Protein Synthesis Solves the Mysteries of Life

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Proteins are involved in numerous biological phenomena. A comprehensive understanding of proteins leads to an understanding of biological phenomena. Therefore, protein synthesis *in vitro* has recently become a significantly valuable technology in various fields such as biology, chemistry, and medicine. Protein synthesis takes place in the living cell. Therefore, a number of researchers have attempted to synthesize proteins in the living cell. However, it is still at a difficult stage due to its complexity compared to DNA synthesis from the original one. In addition, management of protein production is also difficult because the production output of protein depends on the rate of cell proliferation. To overcome these difficulties, a cell-free protein synthesis system has been developed since the 1950s, which can produce proteins simply and efficiently by using cell extract. The advantage of this system is that protein synthesis is independent of cell life and death. Furthermore, active synthesis can be maintained for extended periods, producing more than 1 mg of protein per mL by continuously supplying resources such as amino acids and ATP. Adenosine triphosphate (ATP) can store and release energy for protein synthesis, which can be observed in almost all cells. However, not only protein synthesis but also some reactions which inhibit protein synthesis or are irrelevant to protein synthesis often occur in this system because the cell extract contains factors that induce these reactions. In 2001, Y. Shimizu *et al.* developed a protein synthesis using recombinant elements (PURE) system which can completely eliminate these undesirable reactions in the system[1]. The PURE system is a kind of cell-free protein synthesis method and a powerful tool because this system doesn’t contain factors that induce reactions that inhibit protein synthesis or are

irrelevant to protein synthesis. The PURE system synthesizes proteins from minimum factors which were purified individually, including 20 aminoacyl-tRNA synthetases, T7 RNA polymerase, and ribosomes.

The PURE system has brought about a major innovation in protein synthesis by solving many of the problems that could not be solved by conventional cell-free protein synthesis systems and conventional protein synthesis in batch. In the PURE system, we can alter the system of reactions easily by changing the components because we fully grasp what is included in the reaction solution. Therefore, this system makes it possible to freely create proteins that are difficult to synthesize by the living cell, such as toxic proteins, cytotoxic compounds, and artificial proteins that contain non-natural amino acids that are not included in ordinary proteins. Furthermore, a variety of experiments and analyses that were difficult to do with conventional cell extract have been conducted with the PURE system. For example, through the experiments using the PURE system, T. Niwa *et al*. discovered that about half of the proteins require a type of protein called a chaperone to help form other proteins (chaperones for folding)[2]. Proteins that require chaperones for folding often aggregate with other proteins in the absence of chaperones. They prepared chaperone-free cell solutions with the PURE system and investigated protein aggregation. After examining the aggregation tendencies of thousands of proteins, they showed that about half of the proteins aggregate and require chaperones for folding. Furthermore, researchers have conducted a lot of research using the PURE system such as the synthesis of membrane proteins, interaction analysis of molecular networks in protein synthesis, and protein development for drug discovery. These studies would not be possible without the existence of the PURE system, thus indicating that this system has a significant impact

on society

This PURE system is expected to have a variety of applications in the future. Especially, the construction of living cells will give a significant impact on a lot of fields. In addition, it could be an important step in the search for the origin of life. Researchers attempt to construct living cells by using artificial cells. Currently, two methods, the top-down approach and the bottom-up approach, are known for designing artificial cells. The top-down approach is a method of reconstructing a cell by removing all but the minimal elements necessary to maintain cellular life from the actual cells. On the other hand, the bottom-up approach is a method of creating functional biological systems by constructing complex macromolecules *in vitro* from simple molecules. Of these two methods, I consider that the bottom-up approach, in particular, is an effective way of constructing living cells. This is because this approach can be combined with the PURE system, thereby providing a complete picture of intracellular contents and reactions. It is necessary to provide the minimum functions defined as life, such as the ability to self-replicate and metabolism, to construct a living cell. Each of these functions is currently being studied by the PURE system and could be applied to the design of living cells in the future. Furthermore, there is a possibility that by imparting various functions other than the minimum necessary for life to the artificial cell, biological reactions that have not been elucidated may be elucidated. These results are then expected to be applied not only to the development of basic science but also to medicine, pharmacy, and other fields.

1. Shimizu, Y. *et al.* Cell-free translation reconstituted with purified components. *Nat. Biotechnol.* **19**, 751–755 (2001).
2. Niwa, T. *et al.* Bimodal protein solubility distribution revealed by an aggregation analysis of the entire ensemble of Escherichia coli proteins. *Proc. Natl. Acad. Sci.*

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