Critique of Craig Venter's *Synthetic Life*

Last week various news media were featuring the recent achievement of Craig Venter's laboratory, the creation of a replicating cell that has as its genome an artificially constructed sequence of DNA. In a journal publication, Venter referred to this construction as a *synthetic cell*. The work appeared in http://www.sciencemag.org/cgi/rapidpdf/**science**.**1190719v1**.pdf
The chromosome, or genome, is a 1.08×10^6 base pair (bp) sequence that was assembled in stages from intermediate segments initially constructed 1,080 bp long. These were assembled into segments 10,000 bp long, and then into segments 100,000 bp long, and finally into the final Mbp product. These assembly steps involved using yeast for recombination and then *E. Coli* to make clones. Certain "watermark" sequences were also added to the synthetic genome so that later assays could clearly exhibit the presence of a synthetic genome in a population of replicating cells. The product of this genome assembly process is a modified, watermarked version of the *Mycoplasma mycoides* genome that Venter's group named *M. mycoides* JCVI-syn1.0. This research program was initiated 15 years ago when the Venter group sequenced the genome of *M. genitalium*, a related bacterium with the smallest complement of genes (580,073 *bp*) of any known organism capable of independent growth in the laboratory. The synthetic *M. mycoides* genome was transplanted into an enucleated *M. capricolum* recipient cell. The cell progeny have the expected phenotype for the synthetic genome and are capable of indefinite self-replication.

Without a doubt this is a remarkable achievement, a real *tour de force*, involving many hundreds of thousands of man-hours of effort that involved a number of unsuccessful and frustrating attempts at several stages. Few scientists could have matched this effort in terms of leading a large research group for so long towards a well posed goal and in terms of funding the research (the DOE was the major source). Since I am about to criticize the *ultimate goal* of this research program I want it to be clear at this point that I could not have done what Venter has done in the laboratory. What is Venter's ultimate goal?

Venter's ultimate goal is to construct the *minimal cell genome*. By this he means the genome that contains the least number of genes that support a self-replicating cell population. In this way he hopes to better understand the origin of life and the origin of the genetic code. By achieving this first step with *M. mycoides* and *M. capricolum*, his group is poised to begin a series of experiments in which specific deletions are made in the genome until a minimal genome is

constructed. Unfortunately for this ultimate goal, we already know what the result will be, in essence, and the minimal genome will fail to be informative regarding the origin of life and especially the origin of the genetic code. I will now try to explain these provocative remarks.

E. Coli is a much studied bacterium with a genome ($4.6 \times 10^6 bp$) about 4-5 times bigger than the *M. mycoides* genome. It contains 4,377 genes, of which 4,290 are for proteins and 87 are for RNA's. Most of the proteins are catalysts for metabolism that is organized around a central metabolic core dedicated to energy metabolism: glycolysis, pyruvate conversion to acetyl-CoA, the citric acid cycle, the electron transport chain and the chemiosmotic synthesis of ATP. For an account of the details of these processes and also what I am about to describe regarding origins see my website www.fefox.com and look in the section *Mysterium Tremendum*, as well as the "shortcut" *energy metabolism*. In addition to metabolism, that includes synthesis of many small molecules as well as energy metabolism, there are proteins and RNA's involved with protein biosynthesis. This complex process requires ribosomes that each contain 51 proteins and 3 RNA's, over 30 tRNA's and over 20 aaRS's, the aminoacyltransferRNA synthetases. For details see my website section on *polymer biosynthesis*. So now for the key point. If Venter's group minimalizes the synthetic genome they now have working for them, then it will contain genes for the ribosomal components, the tRNA's and especially the aaRS's. There will also be genes for catalysts of metabolism at a minimal level of function. What is the problem with this outcome?

The problem with the minimal genome that Venter's group will find is that it in no way provides information on how the aaRS's evolved from a simple living system that did not yet utilize tRNA's and aaRS's. The tRNA's and aaRS's did not appear full blown out of nothing but had to evolve from a simpler mechanism. How this happened is the deeper question, the *Mysterium Tremendum*. I have proposed a simpler system, the *primitive RNA translator*, that could have evolved into the contemporary mechanism now functioning in *E. coli*, *M. mycoides*, yeasts and also in humans. This model is an example of the transition from a primitive protein biosynthesis machinery to the complex one now *in esse* and containing ribosomes, tRNA's and aaRS's. No amount of "back-evolving" the *M. mycoides* JCVI-syn1.0 genome will produce this simpler mechanism, or any other simpler mechanism. The minimized *M. mycoides* JCVI-syn1.0 genome will necessarily contain all the genes for the 20 aaRS's. Leaving these out, even one at a time, will not yield a viable cell. Just think about what thwarted the Venter lab for many weeks when their construct only missed a single bp for the essential gene *dnaA*.

Venter's group has achieved a level of genome manipulation that is truly masterly and that will perhaps have many practical applications in replicating cell populations, but it will not have an impact on their *ultimate goal*, understanding how life as we now know it at the molecular level came into being. The back-evolution from the contemporary protein biosynthesis mechanism to a putatively more primitive precursor form of life will be no more successful now than it was for Donald A. Glaser 40 years ago when his laboratory tried to drive *reverse evolution* by supplying everything a simple organism would need at the molecular level in a flow tank apparatus. The hope was that genes would be lost because their protein or RNA products were no longer needed, all metabolic needs being supplied from outside, and only the bare minimum complement of genes needed for life would be retained. This minimum, it was thought, would then inform us about the primitive system and its origins. Glaser's experiments were unsuccessful for technical reasons, but also would have failed anyway for the same reason Venter's back-evolution experiments will fail. Back-evolution of the aaRS's will not occur.

Instead, **conceptualization** of a forward evolution from a primitive precursor mechanism (ur-aaRS's) into the complex contemporary mechanism (aaRS's) is required first (see my *Mysterium Tremendum*), and then one will have to actually synthesize (and assemble) the entire primitive system, cell and all (membrane, metabolism and genome). As Venter has said, his *synthetic cell* is **only** synthetic in so far as the genome is concerned, while the host cell, an enucleated *M. capricolum*, comes ready made. When Don Glaser did his work, the aaRS's had been know to exist for about a dozen years but in 1970 only a few specialists appreciated their importance as part of the evolution of the genetic apparatus. Without the aaRS's all the other base pairing processes are for naught. I remember talking with Don about his experiment but neither of us mentioned the aaRS's at that time. Today, they cannot be ignored and when one thinks about them and their evolution, one sees that back-evolution from a synthetic genome that uses our contemporary protein biosynthesis apparatus (aaRS's) will not yield the ur-aaRS mechanism. The back-evolved genome will have each of the 20 aaRS genes, as well as genes for DNA replication and transcription, for energy metabolism needed to generate ATP, for components of the protein biosynthesis machinery, and for basic metabolism of monomers. A complete list would be nice to have even if we already see qualitatively what the result will be. Finding a working molecular model for the primitive mechanism and demonstrating the evolution of: 1) the protein biosynthesis apparatus, 2) the polynucleotide replication apparatus, and 3) the DNA transcription complex is the ultimate goal. I offer my conceptual model

in *Mysterium Tremendum* as a challenge to the molecular constructionists, including Craig Venter.

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