What would life look like if DNA contained more than four nucleotide bases and proteins more than 20 amino acids? Peter Schultz aims to find out

Creation's Seventh Day

In the casino or in the lab, Peter Schultz loves to take risks. "If I gamble, I usually gamble at high-stakes, high-payoff games," Schultz says. "Science is interesting when it's played at the same level, for the highest stakes with very high risk." For Schultz, a chemist at the Scripps Research Institute and the director of the newly created Genomics Institute of the Novartis Research Foundation (GNF), both in La Jolla, California, that betting system has paid handsomely.

While at his previous home at the University of California (UC), Berkeley, Schultz helped pioneer a fleet of high-speed chemistry techniques to generate molecules by the millions and select the ones that work best as possible catalysts, drug molecules, and even high-temperature superconductors. During the 1990s, he parlayed that experience into a string of start-up companies. Last year he took another big gamble by giving up the comfort of his Berkeley career and financial backing by the Howard Hughes Medical Institute to launch GNF, an outfit Schultz pitches as the "Bell Labs of Biology," which aims to work out the function of the thousands of unknown genes being turned out by the world's genome projects (see sidebar).

Still, his boldest undertaking—and the one that may ultimately have the highest impact—may lie in academic research: With colleagues at Scripps, Schultz is aiming to rewrite the basic chemistry of life. By reengineering DNA, RNA, and the proteins that interact with them, they hope to create synthetic organisms with a chemical makeup fundamentally different from all life that has existed on Earth for the last 3.8 billion years.

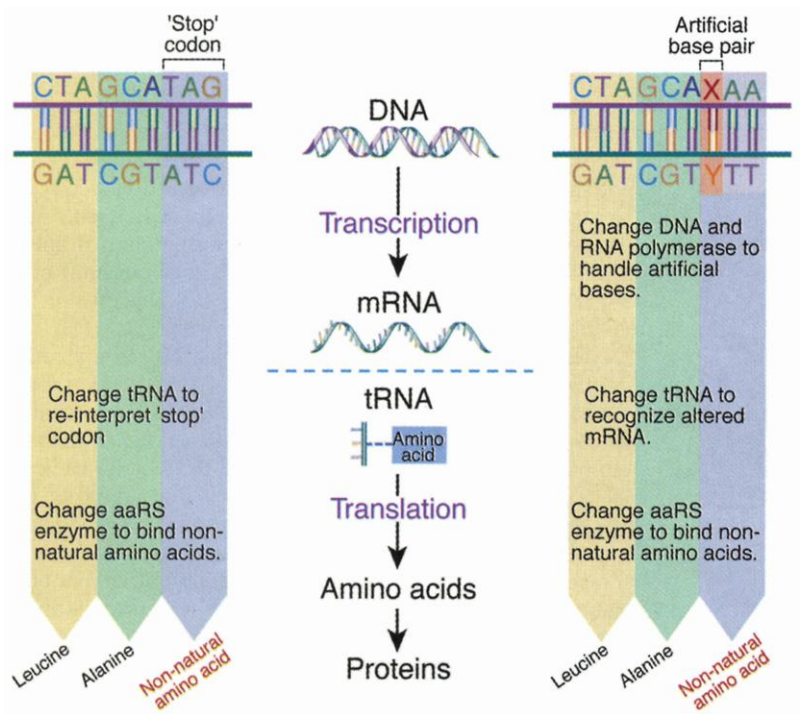
If they succeed, their biochemical reengineering could have a profound effect on everything from basic molecular biology to industrial chemistry. The result—they hope—will be proteins that incorporate amino acids other than the 20 commonly used by life to construct proteins. By adding these amino acids with completely new types of chemical behaviors, Schultz and his colleagues hope to design bacteria to make proteins that work as novel catalysts and drugs, or that carry built-in tracers to help researchers decipher their structures. "The thrust of the work is to expand in a radical way genetic diversity," says geneticist Steven Briggs, who runs the Novartis Agricultural Discovery Institute, a sister organization to GNF. "If Pete can get this to work—and I'm sure he will—it will give us a much bigger toolbox to create medicines and other things to benefit society."

