Shinya Yamanaka

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2010 Balzan Prize for Stem Cells: Biology and Potential Applications

For the discovery of a method to transform already differentiated cells into cells presenting the characteristics of embryonic stem cells.

Institution Administering Research Funds: Kyoto University

Adviser for the Balzan General Prize Committee: Nicole Le Douarin

Molecular Basis During iPS Cell Generation and Its Application

Shinya Yamanaka will use half of his prize to support a five- to six-year research project on molecular mechanisms and application of induced pluripotent stem (iPS) cells at the Center for iPS Cell Research and Application (CiRA), Kyoto University. iPS cells were originally generated from mouse and human fibroblasts by retroviral introduction of four factors, Oct3/4, Sox2, c-Myc and Klf4. iPS cells are similar to embryonic stem (ES) cells in morphology, proliferation, gene expression and most importantly, pluripotency. It is important to develop a method to differentiate various target cells from iPS cells with high efficiency and safety. Synthetic RNA technologies have a promising outlook for controlling such cell-fate conversion. For example, direct injection of synthetic mRNAs into mammalian cells could serve as a powerful tool for gene therapy and regenerative medicine because transfected mRNAs do not integrate into the genome, thus eliminating the risk of cellular damage such as tumor formation. Furthermore, the injection (being irrelevant to transfer to the nucleus and nuclear events) enables rapid and homogenous gene expression in cell clusters. However, precise control of protein production from directly transferred synthetic RNAs has yet to be attained. Thus, elucidating the design principle of functional RNA molecules could be particularly useful for the next generation of stem cell research.

The Center for iPS Cell Research and Application (CiRA) hired one young faculty member, Dr. Saito, on 1 July 2011, to promote the research to control cell fate using synthetic RNA-based gene manipulation technologies. Dr. Saito attempts to take a synthetic biology approach that leads to understanding and controlling cells through the process of 'artificially designed' RNA molecules and RNA-based gene expression systems. Creating artificial RNAs that freely control the functions of cells and applying them to examinations and medical treatments is one of the research goals of this new field. His laboratory will use the unique technology of synthetic biology that designs RNA and/or RNA-protein complexes (RNP) artificially and experimentally evolve them in order to control the fate of target cells depending on cellular environment. In concrete terms, he will engage in the following research projects:

1. Developing a technique to control cell fate with high safety and purity using artificial RNA/RNP molecular complexes.
2. Developing artificial RNA/RNP-based genetic switches that can detect specific protein and/or RNA expression and control ON/OFF of the translation of target genes.

Advances made in fiscal year 2013 included the successful development of synthetic RNA switch extension technology that points to next-generation technology for control of gene expression (Endo K., Nat. Commun. 4:2393, 2013). An outline of these developments is given below.

At present, in order to alter cell fate from iPS cell to differentiated cell, genes have to be induced, for instance by adding growth factor, chemical substances, or other external additives at each stage of the culture process. This means that fate control responsive to intracellular conditions is challenging. The research team introduced above is engaged in the development of RNA switches that will make it possible to control cell fate by adjusting the expression of external genes in response to intracellular conditions (Saito H., Nat. Commun. 2:160, 2011). In their previous research, an OFF switch to repress gene expression and an ON switch to activate expression had to be designed and tuned separately, thus making it difficult to create a switch based on calculating the functions of a module with the target levels of sensitivity and performance.

In their latest work, the Saito group developed a method that allows ready adjustment and inversion of the action of the RNA switch. They have named the resulting device, made of RNA, an "RNA inverter". The newly developed RNA inverter is able to turn

the RNA switch from OFF to ON, switching its function flexibly while maintaining its properties intact. The synthetic mRNA sequence into which this RNA inverter is inserted is rapidly degraded if the target factor is not expressed within the cell. This means that expression of the target gene is switched OFF. Conversely, when the target factor is expressed, the mRNA binds to the target (detection), the mRNA is stabilized depending on the volume of expression (assessment), and the translation of the target external gene is turned on by the inverter (activation). Because the RNA switch is able to independently control gene expression by sensing the intracellular conditions, it should lend itself to a range of applications. It could, for instance, lead to a method for inducing differentiated cells from iPS cells in response to intracellular conditions, or a method of inducing cell death based on exclusive detection of target cells such as undifferentiated cells or cancer cells.

iPS cells and subsequently differentiated target cells/tissues would provide unprecedented opportunities not only for regenerative medicine, but also in disease modelling and drug development. In early 2013, Shinya Yamanaka decided to use his prize to spread iPS cell research over institutes other than CiRA with Dr. Aoi at Kobe University to study recapitulation of several intractable diseases, including cancer by iPS cell technology. In this fiscal year, a new laboratory for the Aoi Group was built at the Kobe University graduate school of medicine. Currently, his team focuses on cancer stem cells, which have been suggested to be the potential for self-renewal and tumorigenesis in certain cancers. To start off, Aoi's group successfully established a novel technology to induce cancer stem cell (CSC) properties in intestinal cancer cells by introducing defined factors and collecting the cells with CSC properties, which leads to a further understanding of cancer disease mechanisms and medical applications.

Researchers:

Hirohide Saito, Associate Professor, Center for iPS Cell Research and Application (CiRA), Kyoto University

Takashi Aoi, Professor, Department of iPS Cell Applications, Graduate School of Medicine, Kobe University

Publications:

Endo K, Hayashi K, Inoue T*, Saito H *. 2013. A versatile cis-acting inverter module for synthetic translational switches. Nature Communications. 4:2393.

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Kashida S, Saito H*. 2014. A Three-Dimensional Design Strategy for a Protein-Responsive shRNA Switch. Methods Mol. Biol. 1111:269-86.
Endo K, Saito H*. 2014. Engineering protein-responsive mRNA switch in Mammalian cells. Methods Mol. Biol. 1111:183-96.

Other Relevant Information

References for the RNA-based gene synthetic biology technologies developed by Dr. Saito:

Saito H*, Fujita Y, Kashida S, Hayashi K, Inoue T*. 2011. Synthetic human cell fate regulation by protein-driven RNA swiches. Nature Communications. 2:160.
Ohno H, Kobayashi T, Kabata R, Endo K, Iwasa T, Yoshimura S, Takeyasu K, Inoue T*, Saito H*. 2011. Synthetic RNAprotein complex shaped like an equilateral triangle. Nature Nanotechnology. 6: 116-120.
Saito H*, Kobayashi T, Hara T, Fujita Y, Hayashi K, Furushima R, Inoue T*. 2010. Synthetic Translational Regulation by an L7Ae-Kink-turn RNP Switch. Nature Chemical Biology. 6: 71-78.

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