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Fundamental Aspects of Synthetic Biology

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Abstract:

Synthetic biology is an emerging interdisciplinary research field. By designing and constructing new or re-designing the existing natural systems, it confers them novel functions, which do not exist in nature. Owing to the predictability and controllability, synthetic biology attracts more and more interest from biologists, physicists, and engineers. Synthetic biology approaches not only can be widely used for biotechnological applications but also can be used to study complex biological systems to address fundamental questions. Here, we reviewed the recent studies following the concept of "build-to-understand", particularly, the studies to understand intracellular network structure, cell physiology, the behavior of multicellular populations, and ecosystems. **DOI:** 10.13345/j.cjb.160477-en

Keywords: synthetic biology; build-to-understand; genetic circuit; genetic network; cell physiology; cellular structure; micro-ecology

1 From "study-to-understand" to "build-to-understand"

“Study-to-understand” is an important concept of Confucianism in ancient China, which advocates to gain insight into the laws of things through the observation and study of things. Traditional biology research is based on a similar methodology. The emergence of synthetic biology allows people to study the complex living organisms from a completely new perspective, which is to understand life through the re-creation or transformation of biological systems [1].

Synthetic biology is a new emerging discipline that is a highly integrated interdiscipline of biology, mathematical science and engineering. Through the "bottom-up" concept, it designs and creates artificial biological systems that do not exist in nature from the "components" to "modules" and then to "systems", or transforms and reconstructs the existing natural biological systems. These biological functions and systems, which are assembled from artificially designed components and integrated by means of signal transduction, gene regulation, and cellular metabolism, are simpler and more controllable than natural biological systems, making synthetic biology systems have broad application prospects in the fields of chemical engineering, medicine, energy, and environmental protection, *etc*. In addition, synthetic biology provides new means for basic life science research. Richard Philip Feynman, the famous physicist and 1965 Nobel Prize laureate, has famously said, "What I cannot create, I do not understand". Synthetic biology is just to study the basic questions in life sciences through the construction of artificial biological systems, which we called "build-to-understand" [2].

2 Understanding the regulation of biological gene network

In the natural biological systems, some prevalent systemic gene network structure may have been preserved and spread in the long natural evolutionary process due to the evolutionary advantages. These systemic network structures can be viewed as a kind of "design principles" of life. Understanding the "design principles" not only helps people build artificial biological systems, but also help biologists understand more profoundly the fundamental nature of life.

In the artificially engineered systems, the network structure units are widely applied, such as negative feedback, positive feedback and feedforward circuits (Fig.

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1A). In the case of negative feedback, for example, Watt added the speed control unit to the steam engine by means of negative feedback when he designed the steam engine. The purpose was to keep the speed of the steam engine within the controllable range. Also, most components in an electronic system have an "input-output" non-linear frequency range and it is often necessary to increase the linearity of the components by introducing negative feedback. Biologists have found that negative feedback network structural units are widely present in prokaryotic and eukaryotic cells. About 40% of *E. coli* transcription factors inhibit the transcriptional activity of their own promoters [3]. However, due to the lack of comprehensive understanding of the components of the native systems, the traditional biology research means cannot clarify the boundary of each network structure unit. It is difficult for quantifiable control of single variables. Therefore, the function of the network structure unit in the whole system is not well known [4]. In recent years, synthetic biology research, through the study of the design of artificially engineered systems, builds artificial negative feedback gene circuits in cells and studies its dynamic process, which gradually reveals the important biological functions of the network structure unit of negative feedback (Fig. 1B, C). For example, Becskei and Serrano in the European Molecular Biology Laboratory found that negative feedback could significantly reduce the gene expression noise caused by the randomization of intracellular biochemical reactions by constructing the transcriptional negative feedback gene circuit that was mediated by the tetracycline inhibitor TetR in *E. coli* [5]. In another example, Balázsi and others in the University of Texas found that negative feedback could improve the linearity of the response of promoters to the signals by constructing synthetic gene circuits in yeast cells [6]. In addition, synthetic biologists represented by Elowitz of California Institute of Technology validated a mechanism of realizing periodic regulation in living organisms by constructing a synthetic genetic circuit and creating a stable, fast, and adjustable "genetic clock" [7]. Quantitative biologists Chao Tang and others in Peking University summarized the basic conditions needed for the formation of a gene circuit with a stable periodic circuit through the analysis of the mathematical models of different genetic circuits [8]. This kind of research is the "build" by the means of synthetic biology, to reveal the law of operation of the natural biological systems, which is "to understand".

