論文 / 著書情報
Article / Book Information

題目(和文)	リポソーム型人工細胞の接合ナノポアタンパク質の構築及び遺伝子発現制御に関する研究
Title(English)	Study on construction of junctional protein nanopores and switchable control of gene expression in liposomal artificial cells
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種別(和文)	論文要旨
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（博士課程）
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論 文 要 旨

THESIS SUMMARY

系・コース： Department of, Graduate major in	生命理工学 系 生命理工学 コース	申請学位（専攻分野）： Academic Degree Requested	博士 （工学） Doctor of
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要旨（英文 800 語程度）
Thesis Summary（approx.800 English Words ）

Multicellular organisms exhibit sophisticated capabilities that go beyond the mere sum of cellular functions through macro-scale structuring. Multicellular structuring in engineered cellular systems is expected to enable the development of advanced and socially beneficial technologies such as chemical artificial intelligence, bio-robots, and in situ drug synthesis and delivery systems that live symbiotically in the human body, but the technology to make cells multicellular artificially is still immature. The development of natural multicellular organisms is based on cell-cell adhesion, molecular communication, and cellular responses to signaling molecules, and the bottom-up construction of artificial multicellular systems requires biological functional modules that enable them.

Toward the realization of artificial multicellularity, various studies on both living cell-based and artificial cell-based artificial multicellular systems have been reported. A living cell-based artificial multicellular system that spontaneously patterns cells and forms layered spheroids by controlling gene expression with synthetic receptors that recognize other cells was reported. The emulsion-based artificial multicellular system was constructed by piling up water-in-oil emulsion in a highly viscous hydrophobic solvent. In emulsion-based artificial multicellular systems, signal transduction and acquisition of positional information by morphogens have been achieved by interconnecting cells with nanopore proteins at their interfaces to allow the migration of molecules. However, liposome-based systems lacked a method to directly connect artificial cells to each other, and molecular communication between artificial cells was limited to systems mediated by diffusion into a solvent.

In the development of multicellular organisms, it is important for cells to alter gene expression in response to morphogens, molecules that provide positional information to individual cells depending on their concentration. Natural cells precisely regulate gene expression to achieve sophisticated biological functions such as efficient energy and material use, functional assembly of proteins, response to external stimuli, and cell differentiation. In order to change or adapt to a new state, cells need to regulate gene expression in such a way that they not only initiate expression but also stop certain cellular functions. Transcriptional control is the most common step in regulating gene expression in cells and engineered cells, but in engineered cells that contain reconstituted cell-free protein synthesis (CFPS) systems that lack RNA degradation systems, the switchable control, including gene expression arrest by transcriptional control, is complicated. Since the reconstituted CFPS system does not have an RNA degradation system and continues to translate from mRNA once synthesized, it is difficult to control the sequence of protein expression because protein expression cannot be stopped by transcriptional control. Therefore, in artificial cells encapsulating

CFPS, control of gene expression by translation control is considered to be the most promising method. Synthetic riboswitch is a translation control system using RNA aptamers that bind to low-molecular-weight compounds and change their conformation. However, there have been few examples of gene expression control using riboswitches in CFPS-encapsulated artificial cells, and there have been no examples of orthogonal and switchable control of two gene expressions.

In this study, I designed spontaneously-assembled nanopore proteins spanning two membranes for molecular communication between adjacent cells and a minimal signal response mechanism using two orthogonal riboswitches for artificial morphogen response, aiming to develop new molecular tools for artificial multicellular systems.

Staphylococcus α-Hemolysin (AH), a protein pore-forming toxin (PFT), is expressed as a soluble protein that forms a heptamer on phospholipid membranes to form nanopores through which small molecules (< 1100 Da) can pass. AH-SpyTag/SpyCatcher, a fusion protein of AH and protein-protein binding domains SpyCatcher and SpyTag, were designed as molecules that spontaneously form nanopores which connect and attach liposome-based artificial cells to each other for minimal artificial multicellular tissue. Western blotting revealed that SpyTag/SpyCatcher can be functionally incorporated into AH by insertion into the C-terminus of AH. When AH-SpyTag/SpyCatcher expressed and purified in E. coli was fed to calcein-encapsulated GUVs (Giant Unilamellar Vesicles), calcein leaked out, indicating that SpyTag/SpyCatcher fusion does not inhibit AH nanopore formation. Cryo-EM tomography revealed the head-to-head 14-meric structure of the AH-SpyTag-AH-SpyCatcher complex as designed.

Next, here I designed a switchable translation control system in response to two substances in a cell-free translation system encapsulated in a liposomal artificial cell. In this system, mutually orthogonal riboswitches AC17-5c and Theo-3c, which respond to two membrane-permeable ligands ASP2905 and Theophylline, respectively, were used to control the expression of GFP and mScarlet-I3. Compartmentalization of the CFPS system with liposomes enabled the addition and removal of ligands by centrifugal washing of the artificial cells. The study demonstrated the initiation and termination of riboswitch-regulated expression of GFP and mScarlet-I3 independently, as well as control over their expression ordering[4]. This technique is not only applicable to artificial morphogenesis but also to the expression of membrane proteins requiring the Sec-Translocon in cell-free translation systems.

Overall, this study presents fundamental technologies for the bottom-up construction of artificial multicellular systems.