TECHNOLOGY FEATURE

LIVING FACTORIES OF THE FUTURE

Scientists are designing cells that can manufacture drugs, food and materials — and even act as diagnostic biosensors. But first they must agree on a set of engineering tools.

SPIBER INC.

Japanese company Spiber Inc. has reprogrammed bacteria to make spider silk, which is being used to make clothing.

BY MICHAEL EISENSTEIN

From an evolutionary perspective, yeast has no business producing a pain killer. But by re-engineering the microbe's genome, Christina Smolke at Stanford University in California has made it do precisely that. Smolke and her team turned yeast into a biofactory that, by starting with sugar as a raw ingredient, makes the potent pain-relief drug hydrocodone[1].

This feat is a prime example of synthetic biology, in which scientists reprogram cells to replicate products found in nature — or even make more-specialized materials that would never normally be produced by a natural organism.

Synthetic biologists are ambitious. "We'd all love to imagine a world where we could adapt biology to manufacture any product renewably, quickly and on demand," says Michael Jewett, a synthetic biologist at Northwestern University in Evanston, Illinois. Groups around the world are engineering yeast, bacteria and other cells to make plastics, biofuels, medicines and even textiles, with the goal of creating living factories that are cheaper, simpler and more sustainable than their industrial counterparts. For instance, the biomaterials company Spiber Inc. in Tsuruoka, Japan, has reprogrammed bacteria to churn out spider silk for use in strong, lightweight winter clothing.

But synthetic biologists are going beyond simply producing materials — they are creating complex systems by 'wiring up' genetic parts into circuits. This approach has already resulted in various living switches and sophisticated sensors. For example, Martin Fussenegger's group at the Swiss Federal Institute of Technology (ETH) in Zurich has built biomedical sensors that can detect disease-relevant metabolites in the blood and trigger the production of therapeutic compounds. In mice, these biosensors successfully staved off gout and obesity, and treated the skin disease psoriasis[2] (see 'Living pills').

This young field has already spawned some success stories, but making and putting together genetic parts currently involves substantial guesswork and unpredictability. For the field to advance, academics and industrial players must agree on a toolbox of reliable genetic parts and the best strategies for assembling them.

To build an artificial product, synthetic biologists begin by selecting DNA parts on a computer and manufacturing them with specialized instruments. The parts can then be inserted into the DNA of microorganisms and cells to reprogram them.

Thanks to the plummeting cost of DNA sequencing, there is now a vast collection of genetic data through which synthetic biologists can sift to find useful genes. "Biology has given us this big, crazy library of stuff to choose from," says Christopher Voigt, a synthetic biologist at the Massachusetts Institute of Technology (MIT) in Cambridge. One leading database, the US National Center for Biotechnology Information's GenBank, contains more than 190 million DNA sequences from 100,000 organisms.

Some of the most widely used genetic parts encode enzymes — proteins that are essential for manufacturing. To transform glucose into hydrocodone, for example, Smolke's team took 23 enzyme-encoding genes from diverse species and put them into yeast[1].

Other favourites in the genetic designer's palette are promoters — stretches of DNA that regulate the activity of nearby genes and cause them to be expressed. When proteins called transcription factors bind to a promoter, the process of transcribing a gene begins. But promoters operate too slowly for some synthetic-biology applications. "We're trying to build things that operate fast — on millisecond timescales," says biologist Pamela Silver of Harvard Medical School in Boston, Massachusetts. Scientists are therefore examining alternative mechanisms that allow gene expression to be controlled directly by signals in the environment, such as toxins or antibiotics.

With myriad synthetic DNA pieces at their disposal, synthetic biologists can indulge their creativity. Voigt is enthusiastic about the possibilities: "The nice thing about biology is that there are lots of ways to do the same thing — and as an engineer, you can pick the way that is easiest to design." But genetic parts must perform consistently if the goal of setting up industrial processes is to be realized. "One of the key problems for biology in general is the lack of reproducibility," says Richard Kitney, chairman of the Institute of Systems and Synthetic Biology at Imperial College London. "In synthetic biology this is totally unacceptable — you have to have reproducibility if you're going to do industrial translation."

