Designer Genomes

Life! I've created LIFE!" shrieks the crazed scientist, eyes wild, hair spiking every which way, deep in the throes of megalomania. The scene is recognizable at once as the melodramatic centerpiece of many a late-night sci-fi flick, both good and bad.

What's more incredible is that such a scene may be playing itself out in a real lab sometime soon. The main difference—aside from the fact that most scientists now comb their hair—will be the creature on the table. Rather than a hulking monster made of body parts pilfered from a graveyard and stitched together by some scientist's fawning lackey, the artificial organism will be a bacterium—a microscopic life-form 1,000 times smaller than the smallest grain of sand.

Spurring this revolution is a new kind of recipe book: in the past five years researchers have determined the complete genomes—the exact sequences of the thousands of nucleotide base pairs that make up the DNA—of 24 different organisms, including yeast and the common intestinal bacterium *Escherichia coli*. As they examine and compare these simple genome sequences, investigators are gaining a fuller understanding of the fundamental instructions for life. Many believe the day is not far off when they will be able to design and create entirely new organisms—new life—from scratch.

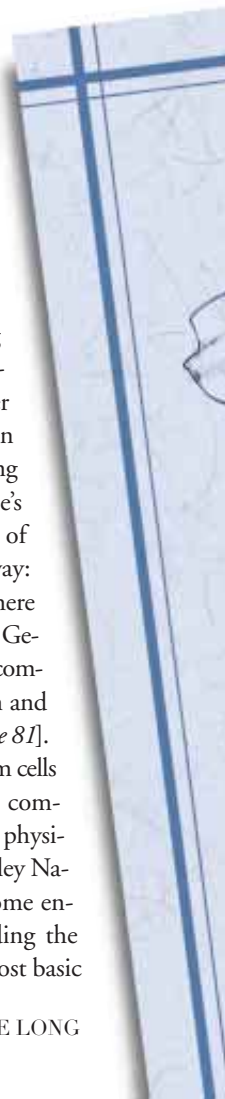
Of course, scientists have been engaged in some form of genetic engineering—introducing single genes into the DNA of microorganisms such as *E. coli*—since the 1970s. They have tweaked bacteria into producing human proteins, engineered corn plants that can make pesticides and grown tobacco plants that clean up mercury from the soil. What makes *genome* engineering different is the scale: researchers are now beginning to outfit microorganisms with new biochemical pathways involving dozens of genes packaged in long stretches of DNA, thereby altering extended segments of the microbes' genomes. Information obtained from the federally sponsored Human Genome Project and other genome-sequencing efforts provides genome engineers with the necessary raw materials—genes and the DNA sequences that control them—as well as a better blueprint of how organisms are put together.

Genome engineering will enable scientists to design microbes that can perform just about any biochemical task—synthesizing increasingly complex molecules or breaking them down. Imagine bugs custom-made to whisk away the "bioorganic halogenated compounds that cover half of New Jersey," says Roger Brent of the Molecular Sciences Institute in Berkeley, Calif.

Engineered microbes may even make molecular electronics a reality, suggests Gerald J. Sussman, a computer scientist and engineer at the Massachusetts Institute of Technology. When computer parts are reduced to the size of single molecules, industrious microbes could be directed to lay down complex electronic circuits. "Bacteria are like little workhorses for nanotechnology; they're wonderful at manipulating things in the chemical and ultramicroscopic worlds," Sussman says. "You could train them to become electricians and plumbers, hire them with sugar and harness them to build structures for you."

How will genome engineers build these marvelous microbial machines? Many will simply modify an existing creature by adding a biochemical pathway cobbled together from other organisms. But even that is a daunting task. Tailoring an existing system to suit one's needs requires quite a bit of knowledge about the pathway: Which steps are slowest? Where are the most likely bottlenecks? Genome engineers are turning to computer modeling to help design and test their systems [*see box on page 81*].

"We want to learn to program cells the same way as we program computers," says Adam P. Arkin, a physical chemist at Lawrence Berkeley National Laboratory. Some genome engineers have started by building the biological equivalent of the most basic



As efforts accelerate to catalogue the lengthy stretches of DNA responsible for life, scientists are getting closer to being able to build living cells from scratch

