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Science

Synthetic Biology

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SPECIAL SECTION

Synthetic Biology

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COVER

Bacteria constructed from toy building bricks represent the potential of synthetic biology to design and construct genetic modules that can be used to introduce new functions into existing organisms or even to engineer new biological systems. A special section highlights how this field is contributing to our understanding of biology and harnessing this understanding to benefit humanity. See page 1235 and www.sciencemag.org/special/syntheticbio.

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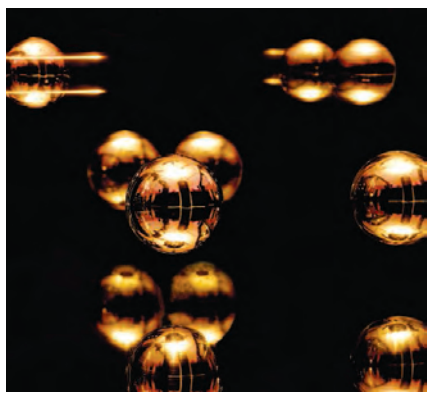

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CREDITS: (MIDDLE) GIO GHIANDONI AND LUIGI PAOLASINI; (BOTTOM) NASA

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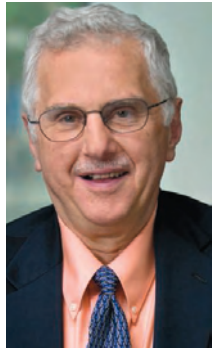
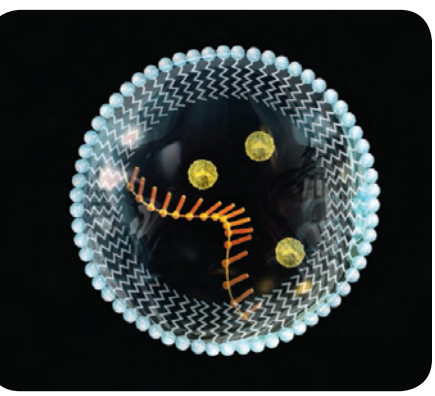


Bruce Alberts is Editor-in-Chief of *Science*.

A Grand Challenge in Biology

RICHARD FEYNMAN, A BRILLIANT NOBEL PRIZE–WINNING PHYSICIST, IS OFTEN QUOTED FOR his statement that "What I cannot create, I do not understand." The remarkable advances in our knowledge of the chemistry of life achieved in the past few decades, published in *Science* and many other journals, could lead nonexperts to assume that biologists are coming close to a real understanding of cells. On the contrary, as scientists learn more and more, we have increasingly come to recognize how huge the challenge is that confronts us. In this special issue, we review the progress made in the decade-old field called synthetic biology, which, as Feynman would advocate, creates biological networks in order to help us understand, and in some cases redesign, living systems. Along with its promise for the biotechnology industry, synthetic biology has the potential to become a powerful new tool for the long-term fundamental research needed to more effectively create breakthroughs in improving human health and welfare and the environment.*



Why do we need this basic research aimed at attaining a deep understanding of the chemistry of life? A complete catalog of the tens of thousands of different molecules present in a human or mouse cell, along with a map of their myriad mutual interactions, is likely to be obtainable with the wide variety of different techniques that are now available. But how can we make sense of such enormous chemical complexity? There are about 21,000 distinct proteins encoded by the human genome. At present, one can only guess the function of nearly half of these gene products. And even when we know the exact function and structure of a particular protein, embedding this protein in the cell often reveals a network of interactions so complex that the biological outcome of any perturbation, such as a drug treatment, is unpredictable. Clearly, there is an enormous amount left to learn.

Fortunately, many living cells are much less complicated than the cells of mammals. Because all living things on Earth are related through evolution, one can bootstrap one's way to understanding human cells by discovering how simpler cells and organisms work. A detailed study of *Mycoplasma genitalium*, a tiny bacterium that causes human disease, suggests that it can grow and divide with a minimal set of only about 430 genes. But no function can thus far be assigned to about 100 of its essential proteins.† This suggests that we may be largely ignorant of some critical functions of proteins, such as their roles in the exquisite spatial organization of the molecules inside cells.

In 1945, the pioneering physicist Max Delbruck started the "phage course" at Cold Spring Harbor Laboratory to recruit a group of talented scientists to work on bacterial viruses. This was the start of modern molecular biology, and it led to remarkable breakthroughs in our understanding of the then-mysterious molecular basis of heredity. Today, we need a focus on producing cooperative groups of scientists who aim at a complete understanding of the simplest free-living cells. Progress is being made.‡ But many more biochemists must get involved in order to reconstruct with purified components the different interacting protein assemblies—the subsystems in cells—so as to elaborate their detailed chemistry. We will also need synthetic biologists to dissect these subsystems, both by rewiring them and by the creation of functions through their transplantation to new settings. And biologists will need the help of mathematicians, computer scientists, and engineers to make sense of the enormously complicated network of molecular interactions found in even the least complex living cells. To make all this possible, governments and foundations must become much more imaginative in allocating resources for the long-term fundamental research needed to prime major breakthroughs in human health and global sustainability.

– Bruce Alberts

10.1126/science.1213238

*N. Nandagopal, M. B. Elowitz, *Science* **333**, 1244 (2011); W. C. Ruder, T. Lu, J. J. Collins, *Science* **333**, 1248 (2011).
†J. I. Glass *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **103**, 425 (2006). ‡For example, see S. Kühner *et al.*, *Science* **326**, 1235 (2009).

