論文の内容の要旨

Morphological Analysis on Two-dimensional Lipid Membrane Transition (二次元脂質膜の形態変化解析)

氏　名　　髙橋　慧

[Background and scope of the current thesis]

The lipid membrane transition modeling for a cell membrane dynamics has drawn much attention as the geometric borderline changing closely related to the definition of life. In order to analyze the morphological transition of the lipid membrane composed of the natural crude lipids or the purified lipids, the current thesis focuses on the establishment of a new **Reconstruction** or **Construction** method based on the two-dimensional lipid membrane. The purpose of this study is to explore the transition of the two-dimensional lipid membrane under a perturbation for comprehending the gap between the life and non-life systems.

[Reconstruction of lipid membrane of amoeba cell and its buckling by cytosol injection]

Amoeba motion is a fundamental mode of the cell motions accompanying cell membrane deformation. To approach this membrane dynamics in the **Reconstruction** method, a new technique for the preparation of two-dimensional lipid membrane composed of crude natural lipids extracted from the amoeba cells was introduced. The micrometer-sized patches of the supported lipid membrane composed of lipids extracted from an amoeba cell, *Dictyostelium discoideum* (*Dictyostelium*), were formed in a chamber by spin-coating. The injection of the cytosol extracted from *Dictyostelium* onto the supported

lipid membrane patches exhibited the membrane buckling with the adsorption and translocation of two proteins, PH-crac protein (tagged with red fluorescence protein (RFP)) and PTEN (tagged with green fluorescence protein (GFP)), which play a role of the signal transduction of phosphorylation and dephosphorylation of phosphatidylinositides during the cell motion of *Dictyostelium discoideum*.

It was found that the spin coating method produced the micrometer-sized patches of *Dictyostelium* lipid with a thickness of several micrometers on the glass substrate by means of an aspiration-type glass holder. Since *Dictyostelium* is a type of amoeba that sprightly shows amoeba motion at room temperature (20 - 23 ℃), the cytosol injection and the observation of the *Dictyostelium* lipid patch were undergone at room temperature. In order to trace the transformation of such lipid membrane film in a three-dimensional manner after the addition of the cytosolic extract, the time lapse observations using a confocal laser scanning fluorescence microscope were performed. Then it was observed that the buckling of the patch occurred after cytosol injection and PH-crac-RFP and PTEN-GFP adsorbed and localized on the patch (Figure 1). The buckling motion is probably due to adsorption of these proteins to the membrane evoking the osmotic pressure. This means that the extracts from the dead cells partly resurged the membrane deformation related to amoeba motion.

This study aimed at reconstituting a system of membrane-assisted biochemical machinery using the lipid and cytosol extracted from *Dictyostelium* separately. This current spin coating method afforded a novel technique to prepare a micrometer-sized lipid patch of the natural crude lipid mixtures which can hardly form well-defined membrane structures, such as single bilayer membranes, supported on a substrate.

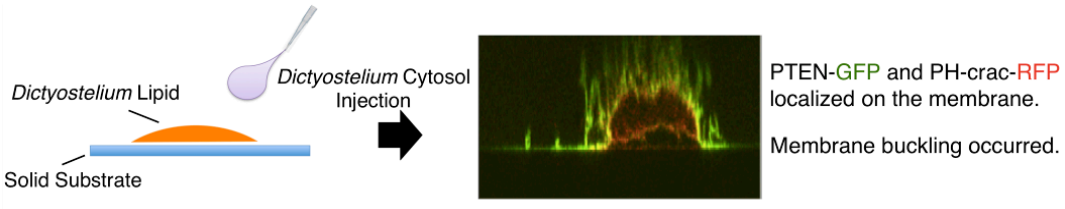

Figure 1. Schematic illustration of the reconstructed two-dimensional lipid membrane using *Dictyostelium* lipid and cytosol extracted separately and fluorescence microscopy image of the membrane buckling.

[Reconstitution of supported lipid bilayer membrane and its transition by cytosol injection]

For exploring about the continuity between life and non-life systems, the membrane transformation involving life phenomenon (extracted cytosol) and non-life phenomenon (supported lipid bilayer (SLB) membrane of purified lipids) has recently drawn much attention. In this chapter, the reconstitution method of supported lipid bilayer (SLB) membrane composed of purified phospholipids and the cytosol extracted from *Dictyostelium discoideum* was developed by using the small unilamellar vesicle (SUV) rupture method.

The SUV rupture method using phosphatidylcholine and phosphatidylinositide produced a uniform membrane which was assigned to bilayer membrane on a glass substrate. The morphology transition of the membrane film after the addition of the cytosolic extract was traced under a confocal laser scanning fluorescence microscope. As a result, the pore deformation on the SLB membrane was

observed. It was found that the phosphatidylinositides included in the SLB membrane tended to disturb the pore formation and expansion (Figure 2). Moreover, the tubular giant vesicles (tGV) generated by the cytosol including the inhibitor medicines of proteins related to the signal transduction of phosphorylation and dephosphorylation of phosphatidylinositides. The generation of tGV had the similarity on the macropinocytosis phenomenon in the scales of time and space. These results imply that such signaling transduction can control not only the symmetry breaking events on the amoeba motion but also the membrane fluidity to avoid the complete breaking of the membrane due to the potential proteins in the *Dictyostelium* cytosol. Since such micrometer-sized transition of the SLB membrane of purified phospholipids which are components of *Dictyostelium* lipid occurred in this reconstituition method, it was indicated that the phosphatidylinositide lipids adjust not only cell motion but also intracellular motility.

