Life Redesigned To Suit the Engineering Crowd

Evolution is not fast or efficient enough for engineers who plan to move blocks of genes around as routinely as they do electronic parts

Marcia Stone

Engineers known as biosynthesists are leading a revolution in molecular biology. Instead of old-fashioned genetic engineering—last generation's revolution—where one gene at a time is moved between microbial species, these engineers have far more radical plans.

At this early stage, for instance, they are mixing genes from several different organisms to build whole new metabolic pathways and novel microbes. Some biosynthesists even expect to rewrite the genetic code altogether, designing creatures that span the divide between nature and machine.

Such issues were the focus during the Second International conference on Synthetic Biology last May at the University of California (UC) Berkeley. Hosted by Jay Keasling of UCB and Graham Fleming from the nearby Lawrence Berkeley National Laboratory, the meeting brought together a diverse group of engineers and scientists from a variety of disciplines, including bioengineering, biochemistry, quantitative biology, biophysics, microbiology, molecular and cellular biology, bioethics, and the biotechnology industry (http://pbd.lbl.gov/sb-conf/agenda.php).

Part of the meeting was devoted to nontechnical issues such as biological safety, ownership, innovation, and perception of the field by nonscientists. Participants also reviewed the SB2 Declaration, an important step towards self-regulation, that is available for comment online (http://openwetware.org/wiki/Synthetic_Biology/SB2Declaration).

Marcia Stone is a science writer based in New York City. More of her work can be seen on http://www.mstoneworks.net.

Genetic Parts—As Lego Sets and Tinker Toys

Engineers at the Massachusetts Institute of Technology (MIT) in Cambridge, Mass., began doing audacious things with genes years ago. Drew Endy, one of synthetic biology's most creative thinkers, developed a set of standardized genetic parts called "BioBricks," based on an idea by Tom Knight, a colleague at MIT. BioBricks consist of short pieces of DNA that can be attached to one another via universal connectors—much like pieces in a Lego or Tinker Toy set. Or, as engineers might put it, they are analogous to transistors, capacitors,

Summary

- Engineers called biosynthesists are reassembling sets of genes to fashion novel metabolic pathways, build new microbes and even rewrite the genetic code.
- Recent feats of genetic manipulation include the development of standardized genetic parts called "BioBricks," and microbes containing a mix of genes enabling them to make a chemical precursor of the anti-malarial drug, artemisinin.
- Biosynthesists are also encoding microbes to produce proteins built from unnatural amino acids, designing bacteria to produce hydrogen-based alternative fuels, and yeasts that make ethanol from cellulose.
- Recognizing that synthetic biology is not risk free, biosynthesists drafted the SB2 Declaration, suggesting ways to monitor and regulate this new field.

and resistors used to build electronic circuits. In this case, however, biologists and engineers are using BioBricks to build logically configured synthetic microorganisms (http://openwetware.org/wiki/BioBricks).

"Evolution gives rise to complicated systems that are difficult to understand and manipulate," Endy says. "Some parts overlap and are unable to function independently of one another. Others have lost their function somewhere along the way, but can't be removed and simply clutter things up. Furthermore, there is no apparent organization or hierarchy to most naturally evolved genetic systems."

Setting aside the slow pace and randomness of evolution, engineers figure that they can intrude and fix things that do not seem right to them. Thus, Endy and his colleagues "fixed" the genome in a simple biological entity—the lytic phage T7 that ordinarily infects *Escherichia coli* — by stripping nonessential and overlapping genetic elements from the phage and replacing them with engineered DNA. The approach was inspired by a practice known as "refactoring" that engineers use to improve the design of computer software. They consider the newly refactored viral genome—designated T7.1, as if the first in a series of software products being released—to be easier to study and manipulate than its natural ancestor, T7. The next phage in this lineage, T7.2, will be even more streamlined.

Endy notes that the ability to "write" fragments of synthetic DNA and piece them together will directly accelerate the engineering of biology, but he emphasizes that traditional biology and the engineering-driven field of synthetic biology are entirely different and complementary. "Our goal as engineers is to build many-component living organisms—microbes or otherwise—that behave as expected, as we want them to behave."

To achieve this end, Endy helped establish a registry of standard biological parts (http://parts.mit.edu). Researchers can go in, find the DNA segments they need, hook them up, and, at least in theory, produce bioengineered cells that do exactly what they are designed to do.

