DIGITAL CELLS

Computer circuits made of genes may soon program bacteria

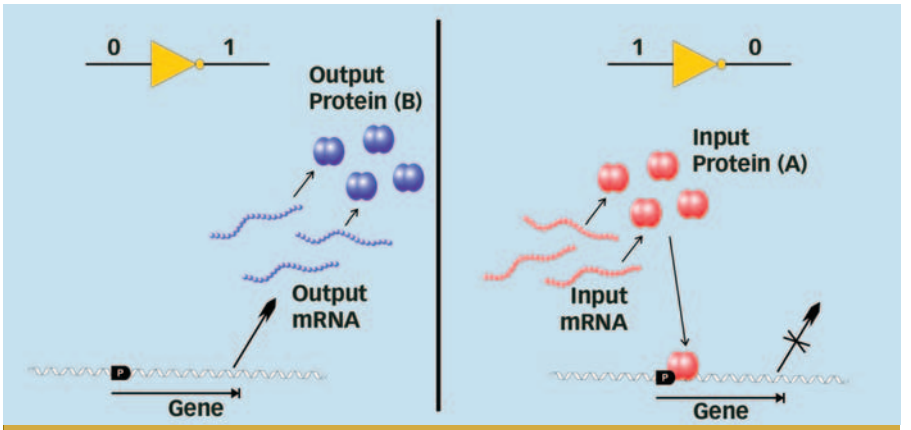
BY ERICA KLARREICH

Imagine a future in which a single drop of water holds a veritable army of living robots; in which people download software updates not for their computers, but for their bacteria; and in which specially programmed cells course through a person's arteries, monitoring blood sugar concentrations and keeping an eye out for cholesterol buildups. These scenarios still belong to the realm of science fiction—but implanting computer programs into living creatures may not be far away. In the past few years, scientists have taken the first steps towards creating a host of cellular robots that are programmed to carry out tasks such as detecting and cleaning up environmental pollutants, tracking down cancer cells in a body, and manufacturing antibiotics or molecular-scale electronic components. These researchers have imported notions of electrical engineering—digital logic, memory, and oscillators—into the realm of biology. Their plan: to create cells with computer programs hardwired into the DNA.

"Eventually, the goal is to produce genetic 'applets', little programs you could download into a cell simply by sticking DNA into it, the way you download Java applets from the Internet," says Timothy Gardner, a bioengineer at Boston University.

The goal is not to produce a Pentium in a test tube. Cellular computers will probably never rival silicon chips in speed and reliability. "We don't use cells because they're a good medium for computation but because they can actually do stuff for us," says Adam Arkin, a bioengineer at the University of California, Berkeley.

Scientists intend to harness the multitude of cellular activities, which go beyond the capacity of silicon devices. Living cells can survive on the flanks of undersea volcanoes and in acidic mine drainage. They operate amazingly efficient factories for producing antibiotics, enzymes, and other useful chemicals, and they generate numerous copies of themselves. Cells can detect minute changes around them, and perhaps most crucially, interact with their environment.



INVERSE LOGIC — A digital inverter that consists of a gene encoding the instructions for protein B and containing a region (P) to which protein A binds. When A is absent (left)—a situation representing the input bit 0—the gene is active. and B is formed—corresponding to an output bit 1. When A is produced (right)—making the input bit 1—it binds to P and blocks the action of the gene—preventing B from being formed and making the output bit 0.

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By cutting and pasting pieces of genetic material, and most recently using artificial evolution as a design tool, engineers are starting to program microbes to carry out behaviors that nature never dreamed of. "We're basically hacking DNA instead of software," says Ron Weiss of Princeton University.

CELLULAR LOGIC Digital circuits, the building blocks of modern computers, encode bits of information in zeros and ones and then manipulate them in exact, controlled ways. Cells, which are basically bags of organelles, proteins, and small molecules, might not at first glance seem promising for such computations.

