A novel synchronization approach using synthetic magnetic Escherichia coli

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**ABSTRACT**

The group headed by Professor Chenli Liu in the Institute of Synthetic Biology, Shenzhen Institutes of Advanced Technology (SIAT) recently developed a microfluidic synchronizer to continuously produce minimally disturbed, normally growing synchronous bacterial cells (ACS Synth Biol. 2019, 8(5): 962–967). This research highlights the main advances made in this work and presents the findings of this study in the context of synthetic biology.

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The active division of cells in a coordinated series of events in cell cycle is a vital process resulting in daughter cells under suitable conditions. The critical phase in cell cycle is the duplication of the chromosome and the subsequent segregation before division, ensuring that each daughter cell has its genome encoding genetic information. A series of regulated and coordinated events take place at specific stages as the cell cycle proceeds. Studies on the cell cycle are essential to unravel the intrinsic regulatory mechanisms of the growth and propagation of cells.

In bacteria, due to the asynchronous nature of bacterial cell division, the cell cycle network remains unclear. The cell sample collected at different stages of cell cycle under normal growth conditions harbors cells in varying stages of cell cycle. To study the dynamics of bacterial cell cycle in detail, there is a need to have single cell analysis or synchronous cultures. Quantitative analysis of synchronously dividing populations of bacterial cells will help us understand the mechanistic details of cell cycle at the population level. The number of events and its chronology can be documented during such an analysis to give insights into bacterial cell division.

In 1956, Barner and Cohen first attempted to obtain synchronously dividing bacterial cells [1]. They used a mutant of Escherichia coli that required thymine for its growth, and in a thymine deficient medium, DNA synthesis could be arrested. After preliminary growth in a thymine deficient medium, supplementation of thymine activated the initiation of DNA synthesis and subsequently synchronous cell division. Also, in 1956, Maruyama and Yanagita adopted mechanical methods to separate cells into mature and immature cells by size [2]. However, chemical and mechanical treatments of cells hindered the progression of the cell cycle and introduced pseudo-phenomena. In the 1960s, Helmstetter et al. developed a synchronization apparatus, called a “baby machine”, to continuously produce synchronously dividing E. coli cells in an exponentially growing culture [3]. Despite the low yield and large medium consumption, such “baby machine” can generate newborn daughter cells which synchronously grow and divide with little interference after inoculation into fresh medium.

The group headed by Professor Chenli Liu in the Institute of Synthetic Biology, Shenzhen Institutes of Advanced Technology (SIAT) recently developed a microfluidic synchronizer to continuously produce minimally disturbed, normally growing synchronous bacterial cells [4]. In this study, synthetic biology techniques were applied to establish a novel synthetic magnetic-capped bacterium. The resulting bacterial cells localize to one pole which is magnetically charged and can be trapped using a magnetic field. Combining with microfluidic techniques, this hands-on microfluidic synchronizer overcomes the limitations of conventional synchronization methods for E. coli such as the hindrance of the progression of the cell cycle and introduction of pseudo-phenomena. Their microfluidic synchronizer provides significant advantages of microfluidic systems to study cell biology.

In their work, a synthetic and inducible “stalk” is constructed in E. coli BL21(DE3) in order to simulate the stalk in Caulobacter crescentus. To construct this magnetic “stalk”, eGFP was heterogeneously expressed and fused with the signal peptide and C-terminal auto-transporter domain of AIDA-I, which is an autotransporter adhesin from an enteropathogenic E. coli (EPEC) belonging to the autotransporter family [5]. This chimeric fusion protein eGFP-AIDA-I under an inducible promoter was expressed and localized to the polar caps on the surface of E. coli BL21(DE3). Then, streptavidin-coated magnetic nanoparticles were mixed with polar displayed eGFP using biotinylated...
anti-eGFP antibody. After one cycle of cell division, only one pole of the bacterial cells will be capped by magnetic nanoparticles. Therefore, assembled magnetic bacterial cells can be arrested by permanent magnet as “mother” cells. Newly divided daughter cells without the synthetic magnetic “stalk” can be eluted by infusing the culture medium (Fig. 1). These strategies sufficiently reduced disturbance, and thus helped to obtain minimally disturbed, normally growing synchronous cells. As the mother cells keep growing in steady-state without any interference, newly divided daughter cells can continue to grow without a lag period. After inoculation of the newly divided daughter cells into a fresh culture medium, a stepwise growth curve indicative of healthy synchronous cell population was obtained [4].

Microfluidic systems provide more control over culture condition environment. In this novel microfluidic bacterial synchronizer, one can easily adjust the culture conditions such as temperature, osmotic pressure, nutrients and concentration of reagents [4]. In addition, the microfluidic device is easy to fabricate and change according to a varying parameter of the experimental design. Integration of baby machines and microfluidic chips sufficiently reduces the consumption of culture medium or other reagents. In the future, it is attractive and promising to integrate bacterial cell synchronization, synchrony quality control and downstream analyses on a microfluidic chip to further study bacterial physiology.

The lack of scientific data on bacterial cell cycle limits the progression of research on bacterial physiology and the applications of microbiology in the fields of medicine, food and industrial production. If we can have a thorough understanding of bacterial cell cycle, engineering them using synthetic biology principles might provide us with more opportunities in the field of bio-production. This study paved a way for using a novel synchronization method to obtain minimally disturbed and well synchronized E. coli cells.

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