Repetitive growth and fission of artificial cells with gene replication

Reconstruction of an artificial cell is one of the biggest challenges in elucidating the border between living and non-living things. Liposomes are compartments that have lipid bilayer membranes, like cells, and therefore are used as an artificial cell model. Many types of biochemical reactions such as gene replication and protein synthesis have been reconstructed in liposomes. However, the encapsulated reactions were temporary because the nutrients in the liposomes were easily exhausted, and the liposomes could not obtain nutrients, such as amino acids, proteins, and nucleotides, from the outer environment. We aimed to develop a method for nutrient supplement. We focused on liposome fusion as the means for supplying nutrients. This method is beneficial because liposome fusion can supply not only the nutrients for inner reactions but also the lipid membrane, enabling the growth of liposomes (Fig. 1).

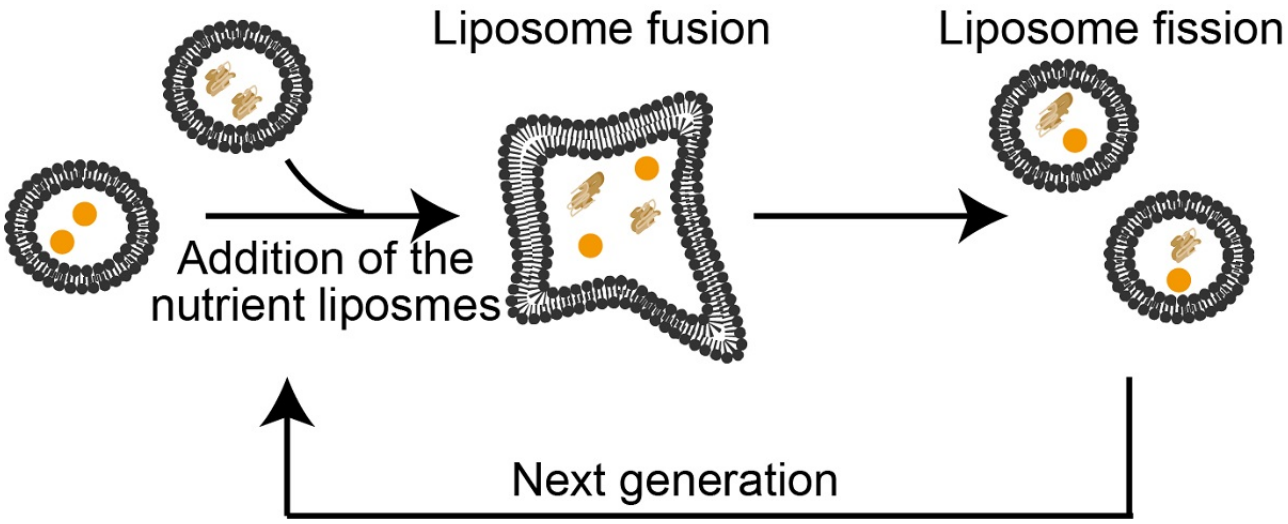

Fig. 1. Concept image of nutrient supply in liposome fusion and fission.

First, we tried to fuse liposomes in a simple manner. The fusion of liposomes can be induced by close contact between multiple liposome membranes and temporary destruction of the membrane. We achieved this by freeze-thawing the liposomes to damage the liposome membrane, following a centrifuge step used for making contact between liposomes. As a result, although approximately half of the liposomes were destructed, fusion and fission were observed in the other half, presumably via the partial defect in the membrane. Liposome fission was likely induced by the excess membrane surface area, which has been discussed in reports regarding liposome fusion. Second, using this system, we tried to supply nutrients such as enzymes, without deactivation of the enzymes. We encapsulated several molecules required for RNA replication reactions into one liposome population, and encapsulated template RNA into another liposome population. After

