chemotactic accuracy did not show the linear dependence on ligand concentration. Based on these results, we will discuss what determines the accuracy of bacterial chemotaxis.

1P-168 細胞性粘菌の仮足生成の統計解析

Statistical analysis of time series of pseudopodia formation in Dictyostelium cells

Tatsuo Shibata (1), Naohiro Akuzawa (2), Satoshi Fujise (2), Akihiro Nagamatsu (2), Masatoshi Nishikawa (3) ((1) *Hiroshima University; PREST, JST; JST CREST*; (2) *Hiroshima University*; (3) *Hiroshima University; JST CREST*)

Pseudopodia are an element of motility of Dictyostelium cells. When there is no external chemotaxis cue, cells exhibit spontaneous motility in a random direction. However, their behavior is not a simple random walk, but shows a characteristic persistent random motion with several time constants. Since the cell motility is generated by the extension and contraction of pseudopodia, such complex behaviors could be described by their properties. Under chemoattractant gradient, the directed migration is also generated by the processes of pseudopodia, which rectify the moving direction. Thus to understand the cell motion and the chemotaxis, we need a quantitative description of pseudopod processes. To these end, we developed an image analysis method to detect pseudopodia formation. We performed time series analysis for the obtained time series data of pseudopodia formation. We will show the results of statistical analysis and quantitative description of the process.

1P-169 lac オペロンの二重安定性における環境応答

Adaptive response to environmental changes utilizing the bistability of the lactose operon

Yoshihiro Shimizu (1), Bei-Wen Ying (1), Junya Ichinose (2), Yoichiro Ito (2), Tetsuya Yomo (3) ((1) *Grad. Sch. Information Sci. & Tech., Osaka Univ.*; (2) *ERATO, JST*; (3) *ERATO, JST; Grad. Sch. Information Sci. & Tech., Osaka Univ.; Grad. Sch. Frontier Biosci., Osaka Univ.*)

Genetic identical cells show different expression patterns even under the same environment, such as cell differentiation during the development process. Multistability (e. g. bistability) of gene expression networks was reported to contribute to such phenotypic variety. Recent studies indicated that cells could transit between bistable states according to environmental variation. Here, we focused on the bistability of the lactose utilization network, a well-known positive feedback loop, in *E. coli*, to investigate whether the transition between two stable states happens in accordance with the environment changes. The gene encoding the enzyme involved in the amino acid biosynthesis was removed from its native operon and reassembled to the other location of the chromosome under the control of the lac promoter. Quantitative analysis of the expression of this gene was monitored by the co-expressed green fluorescent protein. Bistability of this reconstructed genetic loop was achieved by adjusting the concentration of the chemical inducer. Under the bistable condition, a small population of cells raised the expression level of the replaced gene, once the amino acid was removed from the environment. It suggested that cells might transit from the low expression level, one of the bistable states, to the other one of the high expression level, in response to the nutrient starvation, no matter the native operon regulation had been destroyed. Further analysis on population dynamics will be reported.

1P-170 クローディン-3を発現したL細胞に形成されたタイトジャンクション様構造の急速凍結レプリカおよび HAADF-STEM による観察

Tight junction-like structures in L cells expressing claudin-3 protein observed by quick-freeze-replica and HAADF-STEM.

Kazunori Kawasaki (1), Tomoki Kato (1), Mikio Furuse (2) ((1) *National Institute of Advanced Industrial Science and Technology*; (2) *Kobe University Graduate School of Medicine*)

see 1TA5-04

1P-171 分裂期のHela細胞への局所温度勾配の効果

Effect of local temperature gradient on HeLa cells in mitotic phase

Masahiro Motoyoshi (1), Madoka Suzuki (2), Kotaro Oyama (1), Takeshi Itabashi (1), Shin'ichi Ishiwata (1) ((1) *Department of Physics, Faculty of Science and Engineering, Waseda University*; (2) *COBRI, Comprehensive Research Organization, Waseda University*)

see 1TA5-01

1P-172 分裂中期にある紡錘体形状の左右相称制御

Bilateral regulation of spindle shape at metaphase

Kazuya Suzuki (1), Jun Takagi (1), Takeshi Itabashi (1), Shin'ichi Ishiwata (1) ((1) *Department of Physics, Faculty of science and engineering, Waseda University*)

see 1TA5-02

1P-173 線虫初期胚の細胞質流動の力学的基盤の解明のための、流速分布の測定と3Dシミュレーションによる再構築

quantification and 3D simulation reconstruction of the cytoplasmic streaming in *C. elegans* embryo to elucidate its mechanical basis