It could also open a new window on evolution, allowing researchers to explore alternative paths life on Earth may have taken in its infancy and the shape it might take elsewhere in the galaxy. Synthetic life, says Steven Benner, another pioneer in the field from the University of Florida, Gainesville, allows researchers to explore for the first time whether an alternative chemistry of life is truly viable. As Schultz puts it, "If God had worked a seventh day, what would life look like today?"

As scientists, government regulators, and environmentalists squabble over genetically engineering natural DNA and proteins, adding synthetic components to the mix may be asking for trouble. If synthetic organisms do come to pass, researchers will undoubtedly encounter fears that biotechnology's latest twist could lead to new types of superpathogens that will wreak havoc on other forms of life. "I used to joke with members of the [Schultz] group that we would know the project was complete when we saw people protesting outside the window," says David Liu, a former Schultz grad student, who has since gone on to set up his own research group at Harvard.

At present, such protests remain hypothetical. The biggest obstacle Schultz and his colleagues now face is that creating synthetic life, as Stanford University chemist Eric Kool says, "is a very, very hard problem." Getting all the pieces to work "means reengineering 3.5 billion years of evolution," notes Kevan Shokat, a chemist at the University of San Francisco and a former group member in the Schultz lab at Berkeley. If the attempt to make synthetic life forms has one flaw, Benner adds, "it's that it's so ambitious."

That suits Schultz just fine. In fact, the sheer scale of the task may give him an edge over the sparse competition. Coaxing bacteria



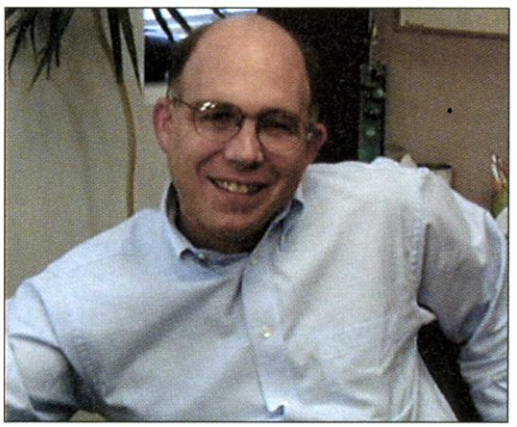
Two paths. Chemists are pursuing parallel approaches to making cells produce nonnatural proteins. In one (left), transfer RNA reinterprets a "stop" codon as a command to insert a nonnatural amino acid. In the other (right), the cue comes from synthetic DNA itself.

ILLUSTRATION: C. CAIN

Tackling Biology With No Holds Barred, at 800 Miles Per Hour

Engineering synthetic bacteria that harbor novel types of DNA, RNA, and proteins isn't a straightforward scientific challenge. It's more the scientific version of a Russian doll, with challenges nested inside problems. While most researchers are comfortable isolating and breaking down one problem at a time, synthetic life is just the sort of puzzle that Peter Schultz thrives on. "I'm not really interested in doing experiments that if somebody picks up the journal and they read it, they forget about it," he says. "I'd rather work on something very hard that may have a high chance of failure. But if it's successful, it has a high impact."

So far, that strategy has served Schultz well. He launched his academic career at the University of California, Berkeley, in the mid 1980s and immediately turned his group toward exploring what made living organisms such powerful synthetic chemists. After studying how the immune system manages to generate antibodies against a limitless variety of unknown targets, Schultz decided that the key to nature's success was its strategy of generating millions of possible chemical solutions to a problem and then screening for the ones that worked best. "We looked at this and said, 'Can we take this strategy and apply it not to what nature did, which is molecular receptors, but to something else?' And the first thing we thought of doing was to make catalysts," Schultz says.



Hyperkinetic. Peter Schultz is bringing rapid-fire methods of combinatorial chemistry to biology.

Schultz and his colleagues set out to evolve antibodies in mice toward new targets, in this case to become catalysts for particular chemical reactions such as a widely used synthetic step called the Diels-Alder reaction. By following nature's lead, they were able to evolve antibodies with unique catalytic abilities and launch a new field of antibody catalysis. As catalysts, antibodies have never managed to outdo enzymes, their protein counterparts that have evolved for the task. Nevertheless, the idea of chemists imitating biology's use of diversity to find new functional molecules was a wake-up call to the chemistry community, and it has become a hallmark of Schultz's scientific approach.