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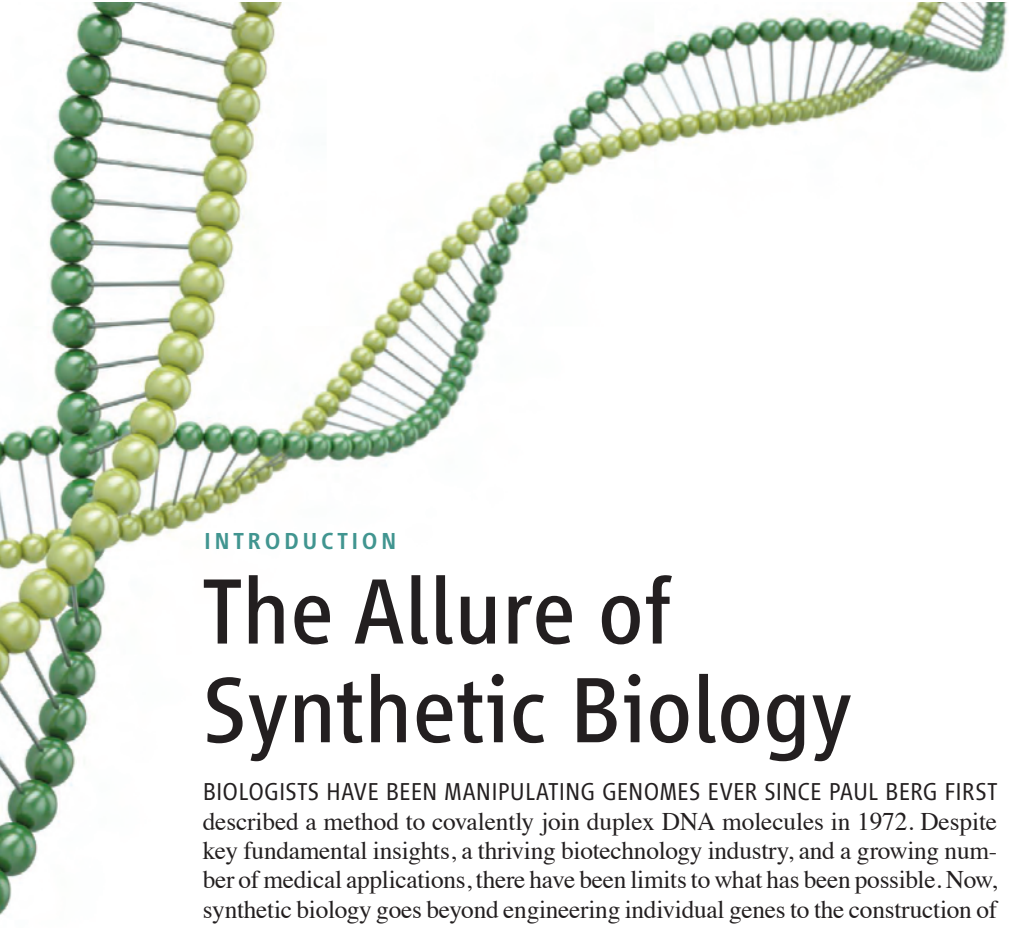

INTRODUCTION

The Allure of Synthetic Biology

BIOLOGISTS HAVE BEEN MANIPULATING GENOMES EVER SINCE PAUL BERG FIRST described a method to covalently join duplex DNA molecules in 1972. Despite key fundamental insights, a thriving biotechnology industry, and a growing number of medical applications, there have been limits to what has been possible. Now, synthetic biology goes beyond engineering individual genes to the construction of DNA-encoded circuits that can be programmed to control cell behavior.

This emerging field brings together biologists, physicists, chemists, and engineers who seek both to understand life and to build new biological functions. For example, Harvard's George Church wants to redesign the genetic code (p. 1236). The potential of synthetic biology has also attracted artists who want to critique it and make use of its techniques (p. 1242), as well as do-it-yourself biologists, some of whom have set up community labs (p. 1240).

Nandagopal and Elowitz (p. 1244) describe how building circuits and studying their behavior in cells can provide insight into biological design principles. Initially, the focus was on creating autonomous circuits, but recently there has been a move toward integration of endogenous and synthetic circuits. This can allow the "rewiring" of cellular circuitry to control biological processes—a goal not only of scientists who seek to understand these processes but also of biotechnicians and clinicians who would like to direct cell behavior to their advantage. Ruder, Lu, and Collins (p. 1248) discuss specific constructs that highlight the potential for moving toward clinical applications. They envision synthetic circuits that detect unhealthy cellular phenotypes and take corrective action. Service (p. 1238) covers progress in algal biofuels, for which synthetic biology has helped to make possible rapid improvements that may ultimately make algae a viable alterative fuel source.

In these examples, the assembly of the synthetic systems is within an existing organism. Schwille (p. 1252) paints a more radical view of synthetic biology, envisioning cells built entirely from synthetic modules. Here, the question is not how a biological system actually functions, but rather what minimal set of elements would support function. With the application of synthetic biology come ethical challenges. Erickson *et al*. (p. 1254) present the industry point of view about the potential of synthetic biology and a possible regulatory framework. This special section, together with the three related research Reports and the profile in *Science* Careers, captures the range of expertise now entering this field and highlights how the construction of new biological systems might be harnessed to serve humanity.

– VALDA VINSON AND ELIZABETH PENNISI

Synthetic Biology

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See also Editorial p. 1200; Reports pp. 1292, 1307, and 1315; Science *Careers content, plus* Science *Podcast p. 1197 and www.sciencemag.org/special/syntheticbio*

Science

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CREDIT: MARIE WU

NEWS

The Life Hacker

He is a pioneer of genome sequencing, but Harvard University's George Church wants to do more than read DNA. He is changing the genetic code itself

BOSTON—"We're going into the inner sanctum," says George Church, gliding through a series of doors and passages, waving his key card to get in. At the center of this labyrinth in Harvard University's Wyss Institute for Biologically Inspired Engineering is a tiny locked room that Church opens with an old-fashioned metal key. Inside is a device that the 57-year-old biologist invented and built with the help of a local robotics company. He calls it MAGE, and it does look slightly magical, as if the contents of a molecular biology laboratory have flown off the benches and arranged themselves into a box. The effect is enhanced as Church—nearly 2 meters tall with an impressive wizard's beard—looms over the desk-sized contraption. But the real magic comes with what MAGE does: Millions of normal *Escherichia coli* bacteria go in one end; a vast menagerie of microbes with new genomes comes out the other end. "I'm hoping this thing will be worth $200 billion," he says.

A statement like that isn't unusual for Church. It sounds brash at first, but laboratories around the world are trying to genetically alter bacteria and other kinds of cells to make industrial chemicals from biomass efficiently, and the potential payoff is huge. Church argues that MAGE, which stands for multiplex automated genome engineering, will be an indispensable tool for doing that.

In a debut of the technology several years ago, Church produced billions of different versions of the *E. coli* genome, identifying one that is five times more efficient at producing the antioxidant lycopene (*Science*, 21 August 2009, p. 928). "That was just a proof of concept," he says. Now he's setting his sights on more lucrative chemicals, such as dyes, and also on enabling MAGE to refashion nonbacterial genomes.