Ritsuya Niwayama (1), Kyousuke Shinohara (2), Akatusuki Kimura (1) ((1) *SOKENDAI; National Institute of Genetics, Center for Frontier Research, Cell Architecture Laboratory*; (2) *Osaka University, Graduate School of Frontier Biosciences, Developmental Genetics Group*)

see 1TA5-08

1P-174 異なる接着強度の基質上における細胞の形・運動・力

Cellular Shape, motion and forces on various adhesive surfaces

Hirokazu Tanimoto (1), Miki Matsuo (1), Masaki Sano (1) ((1) *Department of Physics, Graduate School of Science, the University of Tokyo*)

see 1TA5-07

1P-175 異なる試料調製条件下におけるタンパク質内包リボソームの構造特性

Structural Characteristics of Liposome Occluding Proteins Under Different Preparation Conditions

Yoshihiko Hagiwara (1), Mitsuhiro Hirai (1) ((1) *Graduate School of Engineering, Gunma University*)

For drug delivery system (DDS), molecule-dispersion type microspheres using biological lipids as emulsifiers (LM) were shown to have superior curative effects due to their high adaptability to living body. However, there is a limitation that LM is only available to the use of hydrophobic drugs. As lipid-liposomes can disperse both hydrophilic and hydrophobic drugs, studies and developments to use lipid-liposomes as DDS are now to be pushed forward energetically. On the other hand, in this decade lipid microdomains formed on plasma membrane surface (so-called lipid rafts) have been attracting a huge interest in cell biology since they are assumed to play a role as a platform for various membrane-associated events, such as signal transmission, cell adhesion and so on. We have been studying functional structures of liposomes and micelles of lipid mixtures [1] and their specific interaction with proteins [2], mainly by using small-angle X-ray and neutron scattering techniques. At this annual meeting, we will report on the structural characteristics of uni-lamellar vesicles occluding different type proteins (all-alpha and alpha+beta proteins) under different sample preparation conditions. We have prepared uni-lamellar vesicles by employing a few combinations of different methods (natural swelling, freeze-thaw, ultrasonication, and extrusion). Thermal stabilities of the vesicles containing proteins will be also discussed.

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1P-176 浸透圧下における糖脂質を含んだベシクル構造の pH 依存性について：広角 X 線散乱による研究

Wide-angle X-ray scattering study of pH dependence on the structure of the vesicle containing glycosphingolipid under osmotic pressure

Teruaki Onai (1), Mitsuhiro Hirai (1) ((1) *Dept. of Engineering, Gunma Univ.*)

It is well known that interaction between lipid bilayers and external molecules (ions, polymers and/or proteins) in water solutions has an influence on structural stability of the liposome. By using wide-angle X-ray scattering, we studied the influence of the addition of polyvinylpyrrolidone (PVP) on structure of the liposome composed of the lipid mixtures (monosialoganglioside (GM1)/cholesterol/ dioleoylphosphatidylcholine (DOPC) mixture). PVP have been often used by other authors to measure the force between bilayers, as the osmolyte. Our

previous results suggested that the vesicle structure changes from a uni-lamellar vesicle to a double-shell structure of the vesicle containing another one, by model-fitting analysis of scattering curves. However, stability and formation process of the above structure were not clarified yet. In the present study, we have studied the effect of pH variation on the above structure change. In physiological pH range from 6.5 to 7.5, we have found that the above structural transition under osmotic stress significantly depends on pH. That is, below pH ~7.0, uni-lamellar to double-shell transition have been observed, while, above pH ~7.5, uni-lamellar to multi-lamellar transition have been observed. It might be resulted from the difference of the dissociation degree of the head group of gangliosides depending on pH. We will discuss about the details on the above double-shelled structure.

