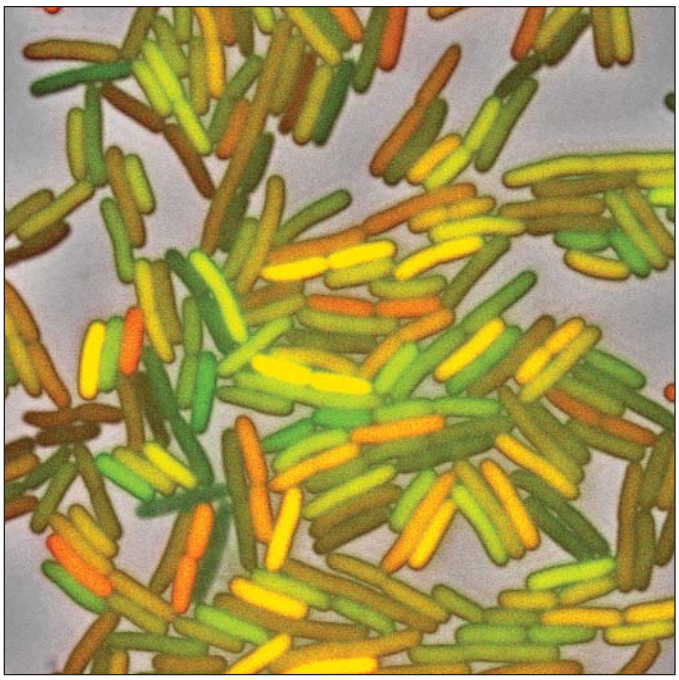
A new breed of bioengineers aims to create microbes from off-the-shelf parts. The parts are coming, but will researchers be able to put them together?

Microbes Made to Order

In the dead of a New England winter, 16 students worked day and night for a month trying to make *Escherichia coli* blink like a lighthouse. No one really expected a blinking bacterium to be all that useful. Instead, the exercise was meant to teach students—and their instructors—how to make reprogramming bacterial behavior more routine. The first class of its kind, held last January at the Massachusetts Institute of Technology (MIT) in Cambridge, also marked the emergence of the hot new field of synthetic biology.

Bacterial blinking circuits are just one element in the MIT researchers' "registry of standard biological parts," which is akin to an inventory that electrical engineers or basement tinkerers might consult when they design a new device, says class co-instructor Drew Endy of MIT. Researchers at MIT and elsewhere are working on sensors and actuators, input and output devices, genetic circuits to control cells, and a microbial chassis in which to assemble these pieces. If they're successful, the registry will help them reach one of the goals of synthetic biology: to allow researchers to "go into the freezer, get a part, hook it up," and have it work the first time, Endy says.

The parts list is itself just one piece of a hugely ambitious plan: to engineer cells into tiny living devices. Some of the engineered devices these researchers envision will function as molecular-scale factories. Others will help detect chemical weapons, clean up environmental pollutants, make simple computations, diagnose disease, fix faulty genes, or make hydrogen from water and sunlight. "We're going to modify the whole behavior of the cell," says bioengineer Ron Weiss of Princeton University in New Jersey. Synthetic biologists aim to build cells from the ground up rather than tinkering with a handful of genes or tweaking a metabolic pathway or two, as do today's genetic engineers.



Blinkers on. A synthetic gene circuit that works like a clock turns on fluorescent proteins that make these *E. coli* flash on and off.

CREDIT: M. ELOWITZ *ET AL.*, *SCIENCE* **297**, 1183 (2002)

The fledgling field, which is attracting engineers and biologists in equal measure, means different things to different people. Engineers view it primarily as an engineering discipline, a way to fabricate useful microbes that do what no current technology can. But many biologists see it instead as a powerful new way to learn about cells. Unlike systems biologists, who analyze troves of data on the activity of thousands of genes and proteins (*Science*, 5 December 2003, p. 1646), synthetic biologists simplify and build. They create models of genetic circuits, build the circuits, see if they work, and adjust them if they don't—learning about biology in the process. "I view it as a reductionist approach to systems biology," says biomedical engineer James Collins of Boston University.