Synthetic biology, with its goal of reengineering cells as industrial machines, is the epitome of ambition. But even in a field of risk-takers, Church stands out. "He always talks about such wild experiments," says J. Christopher Anderson, a synthetic biologist at the University of California, Berkeley, and one of Church's collaborators. "And then he rolls them out. He actually makes some of them work."

Church's scientific risk-taking has paid off. Earlier this year, Church was elected to the U.S. National Academy of Sciences. He is one of four scientists sharing a $20 million grant from the U.S. National Institutes of Health to develop efficient ways to change the genetic makeup of stem cells as a way of treating disease. Church has also helped to create or guide more than two dozen start-up companies and generated 34 biotechnology patents himself—not to mention leading the charge in personal medicine with his Personal Genome Project, in which he and others voluntarily bared their genomes (*Science*, 21 December 2007, p. 1843).

"There are people who are good at identifying the problems for the field, and there are others who are good at doing the experiments," says Jason Chin, a molecular biologist at the University of Cambridge, U.K. Church is rare in that "he does both."

But whether Church can pull off his most ambitious experiment—reinventing the genetic code—is another question. If he succeeds, biotechnology will have a new workhorse cell. And the planet will have a novel life form.

Flunking and thriving

The faint twang in Church's accent betrays his roots in Florida, where he grew up with a series of father figures before heading off to boarding school at 13. He showed a precocious talent for hacking complex systems—not just figuring out how the systems work but subverting them to his will. At the age of 10, he built an analog calculator from spare radio parts. By 16, he was writing his own computer programs—he tried everything from ecological modeling to algorithmic poetry.

By the time he became a graduate student in the 1970s, Church was already an accomplished scientist, with a high-profile paper modeling how proteins bind to DNA. He also wrote the software that helped solve the structure of transfer RNA (tRNA), a molecule that helps make proteins and would later become central to his grand quest.

Yet in 1976, 2 years into his Ph.D. studies at Duke University, Church ran into trouble. He had skipped so many classes to spend more time in the lab that he was about to flunk out. Fortunately, Harvard accepted the distracted student, who buckled down

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and took the required classes. "I'm glad they took a chance on me," says Church, now a Harvard professor.

When Church finished his Ph.D. in 1984, it seemed impossible to read the sequence of a cell's genome, let alone tinker with its content. Church and his Harvard Ph.D. adviser, Walter Gilbert, invented one of the first automatic DNA sequencing methods, widely popular at first but then overtaken by another technology. Just a few years later, Church invented multiplex DNA sequencing, in which many DNA strands can be deciphered in parallel (*Science*, 8 April 1988, p. 185), a method that has inspired countless applications, such as computer chip–like microarrays that track the activity of thousands of genes.

Church took a cue from telecommunications. Thousands of simultaneous telephone conversations can share the same wire because the data streams are uniquely tagged and then combined—"multiplexed"—so they can be teased apart at the other end. Similarly, with fluorescently glowing molecules as tags, and the help of computers to make sense of the data, Church showed that the chemistry of millions of separate molecules, such as strands of DNA, could be tracked and analyzed.

Church's latest ambition is not just to sequence genomes but to completely redesign them. The M in MAGE, the contraption locked away in his lab, is what makes this possible. By multiplexing many simultaneous changes to DNA within a single population of cells, rather than the traditional method of sequentially introducing changes in one generation of cells at a time, Church can edit genomes on the fly, creating a vast diversity of bacteria to evaluate for their commercial utility.

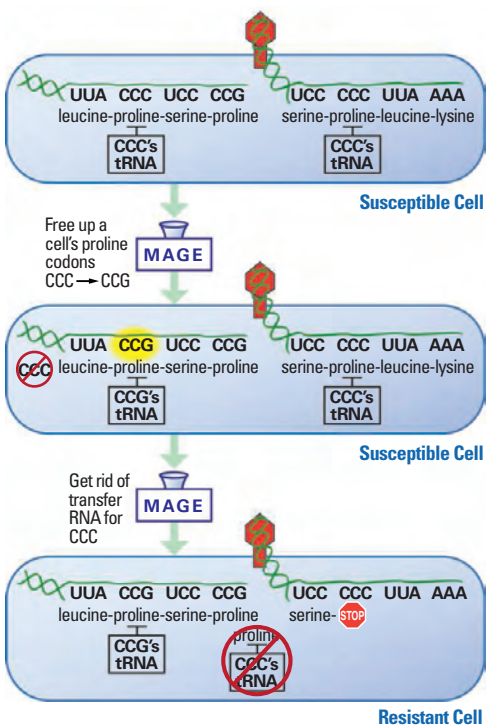
The ultimate hack

Church has even more ambitious plans for MAGE. He wants to hack into a cell's genetic code to make the cell impervious to viruses. That could be a boon to industries that use giant batches of bacteria and other cells to churn out enzymes and other valuable chemicals, Church notes. He points out that in 2009 a virus contaminated drug-producing hamster cells at the nearby biotech company Genzyme. The virus shut down a whole plant, leaving patients stranded.

The complicated, multistep hack that Church believes can make cells virus-proof revolves around the way genes encode their protein-making instructions. Genes are inscribed as a series of DNA base-pair triplets, called codons. The triplet combinations of DNA's four-letter alphabet give rise to 64 possible codons, more than enough for the cell's 20 amino acids, as well as stop signals to mark the ends of genes. To make a protein, specific tRNA molecules read these codons and, until a stop codon is reached, attach the right amino acid to a growing chain.

Viruses take advantage of this system by using the very same codon code in their genes and thus fooling the cell's tRNAs into helping to churn out viruses. But what if Church changed the cell's genetic code and the way tRNA handled that code? With the cell thus rewired, any infecting virus trying to replicate would only make gobbledygook proteins.

The crucial first step is to "free up" a codon in a cell's genome. Because there are multiple codons that specify the same amino acids, one type of codon can be swapped for another that does the same job. If one did this across the entire genome, making a synonymous swap for every single instance of a codon, then the cell no longer needs that codon's tRNA. So there would be no harm done by deleting it. But viruses still depend on the codon, and when they infect this modified cell, that lack of that tRNA would cause viral protein production to hit a premature dead end (see diagram).



Virus immunity. After one of the transfer RNAs is edited out from a cell's genetic code, invading viruses (red) should get nowhere.

All that swapping and deleting is easier said than done, however. "We thought about doing this back in 2003," Anderson says. "But we realized that with traditional methods, it would take forever" because thousands of changes to the genome were required. In addition, such wholesale genome editing might cripple the cell.

