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Starting from scratch

Genetic engineering is old hat. Biologists are now synthesizing genomes, altering the genetic code and contemplating new life forms. Is it time to think about the risks? Philip Ball asks the experts.

Redesigning Life. That was what Steven Benner wanted to call his 1988 conference in Interlaken, Switzerland. A chemist now at the University of Florida in Gainesville, Benner was organizing the meeting to explore the possibilities for making artificial chemical systems that mimic essential features of living things.

But his title caused such a furore among prospective attendees that Benner had to tone it down to Redesigning the Molecules of Life. "Individuals as distinguished as Nobel laureates were convinced that the title would incite anti-recombinant-DNA riots in Switzerland," Benner explains.

Benner's conference helped to define one strand of the emerging discipline known as synthetic biology, a field that is now raising worries that won't be deflected simply by semantics. The expanding toolbox of ways to re-engineer microbes — and even construct new ones — has opened up extraordinary possibilities for biomedical discovery and environmental engineering. But it also carries potential dangers that could eclipse the concerns already raised about genetic engineering and nanotechnology. If biologists are indeed on the threshold of synthesizing new life forms, the scope for abuse or inadvertent disaster could be huge.

In a dramatic demonstration of the potential risks, virologist Eckard Wimmer at the State University of New York at Stony Brook announced in 2002 that his team had built live poliovirus from scratch using mail-order segments of DNA and a viral genome map that is freely available on the Internet[1]. The feat put a spotlight on the possibility that bioterrorists could create even more dangerous organisms — including Ebola, smallpox and anthrax — perhaps endowing them with resistance to antibiotics.

Creative thoughts

Since then, biologists' abilities to engineer life have bounded ahead. Wimmer took three years to build his poliovirus, but last November genome sequencer Craig Venter and his colleagues at the Institute for Biological Energy Alternatives in Rockville, Maryland, announced that they had taken just three weeks to assemble a virus that infects bacteria[2]. At the same time, bacterial cells are being rewired to perform functions they can't fulfil in nature. And researchers are getting close to determining the smallest set of genes necessary to support a living cell, which might make it possible to cook up new life forms.

Almost 30 years ago, concerns that recombinant DNA technology could pose risks to human health and the environment prompted leading molecular biologists to call an unprecedented summit. They gathered at the Asilomar Conference Center in Pacific Grove, California, in February 1975, where they decided to voluntarily forego some kinds of research and to instigate safety measures to prevent abuses of the new techniques.

Is it now time for another Asilomar? Researchers involved in synthetic biology generally agree that more discussion of how to avoid risks is urgently needed, but have yet to take the formal step of calling for a summit. Some concerns were aired at a special session at the First International Meeting on Synthetic Biology, held in June at the Massachusetts Institute of Technology (MIT) in Cambridge, but it did not set out to produce policy recommendations.

The reason we face the question of risk at all is that the potential rewards of pursuing synthetic biology are so great. Protein engineer Wendell Lim of the University of

California, San Francisco, says that if synthetic biology is successful, it may become possible to treat a variety of diseases by repairing defective cell functions, targeting tumours or stimulating growth and regeneration of specific cell types. Other researchers are hoping to engineer bacteria to make complicated drugs or to use sunlight to generate clean-burning hydrogen for cars and power plants.

Synthetic biology is the logical corollary of the realization that cells, like mechanical or electronic devices, are exquisitely 'designed' — albeit by evolution rather than on the drawing board. Their functions are enacted by circuits of interacting genes. As scientists began to map these circuits in the 1990s, they inevitably began to wonder whether they could rewire them.

Glowing report

In 2000, biological physicists Michael Elowitz and Stanislas Leibler, both then working at Princeton University in New Jersey, designed from scratch a genetic circuit that caused oscillating production of a fluorescent protein. Bacteria programmed with the circuit glowed periodically[3]. Other researchers built on this, creating circuits that could be switched on and off by external signals, or that could control bacterial population density[4,5].

Now a growing number of researchers are working on ways to alter the circuitry of cells. Lim, for instance, is retooling some of the proteins that carry signals within and between cells so that they respond to different inputs from the environment[6,7]. And chemical engineer Jay Keasling at the Lawrence Berkeley National Laboratory, has refitted the gut bacterium *Escherichia coli* with the circuitry it needs to synthesize a precursor to the powerful antimalarial drug artemisinin, a product of the wormwood plant that is currently too expensive for widespread use. This meant importing ten genes from other organisms, including wormwood and brewer's yeast, and then carefully tuning their expression levels[8]. If this proves to be a cheap, reliable source of the drug, it could transform the treatment of malaria.