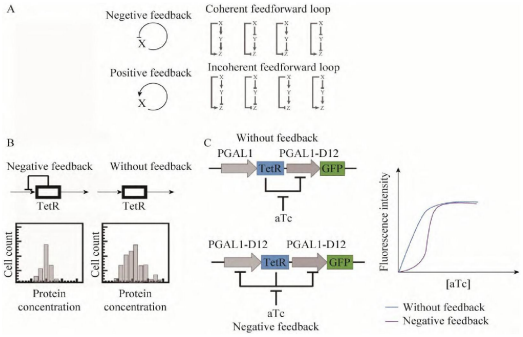

Fig. 1 Network motif in biological system

(A) Typical network motifs, for example, negative feedback, positive feedback and feedforward loop [1]. (B) The negative feedback consisting of tetracycline repressor TetR and TetR-repressive promoter displays significantly lower level of gene expression noise, in contrast to non-feedback control [5]. (C) The negative feedback circuit shows better linearity than the non-feedback circuit [6].

3 Understanding the physiological mechanism of single cells

Cell is the most basic unit of life. Its growth rate, cycle, size and function are subject to strict and quantitative regulation. For a long time, people lack the technical means of quantitative perturbation of the parameters of the study objects, mainly using the qualitative or semi-quantitative means of gene knockout or overexpression to study the functions of the target genes and their effects on the physiological and biochemical processes of cells. In recent years, synthetic biologists and quantitative biologists achieved linear regulation of single or multiple key factors by introducing quantitative and adjustable gene circuits, thereby studying their effects on global physiological activities of cells. The Hwa Laboratory at the University of California, San Diego, studied the cell usage of physiological nitrogen and carbon sources in *E. coli* under different steady-state growth conditions by means of synthetic linearized gene regulation circuits, mathematical models and quantitative experiments. They revealed the growth law mechanism of maintaining the growth rate of *E. coli* by balancing the cell physiological activities such as gene expression and metabolic regulation [9–14].

For a long period of time, it has always been a mystery how cells have set their own volumes and how to achieve the coordinated mechanisms of cell division and intracellular chromosome replication. It is difficult to obtain clear and verifiable conclusions through traditional research methods alone. The linear regulation of the expression levels of key genes in *E. coli* through synthetic gene circuits revealed that altering the expression levels of the *mreB* or *ftsZ* gene could specifically alter the diameter or length of the cells while maintaining the cell growth rate. Furthermore, we analyzed the coordination mechanism

between DNA replication and cell size with the changes of the diameter or length (Fig. 2). The results showed that there was a linear positive correlation between the number of intracellular replication origins and the cell volume, regardless of the change in cell size by changing the diameter or length. Based on the experimental results, we presented the hypothesis of "adder-per-replication origin" to explain the incremental phenomenon that determines the size and steady state of cells in the population, which means that the ratio of the increase of cell volume between the two replication initiation events and the number of intracellular replication origins at the initiation time is a constant value [15]. This study demonstrated the advantages of synthetic gene circuits in studying cell physiology.

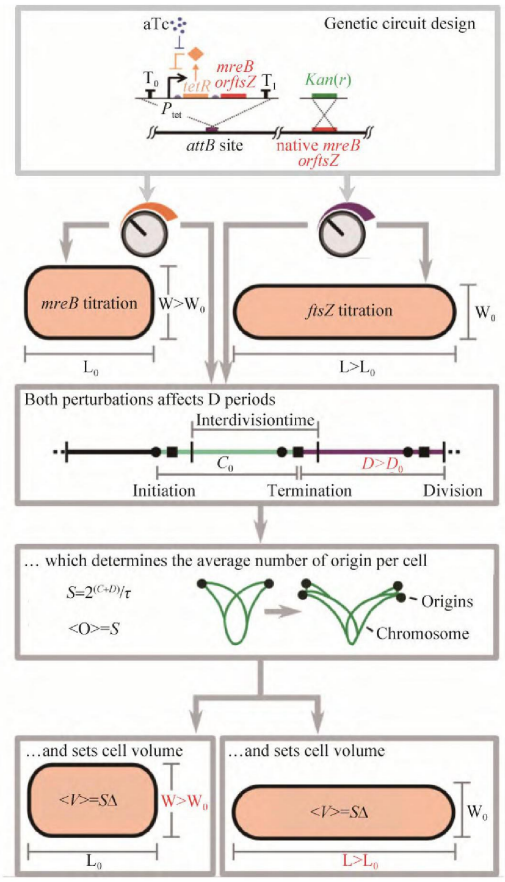

Fig. 2 Schematic illustration of the experiment and the "adder-per-origin" model

The *mreB*- or *ftsZ*-titratable strains were constructed based on P_{tet}-tetR feedback loop. By the designed genetic circuit, *mreB* or *ftsZ* expression levels and cell dimensions were continuously altered. In both case, the *D* period increased with cell width and length. The *C* period and doubling time τ remained constant. The perturbed *D* period sets the average number of origins per cell, which is equal to the scaling factor *S* because replication initiation triggers cell division. The average number of origins per cell then sets the average cell volume [15].