In July, a team led by Church showed that cells can handle at least some genome editing. The researchers freed up one of the three stop codons in *E. coli* (*Science*, 15 July, p. 348). In each of the 314 places in the *E. coli* genome where the "amber" stop codon marks the end of a gene, they used MAGE to replace it with an "ochre" codon, which does the same job. And in unpublished follow-up work, they deleted the gene for the protein that reads the amber codon. These strange new bacteria are alive and well, Church says.

Getting from here to virus resistance will require much more work. Instead of eliminating a stop codon in cells, Church's team has to make at least 3000 replacements to get rid of an amino acid codon, not to mention deleting the gene for the corresponding tRNA.

Whether a cell can survive this massive rewiring remains to be seen. But if the virus-resistance hack works, it may be possible to further modify the cell's code such that its genes cannot be read correctly by other cells should the genes escape into the environment, making Church's new life forms environmentally friendly.

There are other efforts under way to hack the genetic code and teach cells new tricks. Like the DNA sequencing method he helped create 20 years ago, Church's rewired cells may turn out to be an also-ran. "Synthetic biology is such a young field," Chin says. "It's not clear what research will stand the test of time."

Church isn't worried. He continues to tinker with MAGE, trying to make a version that will allow him to edit the genomes of stem cells to treat cancer and other diseases. But his dreams don't stop there.

"I wouldn't mind being virus-free," he says with equal parts mirth and earnestness. It may be too late to reengineer all of his own cells to prevent viral infections, but Church doesn't rule out the possibility of rewiring the genome of a human embryo to be virus-proof. That would be the ultimate life hack.

–JOHN BOHANNON

CREDIT: K. ENGMAN/*SCIENCE*

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NEWS

Algae's Second Try

Fifteen years ago, the United States gave up on algal-based biofuels. Now synthetic biology has helped revitalize the field

IN SCIENCE, AS IN BASEBALL AND COMEDY, timing can be everything. John Sheehan learned that the hard way when he strove to make biofuels from algae from the late 1970s through the mid-1990s. The effort at the National Renewable Energy Laboratory (NREL) in Golden, Colorado, surveyed more than 3000 algal strains for their ability to produce oils that could be converted into diesel and other transportation fuels, then looked for ways to boost oil production in the best ones.

It wasn't enough. Faced with a tight budget, the U.S. Department of Energy (DOE) killed the program in 1996, opting instead to focus its limited funds on turning agricultural wastes and other "cellulosic" material into ethanol.

Fifteen years later, the algae biofuels business is thriving. Since 2000, more than $2 billion in private funds have flooded into the field. In May, Solazyme, an algae biofuels company in South San Francisco, California, raised $227 million on the stock market. Last year, ExxonMobil announced it would invest up to $600 million in the field, with up to half going to Synthetic Genomics, a San Diego, California, startup looking, like Solazyme, to use synthetic biology to create commercial fuelmaking algal strains. And DOE and other U.S. federal agencies have jumped back on board contributing hundreds of millions of dollars more, including $104 million from the recent economic stimulus package to Sapphire Energy in San Diego to build a large-scale algae fuel demonstration facility in New Mexico. Even Sheehan is back in the biofuels game again, studying the environmental impact of biobased fuels at his new home at the University of Minnesota, Twin Cities.

So why the change? In short, better timing, Sheehan says. Biotechnology has made massive strides in recent decades, now making it relatively easy to tinker with algae in ways not possible during the first flurry of interest. "The tools we had to use to manipulate algae were medieval compared to what we have today. Synthetic biology didn't exist in 1996," Sheehan says. As a result, despite algae's advantages, he and others could not overcome the high costs of obtaining oil from these organisms.

But now, companies are using synthetic biology techniques, along with other biotech and engineering advances, to bring those costs down, making algae more efficient by changing the way the organisms use light, increasing the oil content of cells, and improving their efficiency at producing fuel precursors. A spate of recent advances "gives me more assurance that this isn't just folly," Sheehan adds.

It's easy to see why plenty of scientists and investors agree with Sheehan. For starters, with fossil fuels becoming increasingly scarce, expensive, and a source of political instability, the potential market for replacing these liquid transportation fuels is worth trillions of dollars per year. Corn- and sugar-based ethanol production already has a share of that. But because a liter of ethanol has only two-thirds of the energy content as the same volume of gasoline, the alcohol isn't well suited for fueling aircraft and heavy trucks, big chunks of the transportation industry. Plants that produce more energy-rich oils for biodiesel, such as soybean and oil palm, are a better fit in those areas. But these plants produce at most 5930 liters of fuel per hectare per year, according to DOE's 2010 National Algal Biofuels Technology Roadmap.

Fast-growing algae, on the other hand, can produce between 9353 and 60,787 liters per hectare per year of fuel. And some algae companies are convinced that they will ultimately do much better than that. If such a promise comes to pass, algae farms on the scale of Colorado could produce all the gasoline used in the United States each year, a small fraction of the land that would be required for making a comparable amount of biofuels from corn or cellulose (see figure).

Furthermore, unlike most plants, some strains of algae thrive in brackish water, saltwater, or even waste treatment water. Algae farms can also be sited on land unsuited for traditional agriculture. So, in theory, large-scale algal fuel production would not interfere with food production the way other biofuels can. "The fundamental biology makes algae a massive opportunity for humanity," says B. Greg Mitchell, an algae researcher at the University of California, San Diego (UCSD).

But as Sheehan and his NREL colleagues learned early on, algae-based fuels present many challenges, ones that still make them prohibitively expensive. Despite the fact that algae grow quickly, they typically make up only 0.1% of the volume of the water in which they grow. That means collecting a kilogram of algae requires processing 1000 kilograms or more of

New crude? Synthetic biology helps researchers make high-oil-producing algal strains.

