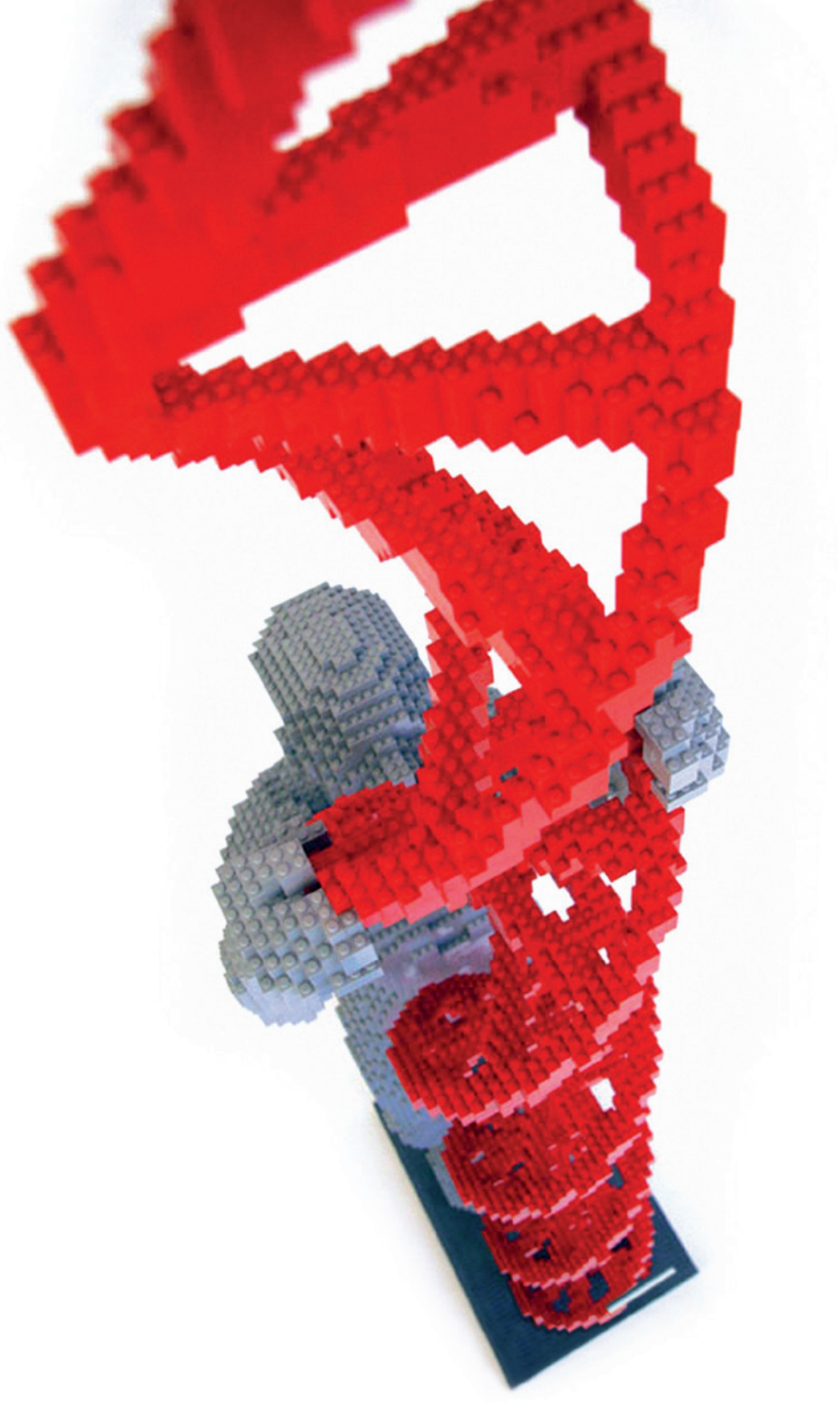

BUILDING BRICKS OF LIFE BY NATHAN SAWAYA. IMAGE COURTESY OF BRICKARTIST.COM

SYNTHETIC BIOLOGY

Bits and pieces come to life

Scientists are combining biology and engineering to change the world.

