

January 17, 2006

Custom-Made Microbes, at Your Service

By ANDREW POLLACK
Correction Appended

There are bacteria that blink on and off like Christmas tree lights and bacteria that form multicolored patterns of concentric circles resembling an archery target. Yet others can reproduce photographic images.

These are not strange-but-true specimens from nature, but rather the early tinkering of synthetic biologists, scientists who seek to create living machines and biological devices that can perform novel tasks.

"We want to do for biology what Intel does for electronics," said George Church, a professor of genetics at Harvard and a leader in the field. "We want to design and manufacture complicated biological circuitry."

While much of the early work has consisted of eye-catching, if useless, stunts like the blinking bacteria, the emerging field could one day have a major impact on medicine and industry.

For instance, Christina D. Smolke, an assistant professor at the California Institute of Technology, is trying to develop circuits of biological parts to sit in the body's cells and guard against cancer. If they detected a cancer-causing mechanism had been activated, they would switch on a gene to have the cell self-destruct.

Jay D. Keasling at the University of California, Berkeley, with part of \$42.6 million from the Bill and Melinda Gates Foundation, is trying to take up to 12 genes from the wormwood tree and yeast and get them to work together in *E. coli* bacteria to produce artemisinin, a malaria drug now extracted from the wormwood tree.

J. Craig Venter, the maverick scientist who sequenced the human genome, wants to create microbes that produce hydrogen for use as fuel.

To be sure, scientists have been putting genes into bacteria and other cells for three decades. The term "synthetic biology" seems to include various activities, some of which are not altogether new.

"This has a catchy new name, but anybody over 40 will recognize it as good old genetic engineering applied to more complex problems," said Frances H. Arnold, a professor of chemical engineering at Caltech.

Some synthetic biologists say they will go beyond genetic engineering, which often involves putting a single foreign gene into a cell. The human insulin gene, for instance, is

put into bacteria, which then make insulin for use as a drug. But there have been genetic engineering projects involving multiple genes, so the number of genes alone is not enough to define synthetic biology.

Rather, the difference seems more about mind-set. "We're talking about taking biology and building it for a specific purpose, rather than taking existing biology and adapting it," Professor Keasling of Berkeley said. "We don't have to rely on what nature's necessarily created."

Also new is an engineering approach - the desire to make the design of life forms more predictable, like the design of a bridge. That could be because many leaders of the field are not biologists by training.

Ron Weiss of Princeton is a computer scientist. Michael Elowitz of Caltech trained as a physicist, and Drew Endy of the Massachusetts Institute of Technology as a structural engineer. Mr. Endy and colleagues at M.I.T. have started a "Registry of Standard Biological Parts." The parts, called BioBricks, are strings of DNA that can perform certain functions like turning on a gene or causing a cell to light up.

In theory at least, these components can be strung together to build more complex devices, just as an electronic engineer might put together transistors, resistors and oscillators to build a circuit. Scientists at the University of California, San Francisco, and the University of Texas used some BioBricks to engineer bacteria so that a sheet of them could capture an image as photographic film does. The microbes were altered so that those kept in the dark produced a black pigment while those exposed to light did not.

Some scientists envision that biological engineers will one day sit at computers writing programs for cells, like software developers. But the code would be written in sequences of DNA, rather than computer language. When finished, the programmer would press the "print" button, as it were, and the DNA would be made to order.

The field is also starting to attract some investment. In June, venture capitalists put \$13 million into Codon Devices, a startup company in Cambridge, Mass., that is developing a way to synthesize long stretches of DNA far less expensively than existing methods. The founders include Professors Church, Endy and Keasling.

Professor Keasling is also a co-founder of Amyris Biotechnologies, which is helping make the malaria drug. And Mr. Venter has started Synthetic Genomics to work on his energy-producing microbes.

What make the engineering approach possible are the inner workings of a living cell. Genes, made of DNA, contain the instructions for producing proteins, which carry out most functions in cells. Some proteins can bind to DNA, turning particular genes on or off. This interplay, which is one way that cells regulate themselves, is not too different from how electronic circuits function, with one transistor turning another on or off.

To make the blinking bacteria, for instance, Mr. Elowitz designed the biological equivalent of an electronic oscillator. It uses three genes that trump one another like the rock, scissors and paper in the children's game. Gene X makes a protein that turns off Gene Y. Gene Y makes a protein that turns off Gene Z. And Gene Z makes a protein that turns off Gene X.

So if Gene X is on, it will turn off Gene Y. But the absence of Protein Y allows Gene Z to turn on. Protein Z then turns off Gene X, allowing Gene Y to turn on, turning Gene Z off, and so on. So the three genes turn on and off in an endless cycle.