CREDIT: COURTESY OF SAPPHIRE ENERGY

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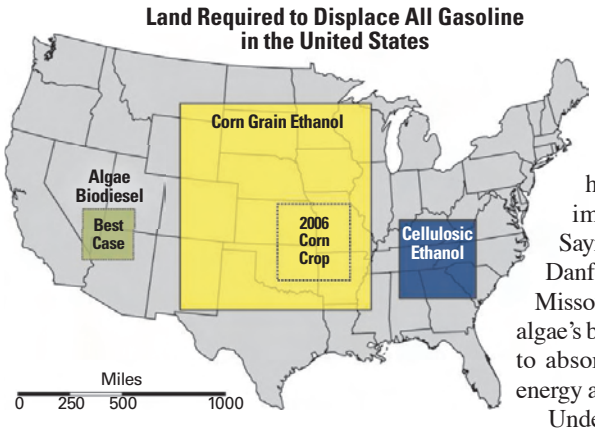

Competitive advantage. Fast-growing algae yield more fuel per hectare than other biofuel producers.

water, an energy-intensive operation. The algae must be harvested and their oil extracted, collected, and processed into the final fuel. Those challenges currently make the cheapest algal fuels cost about $2.25 per liter, more than double today's average gasoline price in the United States, NREL researchers say.

Green design

How individual companies are trying to cut costs depends in part on the type of alga they use and how it's grown. The most popular strategy to grow algae is in sunny, open-air, shallow ponds. But because the algae shade each other, they grow at a low concentration and thus a large amount of energy must be spent concentrating them and harvesting the oils they produce. Competition from other algae strains that blow in and bacterial and viral infections further compromise the efficiency of open ponds.

A separate approach grows the organisms in closed chambers called bioreactors. These chambers can be either transparent, to allow the algae to grow using sunlight, or opaque, in which case the algae are fed sugars or other nutrients to promote their growth. Although bioreactors can grow algae at a greater density, they cost much more than open ponds to set up and operate.

Synthetic biology is helping researchers produce fuels more efficiently in both settings. "We can very quickly do lots of genetic manipulations with synthetic biology," says Alex Aravanis, chief technology officer of Sapphire Energy. "We can go orders of magnitude faster to discover key genes related to yield." A few years ago, the yield had been stuck, with no more than 40% of the alga's weight being oil. Now, with synthetic biology's ability to alter algal metabolic pathways en masse, rather than one gene at a time, "we have the opportunity to drive those efficiencies to unprecedented levels," says Stephen Mayfield, an algae biologist at UCSD.

Some synthetic biology efforts have gone into making indirect improvements in oil yield. Richard Sayre, a molecular biologist at the Donald Danforth Plant Science Center in St. Louis, Missouri, and his colleagues are improving algae's biofuels potential by engineering them to absorb less light, thereby leaving more energy available to nearby algae.

Under natural conditions, individual algae hog the light in an effort to outcompete their neighbors: Their light-absorbing molecular complexes, called antennas, really use just one-quarter of the photon energy they absorb. So Sayre and his colleagues inserted a new set of metabolic instructions to make the algae more community-oriented. They directed the single-celled plants to adjust the size of the antennas such that in bright light, they absorb only enough photons to make as much oil as possible, leaving the rest for neighboring cells. Preliminary studies, presented in August at the American Society of Plant Biologists (ASPB) conference in Minneapolis, Minnesota, show that the strategy increased the population's growth rate by 30%.

That's not the only algae makeover under way. Sayre's team is also boosting metabolism in algae by giving them a human gene for an enzyme called carbonic anhydrase (CA) II, which helps regulate carbon dioxide in red blood cells. In the algae, it converts an inorganic form of cellular carbon into carbon dioxide, which can then be used in photosynthesis to ultimately make oils. The human CA is far more efficient than the algae's own CA. With it, algal photosynthesis rates jumped between 30% and 136% depending on the test conditions, the team also reported at the ASPB meeting.

Gushing with oil

Numerous companies are pushing the limits of algal biology in other, poorly disclosed ways. Harrison Dillon, president and chief technology officer of Solazyme, will only say that his company has engineered algal strains with an oil content of over 80% of their weight. Those strains are grown in bioreactors and fed sugars. Last year, Solazyme produced 416,395 liters of oil and announced that it can produce algae-based fuel for less than $120 a barrel, only slightly more than the recent cost of petroleum.

Sapphire's Aravanis adds that his company is using synthetic biology to come up with and rapidly survey thousands of different genetic manipulations of photosynthetic algae in an effort to make high-oil producers. As part of that program, it has identified several genes, some from algae and some from other organisms, that when inserted into lipid biosynthesis pathways increase oil production enough that if scaled up, the algae could produce an additional 4675 to 9383 liters per hectare per year. These results have yet to be realized in field trials, but the company is currently building a demonstration facility in New Mexico that is expected to produce between 5000 and 10,000 barrels of oil per day by 2018.

Focusing on a different organism, researchers at Joule Unlimited in Cambridge, Massachusetts, meanwhile, are improving the efficiency of photosynthetic bacteria for producing hydrocarbons to make diesel. These bacteria secrete hydrocarbons, so Joule researchers are modifying the bacteria to grow more slowly and instead to divert carbon dioxide almost exclusively into making hydrocarbon fuels. According to Dan Robertson, Joule's head of biosciences, the company now has strains that routinely convert 90% of the carbon atoms that come in as carbon dioxide into fuel molecules secreted by the organisms. The company is currently operating a pilot plant in Leander, Texas, and plans to open a large-scale demonstration plant in New Mexico next year.

Test bed. Open-air "raceway" ponds grow algae cheaply but must contend with infections and predators.

With these and other innovations now taking hold, Sayre is hopeful: "I'm convinced this time around we're much smarter and have a better shot at succeeding."

–ROBERT F. SERVICE

MAP: ADAPTED FROM G. CHARLES DISMUKES *ET AL.*, *CURRENT OPINION IN BIOTECHNOLOGY*, **19** (2008); PHOTO: COURTESY OF SAPPHIRE ENERGY

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A squeeze. A dozen GenSpace members, including *(left to right)* Sung won Lim, Russell Durrett, and Ellen Jorgensen, share two tiny labs.

CREDIT: DAN GRUSHKIN

NEWS

A Lab of Their Own

Do-it-yourself biologists in New York follow their dreams, setting up a community lab that combines synthetic biology with art, fun, and perhaps profit

NEW YORK—It's 4 p.m. on a summery Wednesday afternoon, and four members of GenSpace—two former biotech scientists, an undergrad on hiatus from school, and a person who runs next-generation DNA sequencers at a local medical school—are sitting around on mismatched chairs on the seventh floor of this former Flatbush bank, sipping Magic Hat beer and reflecting on the oddity of becoming minor scientific celebrities. *GQ France* did a photo spread recently on the writers, artists, and biologists who practice biology at GenSpace, and the Guggenheim Museum approached them about collaborating on an exhibit to teach synthetic biology. Low-brow TV producers even pitched the idea of a reality show based at this "community lab," a place where professionals and amateurs alike tinker with life forms and engineer DNA. GenSpace turned the producers down, and things soured with the Guggenheim, but amid any disappointment, members marvel at the continued, and sometimes lurid, fascination they've dredged up. "I've been really surprised at all the attention," says president Ellen Jorgensen.