BY JAMES COLLINS

With a box of Lego, you can create a whole range of different structures. Snap together pieces of various colours, shapes and sizes to create a multitude of structures — a house, a boat, a tower — with different functions. In the world of biology, a growing group of scientists is thinking about parts of cells in much the same way.

Engineers are using genes and proteins as building blocks to create new kinds of cell and new functions for cells. If scientists can build genes from scratch, they can create organisms with new traits. They can create bacteria that can clean up oil spills, rice with genes that keep the plant infection-free, or cells that can churn out new materials. Synthetic biology, the field that revolves around figuring out how to combine genes in new and interesting ways, requires an understanding of biology, creative engineering skills and computing expertise. It is pulling together scientists with different capabilities to solve problems.

The genetic code is like any other language: to be able to write it, you have to learn how to read it and understand it. Before archaeologists discovered the meaning of ancient Mayan hieroglyphics, no one could write in Mayan — it was an uncracked code. Our DNA was once an uncracked code as well, but over the past century, scientists have slowly learned how to read the genetic code that every living cell contains. They have figured out which genes determine which characteristics of cells and organisms, and how changes to genes can alter these characteristics. Now researchers are working towards making new genes or combinations of genes, using the four letters — or nucleotides — that make up DNA.

GENETIC MANIPULATION

Before synthetic biology, genetic engineering was largely just genetics. In 1968, Hamilton Smith, a molecular biologist at the Johns Hopkins University School of Medicine in Baltimore, made a chance discovery that shaped the world of genetics for decades to come. Smith was studying a bacterium, *Haemophilus influenzae*, and the small viral particles called phages that infect bacterial cells. One day, Smith infected the bacteria with a phage called P22. The phage DNA was quickly cut up. Smith was able to isolate the enzyme responsible for the cutting and eventually showed that the enzyme, now known as HindII, always cuts DNA at a particular sequence of nucleotides. HindII was the first restriction enzyme, an enzyme that cuts DNA at a specific sequence.

Restriction enzymes changed genetics because they allow scientists to cut and paste pieces of DNA, although it took two more scientists to realize their potential. In 1972, Herbert Boyer and Stanley Cohen met at a conference about plasmids, small circles of DNA that can move between cells. Cohen was studying how to transfer plasmids from one cell to another. Boyer was studying restriction enzymes and had found

that enzymes such as HindII leave behind 'sticky ends' when they cut DNA. This means that the cut DNA could be joined back together with any other piece of DNA that had the same sticky end. If you cut two pieces of DNA with the same restriction enzyme, you can paste them together.

When the two scientists began sharing their work, they realized that they could use the sticky ends to put new pieces of DNA into the plasmids that Cohen was studying. They had found a way to cut and paste DNA between different organisms. Bacteria were the perfect choice because they are prokaryotic cells. This means they don't have a membrane-bound nucleus; instead of having chromosomal DNA, their genetic information is maintained in a circular plasmid like the ones Cohen studied. Geneticists quickly picked up on the technique, transferring animal and plant genes into bacteria using plasmids and restriction enzymes. This was the beginning of genetic engineering.

Using the technique pioneered by Boyer and Cohen, scientists at the pharmaceutical company Eli Lilly figured out how to insert the human gene for producing insulin into bacterial cells. This turned the bacteria into tiny insulin factories. In 1982, the company filed a patent for the process and the technique drastically changed the production of insulin, which had previously relied on slaughtered animals. Supply could now change with demand, and costs dropped.

Early genetic engineering made possible by restriction enzymes didn't involve much engineering. The field revolved around cutting and pasting DNA — like the gene for insulin — from one organism to another. As engineers began to see the potential in biology, their desire to design things from scratch and build complex devices would revolutionize the field once more.