The availability of such "off-the-shelf" genetic parts is a major step in the hugely ambitious plans of this new breed of genetic engineers, who are attempting to build cells that can detect and clean up chemical weapons or environmental pollutants, diagnose and fix disease, manufacture drugs, and make hydrogen-based fuel from water and sunlight.

The Synthetic Hierarchy

Synthetic elements → Networks → Organisms → Systems

Elements: fundamental building blocks that provide primitive functionality (analogous to switches, oscillators, flip-flops, etc. in the electronics world).

Networks: interacting synthetic elements (regulatory networks of synthetic genes and promoters designed to induce transcription when triggered by external stimuli).

Organisms: living machines assembled from synthetic elements.

Systems: multiple genetic machines working synchronously to achieve a complex goal.

(From A. Bhutkar, Synthetic biology: navigating the challenges ahead. J. Biolaw Business **8**:19–29, 2005)

One Early Goal Is Producing Medicinals in Synthetic Microbial Constructs

Keasling, professor of chemical engineering and bioengineering at Berkeley, agrees that standardized, well-characterized parts are crucial for biosynthesis efforts on an economically viable scale. Backed by the Gates Foundation, he is using synthetic biology to develop an alternative means of synthesizing the antimalarial drug artemisinin (*ASM News*, April 2005, p. 162).

Making artemisinin according to standard chemistry is impractical because it takes so many steps. Hence, Keasling captured genes from the wormwood shrub, *Artemisia annua*, and inserted them into bacteria and yeast, enabling them to manufacture artemisinic acid, which chemists can readily convert into artemisinin. However, instead of inserting the genes the old fashioned way, one at a time, Keasling mixed about a dozen microbial and wormwood genes plus several expression-control elements to make a specialized metabolic unit.

Once this prototype system is up and running, large-scale fermentation will be used to produce industrial quantities of the artemisinin precursor. Keasling anticipates that artemisinin made in this way will be sold for 1/10 of the current

price, driving it down to about $0.20 per dose, which is about the same as the cost of chloroquine—the frontline antimalarial treatment that is becoming obsolete as *Plasmodium* species become increasingly resistant to it.

Making artemisinin mainly inside microbes will not only be relatively inexpensive, it also promises to be more environmentally friendly, according to Keasling. "Traditional synthetic chemistry inherently produces toxic waste products," he says. "In addition, extraction from plants risks environmental destruction, especially if it requires the harvesting of rare plants from the wild. Putting chemical factories inside of living cells helps avoid both of these problems."

This summer Keasling, UC Berkeley, and synthetic biology in general received a big boost from the National Science Foundation in the form of a five-year, $16-million dollar grant to establish a Synthetic Biology Engineering Research Center, known as SynBERC. Participating facilities include UC San Francisco, where much of the pioneering work with recombinant DNA was done in the 1970s; along with MIT, Harvard, Prairie View A&M University in Texas, and the California Institute for Quantitative Research, a cooperative effort among three campuses of the University of California and private industry.

Researchers working under the SynBERC umbrella, says Keasling, will initially focus on developing interchangeable genetic parts that can be mixed and matched among microbes. He envisions a day when biologists will be able to concentrate on difficult science and leave engineering matters and production to others (www.synberc.org).

Another Goal Entails Expanding the Genetic Code

Peter G. Schultz and Lei Wang at the Scripps Research Institute and Salk Institute for Biological Studies, in La Jolla, Calif., respectively, are engineering microbes to encode unnatural amino acids. "While a 20-amino-acid code may be sufficient for life, it is by no means ideal," Wang says. So far, Schultz and Wang have incorporated more than 30 unnatural amino acids into the protein-generating repertoire of *E. coli* by using unique codons and corresponding tRNA/aminoacyl-tRNA synthetase pairs. "The approach has proven remarkably effective, allowing us to add a large number of structurally diverse amino acids into both prokaryotic and eukaryotic organisms," Wang says.

This phenomenon occurs in nature, albeit rarely. For example, *Methanosarcina barkeri* employs an amber suppressor tRNA/synthetase pair to incorporate the amino acid pyrrolysine into proteins. This maneuver has not been lost on Wang and Schultz, who use archaeal tRNA/synthetase pairs in their engineered series of *E. coli* strains. The archaeal synthetases are expressed more efficiently in *E. coli* than are counterpart synthetases from eukaryotic cells.