1P-177 アガロースゲルに埋め込まれた GUV のマイクロリアクター利用 Embedment of GUVs in agarose gels and their application as microreactors

Kanta Tsumoto (1), Masahiro Oohashi (1), Masahiro Tomita (1) ((1) *Mie University Graduate School of Engineering*)

Artificial membrane vesicles are widely used for model systems in order to investigate the properties and behavior of biological membranes. Recently, giant unilamellar vesicles (GUVs) have been supposed to be one of the most interesting tools for constituting cell models by encapsulating sets of biochemical molecules because they have cell-like sizes, structures and morphologies. However, since individual GUVs are generally fragile, fluctuating, and difficult to manipulate without skilled techniques, the development of a method that enables us to handle GUVs more simply would be awaited. Here, we demonstrate embedment of GUVs in agarose gels and the application of them as microreactors to observation on membrane disruption, membrane fusion and entrapped enzyme reaction. First, we determined what kind of and what percentage of agarose was suitable for the embedment; resultantly, GUVs (dioleoyl phosphatidylcholine, DOPC) were embedded in 2% agarose (low melting temperature, m.t. $\leq 65^\circ\text{C}$). Effects of embedment on size distribution, phase behavior and duration of entrapment of GUVs were examined using laser scanning microscopy, and these suggested that the embedded GUVs were not so different from those floating in solutions. GUVs are fixed, so that reagents can be added to the exterior surroundings through diffusion. Thereby, we tried to observe GUV membrane disruption by surfactant, fusion with other liposomes, and entrapped glycosidase reaction in a time course manner.

1P-178 パターン化モデル生体膜における脂質二分子膜の側方収縮 Lateral contraction of phospholipid bilayers in micropatterned model membranes

Takashi Okazaki (1), Kenichi Morigaki (1) ((1) *AIST*)

One of the intriguing questions of fundamental processes in biomembrane activities is dynamics of lipid bilayers such as protrusion and defect formation, caused by localized expansion and contraction of lipid bilayers. Substrate-supported planar lipid bilayers (SPBs) are suitable as a tool for studying this dynamics, because they enable observation with defined areas and controlled lateral pressures. In this study, we investigated contraction of fluid lipid bilayers in micropatterned SPBs. The micropatterned SPBs were generated by photolithographic polymerization of a diacetylene phospholipid. By controlling the density of polymeric bilayer domains with the applied UV irradiation dose, we could integrate three different SPBs, i.e. polymeric bilayers, partially polymeric bilayers, and fluid lipid bilayers. As the temperature of SPB decreased, embedded fluid lipid membranes (DOPC) contracted laterally and defects formed preferentially in the polymer-free regions that were surrounded by partially polymeric bilayers. Localized defects formation suggests different energetic costs for defect formation in polymer-free (fluid) and partially polymeric bilayer regions. These results indicate the possibility of artificially manipulating local membrane morphologies, which allows us to reproduce biomembrane activities caused by lateral dynamics of lipid bilayers.

1P-179 リン脂質／コレステロールの3成分系からなるベシクルにおけるドメイン構造の共焦点蛍光顕微鏡観察 Domain Formation in Ternary Vesicles of Phospholipids and Cholesterol by Observed Confocal Fluorescence Microscopy

Masahiro Hibino (1), Tsuyoshi Shimada (1) ((1) *Muroran Institute of Technology*)

Lipid rafts and functional domains in cell membranes are currently thought to be localized regions in which cholesterol, saturated long-chained lipid, and particular proteins are concentrated. A fundamental attribute of their formation is lateral separation of lipids into coexisting liquid phases. Therefore, we have investigated spontaneous lateral separation in giant unilamellar vesicles (GUVs) containing several different phospholipids and a wide range of cholesterol compositions using confocal fluorescence microscopy (CFM). Here we mostly report interesting phase behavior of ternary mixtures of saturated lipids DPPC, unsaturated lipids DOPC, and cholesterol. As cholesterol is added to DOPC/DPPC mixtures, solid and liquid phase boundaries in GUV membranes can be detected up to ~10 mol% cholesterol concentration. However, as

cholesterol concentration is increased in the vesicles beyond ~10 mol%, coexistence of solid and liquid phases is abolished. As cholesterol concentration is increased at 20 mol%, coexisting liquid phases are observed as bright and dark regions. Beyond 60 mol% cholesterol, domains are no longer observed and vesicles appear uniform. Furthermore, we observed monolayers made from the same lipid mixtures to consider correlations between miscibility in monolayers and in bilayers. These results will help with any fundamental advances in our understanding of lateral heterogeneity within cell membranes.

