

Synthetic life

Synthia or Original Syn?

The J. Craig Venter Institute has proclaimed to have generated the first synthetic living cell. An announcement that deserves a closer look at what it exactly is that they have achieved? Jeremy Garwood reports.

The Prelude

Mycobacterium mycoides colonies formed from cells that once were *Mycobacterium capricolum*. In 2007, Venter's team cloned the *M. mycoides* chromosome in yeast, modified it and finally transplanted it into the related species *M. capricolum*, to create a new type of *M. mycoides* cell. Now, they have succeeded in performing the same species switch using a chemically synthesized chromosome.

Yes, folks, the Craig Venter bandwagon has rolled into action once more to announce the achievement of yet another major breakthrough in the bioengineered world of synthetic biology. Performed at the J. Craig Venter Institute in San Diego, the formal science was described in the article, "Creation of a Bacterial Cell Controlled by a Chemically Synthesized Genome" authored by Daniel Gibson, 22 co-authors and Craig Venter (published online at *Science Express* on 20th May, 2010). Its press conference and media reports went typically worldwide.

However, behind the praise for Venter's vision of 'synthetic biology' lies uncertainty as to what exactly his team has really achieved and questions as to how much Venter's commercial interests and the rest of humanity might gain (or lose) from this technical feat. Nicknamed 'Synthia' by fans, detractors consider it closer to 'Original Syn'.

The Minimum Genome Project

This creation of a living cell controlled by a 100% chemically synthesized genome is, in fact, another step in a larger project to understand the minimal genetics of life, together with hopes that synthetic biology can exploit such knowledge to generate entirely artificial, but economically valuable, life forms.

Craig Venter pinpoints the origins of this ongoing quest for a 'minimal cell' back to 1995 – at his Institute for Genomic Research in Maryland, a team led by the Nobel Prize-winning microbiologist, Hamilton Smith, sequenced the first two complete genomes, both bacteria: *Haemophilus influenzae*, "The first genome of a free-living organism," (1.8 million base pairs) and *Mycoplasma genitalium*. As the latter's name implies, *M. genitalium* is a sexually-transmitted infection, thought to be involved in various human genital diseases. But it also happens to possess the smallest genome of a self-replicating organism. In its 582,970 bp, it possesses 521 genes, of which 482 are protein-coding.

However, once Smith's team had its DNA sequence, they asked, "If this is supposed to be the smallest genome of a self-replicating species, could there be an even smaller genome? Could we understand the basis of cellular life at the genetic level?"

In order to study this, they performed a deletion analysis, eliminating individual genes from *M. genitalium*'s genome to determine, which were necessary to maintain life. After ten years of study, they finally

concluded that 382 protein-coding genes were essential as well as 43 RNA-coding genes (Glass *et al.*, Essential genes of a minimal bacterium 2006, *PNAS* 103: 425).

The synthetic cell ('Synthia')

However, early on, Venter's enterprise also embarked upon a "synthetic route". This entailed chemically synthesizing an entire bacterial chromosome so that they could design the gene content, "understand the essential genes for life" and, incidentally, make some money from exploiting the potential of synthetic biology.

Much of the technology needed to achieve this goal still remained to be developed. In recent years, there has been a succession of reports, including a flurry of papers in *Science* magazine, announcing the project's technical milestones, featuring the synthesis, manipulation and transplantation of large DNA molecules and bacterial chromosomes.

For starters, chemically-synthesizing DNA strands may have become a lot easier and cheaper but these are hardly oligonucleotide primers – to synthesize the genome of *M. genitalium*, each of the 101 DNA cassettes was between 5,000 and 7,000 bp long! These cassettes were then recombined, in the correct sequential order, as bacterial artificial chromosomes in *E. coli* to produce intermediate assemblies of 24 kb, then 72 kbp and 144 kbp, before final assembly of the complete 580 kbp genome in the yeast, *Saccharomyces cerevisiae* (Gibson, 2008, *Science* 319: 1215).

And then you have to work out how to extract the bacterial chromosomes – fully intact – from the yeast. Furthermore, time pressure became a factor – given all the money and publicity invested in the project; Venter and co. became frustrated at the "extremely slow growth rate" of *M. genitalium*. It seems the downside to having a minimal chromosome is that you don't grow very fast! One of biotechnology's hottest projects was being held back for weeks at a time while this uncooperative genital parasite insisted on growing at its own leisurely pace!

In mid-project, an executive decision called for change towards a faster growing species. Enter two "opportunistic pathogens of goats", *Mycoplasma mycoides*, doubling time 80 minutes, and *M. capricolum* (100 minute doubling time). Their one million bp genomes were duly sequenced and shown to have around 75% homology. For transplantation, *M. mycoides* was chosen as the chromosome donor and *M. capricolum*

as the recipient cell, simply because it only worked this way round – *M. capricolum*'s genome was not accepted by the *M. mycoides*'s cell.