"The ability to incorporate unnatural amino acids into proteins directly in live cells offers numerous advantages over the chemical and biosynthetic strategies we now employ, including higher yields as well as greater fidelity and technical ease," says Wang. Among other things, the technique could make it easier to design glycosylated and PEGylated proteins for use as therapeutics. "From the purely experimental perspective, we'll be able to determine if genetically enhanced microbes, those able to encode more than just the 20 amino acids evolution dealt them, have a selective advantage over their natural counterparts," he points out (http://schultz.scripps.edu/ and http://cbpl.salk.edu/~wang/).

Fishing in the Sargasso Sea for Exotic Genes with Novel Uses

Genome-sequencing maverick J. Craig Venter wants to use synthetic biology approaches to do more than design unusual metabolic pathways: He plans to build an entirely new bacterium, one that can generate enough hydrogen to meet future fuel needs. Venter, who is president of the Institute for Biological Energy Alternatives (IBEA) in Rockville, Maryland, claims that he needs two things to accomplish this larger goal: the right microbe to serve in stripped-down fashion as host and the right genes to insert into vacant spaces in its genome.

Earlier, the Synthetic Biology Group at the J. Craig Venter Institute, led by Hamilton O. Smith, chose the bacterium *Mycoplasma genitalium* as its starter host. At least until recently, this bacterium was considered to have the smallest genome of any organism that can be grown in culture (however, see p. 551). Not only is its genome relatively easy to fiddle with; should any of the modified progeny escape the fermentor, they would have a difficult time surviving the

microbe-eat-microbe world outside. For one thing, this bacterium and other *Mollicutes* have no cell walls to protect them. For another, they live in relatively unchanging niches and have little adaptive capability.

The search for appropriate genes to introduce into this modified bacterium—fondly renamed "*Mycoplasma laboratorium*"—took Venter and his scientific crew onto open waters. "There are an abundance of novel genes around the Earth and in its oceans just waiting to be discovered," says Venter, who launched the Sorcerer II Expedition in 2004 to find some of them. Since then, researchers aboard Sorcerer II have been roaming the globe, collecting microbial genes.

Whole-genome shotgun sequencing, a technology developed by Venter at Celera Genomics and traditionally used to identify genomic sequences from one organism at a time, was adapted to analyze DNA from microbial populations collected en masse during the first major stop in the Sargasso Sea. About six million previously unknown genes have been identified so far, including 20,000 with the potential to encode hydrogen-based alternative fuels, according to Venter.

Additionally, the Sorcerer II scientists identified several previously unrecognized bacterial versions of rhodopsin, which, in vertebrates, transduces light energy from retinal cells into the brain. The bacteria of the Sargasso Sea probably use bacteriorhodopsin proteins to monitor light and gauge ocean depths, according to Venter, who is interested in harnessing the energy-conversion potential that rhodopsin proteins provide (see http://www.venterinstitute.org and http://genomicsgtl.energy.gov/biofuels/).

Synthetic Biology for Making Fuels, Touchy-Feely Bacteria, and Proper Signaling

Besides being drinkable, ethanol that is produced by sugar-fermenting *Saccharomyces* yeast is a widely used liquid fuel for propelling vehicles. However, because yeasts produce ethanol from simple sugars, but cannot digest the cellulose, hemicellulose, and other structural molecules that form plant skeletons, there is a great deal of waste when plant material is used as such fuel sources.

But other microbes *can* digest cellulose. So Nancy Ho at Purdue University in West Lafayette, Ind., and her collaborators cloned three of their xylose-metabolizing genes into *Saccharomyces*, enabling it to convert plant matter such as corn stalks, tree leaves, wood chips, and even cardboard boxes into ethanol. Importantly, unlike fossil fuels, burning cellulosic ethanol does not make a net contribution to global warming (https://engineering.purdue.edu/Engr/Research/Focus/LaboratoryofRenewableResourcesEngineeringRunningonE).

Chris Voigt at UC San Francisco is endowing a number of bacteria with sensory capabilities expected to prove useful for pharmaceutical and industrial purposes. Many bacteria respond to their environments with membrane-bound sensors that interact with intracellular response regulators to control gene expression. "These systems are remarkably modular, and new sensors can be built in using recombination to swap protein domains," he says. His goal is to reprogram bacteria to perform complex, coordinated tasks that suit industrial rather than purely microbial end uses (http://www.voigtlab.ucsf.edu).