1P-180 遠心沈降によるジャイアントベシクル融合 Fusion of Giant Vesicles Induced by Centrifugation

Mariko Matsunaga (1), Taro Toyota (1), Masanori Fujinami (1) ((1) *Department of Applied Chemistry and Biotechnology, Graduate School of Engineering, Chiba University*)

1. Introduction

Giant Vesicles have drawn much attention as models of cell-size membrane or artificial bioreactors. However, since the volume of giant vesicles ($> 1 \mu\text{m}$) is as much as 1 fL, the substrates encapsulated in giant vesicles is depleted. Even though this problem is solved in certain conditions of giant vesicles composed of membrane-penetrating proteins[1] or fatty acid derivatives[2], an universal solution is still desired in the case of addition of non-permeable substrates. We have focused on the centrifugation of giant vesicles and developed the fusion process of a mass of giant vesicles.

2. Experimental Result

The giant vesicles were prepared by the W/O emulsion centrifugation method[3]. In order to develop the efficiency of fusion, we have investigated the condition of the preparation, such as components of oil phase, components of phospholipids for giant vesicle membrane, the density difference between inside and outside of giant vesicles, the centrifugation temperature and speed. We also focused on addition of calcium in order to promote the efficiency of fusion. The efficiency of giant vesicles was estimated by flow cytometry which affords us to count the number of fluorescent giant vesicles formed by mixing galactosidase and glucose-caging fluorescent substrate due to the fusion of giant vesicles. Finally we have achieved 83% of the efficiency of fusion. Although the mechanism of the fusion of giant vesicles by centrifugation is not revealed, the current method is expected to be applicable for cycling of enzymatic biochemical reactions inside of giant vesicles.

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1P-181 遠心沈降法によりジャイアントベシクルに内包されたカタラーゼの振動反応 Oscillatory Reaction of Catalase Encapsulated in Giant Vesicles Prepared by W/O Emulsion Centrifugation Method

Taro Toyota (1), Takafumi Kikuchi (1), Masanori Fujinami (1) ((1) *Department of Applied Chemistry and Biotechnology, Graduate School of Engineering, Chiba University*)

Giant vesicles which are closed compartments composed of lipids have drawn much attention for cell-sized bioreactors. Among them, Hideshima et al reported that the bulk O_2 concentration was oscillated when a H_2O_2 solution was added to a dispersion of vesicles encapsulating a catalase solution.[1] However, the mechanism is not fully understood so far. In order to elucidate this phenomenon, we focused on the effect of uniformness of the vesicular membrane.

For comparison with Hideshima's vesicles which do not have uniform lamellarity, the centrifuging method using water-in-oil emulsion[2] was adopted to form single-wall giant vesicles (SWGVs) in this study. The non-encapsulated catalase was inactivated by addition of protease. After bubbling argon gas for deoxygenating the obtained SWGV dispersion for 1 min, the substrate (H_2O_2) solution (1.3 M) was added to the SWGV dispersion and the bulk O_2 concentration was traced by a dissolved oxygen meter. The dispersion of SWGVs encapsulating the catalase provided an oscillatory change of the bulk O_2 concentration. The time-course graph of the O_2 concentration showed a stable base curve and spikes with ca.15 min intervals. In the previous study by Hideshima et al, the base curve of the time-course graph of the O_2 concentration was not stable. The current finding is interpreted as the difference of the number of catalase encapsulating giant vesicles with same lamellarity for the synchronization of oscillatory reaction.

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1P-182 AFM 観察のための平坦化親水基板上的脂質膜の安定化 Lipid membrane stabilized on a flat hydrogel substrate for atomic force microscopy

Akihiro Takagi (1), Hitomi Hokonohara (2), Tomoji Kawai (2) ((1) *ISIR, Osaka Univ; Riken: (2) ISIR, Osaka University*)