However, as cells regulate their activities and respond to the environment, they use many of the same tricks that go into digital circuits, such as on-off switches and feedback loops. What's more, cells house one of the richest known information-processing systems. Their strands of DNA include detailed instructions on how and when to build each of thousands of proteins. A control center for each gene turns it on or off according to the cell's changing needs.

Just as electrical engineers wire together transistors—the basic on-off switches of silicon chips—into complicated circuits, researchers are now stringing together genes and control centers in novel combinations to build what they call genetic circuits, in which the output protein of one gene regulates the next gene.

Silicon circuits perform complex operations using a handful of simple components known as logic gates. Genetic-circuit engineers are now building the same devices inside cells. One such logic gate is the inverter, which outputs a 1 if the input is 0, and a 0 if the input is 1. Another is the AND gate, which takes two inputs, and outputs a 1 only if both inputs are 1s. Amazingly, as simple as these two gates sound, mathematicians can construct any logical operation by hooking up enough of them.

Between 1998 and 2001, Weiss took some of the first steps toward building cellular logic gates when he modeled and built a cellular inverter, an AND gate, and two other gates. In Weiss' inverter, the input bit and output bit are encoded in the concentrations of two proteins—for simplicity's sake, call them protein A and protein B. If the concentration of protein A is high,

the input bit is a 1; if the concentration is low, the input bit is a 0. Similarly, the concentration of B corresponds to the output bit. Weiss constructed in *Escherichia coli* bacteria a loop of DNA containing two important pieces: the gene with the instructions for building protein B, and near that, a segment of DNA to which protein A binds.

To make protein B, a special molecule called messenger RNA assembles itself along the DNA, copying the gene's instructions and carrying them to the cell's protein-making factory. If the concentration of A is high, molecules of protein A will bind to the DNA loop and block the messenger RNA from attaching to the DNA. This prevents the cell from building protein B. If, on the other hand, the concentration of A is low, then protein B will be built in abundance. Thus, in Weiss' circuit, B is high when A is low, and vice versa. Weiss' other gates are constructed in similar, slightly more complicated ways.

By hooking together inverters, engineers can create a wide variety of interesting devices. In 2000, Gardner and his colleagues James Collins and Charles Cantor, both also of Boston University, built a memory device in *E. coli* out of two inverters for which the output protein of one is the input protein of the other, and vice versa.

HIGH AND LOW — With novel circuits genetically engineered into the bacteria, these *Escherichia coli* detect the presence of a substance called a quorum-sensing chemical, which is released by other cells (in a disk at right in these images). The bacterial cells produce a fluorescent protein when the concentration of the quorum-sensing chemical is above a set threshold (top) or below a set threshold (right). The team more recently constructed a circuit that detects the chemical when its concentration falls within pre-selected boundaries.

In the same year, Michael Elowitz and Stanislas Leibler of Rockefeller University in New York City made an oscillator in which three inverters are connected in a loop so that the output protein of each inverter is the input protein of the next inverter. In one test of their system, a fluorescent protein became active whenever one of the proteins was in its low state. The result was a population of gently twinkling cells like flashing holiday lights, Elowitz says. "It was very beautiful," he says.

Weiss' team has just put the finishing touches on a five-gene circuit in *E. coli* that can detect a particular chemical in its surroundings and turn on a fluorescent protein when the chemical concentration falls within preselected bounds. Such circuits could eventually be used to detect environmental toxins, Weiss notes.

"If you spread cells around . . . they will form a fluorescent ring around the [chemical], and the middle of the bull's eye is where the bad guys are," Weiss says.

Ultimately, he says, different cells could be programmed to respond to different concentrations of a toxic chemical and to fluoresce in different colors, so that the cell population would generate a color-coded topographical map of the toxin concentrations.

A DASH OF SPICE It's far easier to describe the schematics of these circuits than to build them for operation inside a cell. For instance, to hook up one gate to the next, the amount of protein produced by the first gate must be the right amount to activate the next gate. And at every step, the output protein must be either very high or very low, to avoid false positives or negatives. It's also necessary to tweak many parameters, such as the strength with which the various proteins and the messenger RNA bind to different parts of the DNA sequence.