In a parallel development, other researchers have been tinkering with the building blocks of genes and proteins themselves. Naturally occurring proteins are built from a standard set of 20 amino acids. Although these are enough to produce protein chains with a staggering array of functions, expanding this repertoire might enable the design of biomolecules with new functions, such as protein-based drugs that resist being broken down in cells.

In 1989, Peter Schultz, a chemist now at the Scripps Research Institute in La Jolla, California, reported that he had found a way to persuade bacteria to incorporate

Tooled up: Steve Benner (above) and Wendell Lim are working to redesign proteins and the DNA in living cells.

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an unnatural amino acid into a specific protein[9]. This produced enzymes with subtly different activities. Since then, Schultz has added more than 80 unconventional amino acids to proteins.

Culture shock

In the same year, Benner persuaded cells to insert a base pair not used in nature into their DNA[10]. A better understanding of the different types of molecules that can function as DNA bases will open a window to the possible chemical ancestors of DNA that might have existed on primordial Earth, and to the possible genetic systems that could support life on other worlds. "I suspect that, in five years or so, the artificial genetic systems that we have developed will be supporting an artificial life form that can reproduce, evolve, learn and respond to environmental change," Benner predicts. "This will help define how life not of earthly origin might appear."

As biologists learn to shape cellular circuits and their molecular components, developments in the automated chemical synthesis of DNA are allowing entire genomes to be designed and assembled. Venter's lightning-fast synthesis of a virus in November was a testament to the expanding capacity of DNA synthesis machines. By some estimates, next year's machines will be able to generate sequences about a million base pairs long — roughly the size of the genome of *Chlamydia*, which causes a common sexually transmitted disease, and a quarter the size of *E. coli*'s genome.

"Bacterial genomes are within the range of current DNA-synthesis technology," says John Mulligan, president of the DNA-synthesizing company Blue Heron Technology in Bothell, Washington. But bacterial genomes must be embedded within a cell and its attendant biochemical machinery, making them much harder to synthesize than viruses. Nevertheless, attempts are under way. In November 2002, Venter made a high-profile announcement of his intention to build a simple bacterium starting with machine-made DNA.

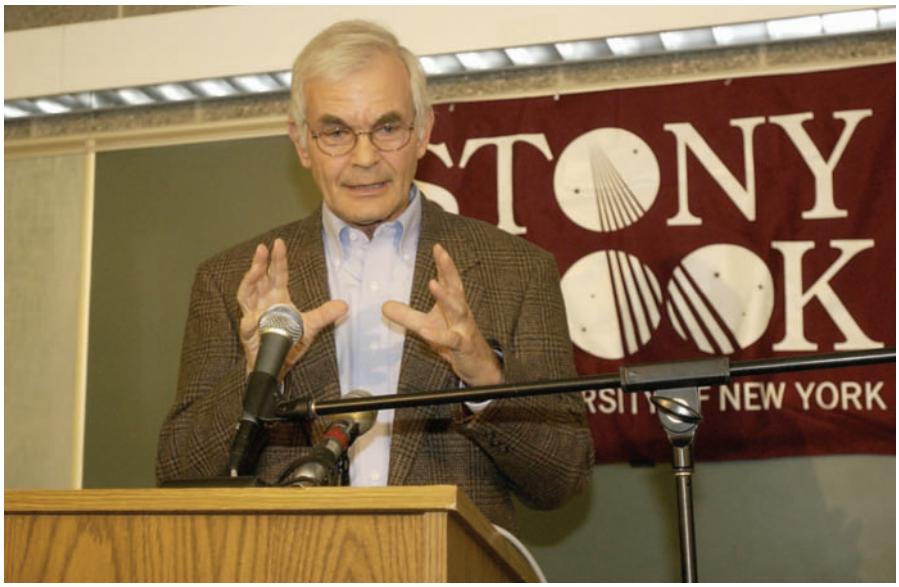
Plain and simple

But building a new bacterial genome is not just a matter of chemistry — you have to design the circuitry too. That's the hard part, so it's good to simplify. "An alternative to understanding complexity is to get rid of it," says Tom Knight, a computer scientist at MIT who brings an engineer's perspective to synthetic biology.

To this end, Knight is studying one of the simplest organisms known, *Mesoplasma florum*, a bacterium that has only 682 genes. The draft genome of this organism was completed last year, and its metabolic pathway has been mapped. The 793-kilobase genome seems to contain very little non-essential DNA, but Knight thinks it can be simplified further. He is now mapping its circuitry and modelling it on a computer to see what else can be removed.