This build-first-test-later philosophy was "pretty heretical" among synthetic chemists, who prided themselves on making just the molecules they designed, says Doug Livingston, a chemist at the Genomics Institute of the Novartis Research Foundation (GNF). The fact that the catalytic antibody experiment worked at all was enough to convince Schultz and drug-delivery entrepreneur Alejandro Zaffaroni to try the same mass synthesize-and-screen approach to finding potential new drugs. In 1988, they launched Affymax, one of the first in what has become a sea of combinatorial drug discovery companies. The strategy proved so successful that it swept through the pharmaceutical industry during the late 1980s and early 1990s and prompted Glaxo Wellcome to buy Affymax a mere 7 years after its inception for $533 million.

Schultz was just warming up. In 1994, working with researchers at UC Berkeley and the Lawrence Berkeley National Laboratory, Schultz showed how the same strategy of generating diversity and then selecting the best could work for materials science. The researchers created a novel library of 128 high-temperature superconductors. That same year, Zaffaroni and Schultz were at it again, this time creating a combinatorial materials company called Symyx Technologies, which has gone on to rack up over $90 million worth of research deals with materials powerhouses such as Dow, BASF, and Unilever to search for novel plastics, catalysts, and even materials for electronic devices en masse.

That flair for combining function with speed was just what leaders at Novartis had in mind when they tapped Schultz last year to create what they hoped would be a new approach to biological research at GNF. That approach, says Schultz, is to go beyond biology's traditional focus on single genes, proteins, or biochemical pathways and use the latest in computer and robotics technology to scan thousands of biochemical targets at once to understand how they work in unison. To do this, he says, requires a delicate marriage between technology and small groups of researchers focused on cutting-edge problems. "The best model for that in recent history is the old Bell Labs," which was a dominant force in physics and materials research for much of the 20th century, says Schultz. "My view is it's time to do this in biology."

Doing that means acquiring a wide array of technology. As a result, Schultz and GNF aim to bring together all the available high-speed tools, such as gene chips for determining the suite of genes active in normal and diseased tissues, high-speed mass spectrometers that are essential for identifying proteins, high-speed robotics for synthesizing compounds and crystallizing large numbers of proteins for rapid structural determination, and powerful computers to make sense of all the data. "A lot of these things can be done in any lab in the world," says Michael Cooke, a cell biologist at GNF. "But in very few places is it done in one [location]." Adds GNF neuroscientist Allen Fienberg, "It's a combinatorial chemist's view of biology. That's kind of [Schultz's] view on the whole place. Nobody else really thinks that way."

Or matches his frenetic pace. Schultz is usually at the office by 5 a.m. and stays on into the evening, dashing between meetings with a Diet Coke in hand, offering quick thoughts on new experiments, ever excited about the results. "You have a crazy idea, you're above the clouds. He pushes you to extend it. He takes you to Mars," says Nada·Zein, a chemist at GNF. Adds Fienberg: "Here's a guy who runs at 800 miles per hour. You have a conversation and he's three thoughts ahead of you. You start to say something and he answers your question saying, 'I know what you're going to say.' "