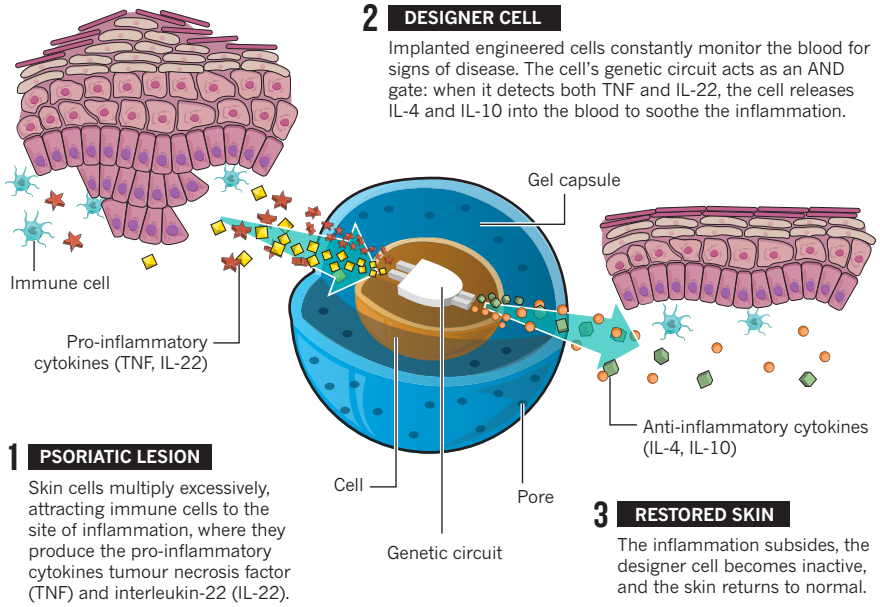
Many researchers deposit their discoveries into shared repositories, such as the Registry of Standard Biological Parts and the Inventory of Composable Elements. But those parts are often poorly defined or lack crucial information about how they were experimentally tested. "The only quality control is in the person who deposited the information," says Voigt.

The US National Institute of Standards and Technology (NIST) launched the Synthetic Biology Standards Consortium in March 2015 with the aim of standardizing the design, documentation and assembly of synthetic-biology parts across academic institutions and industry. In the United Kingdom, Kitney is coordinating a similar effort in which the DICOM (Digital Imaging and Communications in Medicine) standard for sharing medical information will be expanded to include synthetic biology[3]. In parallel, an international team has developed SBOL (Synthetic Biology Open Language)[4] to provide researchers with a standardized vocabulary to describe genetic parts and circuits.

LIVING PILLS

Scientists have made engineered cells that can detect flare-ups of the skin disease psoriasis and dispense on-the-spot treatment. The implanted cells are housed in gel capsules that protect them from the host's immune system and that stop the cells from attacking the host if they malfunction.

ADAPTED FROM REF. 2



CELLULAR SOFTWARE

Thanks to greater automation, it is now simpler and cheaper than ever before to make synthetic DNA parts (see 'Manufacturing DNA has never been easier'). But connecting those parts to form genetic circuits that can work together to provide sophisticated, computing-like behaviours is still a challenge. "Any time you physically connect DNA you're creating a new sequence at that interface — as DNA is so information-rich, you could create a new promoter or change the beginning of the RNA," says Voigt.

Even carefully designed circuits can malfunction and cause unwanted expression of a gene or interference between the genetic elements in the biological circuit — outcomes that cannot be foreseen in computer models. "The community is very much operating in a world where we cannot predict what is going to happen in our systems when we build them," says Reshma Shetty, co-founder of the synthetic-biology company Ginkgo Bioworks in Boston, Massachusetts.

