DOI: http://dx.doi.org/10.7551/978-0-262-31709-2-ch002

Autonomous construction of synthetic cell membrane

Yutetsu Kuruma[1], Hideaki Matsubayashi[1] and Takuya Ueda[1]

[1] Department of Medical Genome Sciences, Graduate School of Frontier Sciences, The University of Tokyo, Japan
kuruma@k.u-tokyo.ac.jp

Abstract

A minimal artificial living cell is a sustainable and reproducible cell-like entity composed of biological components such as proteins, DNA, RNA and phospholipids (Luisi et al. (2006)). The most realistic strategy in producing such an artificial cell is assembling biomolecules that imitate the architecture and the function of biosystems in living organisms (Oberholzer et al. (1995)). Firstly we reconstructed the gene expression machinery with the minimal number of purified translation factors addressing the need for an artificial gene expression system. The PURE (Protein synthesis Using Recombinant Elements) system (Shimizu et al. (2001)), a key tool for bottom-up synthetic biology, enables information encoded in the DNA sequence to be converted to functional proteins and enzymes, and can be used in developing artificial cellular components. Another important and indispensable feature of artificial cells is the encapsulation of genetic information and gene expression system by a lipid bilayer membrane (Ishikawa et al. (2004); Kuruma et al. (2009)). This is also important to sustain an individual from environment. In addition to compartmentalization of biomolecule components, the membrane provides a structural platform for important biological functions such as selective transport of materials, adoption of environment information, production of energy, etc. Actually, many of the vital cell functions reside on the lipid membrane, and these functions mostly rely on membrane proteins.

In this paper, we focused on three important membrane functions, i.e. (i) lipid synthesis, (ii) energy production and (iii) membrane protein synthesis. All these membrane functions were indispensable for sustaining cell alive and must be reconstructed as a consequence of internal metabolic reactions. Each membrane function has the corresponding membrane proteins. Therefore our strategy is to construct the membrane function on artificial membrane vesicles, liposomes, through the gene expression of the corresponding protein components by the PURE system. (i) For the lipid synthesis, we selected eight membrane enzymes involving in the biosynthesis process of major phospholipids from a bacterial genome. These eight enzymes were synthesized by the PURE system in the presence of membrane fraction (membrane Nano-Disc) and measured their activities. The goal of this project is to develop a biochemical network in vitro that aims to produce several kinds of phospholipids necessary for the formation of cell envelop. As the first step, we have succeeded to synthesize two membrane enzymes inside liposomes and individually detected their activities (5). This result would be a fundamental for the construction of a self-reproducible cell membrane. (ii) For energy production, a membrane embedded super molecule complex, FoF1-ATP synthase (FoF1), was synthesized through the eight kinds of component proteins. The function of FoF1 is to produce ATP molecules, which is an energy source of most cellular activities, based on the proton gradient across a membrane. We have succeeded to synthesize FoF1 complex by the PURE system and detected its ATP synthesis activity that driven by an artificially generated proton gradient. Furthermore, the reconstructed FoF1 is coupled with another membrane machinery, bacteriorhodopsin (bR), to construct an artificial organelle. The bR is proton pomp machinery that transports protons to inside of the membrane vesicles due to light stimulation. Therefore, our idea is that if the bR and FoF1 were allocated on a same membrane vesicle, the resulting vesicle is able to generate ATP molecules by light irradiation (Fig. 1). In this design, we have succeeded to detect the production of ATP molecules in the rate of 35 nmol ATP/hr/mL Reaction Solution. If the produced ATP could be used for protein synthesis reaction within the PURE system, this represents an energetically independent system and becomes a practical platform of autonomous artificial cell. (iii) All these membrane machineries are built up based on a spontaneous membrane insertion of the synthesized membrane proteins. However, a certain kind of membrane protein cannot be integrated spontaneously. In that case, the membrane protein needs a help of special membrane machinery, Sec translocon, to achieve the native formation on a lipid membrane. The Sec translocon works as a gate to mediate a membrane insertion and secretion of membrane proteins (Fig. 2). Therefore our idea is to synthesize the component proteins of Sec translocon by the PURE system and construct the Sec translocon on membrane vesicles. Since most of membrane proteins are generated through Sec translocon in living cells, any types of membrane protein can be produced after the construction of Sec translocon. So far, we have succeeded to synthesize three component proteins (SecYEG) of Sec translocon of bacteria and to detect its heterotrimeric complex formation on a lipid membrane. Furthermore, so synthesized SecYEG enables to produce another membrane proteins that cannot be spontaneously integrated into the membrane. This result indicates that, by synthesizing the Sec translocon, other important membrane proteins (machineries) can be continuously produced on the artificial membrane vesicles.