To make the bacteria blink, Mr. Elowitz programmed a gene for the production of a fluorescent protein to be turned on whenever Gene Z was off.

Some newer efforts involve trying to manipulate entire colonies of microbes to cooperate with one another. They take advantage of something called quorum sensing, a natural communications system that bacteria use to determine whether there are enough of them present to mount an attack.

The bacteria secrete a particular chemical into their environment that they and their brethren can detect. When many bacteria are present, the level of this chemical in the environment increases. The concentric circle bull's-eye pattern was made by engineering *E. coli* to respond to a quorum-sensing chemical from a different microbe.

Some bacteria were programmed to produce a green fluorescent protein at high concentrations of the chemical. Others were programmed to produce a red protein if exposed to a somewhat lower concentration.

The bacteria of both types were mixed together and spread on a surface. In the center were placed some microbes that emitted the chemical, which diffused away from the center. The bacteria closest to the center were exposed to a high concentration. Those programmed to respond to high concentrations turned green. Some of the bacteria further away turned red.

The work, published in *Nature* in April, was led by Mr. Weiss of Princeton and Professor Arnold at Caltech. Mr. Weiss, an assistant professor of electrical engineering and molecular biology, is now trying to use similar principles to help control the differentiation of stem cells into different types of tissues in different locations.

"That's how the body develops its organs," he said, "by relying on cell-to-cell communication."

The two scientists also published a paper in *Nature* the same month in which they used quorum sensing to control bacterial populations artificially, by engineering the microbes to turn on a suicide gene if the concentration of the quorum-sensing chemical grew too high. As soon as the first cells started killing themselves, the concentration of the chemical would drop, so the remaining cells could recover.

The demonstrations, however clever, also illustrate problems inherent in designing biological circuits, as opposed to silicon ones. One is that living things are always dividing and evolving.

Indeed, the population-control system breaks down within days because some of the bacteria mutate so that the suicide gene is not switched on.

Those bacteria, having a selective advantage, quickly take over the colony, said Lingchong You, lead researcher on the project at Caltech and now an assistant professor of biomedical engineering at Duke.

Another challenge is that the genes of the circuit can interact with the native bacterial genes in unexpected ways.

There is also great variability among living creatures. The blinking bacteria, for instance, do not light up in unison, but at greatly varying rates. Even a newly formed daughter cell will not blink in sync with its mother cell, despite being almost identical genetically.

"You write the same software and put it into different computers, and their behavior is quite different," Mr. You said. "If we think of a cell as a computer, it's much more complex than the computers we're used to."

For that reason, some scientists say, it might be difficult ever to make biological engineering as predictable as bridge construction.

"There is no such thing as a standard component, because even a standard component works differently depending on the environment," Professor Arnold of Caltech said. "The expectation that you can type in a sequence and can predict what a circuit will do is far from reality and always will be."

The unpredictability could lead to safety risks. What if the novel organisms were somehow to run amok? In addition, the same technology could be used to synthesize known pathogens based on their published DNA sequences.

Scientists have already created a poliovirus from scratch and more recently recreated the 1918 pandemic flu virus.

"It's quite clear this technology could be dangerous" if misapplied, Mr. Endy of M.I.T. said.

The field is starting to grapple with whether it should be regulated and, if so, how. Scientists set up a safety framework for research when genetic engineering was invented in the 1970's.

Much of the concern centers on efforts to make entire microbes. Some scientists call this synthetic genomics as opposed to synthetic biology, though there is considerable overlap.

A big concern is making pathogens by synthesizing their DNA based on published DNA sequences.

The Alfred P. Sloan Foundation has given \$570,000 to M.I.T., the Venter Institute and the Center for Strategic and International Studies, an independent policy research organization, to study the societal implications of synthetic genomes. The group hopes to have a report by midyear, said Gerald L. Epstein, senior fellow for science and security at the strategic studies center.

In March, the Health and Human Services Department set up the National Science Advisory Board for Biosecurity to give advice about research with potentially nefarious uses. That board in turn established a working group on synthetic genomics and synthetic biology that met for the first time in November.

David A. Relman, chairman of the working group, said the challenge was to weigh the promise of the field against the perils.

"We fully recognize the inherent beneficial and very positive attributes of all of this work," said Dr. Relman, an associate professor of medicine at Stanford, "and don't want to stifle it or curtail it or constrain it for no substantive reason."

Correction: Jan. 25, 2006

An article in Science Times on Jan. 17 about the new field of synthetic biology omitted the name of a scientist who helped design bacteria that blink on and off like lights. Besides Michael Elowitz, who was cited in the article, the work was also done by Stanislas Leibler, now of Rockefeller University.