DOI: http://dx.doi.org/10.7551/978-0-262-33027-5-ch031

Ribosome synthesis and construction of a minimal cell using a cell-free expression platform

Filippo Caschera, Michael C. Jewett

Department of Chemical and Biochemical Engineering,
Northwestern University, Evanston, 60208 Illinois,
United States of America

filippocaschera@gmail.com

Abstract

The creation of wet artificial life in the laboratory is a non-trivial challenge for biologists, chemists, and computer scientists (1-4). Such a challenge revolves around the modular integration of complex reactions networks to obtain functional biochemical units able of self-replication, self-reproduction, spatial-temporal control and ultimately open-ended evolution, e.g. minimal and artificial cells (1,5-8).

As a step towards building minimal cells, we have developed a cell-free expression system for bacterial ribosome synthesis named iSAT: integrated Synthesis, Assembly and Translation for *in vitro* construction of *Escherichia coli* ribosomes (9). The ribosomal RNA, transcribed from its natural operon, self-assembles with ribosomal proteins added to the reaction mixture. Afterwards, *in vitro* built synthetic ribosomes translate a reporter gene (10,11). Such system is important to design ribosome with new functions, and for the bottom-up construction of a minimal cell.

Ribosome cell-free synthesis is an essential process for building a minimal cell that can maintain itself (1). Indeed, regeneration of encoded DNA molecular machineries, through a compartmentalized reaction network, will be necessary to ensure gene expression after cycles of self-reproduction (12,13).

In this work, we have sought to improve the efficiency of the iSAT reaction to achieve the break-even milestone of ribosomes that are capable of constructing ribosomes (7,434 peptide bonds are needed to make a complete set of r-proteins). To do this, we prepared and optimized the iSAT reaction system using a robot for liquid handling. The open nature of cell-free expression platforms enables precise settings of each component level for an optimal system's configuration. Previously, high-throughput screening and machine learning have been used for the optimization of a cell-free protein synthesis and a liposomal drug formulation (14-16).

Here, I will present the optimization of *in vitro* ribosome construction using a cell-free expression system, and I will introduce future directions of the project. In particular, I will describe biochemical experimental spaces underlying the cell-free ribosome synthesis. I will show results on the optimization using a liquid handling robot for high-throughput experimentation. Our cell-free protein synthesis platform is the only one enabling *in vitro* ribosome construction, which is relevant to the synthesis of a minimal cell.

Adding effective energy regeneration modules is also important for minimal cell projects. Therefore, I will also present data highlighting the ability to regenerate ATP with a non-phosphorylated energy substrate with the iSAT system. Recently, I developed a novel metabolic scheme for a minimal cell (17). The system is based on the catabolism of polysaccharides and/or a polyphosphate (18-20) to regenerate ATP. Proteins are synthesized using a custom-made amino acids mixture (20).

The system is improved by overcoming a fundamental limitation: the efficient recycling of the orthophosphate (iP), which is the by-product of protein synthesis. As a result, ATP (adenosine triphosphate) is kept at steady state and available for *in vitro* transcription and translation (19). Currently, it represents the most powerful *in vitro* protein synthesis systems (18).

One important feature of a minimal cell is the compartment or container, which more than physically interlock components and sub-systems, confers the necessary genotype-phenotype linkage for evolution (21). The compartment of the minimal cell is based on liposomes (1-3,22), and evolutionary dynamics such as fusion and division, are important for resource feeding and selection respectively (6,8,12).

In summary, the work described is designed to lay the foundation for the construction of a synthetic replicating entity by building up synthetic biological unit operations (*e.g.* cell-free synthesis of constituent parts) and fine-tune the starting blueprint. Indeed, through the bottom-up synthesis of a minimal cell, we are building and understanding complex biological systems.

Filippo Caschera, Michael C. Jewett (2015) Ribosome synthesis and construction of a minimal cell using a cell-free expression platform. Proceedings of the European Conference on Artificial Life 2015, pp. 147-148

References

Journal Article

1.Caschera, F. and V. Noireaux. 2014. Integration of biological parts toward the synthesis of a minimal cell. *Current Opinion in Chemical Biology*, 22C:85-91.

2.Noireaux, V., Y.T. Maeda, and A. Libchaber. 2011. Development of an artificial cell, from self-organization to computation and self-reproduction. *Proceedings of the National Acadedmy of Science U S A*, 108:3473-3480.

3.Luisi, P.L., F. Ferri, and P. Stano. 2006. Approaches to semi-synthetic minimal cells: a review. *Naturwissenschaften,* 93:1-13.