Eventually, talk turns back to biology, and other GenSpace members start drifting in. Indeed, says GenSpace vice president Daniel Grushkin, a science writer, "GenSpace is like a gym membership" in that people come and go 24 hours a day. Grushkin spends the afternoon sketching out plans to use a bacterium to genetically transform the worm *Caenorhabditis elegans* and make it fluoresce. "It's a few steps above a pet rock," he suggests. And amid these discussions of organisms and experiments, all the other distractions fall away. That's why this crew had founded GenSpace, after all—to do their own biology, on their own agenda.

With the lab's debut in December 2010, GenSpace opened a new chapter for the do-it-yourself (DIY) biology movement, which some say parallels the garage computer culture in the 1970s that helped usher in the personal computing revolution. (Some DIY biologists even call themselves "biohackers.") But although the New York crew was the first to commit to a formal lab space for community biology, they're not alone. BioCurious, a DIY biology team near San Francisco, California, founded in 2009, has recently signed a lease for a 220-square-meter office in the Bay Area that will be turned into lab space.

Whether many more GenSpaces will arise is tough to predict. It's hard to quantify the number of active DIY synthetic biologists. Thousands of people trade tips (and jabs) in online forums, and the Web site DIYbio.org has seen its membership grow by orders of magnitude since starting in April 2008. But the number of people doing "wetwork" is significantly smaller, acknowledges Jason Bobe, who co-founded DIYbio.org.

Although not a biologist herself, BioCurious co-founder Eri Gentry says she hunted down lab space to rent because biology students she knew through BioCurious had grown weary of pursuing narrow Ph.D. research topics and wanted to tackle side projects they were passionate about. The setup in most science labs today "doesn't breed creativity," she argues.

That's a common sentiment in DIY bio, and it motivates much of the passion. Scientists are born tinkerers, says Jorgensen, also an assistant professor of pathology at New York Medical College. "This place is made for spare-time tinkering."

Indeed, as James Collins, a synthetic biology pioneer at Boston University, points out, "when we started synthetic biology, most of us were amateurs. We came from engineering, physics, computer science, and other fields." Still, "amateurs" like Collins, although biology neophytes, worked at universities and had access to expensive research equipment. Almost by definition, DIY biologists lack that access, and Collins argues that they will thus have a tough time making significant contributions.

Building communities

GenSpace started in 2009 after several like-minded New Yorkers met online through the Google group DIYbio. For months they puttered around with experiments in Grushkin's living room, but late last year they graduated to their new space: two boxcar-sized labs, each about 10 square meters, and a lounge on the top floor of a building that is primarily an artists' collective. The move to a permanent place was important for doing better science, Jorgensen says: "We kept hitting obstacles easily solved with the creation of a community lab. Suppliers of reagents often won't

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ship to residential addresses, and you need a separate fridge for storage so [microbes] won't contaminate food."

Like a clubhouse, the labs are cobbled together, in part from the impressive piles of junk lying around the building. "A lot of sweat equity went into this place," says Oliver Medvedik, who earned a Ph.D. from Harvard University in biomedical science and has taught there in the past few years but focuses on being GenSpace's director of scientific development. Many of GenSpace's lab benches are countertops salvaged from restaurants. Centrifuges, a PCR machine, and other equipment were donated by Jorgensen's previous employer, a biotech company that laid her off and had to unload things as it downsized. Medvedik even scouted eBay, finding an incubator that he ultimately bought off a truck in Jersey City, New Jersey, for $659.

The research equipment is integral to Medvedik's plans to genetically engineer bacteria to turn colors (perhaps from blue to yellow) in the presence of arsenic, to test groundwater in places like Bangladesh.

Even with a dedicated lab, though, the work Medvedik and others are doing is not easy. All DIY biologists have access to the international Registry of Standard Biological Parts, snippets of genetic code that can be popped into cells and microorganisms, much as resistors or capacitors can be popped into electrical circuits, and that should produce certain molecules or effects each time. But at a "synbio" meeting in July 2010, participants reported that of the registry's 13,413 parts listed then, 11,084 didn't work. As one presenter noted, "Lots of parts are junk." Wary of this, Medvedik and others say they must carefully test each registry part before relying on it.

GenSpace members also pay close mind to biosafety. Medvedik or Jorgensen gives all new recruits a 90-minute safety briefing and lab tour, similar, Jorgensen says, to what typical graduate students get. GenSpace has government and university safety officers on its advisory board, and it stays in contact with FBI agents as well. It even invited agents to one of its "strawberry mayhem" events, at which participants (usually children) mash fruit and extract DNA. The group also screens new members' projects carefully, having recently rejected a proposal involving human pathogens that cause acne.

Besides accepting donations and scrounging for hardware, GenSpace helps make ends meet by offering biology classes to the public. The 12 GenSpace members pay just $100 per month for lab access, but the group charges $300 per student for a 4-week course that includes learning lab techniques such as gel electrophoresis and splicing DNA with restriction enzymes. Jorgensen and Medvedik have taught more than 60 students since January, with more classes planned.

Students range in age from their 20s to their 60s, and most have no real science background. Alumni include a winemaker, biotech investors, and New Yorkers curious about personal genomics. In one class, Medvedik had students engineer *Escherichia coli* to produce pungent banana oil. "Some people want to do real MacGyver stuff" like the TV secret agent, Jorgensen says, whereas others "are fascinated just by running a gel."

Citizen science. The classes for the public that GenSpace teaches have brought in most of the lab's revenue so far.

CREDIT: DAN GRUSHKIN

Different strokes

Likewise, GenSpace members have different motivations for pursuing DIY biology. One of Medvedik's projects involves cultivating a fungus that can digest wood chips or sawdust. It converts those loose materials into a Styrofoam-like matrix, which could find use as an ecofriendly packing material or as insulation. Medvedik is also applying for Bill and Melinda Gates Foundation grants to expand his arsenic-detecting microbe project.