ENTER THE ENGINEERS

Electrical engineers think in terms of circuits, which translate an external command into an action. A light switch is one of the simplest circuits. The external command is the flipping of the switch, and the action is the light turning on or off. Biology has its own form of circuits in the form of proteins that can bind to DNA and turn genes on or off. A gene that is turned on is copied into RNA in a process called transcription. In a second process, known as translation, a protein is then synthesized, based on the instructions in the RNA. A gene that has been turned off, however, does not lead to protein synthesis.

An external signal, such as a change in temperature, can have an effect by turning on and off different genes in a cell. As scientists began to learn how to move genes between cells, engineers began to see how multiple genes could be combined in new ways to create circuits never seen before.

In 2000, my own lab engineered one of the first biological circuits[1]. The genetic toggle switch relies on repressor proteins, which can keep particular genes turned off, and inducing chemicals, which can turn genes on or off. The toggle switch has two competing genes, each of which is turned on in its natural state. When the first gene is on, it produces a repressor protein that keeps the second gene turned off. And when the second gene is on, it produces a repressor protein that keeps the first gene turned off. As a result, both genes can't be on at the same time. If scientists deliver an inducer that turns off the first gene, this allows the second gene to be turned on, keeping the first gene off. If they deliver an inducer that turns off the second gene, this enables the first gene to switch on, keeping the second gene off.

The beauty of the toggle switch is that it gives cells a memory. Before the toggle switch, if scientists wanted a cell to switch a gene from on to off or vice versa, they would have to continuously give it an inducer for the gene encoding that protein. This is like having to hold your finger on a light switch to keep it on, which is not very useful if you want to move around the room. The toggle switch, however, keeps a gene switched on with one single delivery of an inducer. It gives the cell a memory of the state it should be in. For companies that need inducers to turn on the production of a protein inside cells, this method means it can spend less money on inducers.

It also means that cells can act as sensors of factors in the environment. When they are exposed to light, say, or pollution or certain chemicals, cells can retain a memory of that and can give a signal to let people know. For example, Colorado State University biologist June Medford is using engineered circuits in plants to detect explosives. When her engineered plants sense explosive chemicals in the air, the pathway that makes chlorophyll — the green pigment in plants — is blocked. The plants turn white, a clear signal of danger.

In 2005, synthetic biologist Chris Voigt and a team of students at the University of California, San Francisco, created another powerful — and playful — illustration of what it means to program biological circuits with memory. They created a living photograph using engineered bacteria. Each of the bacteria carried a set of genes that made it either produce black pigment or not. If a bacterial cell was growing in the light, the gene was blocked from making pigment. But if it was growing in the dark, the synthetic circuit turned on the pigment-producing gene. By shining a pattern of light and dark onto the cells, the team of scientists created a matching pattern in the growing bacteria.

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The toggle switch and other early examples of engineered circuits showed that the cut-and-paste genetic technology that had been around for almost three decades could be used to give cells functions that hadn't previously existed in any cell. It set the stage for combining not just a few genes, but entire pathways that can make a compound, or a whole genome that can let a cell divide, grow, copy itself and interact with other organisms.

Although some drugs, such as insulin, can be produced in bacteria by inserting a single gene, others require a whole series of genes. In 2005, Jay Keasling at the Lawrence Berkeley National Laboratory in California put a group of genes into yeast to produce a precursor to a drug that treats malaria[2]. The drug, artemisinin, is the most effective antimalarial medication available, but it could previously only be harvested from a herb called Artemisia annua. Supplies depended on crops of the plant. Keasling took the set of genes that produce the artemisinin precursor in the plant and moved the pathway — along with other required genes from different organisms — to yeast, a eukaryotic single-celled organism that is easy to grow in the lab. Switching to Keasling's method for artemisinin production is likely to shave 30–60% off the cost of the drug, making it more affordable in the developing countries that need it. The process is currently in the production phase and drugs made by the process should become available in 2012.