This uncertainty means that many of the steps in engineering a synthetic system need to be tested and optimized. Software tools and robotics are speeding up each part of this process, from building the artificial DNA to inserting it into a microbe. "You can use high-throughput prototyping to just build every variant, and hopefully one of them will hit," says Jay Keasling, a biochemical engineer at the University of California, Berkeley, and a pioneer in the field. The push for automation has led a number of synthetic-biology research centres and firms to install 'biofoundry' facilities in which robotic assembly lines create, test and optimize microbes at a much larger scale than could be done by hand.

Biofoundries are enabling synthetic biologists to embark on ambitious projects. For example, Voigt, who co-directs the MIT-Broad Foundry, cites a collaboration with the Swiss pharmaceutical company Novartis to manufacture a huge range of molecules that are produced by bacteria in the human gut.

Other institutions pursuing the biofoundry model include the SynbiCITE programme at Imperial College London and the National University of Singapore's Synthetic Biology Foundry. The US Defense Advanced Research Projects Agency (DARPA) has also invested heavily in the MIT-Broad facility, including a five-year, US$32-million contract that began in October 2015.

Some biologists remain sceptical about the rush to scale up and automate, and favour a more theory-driven strategy. But Kitney, who co-directs SynbiCITE, considers automation to be an inevitable step in the evolution of synthetic biology. "You can rapidly run a whole series of experiments in parallel to see which configuration works best," he says.

THE PERFECT HOST

Species that are commonly used as model organisms in the lab, such as brewer's yeast (*Saccharomyces cerevisiae*) and the bacterium

Escherichia coli, have also been pressed into service by synthetic biologists. Many breakthroughs in biosynthesis have been achieved with these organisms, such as when Keasling and his collaborators at Amyris, a company that he co-founded in Emeryville, California, in 2003, reprogrammed *S. cerevisiae* to manufacture the antimalarial compound artemisinin[5].

But these common lab organisms are not necessarily suited to being grown on an industrial scale. The hunt for better alternatives has led scientists to search in obscure places. "More and more labs are taking on arcane organisms — I think the *S. cerevisiae* and *E. coli* dominance is dropping," says Voigt.

In some cases, the choice organisms will be those that can withstand harsh manufacturing conditions, says Keasling. "Maybe you're producing something that's toxic but volatile, so if you have an organism that can produce it at relatively high temperatures, you could boil it off while you're producing it." Scientists are also testing whether it is possible to feed microbes with carbon sources other than sugars to make products. Synthetic-biology company Intrexon in Germantown, Maryland, is working with bacteria that feed on methane, a cheaper and more efficient means for producing carbon-based products than is sugar.

MEDICAL CELLS

When it comes to medical applications, synthetic biologists are engineering mammalian cells rather than microbes. Such designer cells could produce drugs in response to disease or take over certain physiological tasks in people with metabolic disorders such as diabetes. But engineering mammalian cells introduces a new set of challenges. "All the tools we have in yeast are just not there in mammalian cells," says Smolke. "We don't have as many promoters, or tools for regulation of gene expression or protein modification."

> ***"It will come to the point where you can just inexpensively synthesize the DNA you need."***

The easiest cells to cultivate are tumour-like, immortalized cell lines, which are inherently 'defective' and therefore not representative of healthy tissues. Conversely, tissue-derived primary cells are hard to cultivate and manipulate, and differences between cell types confound efforts to build toolkits that can be applied across the body. "Something that works in a kidney cell will not necessarily work in the lung or liver," says Fussenegger. To get around this, the ETH team is engineering 'prosthetic gene circuits', which are introduced into host cells that can be implanted at the site of disease.