Living cells exhibit complex signaling behaviors when they process environmental information, and these behaviors are typically mediated by highly specialized protein networks. Voigt's colleagues at UCSF, Wendell Lim and Brian Yeh, are examining how the remarkably diverse array of eukaryotic signaling circuits are organized. They find that these systems are similarly highly modular, noting "that modular regulation can and should be exploited." Plans call for engineering nonnatural signaling proteins and pathways with useful novel behaviors (http://www.ucsf.edu/limlab/people/wendell.html).

"Syn" Biology Is Not Considered Risk Free

As with any technology, synthetic biology is not risk free. And, like its predecessor recombinant-DNA technology, there is a chance that mistakes can reproduce and amplify. Thus, for example, a manufactured microbe could escape from a lab and cause havoc. Perhaps even more worrisome, a malicious biohacker or bioterrorist might recreate a "select agent," such as a pathogen that is dangerous directly to humans or to agriculture, and thereby circumvent legal and physical controls over its use. The scientists who are working in synthetic biology understand that

they need to deal with such safety, ethical, and environmental concerns if their work is to continue. Indeed, researchers at MIT, the J. Craig Venter Institute, and the Center for Strategic and International Studies in Washington, D.C., are already evaluating ways of keeping new life forms under strict control.

To address broader issues, participants at SB2 evaluated a document calling for self-regulatory constraints, using as their model the Asilomar conference of 1975—when biologists called for similar constraints on early recombinant DNA-based research. Companies that can synthesize long stretches of DNA are prime targets of the SB2 Declaration. Engineers and scientists who drafted the declaration cite a recent investigative survey revealing that only 5 of 12 DNA-generating firms systematically check orders from clients to determine whether they are being asked to produce genes encoding hazardous biological materials. The SB2 Declaration calls on such companies to monitor commercial orders and report requests for suspicious sequences to government agencies. To implement this practice, the SB2 draft recommends forming a working group to develop software systems to check all DNA sequences being sold.

Researchers working in this new field of synthetic biology say that they plan to proceed with caution and to exercise oversight and that the SB2 Declaration is a sensible early step in that process. The draft and the ensuing discussion can be accessed online (http://openwetware.org/wiki/Synthetic_Biology/SB2Declaration).

SUGGESTED READING

Anderson, J. C., E. J. Clarke, A. P Arkin, and C. A. Voigt. 2006. Environmentally controlled invasion of cancer cells by engineered bacteria. J. Mol. Biol. **355:**619–627.

Bhattacharyya, R. P., A. Remenyi, B. J. Yeh, and W. A. Lim. 2006. Domains, motifs, and scaffolds: the role of modular interactions in the evolution and wiring of cell signaling circuits. Annu. Rev. Biochem. **75:**655–680.

Chan, L. Y., S. Kosuri, and D. Endy. 2005. Refactoring bacteriophage T7. Mol. Systems Biol. **10:**1038.

Glass, J. I., N. Assad-Garcia, N. Alperovich, S. Yooseph, M. R. Lewis, M. Maruf, C. A. Hutchison, III, H. O. Smith, and J. C. Venter 2006. Essential genes of a minimal bacterium. Proc. Natl. Acad. Sci. USA **103:**425–430.

Ro, D. K., E. M. Paradise, M. Ouellet, K. J. Fisher, K. L. Newman, J. M. Ndungu, et al. 2006. Production of the antimalarial drug precursor artemisinic acid in engineered yeast. Nature **440:**940–943.

Sedlak, M., and N. W. Ho. 2004. Production of ethanol from cellulosic biomass hydrolysates using genetically engineered Saccharomyces yeast capable of cofermenting glucose and xylose. Appl. Biochem. Biotechol. **113–16:**403–416.

Tucker, J. B., and R. A. Zilinskas. 2006. The promise and perils of synthetic biology. The New Atlantis, Spring 2006: http://www.thenewatlantis.com/archive/12/tuckerzilinskas.htm.

Venter, J. C., K. Remington, J. F. Heidelberg, et al. 2004 Environmental genome shotgun sequencing of the Sargasso Sea. Science **304:**66–74.

Voigt, C. A. 2006. Genetic parts to program bacteria. Curr. Opin. Biotechnol., in press.

Wang, L., J. Xie, and P. Schultz. 2006. Expanding the genetic code. Annu. Rev. Biophys. Biomol. Structure **35:**225–249.