Nevertheless, the transplantation of intact bacterial chromosomes into recipient bacterial cells still had to be worked out before the larger, even more expensive, synthetic *M. mycoides* genome was ready. To practise, they extracted the native *M. mycoides* chromosome, then transplanted it into *M. capricolum*, adding a tetracycline antibiotic resistance gene to the *M. mycoides* chromosome. Using this antibiotic selection and polyethylene glycol-mediated transformation, they succeeded in introducing the *M. mycoides* genome into *M. capricolum* cells that simultaneously lost their original genome (Lartigue, 2007, *Science* 317: 632).

Having chosen *M. mycoides* as the chromosome donor, a copy of its genome was now chemically synthesized. This time, the assembly started with 1,078 DNA sequences, each 1,080 bp long. These contained 80 base overlaps at each end to facilitate assembly with adjacent sequences and were recombined into plasmids and grown in *E. coli*. In the next step, ten of these cassettes were enzymatically digested, recombined in vitro with vector and transformed into yeast, generating a total of 109 x 10,080 bp assemblies. These larger sequences were further recombined to produce eleven 100,000 bp intermediates that were finally combined in yeast to give the complete bacterial chromosome of 1,077,947 bp. It was named *M. mycoides* JCVI-syn 1.0.

To add a designer touch and confirm unambiguously that their synthetic genome was indeed present in future cells, Venter's team added a series of "watermarks", DNA sequences, which, if read as the single letters of the corresponding triplet codons, spell out the names of all the authors, their e-mail addresses and quotes deemed suitable for biology's first "synthetic cell" such as "What I cannot build, I cannot understand" (the last words scrawled on the blackboard of the physicist/philosopher, Richard Feynman) or the uninten-

tionally ominous "See things not as they are, but as they might be" (from a biography of Robert Oppenheimer, father of the atomic bomb!).

Defining synthetic cells and artificial life

Unfortunately, in spite of all its signatures, the first attempt to put *M. mycoides* JCVI-syn1.0 into *M. capricolum* cells failed completely. In fact, it took another three months of intensive resequencing of the entire synthetic chromosome to localise the problem – there was one single base pair deletion in the gene for DnaA, a protein essential for the initiation of chromosome replication.

The transplantation now worked. Using a chemically synthesized chromosome, one species of goat pathogenic bacteria was successfully transformed into another!!

How much of an advance does this latest venture from the JCVI represent? There was some initial media confusion as to whether mankind had finally succeeded in creating "artificial life" or "synthetic life" or...

Nature magazine, apparently fully forewarned of the latest publication in *Science* magazine, immediately printed in-depth interviews with eight experts in the field of synthetic biology (*Nature* 2010, 465, 422).

Mark Bedau, editor of the journal, *Artificial Life*, proclaimed it to be "a defining moment in the history of biology and biotechnology". While Arthur Caplan, Professor of Bioethics at the University of Pennsylvania, was so overwhelmed, he declared, "Venter's achievement would seem to extinguish the argument that life requires a special force or power to exist. In my view, this makes it one of the most important scientific achievements in the history of mankind!!"

European experts were less impressed. Martin Fussenegger, Professor of Biotechnology and Bioengineering at ETH Zurich, called it just "a technical advance, not a conceptual one".

And Steen Rasmussen, a biological physics professor at the University of Southern Denmark remarked, "Is it artificial life?

Of course not."

Jim Collins, Professor of Biomedical Engineering at Boston University agreed, "The microorganism reported by the Venter team is synthetic in the sense that its DNA is synthesized, not in that a new life form has been created."

However, Venter's group doesn't claim to have invented artificial life. Instead, they lay claim to the first "synthetic cell". They argue that a cell controlled by a genome assembled from chemically synthesized pieces of DNA is synthetic, "even though the cytoplasm of the recipient cell is not synthetic". This is because the phenotypic effects of the recipient cytoplasm are rapidly diluted as cells carrying the transplanted genome replicate. If one of these original transplanted cells replicates on a plate to form a typical colony, it has already undergone more than 30 divisions, during which time new cellular components are coded by the synthetic chromosome ("the DNA software builds its own hardware"). Therefore, the resulting progeny *cannot* contain any protein molecules that were present in the original recipient cell.

Nevertheless, let it not be forgotten, they still needed to start from a living cell. Besides, the original objective wasn't to create life from chemicals but rather to create economically meaningful life.

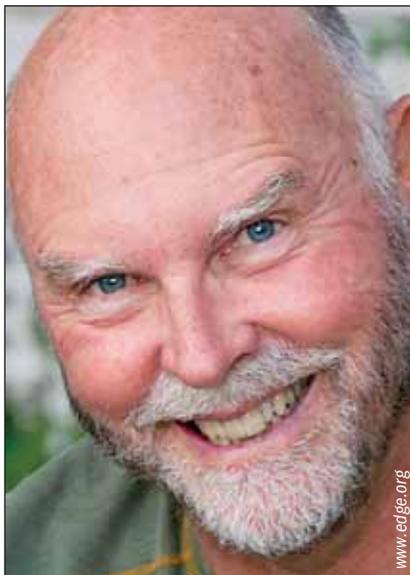
Money matters

In 2005, Craig Venter founded the J. Craig Venter Institute (JCVI) for non-profit genomics research, incorporating The Institute for Genomic Research, the Center for the Advancement of Genomics, the Institute for Biological Energy Alternatives and the J. Craig Venter Science Foundation Joint Technology Center. JCVI has facilities in Maryland and San Diego, employing more than 400 scientists and staff.