Tinkering with genomes can also present problems. Even 'smart' genome-editing tools — such as CRISPR–Cas9, a system for introducing targeted modifications at specific DNA sites — can have unpredictable outcomes. "We don't know enough loci in human cells where you can put things in without any interference," says Fussenegger. His team is exploring whether it is possible to avoid this uncertainty by introducing gene networks that are embedded in synthetic loops of DNA known as plasmids rather than integrated directly into chromosomes. As an extra precaution, his experiments with mice generally make use of engineered cells trapped in implanted capsules, rather than modifying the animal's tissues.

Others want to do away with the cell altogether. Jewett is studying cell-free systems in which bacterial extracts are purified to obtain only the 'useful' parts of the cellular machinery. "You get all the enzymes necessary for energy and cofactor regeneration as well as protein synthesis," says Jewett. "This gives you unprecedented freedom to directly manipulate reaction conditions." This allows researchers to establish chemical conditions that maximize manufacturing productivity without worrying about keeping cells healthy. Jewett's team has shown that this approach can efficiently churn out medically useful proteins such as erythropoietin[6], a hormone that stimulates red-blood-cell production. "It's not yet a replacement for existing technologies, but the yields are sufficient to serve as a complement," he says.

The field is still in its infancy — indeed, the earliest demonstrations of engineered genetic circuits appeared only in early 2000 — and it can be dauntingly complex. Even so, a growing number of scientists grounded in conventional molecular biology are keen to give genetic design a try. Synthetic biologist Ron Weiss at MIT is teaching an online course on the field that proves its popularity. "We've had about 14,000 people sign up," he says.

The pay-off for those entering the field could be huge. "I'm in this space because the frontiers are endless for what biology can do," says Shetty. "It's just a matter of the technology advancing to a point where those new horizons open up." ■

Michael Eisenstein *is a freelance writer based in Philadelphia, Pennsylvania.*

1. Galanie, S., Thodey K., Trenchard, I. J., Filsinger Interrante, M. & Smolke, C. D. *Science* **349,** 1095–1100 (2015).
2. Schukur, L., Geering, B., Charpin-El Hamri, G. & Fussenegger, M. *Sci. Transl. Med.* **7,** 318ra201 (2015).
3. Sainz de Murieta, I., Bultelle, M. & Kitney R. I. *ACS Synth. Biol.* http://dx.doi.org/10.1021/acssynbio.5b00222 (2016).
4. Galdzicki, M. *et al. Nature Biotechnol.* **32,** 545–550 (2014).
5. Paddon, C. J. *et al. Nature* **496,** 528–532 (2013).
6. Sullivan, C. J. *et al. Biotechnol. J.* **11,** 238–248 (2016).

GENES TO ORDER

Manufacturing DNA has never been easier

In the early days of synthetic biology, scientists had BioBricks — a genetic-part format that was conceived by Thomas Knight at the Massachusetts Institute of Technology in Cambridge and designed for modular assembly. It was an attractive concept, but stringing these short bits together to make larger circuits proved to be a laborious and potentially error-prone process.

The task of assembling the pieces is now much easier because newer DNA synthesis machines can churn out strings of several thousand base pairs rather than just a few hundred, which cuts down on the errors introduced by the assembly process. "You can just order a bunch of predictably designed constructs, so you don't even have to think about the modularity anymore," says Pamela Silver of Harvard Medical School in Boston, Massachusetts. The cost of making DNA parts has also fallen dramatically — by as much as 85% between 2009 and 2014 — to the point at which both academic groups and companies routinely outsource the job to specialized providers such as Twist Biosciences in San Francisco, California, Gen9 in Cambridge, Massachusetts, and SGI-DNA in La Jolla, California.

ROY KALTSCHMIDT/BERKELEY LAB

Jay Keasling with a Biomek FX^P lab robot.

Many projects still require constructs that exceed the scale of what can be manufactured in one go, but these longer fragments can now be joined by quick and simple techniques that leave no scar. However, Jay Keasling of the University of California, Berkeley, thinks that even this will soon become a thing of the past. "It will come to a point where you can just inexpensively synthesize the DNA you need, whether it's 10,000 or a million base pairs." M.E.