Using our in vitro gene expression system, cell membrane functions can be partially constructed on the artificial membrane vesicles. More importantly, these membrane functions were autonomously constructed just by adding of the corresponding DNAs. The ability of an artificial cell to au-

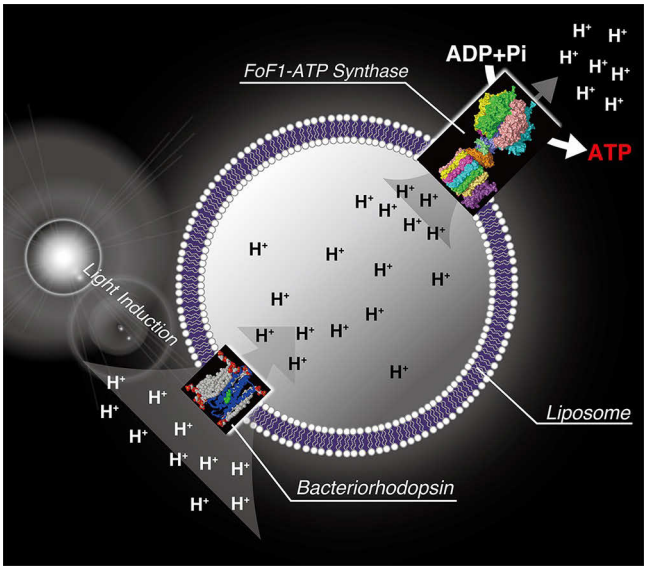

Figure 1: Artificial organelle consists of FoF1-ATP synthase and bacteriorhodopsin for production of ATP.

tonomously produce membrane protein machineries by its internal genetic/metabolic network is consistent with the theory of autopoiesis by Varela and Maturana (Varela et al. (1974)). This is adaptive and complements the definition of life, self-reproduction, which is based on gene replication. We believe that our cell-free approach will become a central device for the construction of artificial cell membranes and a breakthrough for the realization of artificial cells.

References

Ishikawa, K., Sato, K., Shima, Y., Urabe, I., and Yomo, T. (2004). Expression of a cascading genetic network within liposomes. *FEBS letters*, 576(3):387–390.

Kuruma, Y., Stano, P., Ueda, T., and Luisi, P. L. (2009). A synthetic biology approach to the construction of membrane proteins in semi-synthetic minimal cells. *Biochimica et Biophysica Acta (BBA)-Biomembranes*, 1788(2):567–574.

Luisi, P. L., Ferri, F., and Stano, P. (2006). Approaches to semi-synthetic minimal cells: a review. *Naturwissenschaften*, 93(1):1–13.

Oberholzer, T., Albrizio, M., and Luisi, P. L. (1995). Polymerase chain reaction in liposomes. *Chemistry & biology*, 2(10):677–682.

Shimizu, Y., Inoue, A., Tomari, Y., Suzuki, T., Yokogawa, T., Nishikawa, K., and Ueda, T. (2001). Cell-free translation reconstituted with purified components. *Nature biotechnology*, 19(8):751–755.

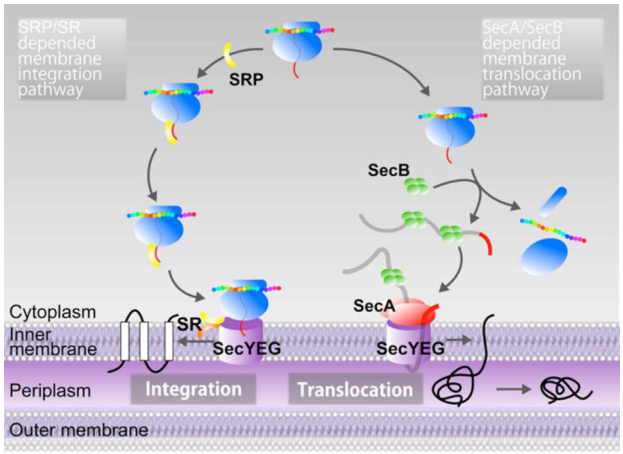

Figure 2: Membrane secretion and integration functions of SecYEG translocon.

Varela, F. G., Maturana, H. R., and Uribe, R. (1974). Autopoiesis: The organization of living systems, its characterization and a model. *Biosystems*, 5(4):187–196.