Protein synthesis with liposome fusion and fission by using the freeze-thaw method

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Abstract

For the experimental construction of artificial cell, it is a challenge to simultaneously supply the nutrients and lipids required for protein synthesis, gene replication, membrane growth, and fission. Inner reactions of liposomes are not permanent because of nutrient exhaustion since liposomes do not have pores or channels on their membrane for acquisition of nutrients. In this study, we demonstrated that the liposome containing in vitro translation system was fused with liposome encapsulating RNA by freeze and thaw for GFP synthesis. The fusion mixed lipid molecules on the two kinds of liposomes, followed by the fission. Consequently, we observed GFP synthesis inside the liposomes after liposome fusion and fission. This freeze and thaw method can be repeated, for the sustainable supplement of nutrients with liposome growth. We hope this method would achieve the ultimate goal of establishing the artificial cells that can acquire nutrients sustainably and proliferate by coupling protein synthesis and gene replication compatible with membrane growth.

Introduction

It is important process to reconstruct life-like compartment with inner biochemical reaction for elucidating the border between life and non-life. The process could give us sights on the origin of life. Repetitive cycles of simple biochemical reaction in liposomes have already been achieved. Recently proliferation of liposomes was achieved with inner DNA replication systems (Kurihara et al., 2015). Also we reported sustainable RNA replication reaction with liposome proliferation by a freeze and thaw method similar to that in this work (Tsuji et al. 2016). Briefly, we mixed two types of liposomes, centrifuged, froze by liquid nitrogen, and thawed at room temperature, and it resulted in liposome fusion and fission. Although these reports succeeded in reconstruction of life-like phenomena, protein synthesis compatible with proliferation of liposomes has not been achieved yet. It has been reported that PURE system, in vitro translation system reconstituted with purified components, could be supplied by liposome fusion and protein synthesis occurred after fusion (Caschera et al., 2011). In this study, we show that PURE system can be supplied to liposomes also by freeze and thaw, and moreover, compatibly with the proliferation of liposomes.

Result

GFP synthesis induced by liposome fusion

We first tried to apply liposome fusion induced by freeze and thaw for supplying PURE system. Previously we reported liposome fusion by freeze and thaw (Tsuji et al., 2016). We prepared liposomes encapsulating RNA which encodes GFP (RNA liposomes), liposomes encapsulating proteins of the PURE system (+nutrient liposomes), and liposomes encapsulating buffer without RNA and the proteins (–nutrient liposomes). After mixing up the RNA liposomes and +nutrient or –nutrient liposomes, we centrifuged the liposomes to produce a liposomal pellet, and fused them by freeze and thaw. Then we incubated the liposomes for 3 hours to induce GFP synthesis. First, we analyzed the size distribution of liposomes before and after freeze and thaw by flow-cytometer (FCM). FCM can measure the size and the fluorescence of each liposome. The results showed that liposome size was hardly changed after freeze and thaw (data not shown) but the lipid markers on the nutrient liposome and RNA liposome were well mixed (Fig 1A, right). The lipid mixing without size change indicates that fusion and fission occurred during freeze and thaw. This result was consistent with our previous report (Tsuji et al., 2016). Second, we measured GFP fluorescence of liposomes by FCM and 8% of the total liposomes showed GFP signals only when +nutrient liposomes were fused with the RNA liposomes (Fig 1A middle, green dots). It should be noted that liposomes with GFP fluorescence appeared only in the region indicating the mixing of the two lipid markers (Fig 1A right, green dots). These data indicate that the two liposomes were fused and inner protein synthesis occurred by mixing of the inner solutions.

Then we observed the liposomes with a confocal laser microscope whether GFP synthesis occurred inside the liposomes. The images show that GFP fluorescence was observed inside the liposomes (Fig 1B). This fluorescence did not appear when RNA liposomes were fused with –nutrient liposomes. Therefore, we concluded that the components of PURE system can be supplied to the liposomes without severe defects of protein activities via freeze and thaw.