READING, COPYING AND WRITING

These examples of synthetic biology rely on being able to read genes, that is, knowing the link between their sequence and their function. But they also rely on being able to make as many copies of a particular gene as a scientist needs. The technology to do this quickly has been around since 1985, when chemist Kary Mullis developed a procedure called the polymerase chain reaction (PCR). PCR is essentially a cycle of heating and cooling that forces certain proteins to copy DNA.

DNA is typically composed of two matching strands that stick together like Velcro. When DNA is heated up, however, the strands come apart. In PCR, a scientist heats up DNA, adds loose nucleotides — the building blocks of new DNA strands — and then cools the DNA. As it cools, an enzyme in the reaction mix attaches the loose nucleotides to form a new strand of DNA to make each single strand double again. As this process is repeated over and over, the number of matching DNA strands grows exponentially.

This basic technique has existed for more than 25 years but scientists are constantly finding better ways to write DNA sequences quickly. The technique has been automated and made faster, and scientists can create longer and longer stretches of DNA. But what if you want lots of different DNA sequences to see which ones work best for what you're creating?

In 2009, Harvard University geneticist George Church unveiled a technique that lets researchers design millions of slightly different versions of a strand of DNA. The method has its roots in PCR but integrates many technologies. A researcher can test the many created strands to find the ones that have a particular function.

Church used the method to find versions of the bacteria *Escherichia coli* that produce higher than normal levels of a chemical called lycopene. Lycopene is a bright red compound found in tomatoes and is studied for its potential to prevent some types of cancer. Church's research showed that cells can be selected for any trait a researcher wishes to screen for, not just lycopene production. Researchers can use the method to make random versions of bacteria and test them for their ability to make biofuels, for example, or to digest oil from an oil spill.

As researchers create longer and longer stretches of DNA using new technologies, it starts to seem possible to build an entire genome, which is the set of all the genes required for an organism to function. Biologist Craig Venter and a group of scientists that included Hamilton Smith — the discoverer of the first restriction enzyme — did just that in 2010. They assembled a DNA strand one million base pairs long and put it into a bacterial cell[3]. To do this, the team wrote one bacteria's entire genome into a digital computer file and then translated the file into small pieces of DNA. They stitched those pieces together to make a synthetic copy of the bacteria's genome, with a few small changes, and inserted it into a different bacterial cell. Although the cell had all its original parts — its cell membrane and all its organelles — all the DNA was replaced. Once the DNA was in the cell, the cell machinery responsible for making proteins started relying on the new DNA for instructions on how to make those proteins. The bacteria was able to metabolize nutrients, copy its DNA and divide into more cells containing the same genetic material.

CHANGING THE WORLD

What can synthetic biology do for us? How can moving genes around cells, creating biological circuits, and writing new genetic programs change the world? Many of the major global problems, such as famine, disease and energy shortages, have potential solutions in the world of engineered cells[4]. To address famine in developing countries, genetic engineers can make inexpensive food crops, such as rice or corn, that contain extra nutrients. They could do this by finding genes in other organisms that efficiently produce vitamin D, for example, and then add those genes to the food's own genome. More than 36 million people a year around the world die from hunger and malnutrition. Deficiencies of specific nutrients, such as vitamin D, cause many of these deaths. Engineering inexpensive plants to include these nutrients could save millions of lives.

Synthetic biology also provides ways to make drugs for diseases such as cancer and infections more cheaply than traditional methods, as we saw in the case of artemisinin. It also offers ways of discovering new drugs. Researchers can engineer cells to light up when a particular gene is turned on or off. If scientists are looking for a drug that turns on a disease-causing gene, they can use a synthetic system to quickly look through massive numbers of chemicals for the right drug, and the one that works will light up the cell. For example, scientists studying tuberculosis, a bacterial disease that kills almost two million people a year, wanted to find a drug that turns off a gene called EthR. When EthR is turned on, antibiotics can't kill the tuberculosis mycobacteria. Synthetic biologist Martin Fussenegger of the Swiss Federal Institute of Technology in Zurich set up a system that makes cells light up under the microscope when EthR is turned off. His team could then scan as many drugs as they want to see which ones make the cells light up.