4 Understanding the formation of multicellular structures

In nature, there are a variety of biological forms, structures, and behaviors that consist of multiple cells. These biological systems have not only the physiological activities of individual cells but also the coordination and communication between cells, which greatly increases the complexity of studying such problems. Synthetic biology can produce artificial biological systems with clear connotation and epitaxial boundaries and quantitatively controllable system parameters by simulating the natural biological systems, providing a completely new perspective to studying the formation mechanism of multicellular biological structures. For example, the Weiss Laboratory in the Massachusetts Institute of Technology has successfully implemented a "striped" biological pattern by introducing cell communication gene elements from the LuxRI quorum sensing system into signal transmitting and receiving cells, respectively. This result confirmed the pattern formation mechanism of the classical Morphogen gradient [16]. The Lingchong You team in Duke University made *E. coli* form a biological pattern with the nuclear-ring concentric structure by constructing a synthetic gene circuit. The basic principle is that *E. coli* colonies can sense the accumulated chemical molecule AHL secreted by them and constitute an integrated feedback circuit under the combined action of nutrients. On the other hand, the core area size and the formation rate and duration of the ring area of the biological pattern formed a non-continuous feedback circuit. The authors used these two different feedback circuits to study the spatial induction mechanism of the cumulative effects, and thus elucidated the self-regulation mechanism of *E. coli* community size [17].

Based on the quorum sensing system, we constructed *E. coli* carrying the synthetic gene circuit to explore the mechanism of the formation of new periodic stripe structure [18]. The engineered strain spontaneously forms a periodic stripe pattern during the growth and expansion process in semi-solid agar space. Based on this system, we proposed a new density-dependent motion capacity model. Unlike other reactive diffusion systems, the density-dependent motion system can form periodic stripes when the front of the density wave reaches a threshold. In addition, according to the prediction of the model, the system has a phase transition from stripe to non-stripe and a finite number of stripes can be formed between the two states (Fig. 3). In the experiment, we validated the prediction of the model by successfully regulating the number of stripes through changing the expression levels of single genes [18–19].

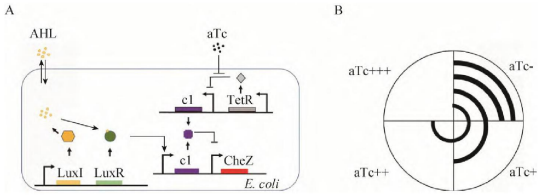

Fig. 3 Stripe pattern formation

(A) Schematic of the circuit. At high cell density, increased AHL level aids to DNA-binding of LuxR, thereby activating the expression of cI. cI suppresses the expression of *cheZ*, which is required for bacterial motility. An extra source of cI is regulated by TetR-repressive promoter. aTc is used to induce the expression of the extra cI. (B) Log-phase bacterial cells were inoculated on semi-solid LB agar plate. The cells formed the stripe pattern after a period of time of growth. The number of the stripe can be tuned by varying the aTc oncentration [17].

5 Understanding the principle of multi-species micro-ecology

Traditional ecology studies are often confined to the observations of number and distribution of populations. With the development of omics, people have more ways to study the multi-species ecosystem at the molecular mechanism level. However, omics studies are based on correlations to deduce the interactions within the population and lack of causality research. Recently, researchers have used synthetic biology to construct artificially designed interactions between species in a bottom-up manner to form a simplified micro-ecological system, thereby studying the dynamic interactions between species in an artificial and controllable external environment.

Cooperative behavior is a confusing question in evolution. Some individuals in the population benefit the rest of the population by harming their own interests. Biologists are puzzled that, according to the principle of survival of the fittest in Darwin's theory of evolution, individuals in the group that harm themselves and benefit others will be eliminated because they do not have competitive advantages. However, it is not true. The Gore Laboratory of Massachusetts Institute of Technology provided a reasonable explanation for the cooperative behavior by constructing a "cooperator" and "cheater" binary micro-ecological system consisting of two artificially engineered yeast strains in combination with the game theory [20]. The study showed that if an individual could obtain even small interest from a cooperative behavior, it could survive even if the individuals around do not cooperate (Fig. 4). In addition, the MacLean Lab at the University of Cambridge, UK, constructed a binary micro-ecology consisting of "Cheats" and "Cooperators" using the yeast strains that were artificially transformed by the Chalmers University of Technology in Sweden [21], which, in combination with dynamic system analysis, explained the rationality of cooperative behavior. The study showed that although the "Cheats" had a competitive advantage in a homogeneous and stable environment, the "Cooperators" and "Cheats" would coexist for a long time under the cyclical changes in resource conditions and spatial heterogeneous resource environments [22].

