【Grant-in-Aid for Scientific Research (S)】

Integrated Disciplines (Complex Systems)

Title of Project : Cellular Programming Using Synthetic RNP Nanosystems

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Research Area : Complex Systems
Keyword : Functional RNA, Synthetic biology, Cellular programming, Regenerative medicine

【Purpose and Background of the Research】

RNA- protein complexes (RNP) are one of the most important biomolecular complexes to form living systems. If we can create synthetic RNP-based gene regulatory systems, it could expand the field of basic life science and medical applications. In this research, we aim to program mammalian cell fate precisely and autonomously, by integrating our developed RNA synthetic biology techniques. To achieve this objective, three research topics are set as below: ① Development of a new method that allows us to precisely detect and purify live target cells, ② Design and construction of artificial RNP nanostructures that control intracellular protein localization and cell signaling, ③ and finally development of experimental evolution techniques to generate functional RNP devices in cells. By achieving these three topics, we aim to establish synthetic RNA-mediated genetic manipulation technologies that control cell fate according to intracellular conditions.

【Research Methods】

① **Development of a new method for detecting and purifying live target cells**:
In order to accelerate regenerative medicine to the clinic, it is important to develop a method that enables to detect and purify target cells safely and precisely, and remove undesired cells autonomously. Therefore, to accomplish this task, we aimed to develop a new method, by introducing synthetic RNA-based gene switches and synthetic circuits into target cell populations.

② **Design and construction of functional RNP nanostructures within cells**:
To control the accumulation and localization of intracellular proteins, we introduce designed RNA nanostructures in mammalian cells. We have recently succeeded in constructing synthetic nanostructures composed of RNA and protein (RNP nano-triangles). Thus, by expanding our RNP molecular design technology, it is possible to generate RNP nanomachines that function in mamalian cells.

③ **Directed evolution system to generate functional RNP devices:**
For realizing the genetic manipulation according to the expression of any target protein factor, it is important to obtain synthetic RNA sequences that bind to any target agent. Therefore, we will develop a new method that enables us to evolve synthetic RNA and control target gene expression (such as a cell death-inducing factor) in live cells.

【Expected Research Achievements and Scientific Significance】

If we can develop new technologies that freely program target cells in accordance with the intracellular environment, it is possible to open up new frontiers in life sciences. In addition, if we can safely and precisely generate a variety of cells derived from pluripotent cells, it will accelerate and contribute to the field of regenerative medicine. Thus, the developed technologies will be a promising tool for both basic life science and clinical stem cell research and medical applications.

【Publications Relevant to the Project】

- Kenji Miki, Kei Endo,…, *Hirohide Saito, and *Yoshinori Yoshida. “Efficient Detection and Purification of Cell Populations Using Synthetic MicroRNA Switches”. ***Cell Stem Cell***, 16, 699-711 (2015)
- Eriko Osada, Yuki Suzuki, Kumi Hidaka, Hirohisa Ohno, Hiroshi Sugiyama, Masayuki Endo, and *Hirohide Saito, “Engineering RNA-protein complexes with different shapes for imaging and therapeutic applications”. ***ACS Nano***, 8130–8140 (2014)

【Term of Project】 FY2015-2019

【Budget Allocation】 124,800 Thousand Yen

【Homepage Address and Other Contact Information】

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