SYNTHETIC BIOLOGY WITH A CELL-FREE TX-TL SYSTEM: GENE CIRCUITS, PHAGE SYNTHESIS AND ARTIFICIAL CELL

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Introduction. Cell-free transcription-translation (TX-TL) systems were developed in the 60s to study the process of protein synthesis and gene regulation in bacteria. After the invention of highly efficient cell-free systems in the 90s, in vitro protein synthesis became used for applications in biotechnology as an alternative to the recombinant protein technology. Over the past decade, cell-free TX-TL systems have become promising platforms for synthetic biology.
I will present a cell-free TX-TL toolbox specifically developed to construct biochemical systems in vitro using a bottom-up engineering approach.

Methods. Our cell-free system is prepared from E. coli (1, 2). Unlike commercial kits, our system uses the transcription and the translation machineries of E. coli. We have optimized the reaction buffer (energy mix) so as to get protein synthesis at a level comparable to bacteriophage systems (1 mg/ml of synthesized proteins).

Results. Our original idea was to reconstruct elementary gene circuits in isolation free of any other genetic material. We constructed a TX-TL toolbox and we demonstrated that elementary circuits can be engineered in test tube reactions (3). Recently we showed that our custom-made TX-TL system can also execute large DNA programs. The bacteriophage T7, composed of about 60 genes, is entirely synthesized in a single test tube reaction from its genomic DNA (4). Replication of the T7 DNA instructions occurs concurrently with phage expression and self-assembly. Our TX-TL toolbox recapitulates the entire chain of biological information to deliver a functional biological entity.
Encapsulated inside cell-sized liposomes, the TX-TL system is used to construct a prototype of artificial cell programmed with DNA circuits (5, 6).
I will present this cell-free synthetic biology platform and our last experiments.

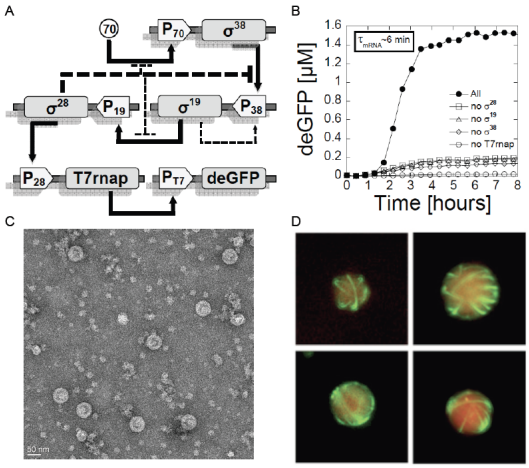

Fig.1 (A) A synthetic 5-stage transcriptional activation cascades. Dotted lines represent the non-specific crosstalks (3). (B) Output signals of the circuit shown in (A). With a global mRNA mean lifetime of 6 minutes, the circuit delivers a specific and biologically relevant output signals. (C) Electron microscopy of T7 bacteriophages synthesized in a cell-free transcription/translation reaction (4). (D) Cell-free expression of the bacterial cytoskeletal proteins MreB and MreC. MreB (labeled with YFP) forms filaments by interacting with MreC at the membrane (5). The vesicle diameter is on the order of 10 μm.

Conclusions. Although originally optimized for specific biological applications, cell-free TX-TL systems are becoming useful platforms for synthetic biology. We have demonstrated that complex biochemical systems can be constructed from DNA programs expressed in test tubes. The ultimate goal is the construction of an artificial cell capable of self-replication.

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References.

1. Shin J., Noireaux, V. (2010). *J. Biol. Eng.* 4:8.
2. Shin J., Noireaux, V. (2010). *J. Biol. Eng.* 4:9.
3. Shin J., Noireaux, V. (2012). *ACS Synth. Biol.* (1):29-41.
4. Shin J., Jardine P., Noireaux V. (2012). *ACS Synth. Biol.* (1):408-413.
5. Maeda Y. et al. (2012). *ACS Synth. Biol.* (1):53-59.