However it's defined, synthetic biology is catching on. A growing cadre is publishing in top journals. Researchers at Lawrence Berkeley National Laboratory (LBNL) in California established the world's first synthetic biology department last June. A European Commission program designed to support "unconventional and visionary research" has issued a request for synthetic biology research proposals. The inaugural synthetic biology conference (Synthetic Biology 1.0) is set for next June at MIT. "I think we're going to see some spectacular new science and engineering," says Eric Eisenstadt, a program manager who oversees synthetic biology funding for the Defense Advanced Research Projects Agency (DARPA). J. Craig Venter, who heads the Institute for Biological Energy Alternatives in Rockville, Maryland, predicts that "engineered cells and life forms [will be] relatively common within a decade."

Rewiring the cell

Nothing is more basic for a parts list than reengineered genetic circuits that direct the behavior of made-to-order microbes. Along with parts, genetic-circuit designers need simple principles to guide their work, just as engineers use Ohm's law of resistance or Kirchhoff's rule on conservation of charge at a junction to guide the design of electric circuits. But biologists are just beginning to grasp the rules.

The library of such principles was inaugurated decades ago when microbiologists François Jacob and Jacques Monod of the Pasteur Institute discovered the first gene circuit—a set of genes that help *E. coli* digest lactose. A regulatory gene called a repressor is normally on, keeping the lactose-digestion circuit inactive. When lactose is present, however, the bacterium turns the repressor off. Such gene circuits can be diagramed with nodes representing genes and arrows indicating which other genes they regulate. "If you squint hard enough, it begins to look like [an electrical] circuit diagram," says bioengineer Jeff Hasty of the University of California (UC), San Diego.

The analogy falls down on the details, however. Electronics engineers know exactly how resistors and capacitors are wired to

each other because they installed the wiring. But biologists often don't have a complete picture. They may not know which of thousands of genes and proteins are interacting at a given moment, making it hard to predict how circuits will behave inside cells.

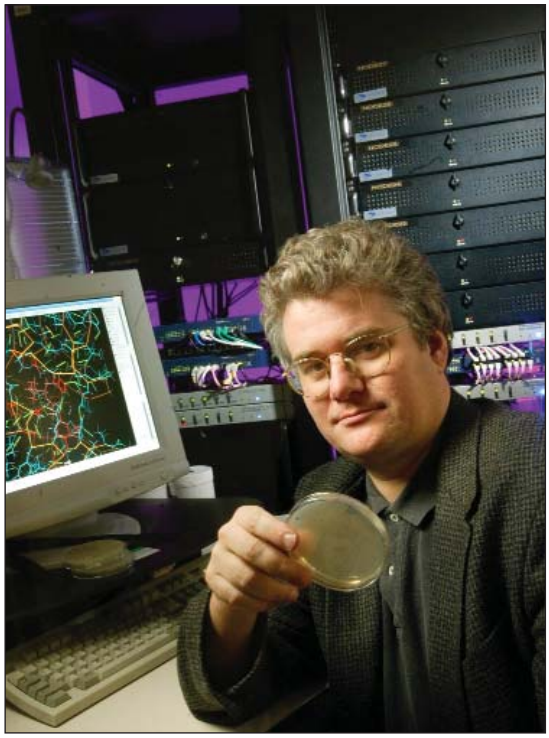
To simplify the problem, physicists Michael Elowitz of the California Institute of Technology (Caltech) in Pasadena and Stanislas Leibler of Rockefeller University in New York City built a genetic clock from scratch—the original blinking bacterium that last winter's MIT students were trying to improve upon. The two, then at Princeton University, designed a circuit of three repressor genes (call them genes A, B, and C), which they dubbed the "repressilator." It worked like the game "Rock, Paper, Scissors": Gene A turned off gene B, gene B turned off gene C, and gene C turned off gene A. Gene C also turned on a jellyfish gene that turned the cell green. In physicists' terms, the device was a limit-cycle oscillator: an oscillator that reestablishes the same behavior after it's perturbed. When they put the circuit into *E. coli*, the cells blinked. The work, reported in *Nature* in 2000, is "the high-water mark of a synthetic genetic circuit that does something," Endy says.

