by invading phytopathogens, such as fungus. Many phytopathogens secrete endo- β -1,4-glucanase to degrade plant cell wall. In response to pathogenic attack, plants produce proteins inhibiting the activity of endo- β -1,4-glucanase to protect plant cell wall. LBP may plays role in the plant's defensive strategy. However, detail about function of LBP is still unclear.

Therefore, we progress structural and functional analyses of LBP. Here, we present the crystal structure of carrot LBP and inhibition assay for an endo- β -1,4-glucanase. We will discuss a structural basis for the inhibition mechanism. Our results will provide to a clue to clarify the function of LBP and pave a way for further analyses.

2P-006 ヒト由来 REV7 – REV3 複合体の×線結晶構造解析
Crystal structure of human REV7 in complex with REV3 fragment

Kodai Hara (1), Toshiyuki Shimizu (1), Yoshiki Murakumo (2), Tomo Hanafusa (3), Haruo Ohmori (3), Mamoru Sato (1), Hiroshi Hashimoto (1) ((1) *Yokohama City University*; (2) *Nagoya University Graduate School of Medicine*; (3) *Institute for Virus research, Kyoto University*)