GenSpace executive secretary Russell Durrett, who graduated with degrees in biochemistry and anthropology from New York University in May 2010 and now has a job running DNA sequencers at Weill-Cornell Medical College, joined GenSpace largely to develop ideas to spin off into a company or sell as inventions. Toward that end, GenSpace announced early on that its members would retain all intellectual property rights. Some biohackers were aghast at this, arguing that it runs counter to the open-source ethos of the computer culture that helped spawn DIY bio, and GenSpace was flamed online.

But what makes sense financially in computing doesn't necessarily work in biotech, Durrett says, because organic parts take far longer to test and develop. His projects right now include designing fluorescent moss. He's also interested in producing cheap PCR machines: At a weekend-long "synbio binge" at GenSpace (an event inspired by "hack-athons" where amateur computer programmers gather and work together for days), he built a homemade PCR machine from plastic piping and a light bulb.

Jan Mun, who took Medvedik's class in May after hearing about it on a digital media listserve, recently joined GenSpace for the sake of her art. She had been culturing mushrooms at her home for an environmental sculpture, but they died; most homes are not antiseptic enough for finicky 'shrooms. GenSpace was her solution, as she could grow them under sterile conditions. "It's very unusual to have access to a molecular biology lab," Mun says, "and it's wonderful that they're open to artists."

Traditionally, there are certain scientific fields, such as high-energy physics, to which only professionals can significantly contribute. In other fields, such as astronomy or ornithology, committed amateurs can do important work.

Synthetic biology is currently the first kind of science, but by teaching classes and opening community labs, groups such as GenSpace and BioCurious strive to make it the second: to welcome Mun's artistic mushrooms alongside Medvedik's humanitarian bacteria or Durrett's entrepreneurial mosses. It's ambitious for such small groups, but Jorgensen welcomes the eclectic mix. DIY bio, she says, "is called a movement because it's just that. It's not organized and means different things to different people." Despite recruits like Mun and spreads in *GQ*, GenSpace isn't quite mainstream yet, but Jorgensen predicts it will be: "We feel the future is community labs." **–SAM KEAN**

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NEWS

Visions of Synthetic Biology

Artists embrace synthetic biology as a tool and an inspiration, but not necessarily as a promising way for the future

Inside Vienna's Museum of Natural History, the Bio:Fiction film festival and its sister art show, Synth-ethic, abound with living fantasia. The world's first art exhibition specifically devoted to synthetic biology, its exhibits are a gamut of interpretations of the emerging field, ranging from the celebratory to the alarmist. One short film sings the praises of a synthetically engineered future complete with glowing trees, a cure for cancer, and a biologically grown spaceship. Another shows how synthetic biology could lead to the devaluing of life. In it, a gamer uploads a superhero's genetic code into a piece of meat through a USB cable, directs the resulting humanoid around with a videogame controller, and eventually suffocates him in a plastic baggie. The art show is similarly diverse, showcasing "Nanoputians"—organic chemicals whose molecular structures resemble human stick figures—a sparkling arrangement of tubes and glassware that recreates the Miller-Urey origin-of-life experiment, and slimy, semiliving "worry dolls": cells on scaffolds to which visitors whisper their concerns about biotechnology.

But it's no accident that the show takes place in a museum of natural history, not art. "They're not just evocative objects," says Synth-ethic curator Jens Hauser. Nor are they simply educational illustrations of synthetic biology. "They're cynical design," using synthetic biology to critique synthetic biology.

As the field has grown during the past decade, so has interest in using its tools for nonscientific purposes. These are early, heady days for a field that promises to revolutionize medicine (see p. 1248), the chemical industry, and genetic engineering, to name just a few. A growing number of artists are attracted to it as a technique and also because of the interesting ethical questions it raises.

Many of these artists work directly with research scientists. Their creations add a cultural counterbalance to the field's tendency to view life like circuitry, a utilitarian perspective that increasingly drives synthetic biology and, they say, informs the public's understanding of it. They find themselves uniquely placed to ask hard questions about the ethical and social issues raised by synthetic biology. While special interests that want to either promote or condemn the nascent science have been eager to fund artistic interpretations of it, they are finding they may not get the results they hoped for.

Yet unlike engineers focused on solving a problem, "artists are the ones in a position to ask questions of 'why?' or 'should we?' " says Richard Pell, an art professor at Carnegie Mellon University in Pittsburgh, Pennsylvania. Continuing in that role is critical, he adds, because synthetic biology "should be thought about much longer than it takes to say 'Frankenfood' or 'cure for cancer.' "

Artists in the lab

With the advent of streamlined genetic and tissue engineering, interest in science-inspired "bioart" has exploded. Synthetic biology itself provides a "wet palette of possibilities" as both a technique and a topic, says Oron Catts, co-founder and director of the SymbioticA program at the University of Western Australia in Perth. SymbioticA has hosted more than 70 resident bioartists since 2000 and even offers a Master of Biological Arts degree. Synthetic Aesthetics, a collaboration between Stanford University and the University of Edinburgh, funds six pairs of scientists and artists to work together exploring one another's world. Programs such as these, as well as the emergent do-it-yourself biology movement (see p. 1240), allow artists to work alongside scientists in order to learn both the molecular techniques and the realities of the field.

Joe Davis, an artist and researcher at the Massachusetts Institute of Technology and Harvard University who has been in the bioengineering business for decades, is a perfect example. In the 1980s, annoyed with what he called the "absurdist" attempts by Search for Extraterrestrial Intelligence efforts to talk with extraterrestrials through radio waves, he encrypted the Arecibo Institute's famous binary message in DNA code, cloned it into spore-producing bacteria, and proposed launching them into space. Although it remained Earth-bound, this "Microvenus" project was his early claim to fame. Nowadays, he works in the lab of Harvard synthetic biology maven George Church (see p. 1236), sitting in on lab meetings, brainstorming with scientists, and reinterpreting ideas. Supported by his own art grants, he sees himself as the quintessential tinkerer, similar to the technically competent backyard rocket builders and radio enthusiasts of the past century.