More recently, Michael Savageau of UC Davis, Alexander Ninfa of the University of Michigan, Ann Arbor, and their colleagues rewired some well-studied bacterial gene circuits to make a less noisy oscillator. With minor modifications, the oscillator also functioned as a toggle switch, turning a circuit on or off. The results, published in *Cell* in April, show that as researchers understand the design principles of genetic circuits, they learn to control their behavior, Savageau says.

So far, most designers have made gene circuits that mimic simple physical devices used routinely by engineers, including toggle switches, oscillators, and feedback loops. But evolution "might have come up with new designs that engineers never thought of," says Savageau. So they have begun drawing design principles from biology. By examining patterns of gene expression in *E. coli*, for example, physicist-turned-biologist Uri Alon of the Weizmann Institute of Science in Rehovot, Israel, and colleagues identified three widespread gene circuit designs. They synthesized a circuit using the most common design, called a feed-forward loop, and installed it in bacteria. As they reported in November in the *Journal of Molecular Biology*, it enables bacteria to turn genes on slowly but off quickly—a property that seems to help the cells filter out molecular noise and activate genes only when they're needed.

Biodesign will truly resemble engineering when researchers can construct models that accurately predict how a gene circuit will behave inside cells, says engineer-turned-biologist Harley McAdams of Stanford University. To do that, Collins, biomedical engineer Timothy Gardner, and their Boston University colleagues used a mathematical method from a branch of engineering called system identification to infer the design of a network—in this case, the SOS pathway that turns on genes in response to DNA damage—by monitoring parts of the network. Given messenger RNA levels produced by some genes, the algorithm correctly determined the entire circuit's wiring. The algorithm, reported in *Science* in July (4 July 2003, p. 102), also predicted which points in the network were blocked by a DNA-damaging drug. Pharmaceutical companies may be able to adapt the method to see if candidate drugs affect parts of the cell aside from their intended target, Collins says.

Despite the recent successes, it will take years for systems biologists to fully understand the logic of gene circuits, in part because there are so many of them. Engineers such as Endy, meanwhile, are happy to get information from any source about genetic modules that can direct the behavior of engineered microbes. They don't plan to wait around for the systems biologists, according to Endy: "Synthetic biology says, 'Screw it. You want modules? We'll *build* modules.' "



CREDIT: DUKE UNIVERSITY PHOTOGRAPHY

Biosensors to go. Homme Hellinga and colleagues retooled bacterial sensor proteins, like the one on the computer monitor, to bind desired chemicals.

Like LEGO bricks

In the eighth-floor playroom of MIT's Artificial Intelligence Laboratory, teams of students in the synthetic biology class designed circuits to improve upon the repressilator, the circuit driving Elowitz and Leibler's original blinking bacteria. One group drew up plans to add a logic gate that would let a

Time for a Synthetic Biology Asilomar?

Synthetic microbes might one day clean up pollutants, produce hydrogen for fuel, improve gene therapy, and more. But synthetic biology also raises some real dangers, say bioethicists such as David Magnus of Stanford University: "The greater control we have over bacteria, the greater potential we have for good but also for harm."

Like today's genetically engineered microbes, many synthetic microbes would be confined to laboratories or biotech factory vats. But some proposed uses of engineered microbes, such as sensing explosives or cleaning up pollutants, would require robust bugs that could survive outside the lab. New methods may be needed to keep them from spreading, Magnus says. "I don't think any engineered species made at this stage should be released, and if it's accidentally released it should ... no longer survive," says J. Craig Venter, head of the Institute for Biological Energy Alternatives in Rockville, Maryland.

