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NEWS AND VIEWS

From synthetic genome to creation of life

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Understanding the world and then changing the world is an everlasting mission of the human being. Life is an important component and one of the most sophisticated elements on the earth. In recent decades, the rapid development of molecular biology, genetics and the related biotechnology and bioengineering kept bringing surprises to scientists worldwide, including the cloning of Dolly (Wilmut et al., 1997) (followed by cloning of a variety of animals), the completion of Human Genome Project (Cheung et al., 2001; Lander et al., 2001; Venter et al., 2001) (followed by the reports of complete genome from other species and by the spread of personalized genome sequencing service), and the induction of pluripotent stem cells (Takahashi and Yamanaka, 2006) (followed by the production of viable iPS mice (Zhao et al., 2009)). Recently, another breakthrough was brought by John Craig Venter and his team, who achieved the first synthetic life—Mycoplasma that only contains synthetic genome (Gibson et al., 2010).

Now, "synthetic biology" has become a keyword for life science area to describe *the design and construction of new biological parts, devices, and systems, and the re-design of existing, natural biological systems for useful purposes* (http://syntheticbiology.org/). Despite the deciphered genetic code and expanding information about genetics and epigenetics, synthesizing life from chemical components is still limited by scientific knowledge and technical practicability. John Craig Venter is a leading scientist in genomic research. With the invaluable contribution of Celera Genomics that he funded in 1998, the Human Genome Project was completed three years ahead of expected date. Later, he funded J. Craig Venter Institute (JCVI), with synthetic genomics as one of the major focuses. After 15 years of investigation and approximately $40 million of investment, JCVI walked out this significant step for synthetic biology.

The synthetic genome started with design of DNA sequence, which highly depends on the accurate sequence of *Mycoplasma mycoides* genome. Venter group spent numerous efforts on comparing different sequencing results, making necessary corrections, and setting watermark sequences to differentiate synthetic and natural genomes. The whole 1.08-Mbp genome was divided into 1078 DNA cassettes, each containing 1080 bp, with 80 bp overlap to adjacent cassettes, and they were individually synthesized by chemical approach. Ten cassettes were recombined in yeast to yield the 10-kb intermediates, which were further recombined to obtain the 100-kb intermediates and the final complete genome. The correct assembly was confirmed by both multiplex PCR and restriction analysis, and the synthetic *M. mycoide* genomes were transplanted into restriction-minus *Mycoplasma capricolum* recipient cells. Bacteria with synthetic genomes were screened by tetracycline and X-gal as designed, and were also verified by multiplex PCR, restriction analysis, whole genome sequencing (the sequenced strain was referred as *M. mycoides* JCVI-syn1.0), etc. Under the experimental condition, the cells with only synthetic genome were able to perform self-replication and logarithmic growth. Proteomics analysis revealed that these cells have nearly identical protein expression pattern as the genome donor, *M. mycoide*, but not the recipient, *M. capricolum*.

Shortly after the announcement of this result, the scanning and transmission electron micrograph of synthetic *M. mycoide* has become a most popular image around the world. This "artificial" life is a hallmark on both conceptual and technical aspects. DNA is supposed to contain all the genetic information for life. It is a complicated process to obtain accurate whole genome sequence and to synthesize an error-free genome with rounds of manipulation before final transplantation. Such work requires high-throughput sequencing facilities, innumerous data processing, sophisticated designing strategy, precise chemical synthesis, multiple steps of quality control, etc. The success of synthetic *M. mycoides* reveals the power of current biotechnology to accomplish such a marvelous project. In addition, it proves the principle of producing cells from computer-designed genome rather than modifying DNA sequence by traditional insertion, deletion or mutagenesis. At current moment, the *M. mycoides* with synthetic genome is almost identical to the naturally existing *M. mycoides*; it opens the door for scientists to make synthetic cells with distinguished, predicted and/or even unnatural properties. Although ethical concerns have emerged, such as its potential application on bioterrorism, the technique will have significant implications in pharmaceutical field,

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environmental science, clean energy, food industry, etc.

In spite that the synthetic *M. mycoides* is called "synthetic cell" and its DNA bases are solely synthesized by chemical approach, the process still relies highly on life components. First, the synthetic DNA fragments, including the 1080-bp DNA cassettes, 10-kb intermediates and 100-kb intermediates, were assembled in yeast cells, in which these pieces were recombined together to finally form a complete functional genome. The pathway and regulation of such recombination in yeast is not well understood. Whether the assembly can be finished in a life-free or a synthetic microenvironment remains to be explored. Second, the synthetic genome required an existing cell, *M. capricolum*, to provide cytoplasm, which contains a variety of life components that could be potentially essential for the function of synthetic genome. Compared to synthesizing DNA, it may be more difficult to reproduce such a complicated cellular environment with all required cellular organs, inorganic elements, organic matters and life macromolecules, such as proteins, carbohydrates and lipids, and the membrane. They play indispensable roles in DNA packaging, transcription, translation, protein expression as well as other cellular functions. Therefore, a synthetic genome itself may not fully represent an "artificial" life; however, this is a significant advancement because it is the genome that contains all inheritable genetic information.

Despite the involved life components, the extensive labor and financial investment, and the safety concerns, this "synthetic" bacterium is undoubtedly a milestone for synthetic biology. *M. mycoides* has the smallest genome in free-living organisms. It may take a long way to fully realize the dream of creating life, but it can be expected that in the near future, the synthetic procedure will be optimized and the cost will be reduced, which will bring benefits to everyday life and the challenges human beings face, e.g., energy crisis, environmental pollution, and increased CO_2.

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