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Scientists have announced the creation of the first man-made cell with almost limitless possible applications. But then, it could also be a double-edged sword.

SYNTHIA: *the first man made cell*

ON 20 May 2010, Craig Venter announced the arrival of *Synthia*. It is a very special cell, belonging to a new species created by man in the laboratory. Unlike other cells, the parent of this cell is not a cell but a computer program. Though it was created in the laboratory from four bottles of chemicals and is not a product of cell division, it can replicate like normal cells to form multiple copies of itself. It is the only cell in the Universe to have a website of its own which has been coded in its genetic code found in its chromosome.

When the world first came to know about it, everyone was shocked. While some scientists considered *Synthia* as a beginning of a revolution, many criticized the creators by saying that it was like "playing God".

But what is so special about this cell? Well, Craig Venter, one of the pioneers of the Human Genome Project was always intrigued by some basic questions:

- What is life?
- Can we digitize life?
- Can we pare it down to its most basic components?
- Can we generate new life from the digital world?

We all know that it is still a challenge for scientists and philosophers to define 'life' in unequivocal terms. Broadly 'life' can be defined as the set of characteristics that have signaling and self-sustaining processes. It carries out metabolism, responds to stimuli, reproduces and adapts to the changing environment. It is a highly complicated process. Amazed by the complexity, Venter wanted to know how 'life' is created.

Nucleic acids are the hereditary material. So the real understanding of life can come from analyzing and digitizing the genetic makeup of a variety of organisms and then finding commonality among them. In 1977, Sanger and his colleagues determined the complete genetic sequence of phage X174, the first DNA genome to be completely sequenced. In the following years, geneticists analyzed the genome of a wide range of organisms, starting from viruses, prokaryotes to the most evolved animal, *Homo sapiens*.

In fact one of the pioneers of genome analysis of humans was Craig Venter himself and the project was famously known as the Human Genome Project, published in 2003. Till date, around 20 million genes have been digitized. While on the one hand, the digitization of genetic codes of different organisms was going on, Dr. Venter tried to find the smallest genome in

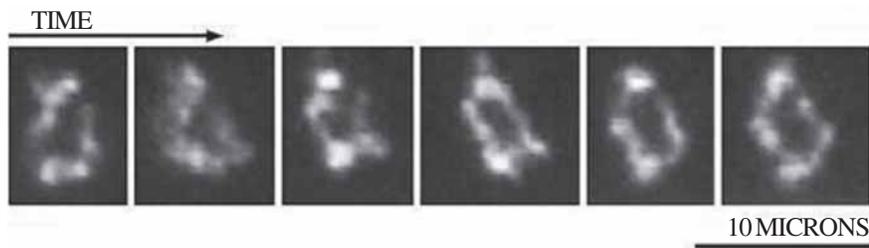
an operating system and tried to understand the basic components of life, an area nobody has ever ventured.

In 1995, Venter's team was able to read the first complete genetic sequence of a self-replicating bacterium, *Haemophilus influenza*, a gram-negative bacteria. In the same year, when Venter's team could decode the genetic make up of *Mycoplasma genitalium*, the smallest bacterium, they launched a project known as the Minimal Genome Project.

Minimal Genome Project

A team of about twenty scientists headed by Nobel laureate Hamilton Smith, DNA researcher Craig Venter and microbiologist Clyde A. Hutchison III was formed. The aim of the project was to determine the minimal instructions or genes needed for a cell to survive and from there add genes that could turn a bacterium into a factory producing compounds useful for humankind.

Mycoplasma genitalium is the smallest known free-living bacterium with the smallest complement of genes of any known organism capable of independent growth in the laboratory. The genome of *M. genitalium* consists of 521 genes (482 protein encoding genes) in one circular chromosome of 582,970 base pairs. Studies showed that it has around 100 non-essential genes that can be 'knocked out'.



Micrograph of synthetic *Mycoplasma genitalium* genome taken over a ~0.6 second period.

But difficulty in knocking out multiple genes from intact chromosome forced researchers to design and build synthetic chromosomes that will be the replica of the original chromosome but will lack the non-essential genes.

But they faced a lot of technical hurdles.