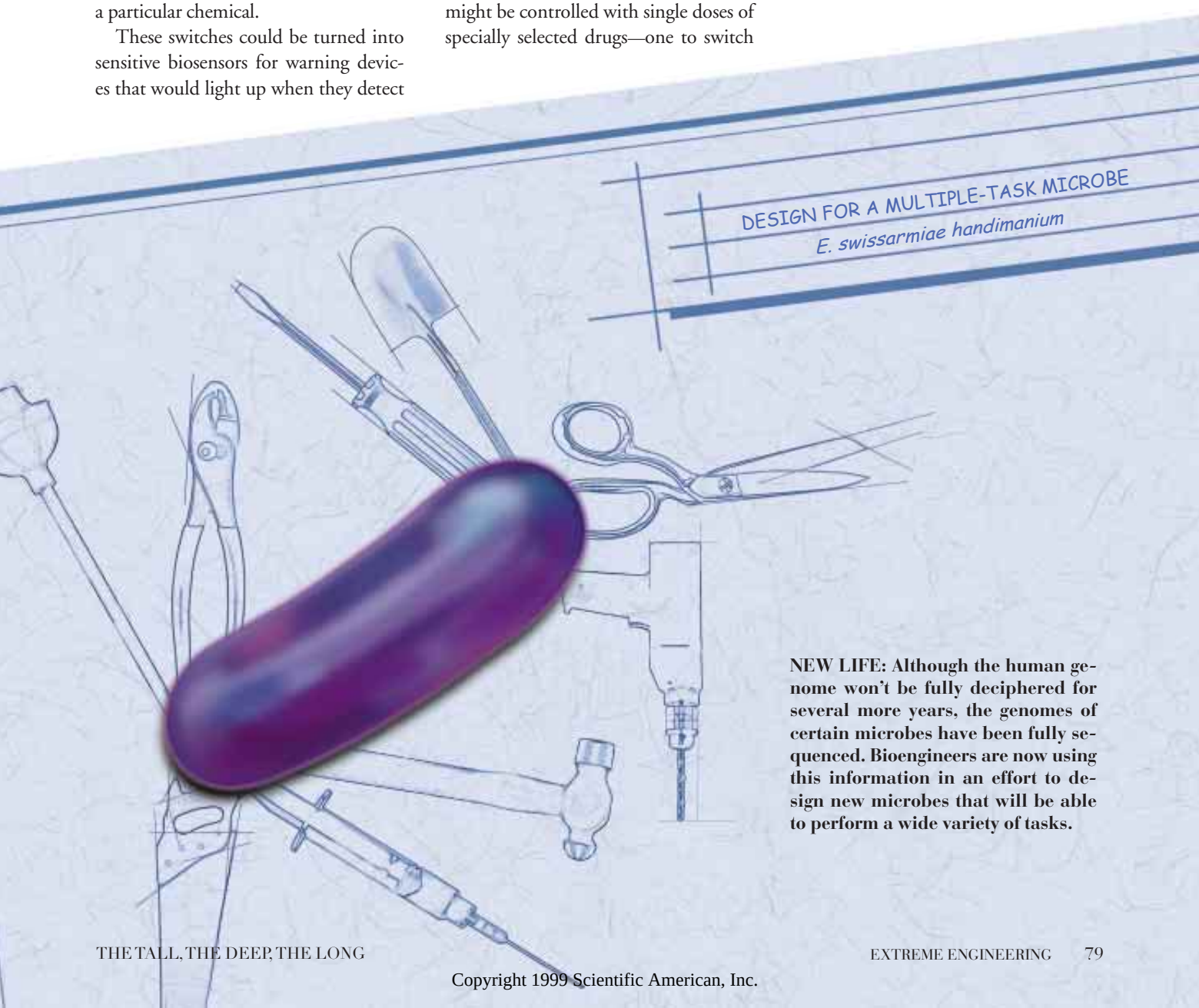
by Karen Hopkin

switch in a computer—a digital flip-flop. Such a cellular toggle switch—made of DNA and some well-characterized regulatory proteins—might be devised to turn on a specific gene when exposed to a particular chemical.

These switches could be turned into sensitive biosensors for warning devices that would light up when they detect bioterrorist weapons such as botulin toxin or anthrax spores, according to James J. Collins, a physicist and bioengineer at Boston University. They could also be used in gene therapy: implanted genes might be controlled with single doses of specially selected drugs—one to switch the gene on, another to switch it off.

"It sounds simple," says Eric Eisenstadt of the Office of Naval Research (ONR), an agency that sponsors such projects. "But believe it or not, it isn't that easy to

LAURIE GRACE



NEW LIFE: Although the human genome won't be fully deciphered for several more years, the genomes of certain microbes have been fully sequenced. Bioengineers are now using this information in an effort to design new microbes that will be able to perform a wide variety of tasks.

do." Selecting the appropriate genes—and configuring them to produce the desired response—is tricky business. Even so, Eisenstadt predicts that such genetic switches will be the "first baby steps" on the way to designing new regulatory pathways and eventually novel organisms.