Making such adjustments via trial and error is prohibitively expensive, Gardner says. Genetic-circuit engineers usually use enzymes to cut pieces of DNA out of one organism's genome, glue the pieces together in different combinations using other enzymes, and then put them in another organism. "It's as if you had to write an article by cutting words out of magazines and pasting them together," Gardner says.

To get around this difficulty, genetic-circuit engineers have turned to mathematical modeling to predict how a circuit will behave before they build it. Together with biologists, mathematicians, and other researchers, they have collectively developed a computer-aided design tool called BioSPICE, named after SPICE, the program that engineers have used for decades to test electronic-chip layouts. BioSPICE simulates a genetic circuit by modeling the speed of the biochemical reactions between the proteins, genes, and other molecules.

Because the circuits generally include complicated feedback loops, the equations that model the reactions tend to be nonlinear. If the quantity of an input protein doubles, the quantity of output protein won't necessarily double. In nonlinear equations, which come up in situations such as weather prediction and population dynamics, tiny changes in parameters can produce large swings in the behavior of a system. This makes the system difficult to analyze. At the same time, nonlinearity is the source of much of the wealth of possibilities for genetic circuits, says Jeff Hasty, a bioengineer at the University of California, San Diego.

"Even in small gene networks with just three or four genes, there is a whole zoo of potential behaviors," he says.

Because many of the parameters of the biochemical reactions are only partly understood, and because the random jiggling of molecules complicates the picture, mathematical simulations give only partial information about whether a circuit will work. Consequently, it's quite common to build a circuit only to find, for instance, that it produces 50 protein molecules when you really need 500, Weiss says. "Then you go back to the model and ask it what parameters to change to get 500 instead of 50," he says. "It's a continual process of simulation, refinement, simulation, refinement, until it works," Weiss says.

Weiss is now bringing another tool—evolution—into the design process. Once a circuit is working almost as he wants, instead of the engineers' refining the design again and again, Weiss permits the DNA to mutate and lets a lab-made version of natural selection do the hard work. In the Dec. 24 *Proceedings of the National Academy of Sciences*, Weiss and Frances Arnold of the California Institute of Technology in Pasadena report their team's work that used evolution to fix up a faulty inverter that Weiss had previously built.

The team took copies of the inverter and introduced small ran-

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dom changes, then put the mutated DNA circuits into cells and measured how well they performed. The engineers kept the circuits that worked better than the original and threw away the others. After only two rounds of mutation, the team had a working inverter.

"Without feeding the circuit any information, we evolved the original protein into a non-natural protein that acts as a very good digital logic inverter," Weiss says.

BRICK BY BRICK As with electronics, the real power will come from assembling cellular logic gates into large circuits. To do that, many technical challenges must be overcome. For example, each new gate in a circuit must usually be turned on and off by different proteins than those that control the previous gates. What's more, the DNA must be carefully designed so that the proteins produced at each stage don't accidentally interfere with other parts of the circuit. Right now, only a few proteins are understood well enough to be useful to genetic circuit engineers, says Drew Endy of the Massachusetts Institute of Technology (MIT).

"There are only about half a dozen parts that have been captured from the wilds of nature," he says.

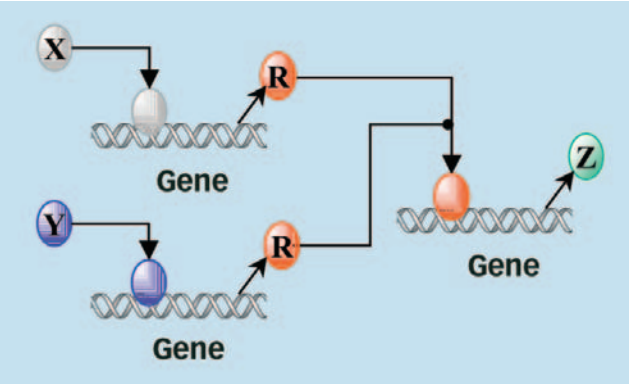
Endy and Thomas Knight of MIT are working to create a library of what they call BioBricks, standardized building blocks that genetic circuit engineers could link. "In electrical engineering, there's the idea of the specification sheet, which tells all the important properties of a component, like the environment in which it will work, the extreme conditions in which it will break, its size, accuracy, reliability, and so on," Knight says. "We'd like to make a similar set of biological components.