All of these technologies combined are raising issues similar to those that sparked the Asilomar summit. Back then, molecular biologists realized they had all the tools to genetically modify bacteria — and possibly higher organisms — in just about any way imaginable. The hope was that bacteria could be engineered to produce drugs such as human insulin cheaply, and indeed they soon were. The worry was that no one knew how modified bacteria might fare in the environment — whether, for example, they might be toxic, or resistant to antibiotics.

Synthetic biology is now raising the bar. Should limits be set on what is attempted? If so, what should they be and how should they be enforced? And what steps can be taken to ensure that a rogue organization, or even a state-sponsored bioweapons



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Past the post: Eckard Wimmer announces the complete synthesis of poliovirus from mail-order DNA.

programme, does not use the technology to synthesize a dangerous microbe?

Roger Brent, president of the Molecular Sciences Institute in Berkeley, California, suggests that one option might be for DNA synthesis to require a licence. But more importantly, Brent says, synthetic biology should avoid developing a hacker subculture like that which spawns computer viruses. Rogue computer hackers hope to earn respect from their peers by producing particularly clever or insidious virus programs. Brent urges researchers in the field to encourage responsible lab culture by not engaging in showy stunts with no research purpose.

Assembly lines

Even though licensing is currently not required, some DNA synthesis companies have taken their own steps to avoid inadvertently aiding irresponsible work. Molly Hoult, senior vice-president of Blue Heron, says that all the company's orders for DNA are cross-checked against a database of "biological nasties". If a match turns up, the company tries to find out more about the customer's research before completing the order. If it can't easily be checked out, Blue Heron simply turns the order down. "We walk away from some business," Hoult says.

Such self-policing could become the norm, and scientists might even be asked to cooperate more closely with intelligence agencies to prevent the abuse of synthetic biology. An unclassified report by the CIA released last November warned that synthetic biology could produce engineered agents "worse than any disease known to man" and suggested "a qualitatively different working relationship between the intelligence and biological sciences communities". In particular, the bioscience community might function as a "living sensor web" that reports to the government on technical

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The CIA is calling for a change in the way the intelligence service and scientists work together.

advances that could be used as weapons[11].

But it is not clear whether the risk of bioterrorism will be the most important concern with synthetic biology. Ron Weiss, an electrical engineer at Princeton University who spends his time rewiring bacteria, points out that adding antibiotic-resistance genes to harmful bacteria is relatively straightforward and has been possible in principle since the 1970s — yet it has not become a major focus of biowarfare. It would be easier and cheaper simply to breed and release existing harmful organisms than to make new ones. "If I was a terrorist," says Weiss, "this isn't the way I'd get maximal damage for my buck."

It is much harder to anticipate the unintentional dangers of synthetic biology. For example, if new strains of bacteria were developed with unprecedented capabilities, how could they be kept under control?

One way might be to use built-in safeguards. For instance, the innate ability of bacteria to respond to high population density, a feature known as quorum sensing, could be co-opted to activate a self-destruct mechanism. Another option might be to build gene circuits that function like the logic gates of computers to count the number of times a cell divides. After a preset number, the cell would die.

Initial attempts have been made. Unfortunately, Weiss has found that mutant strains evolve after just a few days that can evade his population-control mechanism[5]. But he thinks this can be solved by creating several layers of defence. After all, such redundancy seems to be built into naturally occurring quorum-sensing bacteria, which do not mutate to evade their own population controls. "Nature does this already," Weiss says.

Into the unknown

Yet as synthetic biology develops, it will be hard to anticipate all the possible problems, whether malevolent or inadvertent. "The repertoire over the coming decade is limitless," says George Poste, a bioterrorism expert and director of the Biodesign Institute at Arizona State University in Tempe. "You'll never identify all the risks." Poste says that he is not particularly concerned about immediate dangers, as most researchers are still working with biological materials isolated from cells, so nothing is likely to escape from the laboratory. But "fast-forward two decades and it may be quite different", he adds.

To help quantify risks as they emerge, Poste proposes developing what he calls a 'calculus of risk' — an equation that can enumerate a 'risk factor' for new developments and sound an alarm bell when a certain risk threshold is reached. It's a necessarily crude tool — Poste's equation includes poorly quantified factors such as the projected time it would take to convert a new technology for malevolent use — but it might at least help to distinguish remote risks from more immediate ones.

The difficulty of putting a finger on the risks might leave researchers attending an Asilomar-style conference clutching at shadows. So for now the talks will remain informal. "This definitely merits a lot more discussion," says Weiss. "We don't understand the issues sufficiently yet."

Sooner or later, synthetic biology may find itself facing dangers that are far more than hypothetical. As Poste puts it: "Biology is poised to lose its innocence." ■

Philip Ball is a consultant editor of *Nature*.

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