Engineered systems can also help with carefully timed drug delivery. In 2000, Michael Elowitz and Stanislas Leibler at Princeton University developed a synthetic cellular circuit with three genes[5]. The circuit is arranged so that the genes are turned on and off in sequence, which is useful when we want to turn a gene on but only some of the time. Many genes in the body are not on at the same levels all the time — some turn off when you sleep, or after you eat, for example — so this development could provide a way to deliver compounds in natural fluctuations.

Genetic engineering also helps scientists discover new ways to make bacteria produce energy from raw materials. As worldwide oil supplies diminish and the cost of gas increases, everyone is affected. Our society relies on energy to do almost everything, from lighting and heating our homes and workplaces to driving around and even growing food. Church's method of creating many different versions of genes at once allows genetic engineers to find changes to genes that lead to cheaper, more efficient energy production.

"What if we could engineer humans with sonar, like that used by bats, to help us navigate in the dark?"

Venter's team and other scientists around the world are trying to engineer photosynthetic bacteria that use light and water to create hydrogen gas. This could be a whole new source of energy. Governments worldwide have set targets for biofuel production by 2050. Today, biofuels — organic fuels derived from microorganisms, plants and animals — represent just 3% of global fuel consumption for road transport. The goal for 2050 is a 27% share of the fuel for transport. The International Energy Agency says that meeting this goal will require a 30-fold increase in biofuel production capacity by 2030. Could synthetic biology help meet those needs?

As scientists improve their ability to read and write DNA, the possibilities continue to expand. What if we could engineer humans with sonar, like that used by bats, to help us navigate in the dark? What if we had genes that enabled us to get energy from sunlight, like plants do? Pamela Silver's group at Harvard Medical School has inserted cyanobacteria, the microbes responsible for nearly half the world's photosynthesis, into zebrafish embryos as a first step towards achieving that goal. Zebrafish are perfect for this study because they are transparent, and the cyanobacteria were made fluorescent to make it easier for the scientists to see where they were growing. Both the fish and the cyanobacteria grew well in the experiment. It is clear that genetic engineering offers new ways of interacting with our environment, including some we can barely imagine.

PUBLIC CONCERN

When Venter and his colleagues announced the construction of cells with entirely synthetic genomes in 2010, public figures including President Barack Obama and Pope Benedict XVI commented on the breakthrough, discussing the potential benefits to society as well as the risks to keep in mind. Although it was a major scientific success, the project raised lots of questions about how synthetic genomes could be used, for good or bad, and what the political and societal implications were. After all, synthetic cells could be seen as a new form of engineered life.

The consequences are hard to predict. Some people worry that an engineered life form could change genetically or grow out of control — could an engineered virus gain a mutation that harms humans and infect the population? Another concern is that synthetic microbes could be purposefully engineered for bioterrorism. Is it safe to develop synthetic microbes, even with a particular benefit in mind? Could synthetic plants or animals wipe out native populations, and if so, what would that mean for biodiversity?

There is no short answer to the question of how synthetic biology should be regulated. For now, though, synthetic cells are limited to lab benches. The circuits we have created from scratch are small and are made up of only a handful of genes. A living cell has thousands of genes, and circuits on that scale are currently beyond the reach of synthetic biology. We have a list of parts — the genes and their regulators — but we don't know how they all interact. Assembling a living cell from a list of genes would be like assembling a jumbo jet from a list of mechanical parts. You need a manual, and the complexity and messiness of biology means we are a long way from having a manual for how a cell works. Many areas are still in need of clarification and elaboration. ■

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