To deal with the money-making side, Venter founded the company, Synthetic Genomics Inc. (SGI). SGI has funded the work at JCVI in exchange for exclusive assignment of intellectual property rights. Overall, the research on the first "synthetic cell" has cost some \$40 million, \$30 million of it being provided by SGI since 2005.

To capitalise on this investment, SGI has filed 13 patent application families on the "unique synthetic genomics inventions of the JCVI team". However, there have been concerns that granting these patents could result in a monopoly on synthesized life.

Following the announcement of Synthia's birth, John Sulston, Professor at Manchester University, told the BBC that he had



A smile for the media? J. Craig Venter

“read through some of these patents and the claims are very, very broad indeed”. If accepted, the patents “would bring genetic engineering under the control of the JCVI. They would have a monopoly on a whole range of techniques”.

As a former director of the Sanger Centre in Cambridge, Sulston was a key figure in the publicly-funded Human Genome Project that, in 1998, unexpectedly found itself directly challenged by the complete sequencing of the human genome by Craig Venter’s privately-funded company, Celera Genomics. Sulston has had experience battling patent applications deriving from Venter initiatives – Celera filed 6,500 of them for whole or partial human genes! The ensuing scandal appeared to be solved when, in 2000, US President Clinton declared that the human genome could not be patented. However, in 2010, the US National Human Genome Research Institute notes, in a continuing legal debate, “There are now patents (held by various companies) associated with around a quarter of the genes in the human genome.”

Gordon Wright, a European patent attorney at Elkington and Fife, agrees with Sulston that SGI’s patent applications are “fantastically broad”. For example, the patent relating to insertion of a synthetic genome into a cell “is not restricted to cell type and it is not restricted to the size of the genome. And it applies to prokaryotic and eukaryotic organisms”. However, Wright says that it is highly unlikely that such broad patents will ever be granted in either the USA or Europe. Instead, it seems more likely that the JCVI will succeed with patents on specific modifications that they might make to these overall techniques.

“Craig Venter is now God...”

At least, that’s how Katie Fehrenbacher titled her article on the website www.earth2tech.com. Her argument is that Venter’s approach to synthetic biology might save the planet by generating synthetic life forms that can do potentially useful things like consume atmospheric carbon dioxide while turning themselves into “transportation fuel”.

In fact, Venter has been successfully attracting big investors by making similar promises. He and his companies have explained that through their “digital biology” approach, they can potentially do anything with life. Hamilton Smith, now JCVI’s director of synthetic biology and bioenergy, makes it sound simple, “If you purchase a computer at a store, it doesn’t do anything unless you install an operating system on

it. We’re, in effect, designing the software and installing it into a cytoplasm. The cell then does what we have designed it to do.”

SGI’s business focus is centred on bioenergy, with spin-offs in the areas of food production, clean water and vaccine development. “Many aspects of JCVI’s synthetic genomics work have been integrated into the SGI business programmes. SGI plans to revolutionise many industrial processes by designing new cells that synthesize the desired commercial products.”

Ari Patrinos, SGI President, continues, “With the growing impact of climate change, increased global demand for energy and the potential for environmental impact issues surrounding the drilling for oil and mining for coal, it is clear that new technologies are needed in these areas. Synthetic genomics research from JCVI and the applied science at SGI have the potential to address these issues.”

...and how this affects climate change”

Attracted by the prospect of growing oil in a tidy container rather than sucking it out of leaky undersea pipelines, oil giant BP is already one of SGI’s big investors. ExxonMobil has also recently announced plans to invest more than \$600 million in a new photosynthetic algae biofuels programme with Venter. The idea here is to collect and test thousands of strains of algae to find the most efficient and economical strains for production of transportation fuels. While Venter’s team focuses primarily on “microengineering”, Exxon will help with the macroengineering for production systems and integration needed for commercialisation.

Venter claims that the technology involved in creating Synthia is a step toward manufacturing a synthetic biofuel superbug, “This area of research will enable us to create new fuels to replace oil and coal.” In fact, he goes even further and states, “We can use living systems to increase our chances of survival as a species.” By driving around more?

Meanwhile, his group’s announcement has led to renewed calls for a global moratorium on synthetic biology, for example, from the editor of *Nature* (*Nature*, 2010, 465, 397) and the technology watchdog, ETC Group (www.etcgroup.org). Some experts continue to claim that the current technology for making synthetic cells is too expensive and complex to copy. But what future risks could arise, whether by bioerror or bioterror, unforeseen accident or malevolent design?