suggest that surface properties of PLGA-NPs are important for endocytosis-like uptake into a GUV.

3PT140 トランスポータン 10 が DOPC 膜の巨大リボソームの膜透過性と構造に与える効果

Effects of Transportan-10 on Membrane Permeability and Structure of Single Giant Unilamellar Vesicles of DOPC membranes

Hiroataka Ariyama, Masahito Yamazaki (*Integrated Bioscience Section, Graduate School of Science and Technology, Shizuoka Univ.*)

Transportan 10 (TP 10) is one of the cell penetrating peptides. So far the permeation of TP 10 into cells and the interaction of TP 10 with lipid membranes using LUV suspension have been investigated. However, the detail characteristics and mechanism of the interaction and its permeation through membranes remain unclear. In this report, we have investigated the effect of TP 10 on membrane permeability of lipid membranes using the single giant unilamellar vesicle (GUV) method.

First, we investigated the interaction of TP 10 with single GUVs composed of dioleoylphosphatidylcholine (DOPC) and PEG2K-DOPE (molar ratio, 98:2) containing the fluorescent dye, calcein, in a physiological buffer using phase contrast, fluorescence microscopy. The interaction of 0.8 μ M TP 10 with single DOPC-GUVs induced the rapid monotonous leakage of calcein from the inside of the most GUVs, but in some GUVs step-wise leakages were observed. During the calcein leakage, the size of most GUVs decreased a little (less than 5 %), but in some GUVs their radius decreased greatly and some small high-contrast particles appeared on the GUV membranes. The fraction of leaked GUV increased with time, and also with an increase in TP 10 concentration. Second, the effect of cholesterol on the TP 10-induced leakage of calcein was investigated. 0.2 μ M TP 10 did not induce calcein leakage from DOPC/cholesterol (6/4)-GUVs. On the basis of these data, we discuss the mechanism of the interaction of TP 10 with DOPC-GUVs and DOPC/cholesterol-GUVs.

3PT141 F-BAR によるリボソームのチューブレーションのリアルタイム観察 Real-time observation of liposome tubulation by F-BAR

Yohko Takiguchi¹, Toshiki Itoh², Kingo Takiguchi¹ (¹*Grad. Sch. Sci., Univ. Nagoya*, ²*Grad. Sch. Med., Univ. Kobe*)

The Fer-CIP4 homology-BAR (F-BAR) domain has been identified as a biological membrane-deforming module. The F-BAR domain has been reported to transform lipid bilayer membranes into tubules. However, the process of tubulation still remains unknown. Here we monitored the entire tubulation process induced by the F-BAR domains or by the full lengths of four different F-BAR domain proteins, PSTPIP1, FBP17, CIP4 and Pacsin2, using direct real-time imaging, and show that each F-BAR domain or protein induces tubules through a unique kinetics from model membranes.

FBP17 and CIP4 develop many projections simultaneously throughout the surface of individual liposomes, whereas PSTPIP1 and Pacsin2 develop only a few projections from a narrow restricted part of the surface of individual liposomes. The results provide striking evidence that a nucleation process is involved in the F-BAR-induced tubulation, and individual F-BAR domains have a unique nucleation rate and/or property even though essentially they have the same crescent-shaped structure. The differences in process of tubulation induced with those F-BAR domain proteins may reflect their unique physiological roles, and function favorably to build networks of various and robust membrane trafficking processes observed in cells.

3PT142 PEG 担持ジャイアントベシクルにおける膜組成と分裂様式の関係 Correlation between Membrane Composition and Mode of Division of PEG-grafted Giant Vesicles

Yumi Kan¹, Kensuke Kurihara², Taro Toyota^{2,3}, Masayuki Imai⁴, Tadashi Sugawara^{3,5} (¹*Ochanomizu University*, ²*The University of Tokyo*, ³*Research Center for Complex Systems Biology*, ⁴*Tohoku University*, ⁵*Kanagawa University*)