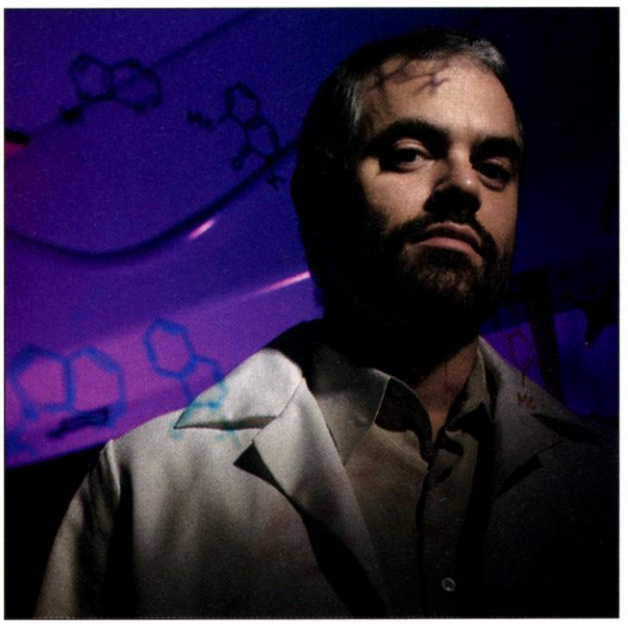
What nobody yet knows is whether the GNF experiment will succeed. The nonprofit research institute was launched 2 years ago with a promise of $250 million over 10 years from the Novartis Research Foundation—a philanthropic arm of the pharmaceutical giant. That number looks sure to swell as GNF plans to spend up to $35 million this year alone and just broke ground on a new $83 million campus a stone's throw from its current temporary site. "We're still in the honeymoon period," says Nicholas Gehakis, a GNF molecular biologist. "Everyone is struggling hard to live up to ... expectations." But the clock is ticking. Says GNF robotics engineer Bob Downs: "If we don't perform they'd shut us down, and we'd deserve it."

–R.F.S.

CREDIT: ANDY MARTIN

to work with new nucleotides and amino acids requires expertise in molecular biology and genetics, along with physical, synthetic, and combinatorial chemistry. Not many research labs bring together all those specialties. But with roughly 40 members spanning an ever-changing array of disciplines, Schultz's lab does. "I think if anyone can do it, Pete Schultz's lab is the place where it can get done," says Christopher Switzer, a chemist at UC Riverside who has also worked to add new nucleotide bases to DNA.

What makes Schultz's goal conceivable is the basic simplicity of life itself. For all their diversity of form, all living organisms make use of the same fundamental chemical machinery: DNA and RNA to store genetic information that encodes for proteins, which carry out vital cellular chemical reactions. All DNA and RNA is made up of four nucleotide bases. All proteins draw on the same 20 amino acids (a 21st, selenocistine, crops up in exceptional cases).



Radical approach. Floyd Romesberg's lab aims to overhaul the cell's protein-making machinery from DNA on down.

Nature and synthetic chemists, however, are capable of making hundreds of amino acids that play no part in the makeup of living creatures. In the mid-1980s, Schultz began to wonder whether it was possible to incorporate nonnatural amino acids into the chemistry of life. Protein chemists had developed machines capable of synthesizing short proteins out of both natural and nonnatural amino acids. But coaxing cells to do the same thing would be vastly more difficult. Billions of years of evolution had honed their machinery to convert DNA into proteins by a strict series of steps. The machinery first turns DNA into messenger RNA (mRNA), which leaves the nucleus and travels to ribosomal protein factories in the cytoplasm. In the ribosomes, the mRNA forms a template onto which short molecules of transfer RNA (tRNA) can ferry in amino acids and link them into the sequence of a protein. At each step, protein-based machines transcribe one code to the next: RNA polymerase converts DNA to mRNA; aminoacyl-tRNA synthetases link amino acids onto tRNA molecules; and after those tRNAs link up with their mRNA counterparts, the ribosomes assemble amino acid cargo on the tRNAs into proteins.

To create proteins containing nonnatural amino acids meant tweaking those protein-based machines to get them to work with amino acids that billions of years of evolution had trained them to avoid. Rather than reengineer the entire protein synthesis apparatus at once from DNA onward, Schultz's team opted to climb one mountain at a time. For starters, 11 years ago they came up with a test tube–based method to trick the protein assembly apparatus of the bacterium *Escherichia coli* into accepting nonnatural amino acids.

To do so, they needed to hijack a normal DNA signal and persuade the bacterium to read it as a command to insert a nonnatural amino acid. The signals in DNA come in the form of triplets of nucleotide letters in genes. DNA's four nucleotide letters—A, C, G, and T—can occur in 64 different combinations of three: ATC, ATA, and so on. Because these 64 triplets need only code for the insertion of 20 amino acids, different combinations sometimes code for the same thing. Both TTA and TTG, for example, code for the amino acid leucine. Similarly, three different DNA trios, or codons, serve as the "stop" signs that signal the ribosome to stop adding amino acids to a protein. When the cellular machinery transcribes DNA into mRNA, the letters change, but the stop signals remain in place. Schultz's team modified a certain type of tRNA to recognize one of those mRNA stop signs and insert an nonnatural amino acid into a growing protein when it did.