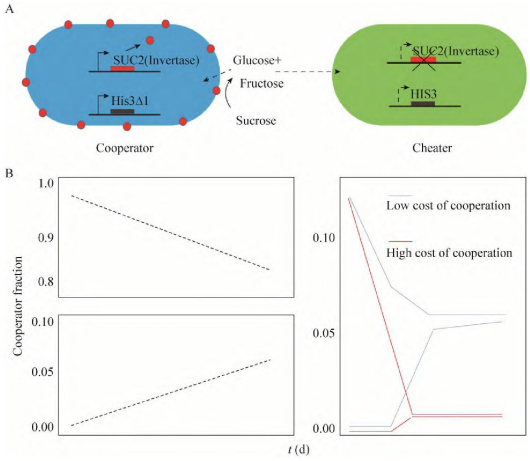

Fig. 4 Cooperator and cheater

(A) Schematic of the experiment that utilizes the sucrose metabolism in yeast. The cooperator expresses invertase, which hydrolyzes sucrose in the periplasmic space. The vast majority of the glucose and fructose created by sucrose hydrolysis diffuse away and can be consumed by a mutant cheater, which does not produce the invertase. The cooperator strain has a defective HIS3 gene, so it is histidine auxotroph. The researchers can tune the cost of cooperation by tuning the concentration of histidine [20]. (B) In sucrose culture, the cooperator and the cheater can coexist. The data suggest that cooperator and cheater reach an equilibrium with the consuming of histidine, regardless of starting fractions [21].

Antibiotic resistance is an increasingly serious problem that mankind faces. There are several mechanisms that lead to drug resistance, such as horizontal gene transfer, self-modification of drug targets in bacteria, and drug efflux pumps in cells. It is generally believed that antibiotics increase the rate at which bacteria share their resistance genes and promote the spread of drug resistance. However, researchers transformed bacterial strains with the synthetic biology methods and discovered a new way of bacterial drug resistance that challenges the conventional understandings. The Lingchong You team in Duke University found that antibiotics had no significant effect on the efficiency of horizontal gene transfer by monitoring the dynamic changes in drug resistance of strains modified by synthetic biology in a newly developed high-resolution microfluidic device for a long time. The results suggested that the effect of adding antibiotics in the process of generating drug resistance might be overestimated [23].

6 From "to-understand" to "build"

As mentioned above, synthetic biology is a comprehensive discipline that introduces engineering ideas

into biological systems. The goal is to design organisms in accordance with the wishes of the researchers and to serve the needs of scientific research and social livelihood. At present, a major bottleneck in synthetic biology research is the lack of the ability to design a completely new biological system or organism. The synthetic biology research through the concept of "build-to-understand" will continue to accumulate the knowledge about the principles of natural and artificial biological systems, so that people will gradually, from simple to complex, master the ability to design new biological systems. Another bottleneck that needs to be addressed urgently in synthetic biology research is the lack of available functional components. Although there are a wide variety of regulatory and functional genes in the natural biological systems, most of them do not meet the requirements of practical applications or people lack sufficient knowledge of them. Therefore, in practical synthetic biology research, there are few stable and reliable molecular components for researchers. One of the nice solutions is to create the biological components with custom functions by means of artificial guided evolution. Recently, a phage-assisted continuous evolution system developed by the Liu laboratory in Harvard University could help researchers obtain biological elements with the desired functionality in a very short time [24].

The solutions to these bottlenecks will lead us from "build-to-understand" to "build-by-understanding". At present, the international counterparts have already carried out pioneering work in this regard. For instance, the Lu Laboratory in the Massachusetts Institute of Technology and the Fussenegger Laboratory of the Federal Institute of Technology in Zurich have built biological computer systems in bacterial and mammalian cells, respectively, so that cells can use the logic gates formed from synthetic gene circuits to calculate the digital or analog input signals [25–26]. In the future, with the clarifications of the standard principles of the components and modules, the establishment of the corresponding design methods, the advent of the system design and simulation software in synthetic biology with simple and easy operations, improved throughput and lowered cost for the synthesis of large DNA fragments, coupled with the maturation of automated experimental platform, synthetic biology is expected to form a set of standardized processes including the computer-assisted design of gene circuits, the synthesis and splicing of components and modules, the experimental verification of the functions of gene circuits, the fitting of experimental data to mathematical models, the adjustment of parameters of components and modules, and the further optimization of gene circuits. By then, synthetic biology will be gradually mature to form a new practical engineering discipline that is similar to today's mechanical engineering and electronic engineering.

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[致谢]

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(Translated by 白小东)

[尾注]