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In vitro Synthesis of Membrane Protein Machinery toward the Construction of Artificial Cell

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Abstract

Cell membranes are essential part of living cells. They are important as the envelope which encapsulate the biochemical systems within them and distinguish "self" components from "not-self" surrounding environment. Furthermore, cell membranes function as an interface which exchange materials between inside and outside of the cell, sense external environment, and transmit signals to inside systems to response the circumstance. In the field of synthetic biology, liposome (lipid membrane vesicle) has been widely used as a model of cell membrane. Although liposome is certainly a good model as for cell envelope, it is not satisfying the biochemical functions of cell membranes. Since the most of cell membrane functions are led by membrane embeded proteins, we should combine membrane proteins with liposome to construct more feasible artificial cell membranes. In this research, we aim to equip membrane machinery on liposome membrane using in vitro gene expression system.

We show that a membrane machinery Sec translocon[1], which conducts membrane secretion and insertion of protein (Figure 1), has been synthesized onto the liposome membrane from its template DNAs. The gene expression was performed with the cell-free protein synthesis system, PURE system[2]. The PURE system is a reconstructed transcription/translation system that actualizes the phenomenon of Central Dogma (DNA-RNA-protein) in vitro with the minimal number of factors. Synthesized Sec proteins spontaneously localized at lipid bilayer and about 80nM Sec translocon were produced in functional state. This indicates that 2-3 Sec translocon were allocated to one liposome membrane based on a sequence of statistical calculations. Although the population density of the produced Sec translocon was not so high, a substantial peptides secretion activity of the Sec translocon was detected by biochemical assays. The specific activity of the synthesized Sec translocon was comparable to that of native Sec translocon isolated from cells in the function of protein secretion. In addition, the synthesized Sec translocon was able to conduct membrane insertion of a multi-spanning protein. These results indicate that the artificially synthesized Sec translocon is functional both in secretion and insertion. It should be noted that the formation of Sec translocon was achieved in self-assembly process.

Our results demonstrate that the functional Sec translocon has been constructed in totally synthetic manner. Although the protein synthesis in this study were performed on the outside of the liposomes, the same reaction would be occurred inside liposomes, for instance giant unilamellar vesicles that can effectively encapsulate a cell-free system and DNA[3]. More importantly, our results raise a possibility that various membrane proteins can be subsequently produced in liposome membrane by primarily constructed Sec translocon, and eventually non-functional liposomes will gain divers bio-functions that are essential for a living artificial cell.

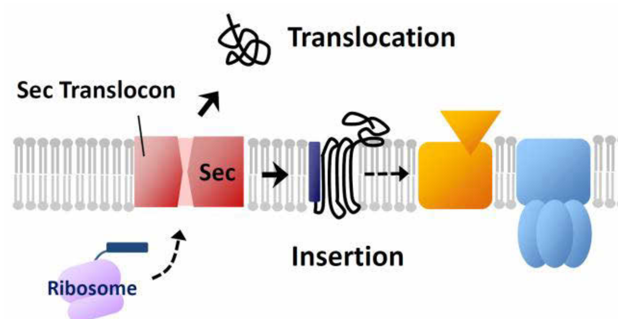

Figure 1: Sec translocon mediates translocation of secretory proteins and insertion of membrane proteins.

References

1. du Plessis DJ, Nouwen N & Driessen AJ (2011) The sec translocase. *Biochim Biophys Acta* 1808(3): 851-8652.
2. Shimizu Y, *et al* (2001) Cell-free translation reconstituted with purified components. *Nat Biotechnol* 19(8): 751-755.
3. Stano, P, *et al* (2011) Compartmentalized reactions as a case of soft-matter biotechnology: synthesis of proteins and nucleic acids inside lipid vesicles. *Journal of Materials Chemistry* 21: 18887-18902.