4.Jewett, M.C. and A.C. Forster. 2010. Update on designing and building minimal cells. *Current opinion in biotechnology,* 21:697-703.

5.Shin, J. and V. Noireaux. 2012. An E. coli cell-free expression toolbox: application to synthetic gene circuits and artificial cells. *ACS synthetic biology,* 1:29-41.

6.Caschera, F., T. Sunami, T. Matsuura, H. Suzuki, M.M. Hanczyc, and T. Yomo. 2011. Programmed vesicle fusion triggers gene expression. *Langmuir : the ACS journal of surfaces and colloids,* 27:13082-13090.

7.Caschera, F., S. Rasmussen, and M.M. Hanczyc. 2013. An Oil Droplet Division-Fusion Cycle. *ChemPlusChem, 78*:52-54.

8.Caschera, F., P. Stano, and P.L. Luisi. 2010. Reactivity and fusion between cationic vesicles and fatty acid anionic vesicles. *Journal of colloid and interface science,* 345:561-565.

9.Jewett, M.C., B.R. Fritz, L.E. Timmerman, and G.M. Church. 2013. In vitro integration of ribosomal RNA synthesis, ribosome assembly, and translation. *Molecular systems biology,* 9:678.

10.Fritz, B.R. and M.C. Jewett. 2014. The impact of transcriptional tuning on in vitro integrated rRNA transcription and ribosome construction. *Nucleic acids research,* 42:6774-6785.

11.Liu, Y., B.R. Fritz, M.J. Anderson, J.A. Schoborg, and M.C. Jewett. 2014. Characterizing and Alleviating Substrate Limitations for Improved in vitro Ribosome Construction. *ACS synthetic biology*.

12.Szostak, J.W., D.P. Bartel, and P.L. Luisi. 2001. Synthesizing life. *Nature* 409:387-390.

13.Hanczyc, M.M., S.M. Fujikawa, and J.W. Szostak. 2003. Experimental models of primitive cellular compartments: encapsulation, growth, and division. *Science,* 302:618-622.

14.Caschera, F., M.A. Bedau, A. Buchanan, J. Cawse, D. de Lucrezia, G. Gazzola, M.M. Hanczyc, and N.H. Packard. 2011. Coping with complexity: machine learning optimization of cell-free protein synthesis. *Biotechnology and bioengineering,* 108:2218-2228.

15.Caschera, F., G. Gazzola, M.A. Bedau, C. Bosch Moreno, A. Buchanan, J. Cawse, N. Packard, and M.M. Hanczyc. 2010. Automated discovery of novel drug formulations using predictive iterated high throughput experimentation. *PloS one, 5*:e8546.

18.Caschera, F. and V. Noireaux. 2014. Synthesis of 2.3 mg/ml of protein with an all Escherichia coli cell-free transcription-translation system. *Biochimie,* 99C:162-168.

19.Caschera, F. and V. Noireaux. 2014. A Cost-Effective Polyphosphate-Based Metabolism Fuels an All E. coli Cell-Free Expression System, *Metabolic Engineering*, 27:187-189.

20.Caschera, F. and V. Noireaux. 2015. Preparation of amino acid mixtures for cell-free expression systems. *Biotechniques*, 58:40-43.

21.Uno, K., T. Sunami, N. Ichihashi, Y. Kazuta, T. Matsuura, and T. Yomo. 2014. The evolutionary enhancement of genotype-phenotype linkages in the presence of multiple copies of genetic material. *Chembiochem,* 15:2281-2288.

22.Ichihashi, N., T. Matsuura, H. Kita, T. Sunami, H. Suzuki, and T. Yomo. 2010. Constructing partial models of cells. *Cold Spring Harbor Perspectives in Biology,* 2:a004945.

Proceedings Paper

16.Caschera, F., M.M. Hanczyc, and S. Rasmussen. 2011. Machine learning for drug design, molecular machines and evolvable artificial cells. *GECCO '11 Proceedings of the 13th annual conference companion on Genetic and evolutionary computation*, 831-832.

17.Caschera, F. and V. Noireaux. 2014. A novel in vitro metabolic scheme for the construction of a minimal biological cell. *ALIFE 14: The Fourteenth Conference on the Synthesis and Simulation of Living Systems, 14*:3-5.

Filippo Caschera, Michael C. Jewett (2015) Ribosome synthesis and construction of a minimal cell using a cell-free expression platform. Proceedings of the European Conference on Artificial Life 2015, pp. 147-148