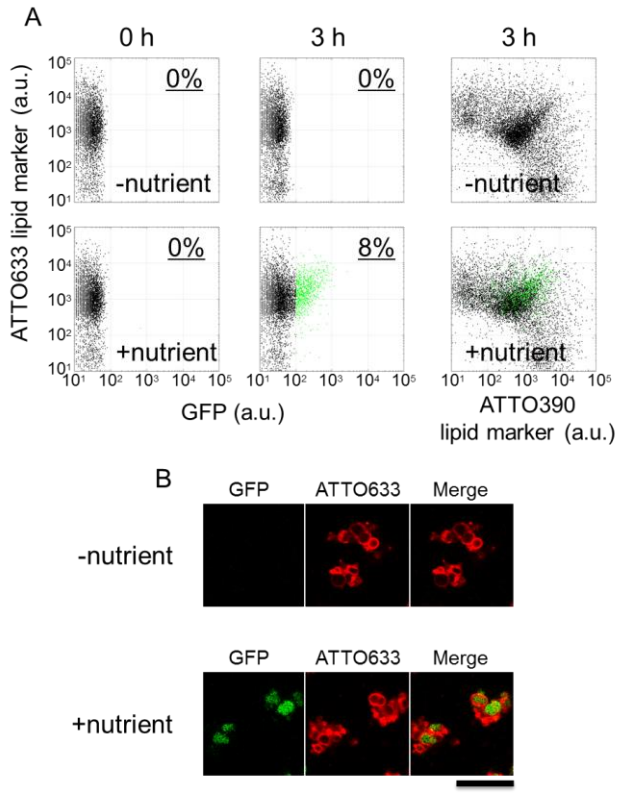

Figure 1 GFP synthesis in liposomes

(A) FCM analysis of GFP synthesis after liposome fusion. Vertical axes of all plots indicate the fluorescent intensity (F.I.) of ATTO633 lipid marker. Horizontal axes of left two columns show F.I of GFP and right column shows F.I. of ATTO390 lipid marker. Green dots indicate liposomes synthesizing GFP (F.I. >100). (B) Microscopic images of 3h incubated samples (scale bar 25μm).

Discussion

We reported the establishment of protein synthesis inside the liposomes by supplying nutrients from outer environment via liposome fusion. This study showed that our liposome fusion method by freeze and thaw can be applied for supplying PURE system. Therefore, we can design the artificial reaction system by introducing the requisite genes and will be able to reconstruct the flexible and extensible life-like structure.

In this report, only 8% of liposomes synthesized GFP, whereas the liposome fusion was observed in higher efficiency in our previous report (50%, Tsuji et al. 2016). This difference was in part because 39 elements were required for GFP synthesis, whereas only 5 elements were required for the previous work. Yet, it is noteworthy that macromolecules such as proteins and tRNAs, which are difficult to pass through the membrane, can be supplied by the freeze and thaw. By developing the gene replication system compatible with the presented freeze and thaw techniques, we will be able to perform “genotype-phenotype linked natural selection” in artificial cells, as a simplest form of “evolvable” protocell model.

Materials and Methods

Materials

1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) was purchased from Avanti Polar Lipids (Alabaster, AL). Liquid paraffin (0.86-0.89 g/mL at 20°C) was purchased from Wako (Osaka, Japan). 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE) labeled with ATTO 633 (ATTO633) and DOPE labeled with ATTO 390 (ATTO390) were purchased from ATTO-TEC (Siegen, Germany).

GFP synthesis in liposome

The GFP synthesis in liposomes was induced by supplying the PURE system (Kazuta et al. 2014) via the freeze-thaw method. Liposome preparation and liposome fusion were performed as described in previous report (Tsuji et al., 2016). Liposome was prepared by using POPC. RNA encoding GFP (gfp-RNA) was prepared as described in the previous work by Kazuta et al. The RNA liposomes in this experiment were prepared by encapsulating 0.3 mM of each amino acid, 0.8 mM tRNA mix, 3.75 mM ATP, 2.5 mM GTP, 1.25 mM CTP, 1.25 mM UTP, 100 mM HEPES-KOH (pH 7.6), 280 mM potassium glutamate, 1.5 mM spermidine, 19 mM magnesium acetate, 2.5 mM phosphocreatine, 1.5 mM dithiothreitol, 0.01 μg/μl 10-formyl-tetrahydrofolate, 200 mM sucrose, and 2000 nM gfp-RNA. The nutrient liposomes were prepared by encapsulating all constituents of the PURE system, including ribosomes and other proteins required for protein synthesis. The outer solution before freeze contained same components of inner solution of RNA liposomes without RNA and sucrose. Instead of sucrose, 200 mM glucose was added to the outer solution. The outer solution during incubation contained the same components of the outer solution before freeze except that tRNA was not included. After freeze and thaw, liposome solutions were incubated at 37℃ for 3 hours. The RNA liposomes and nutrient liposomes were labeled with the fluorescent lipid markers ATTO390 and ATTO633, respectively.

FCM and confocal microscopy analysis

We performed FCM analysis and microscope works as previously reported (Tsuji et al., 2016).

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