Genome engineers trying to make such switches at least have a pattern to copy; nature serves as both teacher and supplier. "Cells switch genes on and off all the time," observes M.I.T.'s Thomas F. Knight, Jr., a computer scientist turned bioengineer. By taking advantage of nature's designs, genome engineers are starting off with circuits and components that have been "evolutionarily validated" as parts that work well, Brent adds.

Some researchers are harnessing the powers of evolution even more directly. They are using the principle of natural selection (in this case, survival of the fittest) to generate improved enzymes and perhaps whole organisms. In a process described as DNA shuffling, Willem P. C. Stemmer and his colleagues at Maxygen in Redwood City, Calif., isolate the genes for a particular enzyme from a handful of microbes. They break the genes into fragments and randomly introduce mutations to provide added variety. Then they shuffle and stitch the fragments back together.

By then screening for the mutant enzyme that is the fastest or most stable, investigators wind up with a hybrid that might be thousands of times more efficient than any of its parent enzymes, says Maxygen's Jeremy Minshull. Stemmer and his colleagues plan to apply a similar technique to shuffling not just single genes but whole genomes, which should yield bacteria optimized for whatever properties they desire—the ability to detoxify New Jersey, for example.

Andrew D. Ellington and his associates at the University of Texas use selective pressures to steer bacteria toward something even more unnatural—accepting and using amino acids that do not occur in nature and that are normally poisonous to living organisms. Ellington hopes that these funky bugs, which he calls Un-coli, will perform novel chemical reactions. Such as? "We don't know," he chirps with glee. "That's what makes this fun."

VICTORIA ROBERTS

"Our daughter cell may have my ability to take up inorganic ions, but she's got your wonderful talents at amino acid metabolism."

FUNCTION OF ESSENTIAL GENES	NUMBER OF GENES
TRANSLATION: Assembly of amino acids into a protein, based on the blueprint provided by the sequence of nucleotides in a molecule of messenger RNA	95
ENERGY: Production of enzymes necessary to allow the microbe to extract energy from nutrients such as simple sugars	34
NUCLEOTIDE METABOLISM: Synthesis or recycling of the four chemical bases that make up a strand of DNA or RNA	23
REPLICATION: Creation of a duplicate copy of the bacterial DNA chromosome, without which the microorganism could not reproduce	18
CHAPERONES: Production of molecules that guide, or "chaperone," the correct assembly of newly produced proteins	13
TRANSCRIPTION: Conversion of a strand of DNA into a sequence of RNA, from which a protein could be manufactured	9
RECOMBINATION AND REPAIR: Detection and repair of breaks or errors that can occur in replicating DNA for reproduction	8
COENZYME METABOLISM: Synthesis and use of small-molecule co-factors that help some proteins to perform their tasks	8
EXOPOLYSACCHARIDES: Production of complex sugars that form part of the cell wall or external capsule	8
AMINO ACID METABOLISM: Synthesis or scavenging of the amino acids that are the building blocks of proteins	7
LIPID METABOLISM: Production of lipids that store energy and form the bulk of the cell membrane	6
UPTAKE OF INORGANIC IONS: Production of the channels that permit the cell to respond to changes in its environment and to import salts and metals	5
SECRETION AND RECEPTORS: Synthesis of molecules that enable cells to export proteins and respond to external signals such as the presence of nutrients	5
OTHER CONSERVED PROTEINS: Synthesis of additional proteins or RNAs with essential but as yet unknown functions	18

Adapted from "A Minimal Gene Set for Cellular Life Derived by Comparison of Complete Bacterial Genomes," by Arcady R. Mushegian and Eugene V. Koonin, in *Proceedings of the National Academy of Sciences USA*, Sept. 17, 1996.

BASIC GENES: By comparing the genomes of the microbes *Hemophilus influenzae* (1,700 genes) and *Mycoplasma genitalium* (500 genes), scientists may have determined the 257 genes essential for life, at least for microbes.

Rather than tinkering with existing bacteria, other scientists are talking seriously about building a creature from scratch, the ultimate engineering feat. Their approach is to start small, and several groups of investigators are trying to determine the minimal set of genes necessary for a cell to survive and reproduce.

One way to ascertain which genes are essential for life is to examine those present in microbes that have been fully sequenced and see which ones nature has elected to preserve. Eugene V. Koonin and Arcady R. Mushegian of the Nation-

al Institutes of Health's National Center for Biotechnology Information have done just that. They compared two fully sequenced microbes: *Hemophilus influenzae,* with 1,700 genes, and *Mycoplasma genitalium,* with 500 genes—the smallest bacterial genome sequenced to date.