To begin with, till then the largest synthesized DNA contained only 32,000 base pairs. Thus, building a synthetic version of the genome of the bacterium *M. genitalium* that has more than 580,000 base pairs presented a formidable challenge. Moreover longer the DNA more

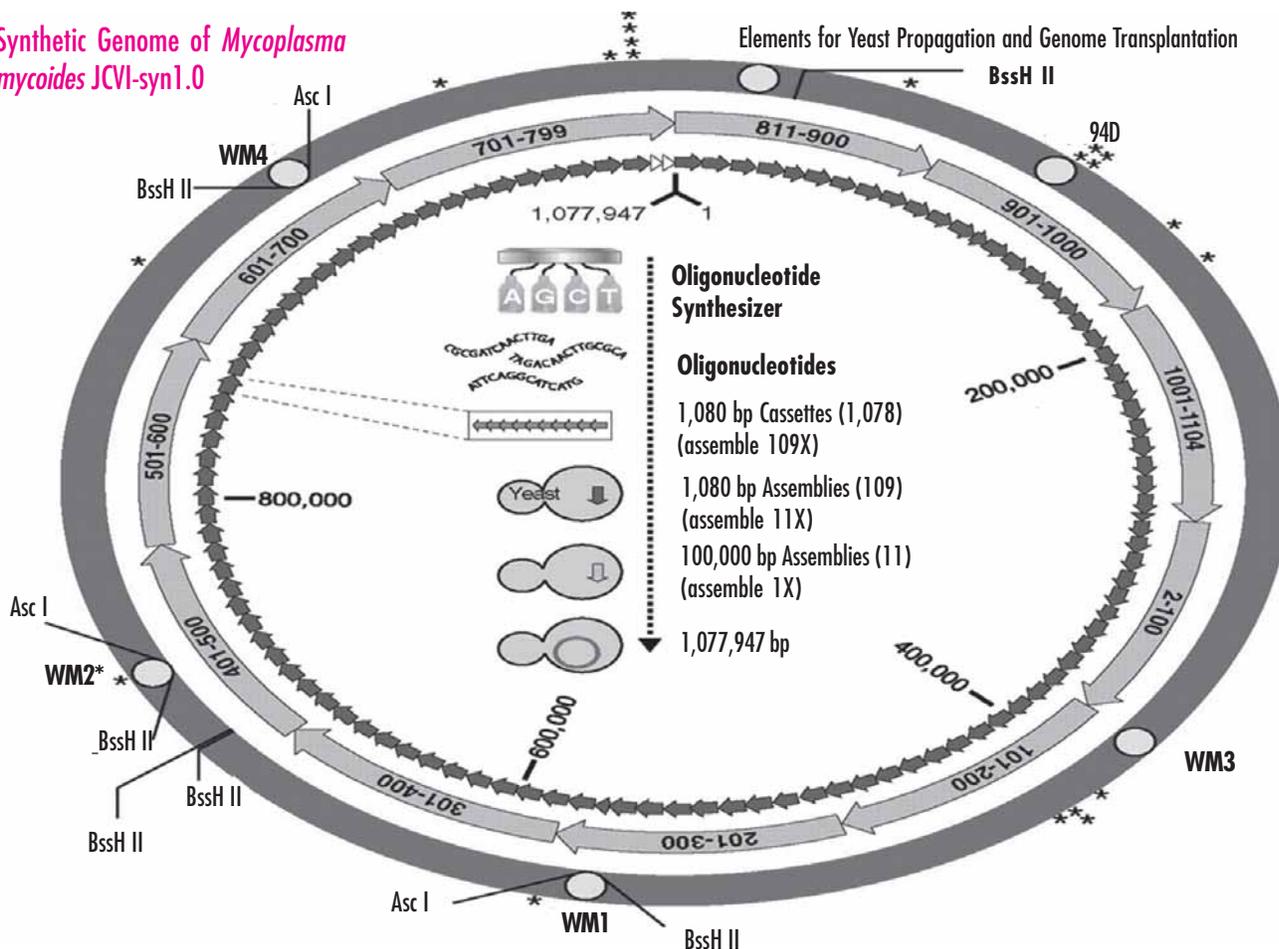
is its tendency to break and more will be the errors.

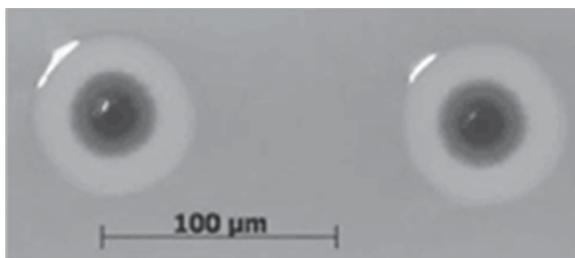
In 2003, Venter, Smith and Hutchison took the first significant stride in the development of an 'error-free' synthetic genome of a single-stranded (ssDNA) virus, bacteriophage ϕ X174 (phi X), which has 5,386 base pairs. They did so using short, single strands of synthetically produced, commercially available DNA (known as oligonucleotides) and using an adaptation of polymerase chain reaction (PCR), known as polymerase cycle assembly (PCA), to build the phi X genome.

Like any new scientific development, this technology of cell designing is a double edged sword. Misuse of this technology can result in creation of superbugs that can even challenge our existence.