Bioengineers, like other engineers, should be drilled to put public "health, safety, and welfare" first, says engineering ethicist Aarne Vesilind of Bucknell University in Lewisburg, Pennsylvania. But the new field ups the ante. "A sleazy bioengineer could develop something ... that affects the entire global ecosystem," Vesilind says. He and others say that synthetic biologists and ethicists should hold a summit meeting to define the bioengineers' "responsibilities to society," perhaps modeled on the 1975 Asilomar Conference, at which biologists defined safeguards needed to contain genetically engineered microbes. "These guys have got to get on it," Vesilind concludes, "because otherwise it's going to get away from them." **–D.F.**

chemical switch the blinking on or off. Another designed a system to flash more often. A third group planned a "synchronator" that would make all the cells blink in concert.

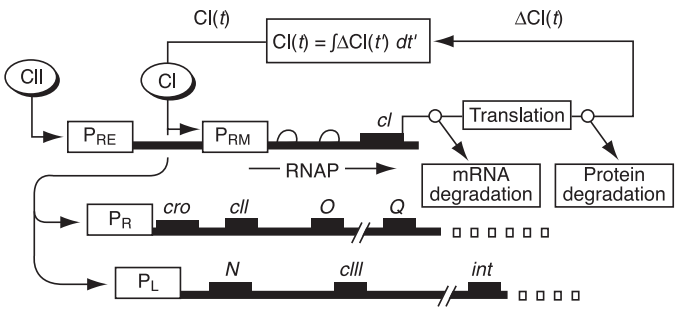
Each module was composed of parts from MIT's standard synthetic biology parts list, a data book dubbed "BioBricks." It's based on a parts list called the transistor-transistor logic data book, from which electronic circuit designers select compatible, LEGO-like modules for complex circuits. Each BioBrick is a piece of DNA; each can be spliced to any other BioBrick. Each either makes up or encodes a functional element familiar to any molecular biologist: promoters and terminators to start and stop transcription, antisense RNAs to block gene expression, ribosome-binding sites that spur cells to make protein from messenger RNA, and reporter genes that make cells glow green.

As often happens when engineers test electronic circuit designs, the MIT students were forced to improvise when their grand plans collided with reality. Each group was allowed to play with a budget of 5000 base pairs of DNA; all of them ran over budget. They learned to economize and share parts. Future synthetic biologists, like engineers, will also have to learn to specialize, says course co-instructor Gerald Sussman, with some designing circuits, others fabricating them, and still others making the larger components. "Eventually we'll be able to design and build in silico and go out and have things synthesized," says Jay Keasling, head of LBNL's new synthetic biology department.

Synthetic biologists eventually aim to make bacteria into tiny programmable computers. Like electronic computers, the live ones would use both analog circuits and digital logic circuits that perform simple computations. Rudimentary components are already taking shape. Princeton's Weiss, MIT's Tom Knight, and their colleagues made an amplifier and other analog circuits. They also made a set of genetic on-off switches that can perform basic Boolean computations and used them to fashion eight genetic circuits that work as logic gates, including a NOT gate and an AND gate. And chemist Milan Stojanovic of Columbia University and computer scientist Darko Stefanovic of the University of New Mexico in Albuquerque created a digital logic circuit made of DNA that's unbeatable at ticktacktoe. Such computers would never rival the raw computing power of their electronic cousins, Weiss says, but they'd be able to direct the operation of engineered cells.

Peripheral components are being developed, too, such as engineered cells that can sense chemicals in their environment and respond with a signal. Bacteria are already good at sensing the molecules they care about, but biochemist Homme Hellinga of Duke University in Durham, North Carolina, and colleagues have devised an algorithm to direct natural biosensor proteins to bind whatever chemical the designers want. The method, reported in *Nature* in May, allowed the team to reengineer a single *E. coli* sugar-binding protein to bind the explosive TNT; a metabolite called lactate; or serotonin, a compound brain cells use to communicate. The team members plugged the redesigned protein into an engineered gene circuit, which they stuck into a bacterium to create a bug that glows green when it sees its target chemical. Similar microbial biosensors could detect underwater ordnance or environmental pollutants or be used in medical diagnostics. "We'd be the ones who give you different components for an electronic breadboard, beyond what nature offers," Hellinga says.