Recently we realized a model protocell using a giant vesicle (GV) as a compartment, comprised of phospholipids, cationic synthesized membrane molecule (V) and an amphiphilic catalyst (C). Encapsulated DNA in GV was amplified by polymerase chain reaction, and then the GV grew and divided when the incorporated membrane-precursor was converted to V by the assistance of C. We also revealed that the attractive interaction between amplified DNA and the inner surface of the GV membrane accelerated the GV

division. Here we prepared a new model protocell including 5 mol% of polyethylene glycol (PEG5000)-grafted phospholipid: a PEG-chain of the phospholipid not only increased the tolerance of GV to high ionic strength and high temperature but also affected the interaction between DNA and the GV membrane containing the cationic membrane molecule V. In order to elucidate the morphological change of the GV membrane throughout the self-reproduction process, we stained the membrane by Texas Red-tagged phospholipid and observed the dynamics in terms of a confocal laser scanning fluorescence microscope. Whereas the division mode of non PEG-grafted GV was a budding-type exclusively, the birthing-type deformation occurred mainly in the case of PEG-grafted GV. We speculated that the interaction between the amplified DNA and the PEG-grafted inner surface of the membrane influences the division mode of GV self-reproduction. In this symposium, we will discuss the effect of the length and the content of PEG-grafted phospholipid on the mode of GV division.

3PT143 静置水和法による均一径リボソームアレイ Size-controlled Giant Liposome Array System with Gentle Hydration

Toshihisa Osaki¹, Koki Kamiya¹, Kaori Kuribayashi², Ryuji Kawano¹, Shoji Takeuchi² (¹*Kanagawa Academy of Science and Technology*, ²*Institute of Industrial Science, The University of Tokyo*)

This work presents a methodology that realizes size-controlled, solvent-free giant liposome arrays with simple gentle hydration technique. Gentle hydration and electroformation have been the most commonly used methods to prepare giant liposomes, yet they have difficulty controlling the size distribution of the formed liposomes as well as their shape and lamellarity. We therefore set the goal of developing an alternative method that allows the formation of uniform-size giant liposomes with high reproducibility. Our system consists of a dried lipid pattern on a substrate that is a micropatterned polymer thin film on an ITO glass slide. An electrospray deposition (ESD) method was applied to obtain the selective lipid-deposition at the polymer pattern where the ITO surface was exposed. With a simple hydration process of the dried lipid, we succeeded in formation of giant liposomes on top of the pattern. The diameter of the liposomes was similar to the patterned size, achieving a narrow range of size distribution. We also report the applicability of the system for various biological assays such as object encapsulation into the liposomes, buffer exchange during the assays, and reconstitution of membrane proteins into the liposome membranes.

3PT144 REMD シミュレーションによる粗視化された脂質膜の相転移の研究 Study on phase transition of coarse-grained lipid bilayer by REMD simulations

Tetsuro Nagai, Yuko Okamoto (*Grad. Sch. Sci., Nagoya Univ.*)

A replica-exchange molecular dynamics simulation of a lipid bilayer system was performed in order to study sol-gel phase transitions. The replica-exchange molecular dynamics method enables one to enhance conformational sampling efficiency and to study the system at a wide temperature range at once. We employed a coarse-grained model MARTINI. The results show abrupt changes in internal energy, bilayer thickness, and area of bilayer around 296 K. A peak in specific head capacity was also observed around this temperature. These suggested that the sol-gel phase transitions. The bilayer has two states in the gel state. One is a tilted gel state and the other is an un-tilted gel state. A previous work with MARTINI force field reported only the un-tilted gel state. This indicates that conformational sampling efficiency is not trivial even with coarse-grained model, which has smoother energy landscapes and reach longer time scales than atomistic model. The un-tilted gel phase is a little thicker than the tilted gel phase, while the area of lipid bilayer is quite similar. We will show more detail results in the presentation.

3PT145 長鎖リン脂質 DMPC と短鎖リン脂質 DHPC で構成される複雑な相挙動のダイナミクス

Dynamics of complicated phase behavior on the mixtures consisting of long-chain phospholipids DMPC and short-chain phospholipids DHPC

Ryota Kobayashi, Tetsuhiko Ohba (*Department of Physics, Tohoku University*)

The binary phospholipid mixtures of long-chain DMPC and short-chain DHPC show a variety of complicated phase behavior, depending on the mixing ratio of two lipids, the total lipid concentration in water, and temperature. At low