Koonin and Mushegian conclude that only 250 or so genes are required for life. J. Craig Venter and his colleagues at the Institute for Genomic Research (TIGR)—the team that sequenced *H. influenzae* and *M. genitalium*—venture that it's closer to 300. An organism with these 250 or 300 genes—whatever they are—would be able to perform the dozen or so functions required for life: manufacturing cellular components such as DNA, RNA, proteins and fatty acids; generating energy; repairing damage; transporting salts and other molecules; responding to chemical cues in their environment; and replicating. Although each of these functions requires multiple genes, the whole shebang could be carried in a genome some 300,000 nucleotide bases in length—about half the size of *M. genitalium*'s.

To determine which genes are truly indispensable, some researchers have been deleting them one by one. Venter's TIGR team is knocking genes out of *M. genitalium.* Other groups are performing similar elimination experiments in *E. coli* and yeast. Pharmaceutical companies are using *E. coli* mutants generated by George M. Church of Harvard Medical School to identify new targets for antibiotics—genes that appear to be essential for bacteria but are not found in humans.

Knowing which genes are necessary is one thing, but how do you turn that information into life? Today's DNA synthesizers are not capable of whipping up genome-size chunks of DNA. But researchers are working on techniques for synthesizing large rings of DNA that hold the genes for a single biochemical function—say, all the enzymes necessary to produce ATP, the molecule that cells use for energy. Glen A. Evans and his colleagues at the University of Texas Southwestern Medical Center can churn out DNA 10,000 to 20,000 nucleotide bases in length; they'd like to make pieces 10 times as long.

The Drawing Board of Life

When engineers set out to build digital circuits," observes Roger Brent of the Molecular Sciences Institute in Berkeley, Calif., "they don't touch soldering iron to circuit board until they've modeled the system computationally." Like good engineers, biologists who aim to design new metabolic pathways—or novel organisms—are coming to recognize the value of a good computer simulation.

One such model has already yielded some provocative predictions. The institute's Drew Endy has been perfecting a computer program that simulates the life cycle of T7—a virus that attacks *E. coli.* Endy's program is based on detailed knowledge of T7's biology—such as when and how strongly each of the virus's roughly 50 genes is turned on and which RNA and proteins result. In his model, Endy divided the virus's genome into its individual genes and the DNA elements that control them. He then shuffled all the pieces and asked whether these new viruses could survive in his virtual world. Most didn't do too well: they failed to produce as many progeny as the original parent virus in the same amount of time. But a few did better than the real-world T7, which suggests that the virus's genome may not be configured for optimum reproduction.

Why didn't evolution select the better breeder? According to Endy, the real T7 may work better in varying environments than its computer model cousins. For instance, when Endy makes food scarce in his model, he finds that almost none of the mixed-up viruses fare as well as the T7 that nature engineered, in terms of the number of offspring. "This cries out to be tested experimentally," Brent notes. A new DNA synthesizer should allow Brent and Endy to generate these jumbled genomes and put their predictions to the test. ***—K.H.***

With the proper genes in hand, all that would remain would be for scientists to stuff the pieces of DNA into an empty cell sac—most likely an animal cell from which the nucleus had been removed. The proteins left in the gutted cell, Evans and others hope, would begin making the molecules necessary to jump-start this new form of life.

Of course, producing novel life-forms will raise many concerns, from ecological to ethical. Potential problems have already surfaced in the genetically engineered plants of today. For example, corn that produces its own insecticide may kill harmless bugs (like monarch butterflies). Minimal-genome microbes, however, might not even be able to survive outside the lab. "I doubt this minimal life-form will be lurching around frightening villagers," comments Thomas H. Murray of the Hastings Center for Bioethics.

Then there is the philosophical question: If scientists can actually create life, are they playing God? "People usually raise that point as a way to forestall discussion of the real issues," says David Magnus of the University of Pennsylvania Center for Bioethics. He and his colleagues have been considering the ethical implications of synthesizing cells from the ground up—an event he guesses will grab headlines in the next five years. After two years of contemplation, the group has concluded that the potential benefits of engineering life—which Magnus says include better gene therapy techniques and an enhanced understanding of cell biology—outweigh the possible dangers. But these issues, he asserts, should be addressed by scientists and society.

That discussion had better start soon, because genome engineers are closer than even most scientists realize to making creatures unlike anything ever seen on Earth. What this brave new bioengineered world will look like is hard to say. "But it's going to be awesome," ONR's Eisenstadt predicts. "I mean, it's life." SA

About the Author

KAREN HOPKIN is a recipient of the M.I.T. Knight Science Journalism Fellowship. She has no immediate plans to move to New Jersey.