Artificial cell models of membrane protein synthesizing liposome

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In vitro gene expression installed in a liposome can be regarded as a bootstrap sequence for an artificial cell-model (ACM). Recently, we reported that functional protein synthesis was observed in cell-sized lipid vesicles following encapsulation of a gene-expression system [1]. Interestingly, at the early stage of the reaction, the expression efficiency inside the vesicle was remarkably higher than that in the solution outside. This fact supports the hypothesis that the internal space of the cell-sized liposome is suitable for protein synthesis reactions. Most of the research on *in vitro* gene expression systems has been devoted to the synthesis of water-soluble proteins. The synthesis of membrane proteins is still limited due to the low solubility and folding problem. We are trying to investigate the direct constitution of nascent membrane proteins into giant liposomes.

First, as a first example, we adopted apo-cytochrome b5 (b5) having single anchoring (or transmembrane) sequence [2]. Giant liposomes were added to a translation reaction cocktail of an *in vitro* protein translation system. The b5 and its fusion proteins were synthesized and directly localized on the liposome membrane. It is likely that the hydrophobic sequence of the nascent peptide attaches the liposome membrane immediately. After the translation reaction, the proteo-liposomes were isolated by simple centrifugation. From the isolated liposome fraction, the membrane protein was found as a single band by SDS-page analysis. b5 conjugated dihydrofolate reductase was synthesized in the same procedure and the protein was directly displayed and functioned on the liposomes. In the case, b5 acts as a "hydrophobic tag" for recruitment the enzyme to the liposome surface.

Next, gap-junction protein connexin (Cx) was examined [3]. Cxs are a critical component of cellular gap junctions (GJs), forming structures that mediate intercellular communication. Each GJ consists of two docked hemichannels (connexons; 6mer of Cx) between two neighboring cells. A connexon is comprised of six connexin proteins in self-assembly. Small molecules (<1.8kDa) can be directly transferred from one cell to a neighboring cell through GJs. Cx-containing liposomes were prepared by natural swelling method using *in vitro* transcription/translation systems with plasmids encoding connexin. The expressed Cx was directly constituted to the liposome membrane upon in vitro synthesis, leading to pure membrane protein-containing liposomes. The localization was investigated by immunostaining methods. The function of the expressed Cx was determined by dye transfer assay. Cx-liposome

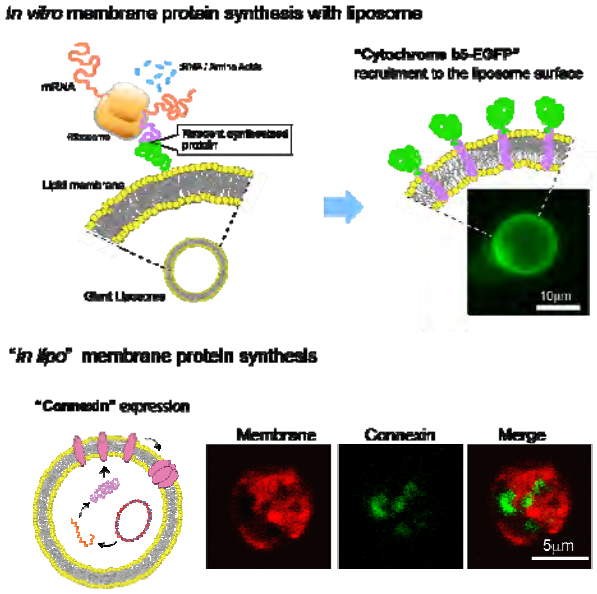

Figure 1. Schematic illustration of membrane protein synthesizing liposomes.

containing calcein dye (Mw. 623 Da) was added to the cultured Cx-cell. After two hours incubation, the fluorescence signal was observed in the cellular layer. The transfer phenomenon was critically dependent on expression of the gap-junction protein connexin on the liposome. Thus, we conclude that the gap-junction between liposome and cell was established.

Functional membrane protein expression with liposomes is a crucial step for constructing a more realistic cell-model. In conclusion, the presented ACM of membrane protein expressing liposome will become a useful method for understanding the complicated biomembrane function from the elemental principles.

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References

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