Command center. Students and instructors in MIT's first annual synthetic biology class labored to make microbes that do their bidding.



Circuit logic. To control synthetic microbes, scientists are reengineering genetic circuits like this one.

Designers are working on programmable cells that would need the equivalent of a keyboard to receive input. So far researchers have used chemicals to send signals to reengineered microbes, but the MIT team is exploring ways to use light as well. And to provide a readout on what's going on inside cells—the equivalent of a monitor—the researchers will need something beyond the fluorescent jellyfish protein that's been used until now. Ideally, engineered cells would communicate with one another, allowing them to act in concert, Weiss says. To do that, he and his colleagues are rigging *E. coli* with quorum-sensing proteins, which other bacteria use to send and receive signals.

All together now

Like a computer or a car, engineered microbes require a chassis. One would-be organism frame builder is Venter, who directs a high-profile effort to engineer synthetic microbes that can make hydrogen from sunlight and water (*Science*, 14 February 2003, p. 1006). His strategy is to install special components and a new genetic agenda into a microbe with a stripped-down genome.

But getting installed functions to work reliably—and safely—will be a tremendous challenge (see sidebar on p. 159). For starters, devices put together in the lab may not work well in cells, where they'll sit in close quarters with hundreds or thousands of other biological parts, says DARPA's Eisenstadt. But Weiss, Caltech chemical engineer Frances Arnold, and their colleagues have come up with a possible way around that problem. Last year in the *Proceedings of the National Academy of Sciences*, they reported fine-tuning the connections of a genetic circuit using directed evolution, a test tube method by which researchers mutate microbial genes until the bugs perform the way the researchers want. The method allowed two previously incompatible parts of the circuit to work together.

Making the components compatible isn't enough, McAdams says. Synthetic biologists will have to test their prototypes for robustness—whether they work under a wide range of conditions. They'll need computer programs that predict how designed circuits would behave in the cell, he adds. And once circuits are installed, Savageau says, researchers will need the equivalent of a voltmeter to test how well the circuit is working. "What you'd like is some magic instrument where you could look at the concentration" of proteins, RNAs, and

CREDITS: (TOP TO BOTTOM) MIT; H. MCADAMS AND L. SHAPIRO, *SCIENCE* **269**, 650 (1995)

metabolites, he says. That doesn't exist, but with microarrays and other technology, "it's coming," he says.

Despite many early successes, synthetic biologists might be getting ahead of themselves. Much more needs to be known about the basics of cellular "device physics"—including where proteins are located, how fast they turn over, and what other proteins they talk to, says Eisenstadt. "We'd like to be building life forms from first principles," says Venter, "but it's kind of hard when you don't know all the first principles." And after all is said and done, researchers may never be able to make a synthetic cell at all, Venter says: "People should not accept as a fait accompli that this will work."

Back at MIT, it's still not clear whether last year's bacterial class projects will blink. The modules were made, and Endy and co-instructor Knight's teams are still installing them in *E. coli* to test them. But whether they work or not, the MIT engineers are pressing on. For the second annual synthetic biology class, which kicked off this week, they'll challenge the students to make bacteria communicate with their neighbors on a petri dish to turn genes on or off. The goal this time: genetically encoded polka dots.