The significance of this study is that components of the two-dimensional lipid membrane were reduced extremely than that of the *Dictyostelium* cell membranes and the cytosol of *Dictyostelium* cell was used just as it is. The findings imply that phosphoinositide lipids have potential to control the cytoplasm. Hence this technique is the "**Pre-reconstruction"** method combining the constitution of membrane with the reconstitution of the cytosol and contributes to us for the membrane deformation analysis.

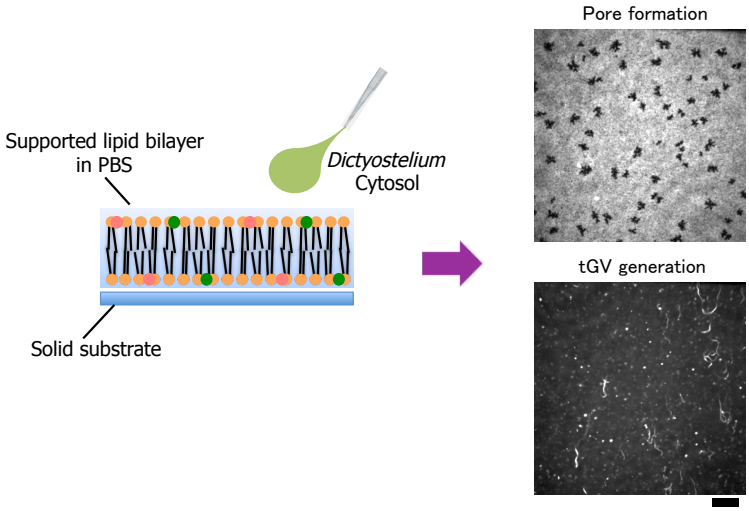

Figure 2. Schematic illustration of the constructed two-dimensional lipid membrane composed of purified phospholipids and cytosol extracted from *Dictyostelium* and fluorescence microscopy images of the membrane transition. Bar = 10 μm.

[Constitution of lipid membrane of novel DNA-lipid conjugate and its transition by heat stimulation]

Finally, this study focused on the construction of a lipid membrane bearing the information factors. DNA molecule is stable in an aqueous circumstance, but so stable that it does not have chance to change the circumstance such as lipid membrane. Then the stem-loop structure of DNA-RNA chimera molecule was conceived and designed here. The chemical conversion of DNA-RNA chimera modified with oleic acid was expected to induce its membrane transition in the micrometer scale. The products of the hydrolyzed DNA-RNA chimera modified with oleic acid (**1**) are two types of DNA-lipid conjugates (**2**, **3**). The two-dimensional (2D) lipid membrane of DNA-RNA chimera lipid **1** was prepared by the casting method and wetted by the buffered solution. The heat-induced transition of the 2D lipid membrane of **1** was traced under a phase contrast microscope. The transmission electron microscopy and

the electrocataphoresis were examined for further revealing the membrane transition in the molecular level.

The microscopic observation revealed that 2D-3D membrane transition was induced by heat stimulation (Figure 3). In order to evaluate the chemical conversion of DNA-RNA chimera lipid **1** in the 2D-3D transition of the casted lipid membrane, the electrocataphoresis was examined. As a result of transmission electron microscopy and the electrocataphoresis, it can be said that, the buffered solution of DNA-RNA chimera lipid **1** and that of 1:1 mixture of DNA-lipid conjugate **2** and **3** exhibited nanometer-scale self-assembly and their structures were more complicated than that of only DNA-lipid conjugate **3**.

As the **Construction** method, the significance of the current results is one implication that a life system needs DNA to compose and keep its territory in three-dimensional manner as well as to make the hereditary information robust for the system. The findings are the first experimental result of micrometer-sized molecular self-assembly linked to the information materials. This molecular design and the experimental strategy are applicable to energy estimation on the emergence or the constitution of 3D body structure and its transition containing the information materials taking the minimal condition of life into account.

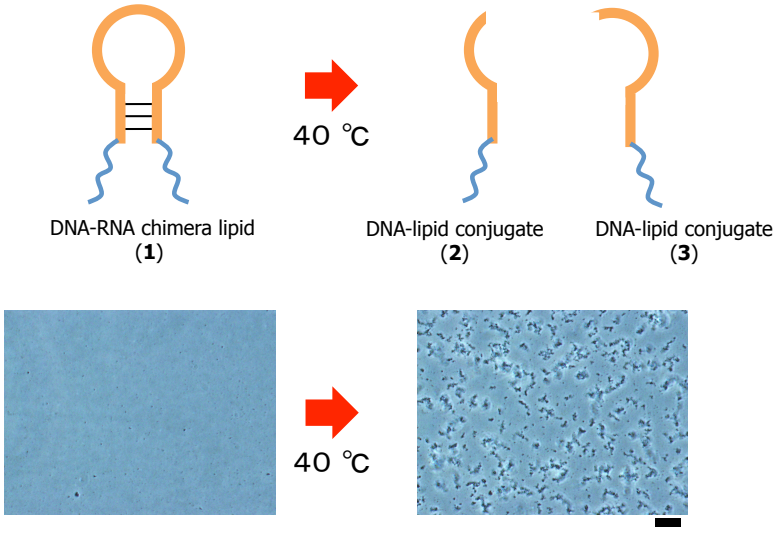

Figure 3. Schematic illustration of a newly designed DNA-lipid conjugate and its chemical conversion and the microscopy images of the emergence of 3D body in its specimen by the heat stimulation. Bar = 15 μm.

[Conclusion]

In this study, the new methods for the membrane transition of two-dimensional lipid membranes were proposed for approaching the nature of membrane in life system. These **Reconstruction** and **Construction** methods and findings will contribute to synthetic biology and soft matter science for comprehending the role of membrane deformation in life system.