"We want to move away from the situation where you build the system and pray that it will work, toward the situation where you build the system and, unless you've done something stupid, it will work," he says. "It's going to be a long road, but we've got to get started."

Building circuits with many components will probably take years of work, researchers agree. "We're still making baby steps, and it's not clear just what we'll achieve," Arkin says. "But . . . if we keep pushing the boundary, we're going to get someplace."

Genetic-circuit engineers can look for inspiration in the shining example set by natural cells themselves. "Obviously, evolution has been able to figure things out to the point where it can get really complicated behavior from biological systems," says Michael Simpson of Oak Ridge National Laboratory in Tennessee.

If researchers can figure out how to tap into this richness, cellular robots may not be far away. ■



AND MORE — In this biological AND gate, the input proteins X and Y bind to and deactivate different copies of the gene that encodes protein R. This protein, in turn, deactivates the gene for protein Z, the output protein. If X and Y are both present, making both input bits 1, then R is not built but Z is, making the output bit 1. In the absence of X or Y or both, at least one of the genes on the left actively builds R, which goes on to block the construction of Z, making the output bit 0.

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OF NOTE

ZOOLOGY

Chicks open wide, ultraviolet mouths

The first analysis of ultraviolet (UV) reflections from the mouths of begging baby birds has revealed a remarkable display that birds can see but people can't.

The colors of chick mouths have attracted much scientific interest, says Sarah Hunt of the University of Bristol in England. An old theory held that the bright yellows and reds create conspicuous targets for parents delivering food in dim nests. Newer evidence shows that health influences mouth color, which suggests that various shades give parents a quick clue to a chick's condition and need for food.

To get a better idea of what the birds are seeing, Hunt and her colleagues measured UV reflection from chick gapes and nests for barn swallows, blackbirds, house sparrows, and five other European species. The gapes reflect a lot of ultraviolet, but the nests don't, Hunt and her colleagues report in an upcoming *Proceedings of the Royal Society of London B.* The big UV difference between nest and chicks suggests that it's time to dust off the old conspicuous-target theory, says Hunt. —S.M.

ENVIRONMENT

Traces of lead cause outsize harm

Minute amounts of lead in blood are worse for children than scientists had realized, according to new research. Data now suggest that lead affects development of kids' thinking skills at concentrations below 10 micrograms per deciliters (μg/dl) of blood. Higher concentrations had previously been recognized generally as harmful to the brain.

In fact, microgram for microgram, lead may pack more punch below 10 μg/dl than it does at higher concentrations, according to Richard L. Canfield of Cornell University and his colleagues. They periodically measured blood-lead concentrations in 172 children beginning when the kids were 6 months old and continuing until they were 5 years old. They gave each child an intelligence test at age 3 and at the end of the study.

Putting all these data together, the researchers found that for blood-lead concentrations between 1 and 10 μg/dl, the average effect of each additional 1 μg/dl was a drop of 0.82 IQ point. However, each 1 μg/dl blood lead above 10 μg/dl translated into only a 0.13-point loss, the researchers report in the April 17 *New England Journal of Medicine.*

A recent study found that 2.2 percent of U.S. children age 1 to 5 have blood-lead concentrations greater than 10 μg/dl, nearly 10 percent have concentrations of at least 5 μg/dl, and 90 percent have at least 1 μg/dl (*SN: 2/22/03, p. 120*). —B.H.