Intelligent Design?

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The First Artificial Cell—A Revolutionary Step in Synthetic Biology?

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After being the first scientists to succeed in sequencing the human genome,[1] Craig Venter and his team have once again reported an outstanding achievement: the creation of the first self-replicating artificial microorganism.[2] How did they achieve this and what is the implication for chemistry?

Their starting point was the complete sequencing of the genome of the microorganism *Mycoplasma genitalium*,[3] one of the smallest known genomes. Chemical synthesis of this genome (582 970 base pairs) by the Venter team represented the first synthesis of an entire genome.[4] This in itself was a major achievement, as a number of sophisticated techniques needed to be developed to assemble the full length genome. First, very short DNA strands (10^4 oligonucleotides, each composed of 50 nucleotides) were assembled into 101 cassettes (each ~6 kb in length), followed by their combination into larger stretches by using in vitro enzymatic methods. This was followed by in vivo recombination—first in *Escherichia coli* and finally in the yeast *Saccharomyces cerevisiae*.

To create a self-replicating artificial organism from the "dead" DNA synthesized chemically, Venter and co-workers next needed to invent methods for extracting intact chromosomes from yeast and transplanting this genome into the newly emptied host cell. For this, a subspecies of *Mycoplasma capricolum* was chosen as the recipient. The *M. mycoides* JCVI-syn1.0 genome synthesized in the most recent study was 1.08 million base pairs (Mbp) long. This again was created from synthetic DNA oligonucleotides by combination of cassettes first from 10 kb fragments, then assembled to 100 kb intermediates, and finally by completing the full length genome in yeast (Figure 1). The synthetic genome was then transplanted into the recipient cell *Mycoplasma capricolum*. It was subsequently shown that the cells containing the synthetic genome were self-replicating (and hence, the synthetic DNA software built its own *M. mycoides* hardware within the *M. capricolum* surrogate host), showed logarithmic growth, and was a typical *Mycoplasma* phenotype. This was verified by scanning and transmission electron micrography.

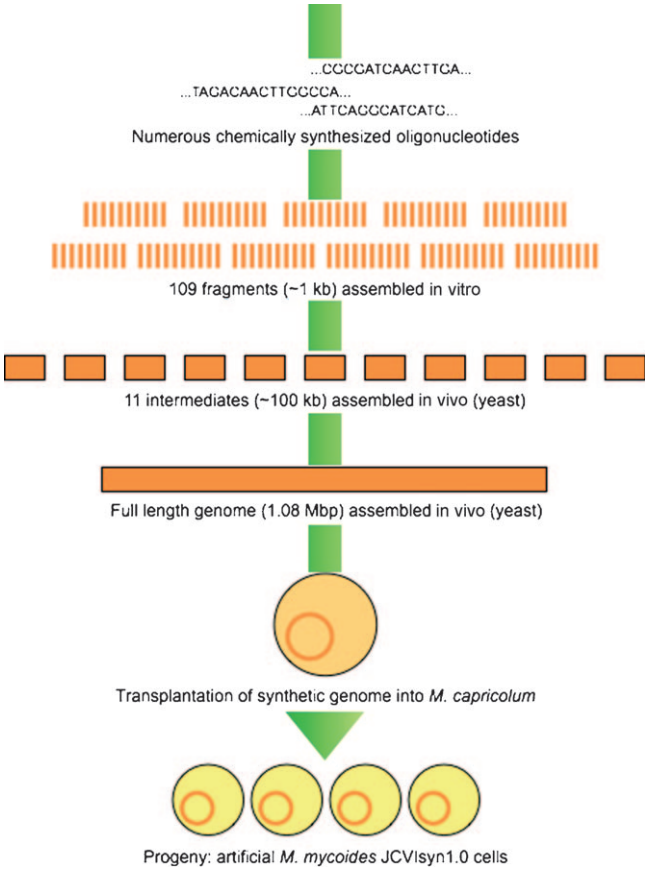

Figure 1. Procedural method for the creation of the artificial cell.

Furthermore, proteomic analysis confirmed that the protein patterns were almost identical, except for the proteins that were altered or deleted in the artificial genome so as to distinguish wild-type from synthetic strains. Further important quality checks were the introduction of "watermarks" for specific amplification by four primer pairs and restriction pattern analysis, both designed to work only for the synthetic genome.

The success by Venter and co-workers is without doubt outstanding, although its relevance is already under great debate.[5] Opinions range from putting this discovery in line with those of Galileo, Copernicus, Darwin, and Einstein,[5c] to much more critical views that this process is hard to apply in

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most laboratories, that current tools in metabolic engineering are already of more use, and that the artificial cell can be simply considered as a normal bacterium with a prosthetic genome.