Using that system, the Schultz team has added more than 80 different nonnatural amino acids to proteins. But the method has big drawbacks. One is that it uses synthetic chemistry to attach the nonnatural amino acids to the tRNA molecules that recognize the stop codons—an expensive, time-consuming procedure. Once the complexes are synthesized, the researchers simply add them to a mix of cellular components in a test tube and hope that some of the cellular machinery can incorporate them into proteins. But this hit-or-miss approach is inefficient, and very little of the protein with nonnatural amino acids winds up being made. Says Liu: "Translating a protein in vitro is not a high yielding process."

It would be far more efficient if all that work were done inside a living cell. "What we really want to do is build an organism—a living organism—where you can add a 21st amino acid to the growth medium and it takes up that amino acid and puts it selectively into a protein," says Schultz. Schultz and his collaborators are working on two separate tracks to the problem, at least one of which could hit the jackpot sometime in the next year, Schultz believes.

The group's main effort in creating a synthetic organism builds on the earlier success with stop codons in *E. coli*. Instead of linking the amino acids to the tRNAs themselves, the researchers are trying to adapt the cells' natural machinery to do that job. That machinery in this case is a set of proteins known as aminoacyl tRNA synthetases (aaRSs). An aaRS is a two-part molecule. One end recognizes a particular triplet sequence in tRNA, and the other end binds to the appropriate amino acid. Aminoacyl tRNA synthetases serve as the go-betweens that connect the genetic information in DNA and RNA to the chemistry of proteins.

To coax aaRSs into handling amino acids different from the ones they've evolved to work with, Schultz and colleagues systematically change the chemical structure of the enzymes and then test whether they will grab hold of nonnatural amino acids and insert them when they see the mRNA signal for their preselected stop codon. At a combinatorial chemistry meeting last April in Tucson, Arizona, Schultz reported that his team has achieved some success in this effort. The machinery is inefficient: So far it performs its task only about 1% of the time it is signaled to do so. Still, Schultz says, "we've got our foot in the door. I think it's no longer a question of will it work, but how long it will take."

Paul Schimmel, an expert on tRNA synthetases at Scripps who does not work with the Schultz group on their project, is more skeptical. Aminoacyl tRNA synthetases, Schimmel points out, also play an important role in editing out mistakes in the sequence of amino acids. So even if nonnatural amino

acids initially get attached to a tRNA, they may get plucked off during the editing process. "It's hard to imagine they won't have problems" with this, he says.

Meanwhile, a more radical approach to reengineering life's chemistry is progressing rapidly as well. For this work, Schultz has teamed up with the lab of chemist Floyd Romesberg of Scripps, who worked as a postdoc for Schultz at Berkeley before moving to Scripps to start his own lab in mid-1998. Instead of using DNA's stop signals as a message to insert nonnatural amino acids, Romesberg's team writes all-new messages by expanding the number of letters in DNA.

The advantage of this strategy, Romesberg says, is that it gets around the limitations of the use of stop codons. A stop codon can code for only one nonnatural amino acid at a time, and because stop signs are scattered throughout the genome, nonnatural amino acids could wind up being inserted where they are not wanted. By adding new letters to DNA, researchers could write a whole new set of codons, encoding for novel amino acids wherever they wanted them in the genome. (Schultz's group, meanwhile, is trying to achieve the same goals by creating codons for nonnatural amino acids that are four bases long instead of three.)