–DAN FERBER

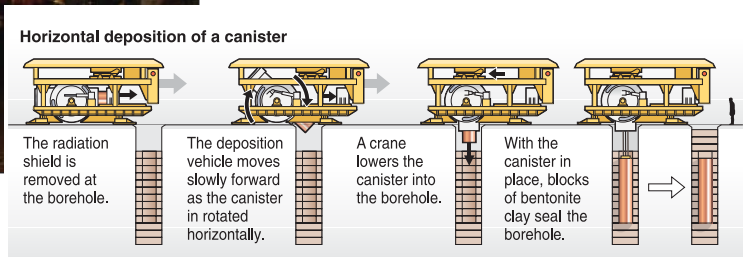
Nuclear Waste

Deep Repositories: Out of Sight, Out of Terrorists' Reach

The threat of terrorism and shifting economics are spurring efforts to entomb nuclear wastes deep underground; Sweden is helping pave the way

ÄSPÖ, SWEDEN—A thin stream of water trickles down the rough-hewn black granite of a tunnel deep beneath the Simpevarp Peninsula on the Baltic Sea. It's in this kind of crystalline bedrock that Swedish authorities intend to imprison the most pernicious isotopes of uranium, plutonium, and other radioactive elements, some of which will remain dangerously hot for 100,000 years. If local residents agree, thousands of tons of spent-fuel assemblies accumulated by the country's 11 civilian nuclear power plants will be loaded into copper canisters and entombed for perpetuity. Experiments here at the Äspö Hard Rock Laboratory are intended to show that the crypt will withstand everything from crushing pressures to the unremitting heat of the nuclear material to the most difficult problem of all: relentless attack by moisture.

Deep heat. Sweden's Äspö Hard Rock Laboratory is testing a machine that deposits canisters of highly radioactive waste in deep boreholes.



CREDITS: (LEFT TO RIGHT) R. STONE/*SCIENCE*; MATS JERNDAHL/SKB

Äspö and other unusual labs of its ilk are prepping nuclear scientists around the world for some of the most important and costly engineering projects ever undertaken: the construction of geological repositories for spent nuclear fuel. For more than 2 decades, the prospect of high-level waste underfoot has sparked determined opposition from the general public. Indeed, as protests last year in Italy and South Korea show, repositories continue to be a hard sell. And in Europe, many countries are reluctant to be the first to open a repository for fear that they will end up taking waste from their neighbors.

But the tide may be turning in favor of building repositories. The 11 September terrorist attacks have highlighted the potential vulnerability of aboveground storage of vast quantities of spent uranium fuel laced with plutonium and other radionuclides. "The risks we face from terrorism and nuclear proliferation are immediate," contends Kenneth Brill, U.S. ambassador to the International Atomic Energy Agency (IAEA) and the Vienna office of the United Nations. Such concerns spurred the U.S. Congress in July 2002 to override the state of Nevada's objections and approve Yucca Mountain as the U.S. national repository. Across the globe, at least two dozen national efforts are now in motion.

Another watershed is that specialists have embraced eternal entombment as the best option. "All experts in the world agree this is the safest solution," claims Bernard Frois, director of energy, transport, environment, and natural resources at France's science ministry. Indeed, geological repositories are "the only sustainable solution achievable in the near term," IAEA director-general Mohamed ElBaradei told a conference* in Stockholm last month. A recent report† from Harvard University's Managing the Atom Project argues, moreover, that entombing spent-fuel rods is far more cost-effective than reprocessing them to extract fissile material such as plutonium—and that it will remain so for decades. Yet there remains a Catch-22, ElBaradei says: Although public skepticism hampers efforts to build repositories, one or more in successful operation would dramatically boost public confidence.

When the first repositories open, they will become potent symbols with starkly contrasting meanings. In places such as the United States and Russia, a solution to the long-standing dilemma of what to do with highly radioactive waste could breathe new life into an industry suffocated by Three Mile Island and Chornobyl. "We believe there will be a second nuclear era," says Thomas Sanders, manager of the Global Nuclear Future program at Sandia National Laboratories in Albuquerque, New Mexico.

* "International Conference on Geological Repositories: Political and Technical Progress," 7–10 December 2003.

† *The Economics of Reprocessing vs. Direct Disposal of Spent Nuclear Fuel*, December 2003. bcsia.ksg.harvard.edu/BCSIA_content/documents/econ_reprocessing_m_bunn.pdf