In a similar manner, to get an error-free synthetic sequence, they resequenced the native *M. genitalium* genome. After obtaining this correct version of the native genome, the team specially designed fragments of chemically synthesized DNA to build 101 "cassettes" of 5,000 to 7,000 base pairs of genetic code. From here, the team devised a five-stage assembly process where the cassettes were joined together in subassemblies to make larger and larger pieces that would eventually be combined to build the whole synthetic 582,970 base pair genome of *M. genitalium* JCVI-1.0.

Synthetic Genome of *Mycoplasma mycoides* JCVI-syn1.0





Images of *M. mycoides* JCVI-syn1.0

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The team experimented with yeast and found that it tolerated the large foreign DNA molecules well, and that they were able to assemble the fragments together by homologous recombination. By 2008, the synthesis of *M. genitalium* genome in the laboratory was completed.

But the final challenge was to transplant these artificial chromosomes in a bacteria. A major breakthrough came in June 2007 when Carole Lartigue announced the results of her work on genome transplantation methods allowing them to transform one type of bacteria into another type dictated by the transplanted chromosome. They successfully changed one bacterial species, *Mycoplasma capricolum*, into another, *Mycoplasma mycoides* through genome transplantation.

But the team faced two major problems with *M. genitalium*. It is an extremely slow growing bacteria, often taking weeks to conduct a single experiment and the synthetic genome could not be transplanted into another bacteria for the nuclease enzyme present in the bacteria destroyed the synthetic genome. Both these factors were delaying the project.

Without wasting any time they immediately focussed their attention on *Mycoplasma mycoides*, which is a fast growing bacterium. But its genome is over a million base pairs long. In order to

synthesize it artificially, researchers inserted the shorter sequences of synthetic DNA into yeast, whose DNA-repair enzymes linked the strings together. They then transferred the medium-sized strings into *E. coli* and back into yeast.

After three rounds of assembly, the researchers had produced the genome of *Mycoplasma mycoides*. Not only that, in order to distinguish this genome from a naturally occurring genome, they added four "watermarked" regions, specifically designed segments of DNA that use the "alphabet" of genes and proteins that enable the researcher to spell out words and phrases. The watermarks are an essential means to prove that the genome is synthetic and not native, and to identify the laboratory of origin.

Encoded in the watermarks is a new DNA code for writing words, sentences and numbers. In addition to the new code there is a web address to send emails to anyone who can successfully decode the new code, the names of 46 authors and other key contributors and three quotations: "TO LIVE, TO ERR, TO FALL, TO TRIUMPH, TO RECREATE LIFE OUT OF LIFE." - JAMES JOYCE; "SEE THINGS NOT AS THEY ARE, BUT AS THEY MIGHT BE." - A quote from the book, "*American Prometheus*"; and "WHAT I CANNOT BUILD, I CANNOT UNDERSTAND." - RICHARD FEYNMAN.

Fortunately, after many failed attempts to transplant this genome in *M. capricolum*, they finally did so with little tinkering. They could finally get the blue colony of bacteria that was rapidly growing on a lab plate over the weekend, a testimony to their success. They sequenced the DNA in this colony, confirming that the bacteria had the synthetic genome, and checked that the microbes were indeed making proteins characteristic of *M. mycoides* rather than *M. capricolum*. Interestingly, the original genome of *M. capricolum* was lost.

This was a defining moment for the team of 20 scientists, working tirelessly for fifteen years with a total cost of around £40 million to create this unique man-made cell.

Applications

According to Craig Venter, the potential of this new technology is limitless. Making new chemicals or food ingredients and cleaning up water are possible benefits. Most importantly, it has changed our view of life. It has shown that the moment we change the DNA or the software of the cell, the cell starts behaving differently, in accordance with the new software. Again for the first time it has proved that if a computer program in the form of a genetic code can be synthesized and then transplanted in a compatible cell, we can get a new cell with the desired features. So, basically we can now design cells according to our wish.

Already the industry has shown a lot of interest. ExxonMobil announced a \$600 million collaboration with Synthetic Genomics to research and develop next-generation biofuels. It will be a fourth-generation fuel where algae like *Methanococcus* will be coaxed to produce oil by capturing carbon dioxide, in a magnitude never seen before. In fact, the model organism is expected to get ready in a year. If the above experiment succeeds, then in the near future we may not need to extract oil out of the ground at all.

Pharmaceutical giant NOVARTIS have signed a deal to develop new vaccines like flu vaccine because this technology can reduce the time scale for vaccine development by 99%.

In the near future, the technique can be applied to single-celled eukaryotes like yeasts that can generate ethanol on a commercially viable scale.

Like any new scientific development, this technology of cell designing is a double edged sword. Misuse of this technology can result in creation of superbugs that can even challenge our existence. But that should not stop us from welcoming this new technology that has almost revolutionized our idea of life.

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