The price of that flexibility is complexity. In addition to coming up with the novel DNA bases, the researchers must reengineer the proteins that copy DNA and transcribe it to RNA—enzymes known as DNA and RNA polymerases—to work with these new bases. Again, part of the task is well in hand. At the American Chemical Society meeting in San Francisco in April, Romesberg and Yiqin Wu, a postdoc supported by both the Romesberg and Schultz labs, reported that they had come up with a new DNA base that they can add to the DNA chain. This base, called 7-propynyl isocarbostyril, or "PICS," pairs with itself, forming a new rung in the DNA ladder alongside pairs of A and T as well as G and C. The Romesberg-Schultz team is not the first to have inserted new letters into DNA. But previous nonnatural bases had a way of prompting the natural bases in DNA to pair incorrectly. The double PICS base pairs, Romesberg says, don't prompt such mispairings. Romesberg's lab also reported that researchers there have isolated a DNA polymerase that can copy a single strand of DNA containing the novel code, forming the standard double-stranded DNA—a key step toward creating cells that can pass down the novel changes in their genetic code.

Next the researchers will attempt to carry out the same sleight of hand with RNA polymerase, which converts the DNA strand into RNA. Because RNA polymerases are nearly identical with DNA polymerases, Romesberg is confident that this challenge will fall quickly. Finally, the researchers will then have to find tRNAs and aaRSs to recognize the novel bases and insert the nonnatural amino acids. But here again, because Schultz's lab has already paved that road, the team members are confident that they can get all the pieces to work together.

If synthetic life does indeed materialize one day soon in a petri dish in the hills north of San Diego, Schultz expects it will quickly attract interest from scientists conducting basic research into the behavior of proteins. Researchers could add fluorescent amino acid tags to proteins to signal their location in cells, thereby providing clues to their function. They could also insert amino acids bearing heavy atoms that can be used to help protein crystallographers work out the three-dimensional structure of proteins.

Beyond these tools for understanding proteins, Schultz believes researchers will be eager to outfit proteins with new functional groups, such as a versatile one known as a ketone, that will serve as hooks for organic chemists to add new chemical functions to proteins. Such souped-up proteins could serve as better drugs and improved enzymes for industry. "This is a gold mine for chemists," Schultz says. "There are so many things you can think about doing."

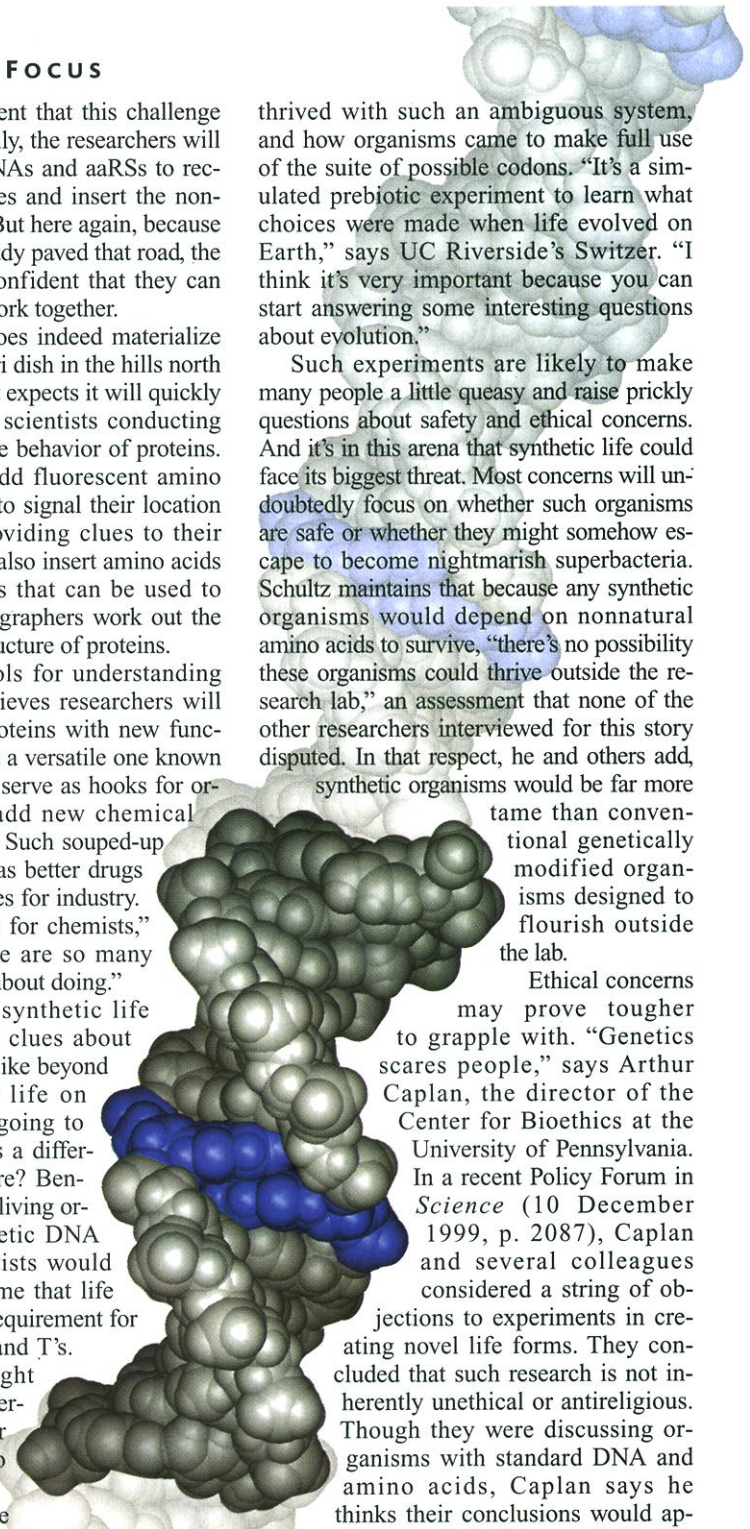
If nothing else, synthetic life should provide new clues about what life might look like beyond Earth. "If you see life on Mars, how are you going to recognize it" if it has a different chemical structure? Benner asks. By creating living organisms with synthetic DNA and proteins, scientists would know for the first time that life has no fundamental requirement for using A's, G's, C's, and T's. That realization might set the stage for understanding still deeper patterns common to life everywhere.

