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In Vitro Reconstruction of Functional Membrane

Yutetsu Kuruma[1,2], Hideaki Matsubayashi[2], and Takuya Ueda[2]

[1]Earth-Life Science Institute, Tokyo Institute of Technology
[2]Gradient School of Frontier Sciences, The University of Tokyo
kuruma@elsi.jp

Abstract

One feasible strategy in constructing an artificial cell is the assembly of biomolecules to fulfill the basic cellular reactions which necessary for cell alive (Luisi et al., 2006). In this strategy, membrane vesicle has been widely employed as a model cell compartment allowing internal biochemical reaction such as gene expression (Fujii et al., 2013), lipid biosynthesis (Kuruma et al., 2009), and so on. Although difficulties in constructing biochemically functional membrane composed of lipids and membrane proteins have limited the construction of a viable artificial cell, it has been reported that some kind of membrane protein can be spontaneously integrated into a lipid membrane during the translation reaction by ribosome (Ashley et al., 2012). This phenomenon can be efficiently applied for the construction of membrane protein architecture on the vesicle membrane. On the other hand, not all membrane proteins can spontaneously integrate in the native-like conformation. For example, if a membrane protein contains a large hydrophilic domain at the other side of membrane, this type protein requires a membrane translocation channel, which called as Sec Translocon (Luirink et al., 2005). Therefore, if the Sec translocon could be synthesized within the vesicle, any membrane protein should be synthesized as downstream reaction in the regulated membrane integration. In either spontaneous and Sec dependent membrane integrations, the important point is that membrane proteins have to be synthesized in the presence of vesicles in totally artificial way. In this sense, in vitro protein synthesis system, called as "cell-free system", is used as an underlying technology (Shimizu et al., 2006). A cell-free system enables protein synthesis by just adding an interest gene into the cell-free reaction mixture. Thus, the cell-free system encapsulated in vesicles can synthesize membrane proteins inside and the synthesized membrane proteins are spontaneously localized onto the vesicle membrane. This is somehow similar to what a real living cell is doing. So far, we have synthesized several membrane enzymes using the technologies of cell-free system and vesicle manipulation (Kuruma et al., 2009; Kuruma et al., 2005; Ozaki et al., 2008; Kuruma et al., 2012). For instance, two membrane enzymes, which involved in phospholipids biosynthesis pathway, synthesized within vesicles produced new lipid molecule (Kuruma et al., 2009). The membrane localization of such internally synthesized membrane protein can be visualized under the microscopy observation when a tandemly conjugated αHemolysin-GFP chimeric protein was synthesized in giant unilameller vesicles (Fig. 1). This method will provide new aspects in artificial cell study and dynamic analysis of membrane protein (Shimizu et al., 2014).

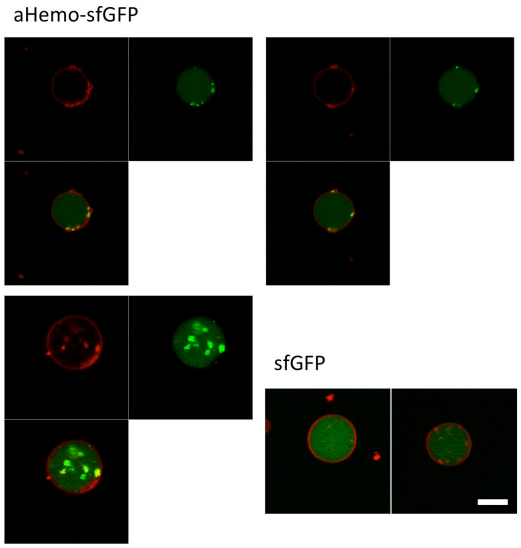

Fig. 1. Protein synthesis of αHemolysin-GFP inside giant unilameller vesicle.

Addition to the internal protein synthesis approach, reconstruction of biological reactions involved around vesicle membrane is also important for the development of artificial cell, and also for the deep understanding of the basic structural and dynamic organization of living cells. So far, cell division machinery (Osawa et al., 2008) or cytoskeleton structure (Maeda et al., 2012) has been partially reconstructed on the vesicle membrane. Although these membrane functions are important, self-production of ATP is crucial for autonomous cell alive. About this point, we have reconstructed an artificial organelle, which consists of ATP synthase and bacteriorhodopsin (bR). bR is a proton pump membrane machinery stimulated by irradiation of light. The generated proton gradient between outside and inside of vesicle drives the ATP synthase that was also integrated in the vesicle membrane. We have reconstructed this cell-like device and succeeded to detect ATP production depending on light irradiation.

We would like to present recent results on the construction of artificial cell by means of biomolecules and membrane vesicles, focusing on cell membrane functions.

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