Indeed, the requirement of a tedious chemical synthesis of an entire genome in the first place, to later modify the genome to include, for example, genes required for the biosynthesis of novel products in the host cell, will remain challenging. Insertion of only a handful of foreign genes into a host organism to establish a new pathway is currently technically rather simple, but the devil is in the detail: Are all proteins functionally expressed? How can they be regulated? How can one steer the localization of the proteins in the different compartments of the host cells? Is the carbon flux going into the desired direction? Are there enough reduction equivalents available etc.? Scientists active in metabolic engineering and synthetic biology still struggle with these issues, despite extensive achievements in "omics" technologies (genomics, proteomics, metabolomics, fluxomics etc.), bioinformatics, molecular biology, and related disciplines.

Only the future will tell us whether the achievement by the Venter team is just a step forward or a great leap that eventually represents the major breakthrough of the 21st century. Looking back into history, the first successful transformation of foreign DNA into the microorganism *E. coli*[6] set the basis for modern biotechnology and was the foundation of a whole industry. Only a few years passed until the first commercial products such as insulin or detergent proteases were obtained recombinantly on an industrial scale. Expectations were very high when the human genome was first fully sequenced,[1] and scientists believed that we were very close to curing numerous gene-related diseases. Ten years later, we learned that reading the blueprint of the human genome does not mean that we fully understand its content, let alone its interpretation. Similarly, assembling an airplane from hundreds of thousands of pieces does not tell us why it flies and how we can fly it. Furthermore, what happens if we exchange or add parts?

Linking the discovery by the Venter team to chemistry, I see several parallels to organic total synthesis (in both the means and the ends of the research). Driving forces in the multistep total synthesis of complex natural products[7] are: 1) to be the first scientist to accomplish the total synthesis, 2) to synthesize a product that is identical to the natural compound, and 3) to establish the ability to modify the synthetic route to produce natural product analogues with, for example, broader applicability as a drug.

A very important "by-product" of the numerous examples of total synthesis in history has been the development of a plethora of novel synthetic methods to achieve exact structure and stereochemistry. For example, the first total synthesis of vitamin B12 yielded new bond-forming strategies, hypotheses about biogenesis, and the principles of orbital symmetry conservation.[8] In a similar manner, Venter's success required the development of sophisticated methods for the chemical total synthesis of the artificial genome, methods to combine the DNA fragments in vitro and in vivo, advanced quality control methods, the genome transplantation method and, finally, the means to create a self-replicating artificial cell.

The analogy between organic synthesis and synthetic biology goes further: even the smallest of errors is not tolerated. A substantial delay in the Venter example was caused by deletion of a single base pair (that is less than 1 ppm or 0.0001 % in the 1.08 Mbp genome) that occurred in an essential gene and caused a frame shift, thereby halting the project until the single error was narrowed down. Similarly, a single wrong absolute configuration of one chiral center in a complex natural product (namely, the erythromycin core structure contains 10 chiral centers, hence $2^{10} = 1.024$ diastereomers are possible) might diminish or even destroy the biological function of the compound.

All the methods developed by the Venter team along the way to success will be very helpful for other researchers, even if they only wish to install a complex biological pathway (such as a polyketide synthase pathway) in a host organism. A recent example of the microbial production of biofuels and chemicals from fatty acids[9] has shown that we are not far away from a substantial change in the future supply of energy, fuels, and chemicals, where modern biotechnology fostered by synthetic biology may become the key technology. A successful transfer of the methods developed by the Venter team to a more complex standard host organism, such as *E. coli* or yeast, will certainly boost this development. Obstacles such as the chemical synthesis of the significantly larger genomes of these microorganisms are likely to be overcome, bearing in mind the rapid decrease in costs for the synthesis of the DNA fragments and faster quality control by sequencing in combination with further automation. Metabolic engineering using well-studied host microorganisms will, for sure, remain the method of choice, at least for the near future.

The anticipated risks already discussed for the potential abuse of the technologies developed by the Venter team can be currently considered rather modest as only a handful of researchers worldwide have the capacity to work in this area. In addition, standard methods for (random or targeted) mutagenesis and metabolic engineering are at hand and much easier to apply by "bad guys". I am also convinced that we are still very far away from the ability to create higher species, such as mammoths or even Neanderthals, from synthetic DNA—"options", which should be banned anyway for ethical reasons.

In conclusion, the Venter team have undoubtly provided a major technological breakthrough, but it waits to be seen whether this will indeed become the key approach to provide mankind with fuels, energy, and chemicals in the near future, and be competitive with current methods of synthetic biology and metabolic engineering.

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