Synthetic life could also provide novel insights into life's distant past on this planet, says Schimmel. For example, he says, today's organisms have evolved to have tRNAs to recognize each of DNA's 64 separate codons. Early in life's history, however, individual tRNAs probably have worked with more than one codon each. By reengineering tRNAs to carry out new functions, Schimmel says, researchers may be able to explore how early organisms could have thrived with such an ambiguous system, and how organisms came to make full use of the suite of possible codons. "It's a simulated prebiotic experiment to learn what choices were made when life evolved on Earth," says UC Riverside's Switzer. "I think it's very important because you can start answering some interesting questions about evolution."

Such experiments are likely to make many people a little queasy and raise prickly questions about safety and ethical concerns. And it's in this arena that synthetic life could face its biggest threat. Most concerns will undoubtedly focus on whether such organisms are safe or whether they might somehow escape to become nightmarish superbacteria. Schultz maintains that because any synthetic organisms would depend on nonnatural amino acids to survive, "there's no possibility these organisms could thrive outside the research lab," an assessment that none of the other researchers interviewed for this story disputed. In that respect, he and others add, synthetic organisms would be far more tame than conventional genetically modified organisms designed to flourish outside the lab.

Ethical concerns may prove tougher to grapple with. "Genetics scares people," says Arthur Caplan, the director of the Center for Bioethics at the University of Pennsylvania. In a recent Policy Forum in *Science* (10 December 1999, p. 2087), Caplan and several colleagues considered a string of objections to experiments in creating novel life forms. They concluded that such research is not inherently unethical or antireligious. Though they were discussing organisms with standard DNA and amino acids, Caplan says he thinks their conclusions would apply to synthetic life as well. "At the end of the day, I don't see any fundamental amorality to making synthetic DNA to regulate a synthetic life-form."

All the same, Romesberg is bracing himself for controversy ahead. "There are going to be people who don't like this," he says. "New ideas are often scary until you demonstrate something good that comes of it." But if Schultz's high-stakes reengineering project works out, demonstrating a useful payoff will be the easy part. **–ROBERT F. SERVICE**



Alien zone. Blue band in this computer-generated DNA model shows where chemists inserted nucleotides not found in nature.

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