mixing the two populations and freeze-thawing, RNA was replicated in the fused liposomes, implying that the nutrients were successfully supplied (+nutrient) (Fig. 2A). However, RNA was not replicated when we added the population of empty liposomes in which nutrients were not encapsulated (-nutrient) (Fig. 2A). Third, we tried to supply nutrients iteratively by repeating the freeze-thaw operation. We used RNA as a model for genetic molecules and succeeded in repetitively supplying the nutrients of RNA replication via liposome fusion for 10 iterations (Fig. 2B). As a result, RNA replication in the liposomes occurred after every freeze-thaw operation. The replication efficiency of the first generation was higher than that of the other generations because the initial amount of RNA molecules was very low. This result indicates that the liposomes were repetitively fused and that the inner RNA was sustainably replicated. Additionally, the number of liposomes replicating the RNA increased during the 10 generations. Thus, we succeeded in 'culturing' the artificial cells in the same manner as that performed for native cells.

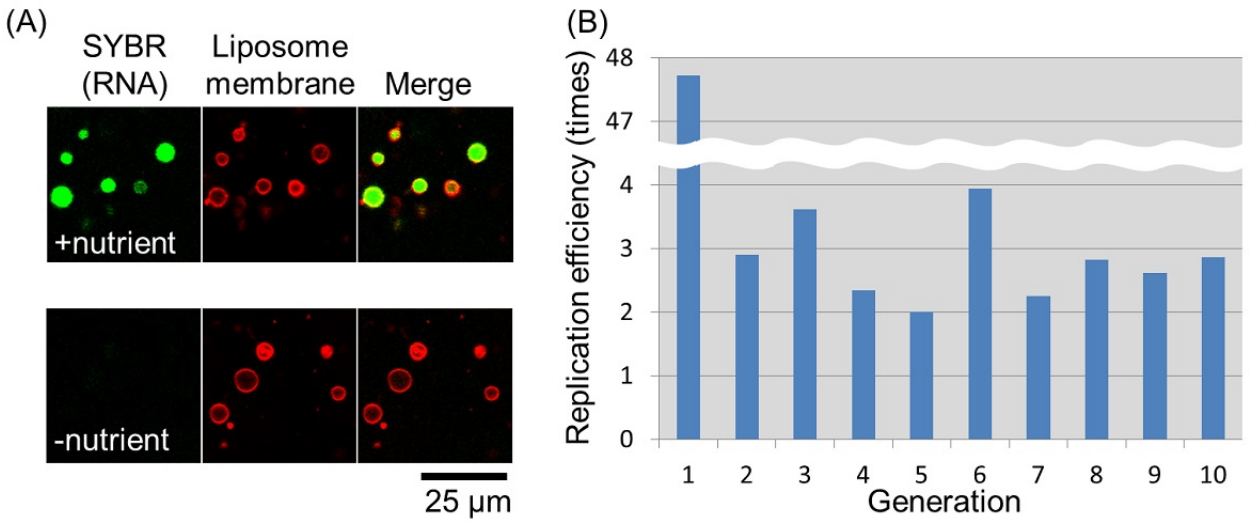

Fig. 2. (A) Confocal microscopic images of liposomes. Green indicates RNA that was stained by SYBR Green II. (B) Bar graph of RNA replication-fold in each freeze-thaw operation. Replication efficiency represents ratio of the total amount of RNA in the liposome solution after replication to the amount of RNA before replication.

In conclusion, we established the proliferation of artificial cells that replicate the genetic material within a lipid bilayer membrane. Through design of the nutrient constituents, this method may enable us to reconstruct more complex artificial cells that can synthesize proteins and replicate genes. Continuous proliferation of these artificial cells may result in the accumulation of mutations in the encapsulated RNA because they do not have error repair systems. This error prone system may induce the evolution of genes in artificial cells. Thus, novel types of cells may be derived from artificial cell lines in the near future.

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Publication

Sustainable proliferation of liposomes compatible with inner RNA replication.
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