A crystal radio was precisely what Davis displayed in the Synth-ethic art show—one

CREDITS (LEFT TO RIGHT): ALEXANDRA DAISY GINSBERG (3); JOE DAVIS ©PIETER BAERT

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Mixed media. Artists' reactions to synthetic biology, from left: Daisy Ginsberg imagines the medical implications of synthetic biology as organs coated in biological crystals and a diagnostic suitcase of colorful poo. Joe Davis powers a crystal radio using bacterial nanowires. Tuur van Balen builds a window trap for pigeons to catch them and turn them into soap dispensers. And Oron Catts grows cells into the shape of "worry dolls" ready to listen to concerns about biotechnology.

built of bacteria that naturally create their own communication lines, or nanowires. Engineered with a modified gene from a sea sponge that builds its own skeleton from silicon in seawater, the silicon-producing bacteria grow to form an electrically conductive circuit and are hooked to an antenna and speakers. The "radio" still has a few kinks, he says; he hopes to get it working soon. He and others in Church's lab are now trying to clone the modified gene into silkworms to see if the caterpillars will spin glass cocoons as art pieces.

Davis wishes more artists were willing to spend extended time in labs—where they experience both the excitement and constraints of cutting-edge science. Too many bioartists, he says, are more interested in shocking people than seeing what science is really about.

Yet bioengineers are not always welcoming of the input—and potential criticism—of artists. The International Genetically Engineered Machine competition (iGEM), an annual program in which undergraduates create useful life forms from standardized genetic components, is often touted as the future of synthetic biology. But in 2009, art infiltrated this bastion of utilitarianism when a team from Bangalore, India, entered *Escherichia coli* they had engineered to produce the smell of rain before a monsoon. "It was the angle I'd always hoped to find at iGEM," Pell says. Not everyone agreed, however, leading to a minor debate among the judges about whether such an impractical creation belonged at iGEM. In the end, the team got a "Best Presentation" award, and several other art pieces have since been entered.

Catts says that this kind of creativity and "irrational design" have been providing a much-valued counterweight to the stolid logic of the field's many engineers and computer scientists. "There's a nice amount of mutual respect when a field is still embryonic and territories haven't been carved out yet," Pell says. But as synthetic biology matures and becomes a lucrative area for investors and entrepreneurs, he expects there will be growing pressure on artists to present particular perspectives on the field. He fears this sweet period of artists freely cooperating with scientists may be nearing its end.

Shades of ethical gray

Eager to avoid the mistakes made with the introduction of genetically modified organisms, which drew irreparable backlash from the public, the scientific world, particularly in Europe, hopes to enlist the aesthetic contributions of bioartists to their cause. Institutions such as the U.K. Royal Academy of Engineering, in discussions about how to engage the public, have called on artists to help illustrate synthetic biology in outreach programs. And it's common practice for European companies, including some biotech firms, to include artists in their public outreach budget—with, Catts says, unspoken PR expectations.

So Catts has been hard at work fighting what he sees as a concerted and premeditated effort to co-opt artists into helping engineer public acceptance of synthetic biology. "I think they've got a misconception about the role of artists in society," he says. "It's art's place not just to make sense of [science] but to critique it."

But insofar as artists are interpreters, informing a society that gets its science in sound bytes, their messages span the range. For each shock artist who makes dire predictions and illustrations of "spider-goats"—inspired by a scheme to put a spider gene into goats—there exists what Catts calls a "technofetishist" who revels in humans' ability to modify the world and themselves.

Yet most of those who have talked to scientists and learned about synthetic biology inhabit a middle ground. "It's an ethical gray zone I like to explore in my work, and I like people to engage with," says designer Tuur van Balen of the Royal College of Art in London.

Humor also plays a role: One of Van Balen's projects, Pigeon d'Or, consists of a window trap with pigeons. He envisions them eating a gut bacterium that he would "engineer" to produce a biological soap that could pass through the pigeon gut intact, spreading sudsy excreta. The idea? Feeding the bacteria to pigeons could draft them as the ecosystem's windshield washers. This absurd flight of fancy should make people stop and think about how synthetic biology might turn ecology on its ear.

The question of how synthetic biology will affect larger organisms and ecosystems intrigues Alexandra Daisy Ginsberg, one of the founders of Synthetic Aesthetics. "There's something not so threatening about microbes," she says. So she decided to make "something visceral: What will synthetic biology actually look like?" she asks. One of her projects, Synthetic Kingdom, explores environmental health effects. For instance, future organisms designed to make telltale red crystals when exposed to carbon monoxide might inadvertently colonize human lungs. In smokers, this could produce an artistic result: red lungs.

Another Ginsberg piece, *E. chromi* (see image), imagines a future in which we ingest synthetic bacteria that turn our feces different colors according to the diseases we have. The project is a response, Ginsberg says, to the personalized medicine that synthetic biology promises. This "suitcase of poo" has won numerous art awards and is now being displayed in the Museum of Modern Art in New York City. For Ginsberg, who says she's "frustrated by misinformed visions" of the future, getting people to think about the technology's day-to-day implications is the most important issue.

Her fellow artists also want to be thought-provoking. "I'm not a science communicator," Van Balen says. "I don't want people to see my work and learn what synthetic biology is; I hope their reaction would be to walk away and scratch their heads and be a bit puzzled."

–SARA REARDON

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REVIEW

Synthetic Biology: Integrated Gene Circuits

Nagarajan Nandagopal[1] and Michael B. Elowitz[2]*

A major goal of synthetic biology is to develop a deeper understanding of biological design principles from the bottom up, by building circuits and studying their behavior in cells. Investigators initially sought to design circuits "from scratch" that functioned as independently as possible from the underlying cellular system. More recently, researchers have begun to develop a new generation of synthetic circuits that integrate more closely with endogenous cellular processes. These approaches are providing fundamental insights into the regulatory architecture, dynamics, and evolution of genetic circuits and enabling new levels of control across diverse biological systems.

Cells use genetic circuits of interacting genes and proteins to implement diverse functions including growth and division, signaling, and differentiation. Most of our knowledge of these circuits comes from top-down approaches based on genetic or pharmacological perturbations of model systems. Despite the increasingly comprehensive interaction maps these approaches are producing, it remains challenging to answer fundamental questions about gene circuit design, such as why one circuit architecture may have been selected over another or how a given circuit will respond to changes in its inputs (*1*–*3*). In addition, it remains difficult to engineer circuits for use in biotechnological or biomedical applications (*4*).

Synthetic biology offers an alternative bottom-up approach to understanding biological circuits, based on designing and constructing simple synthetic gene circuits from well-characterized genes and proteins and then analyzing their behavior in living cells (*1*–*5*). It thus reflects a shift in genetic engineering from the level of an individual gene to the level of a gene circuit. Over the last decade, synthetic approaches have provided key insights into gene circuit design principles (*1*–*3*, *6*, *7*).

This field began with the goal of creating autonomous genetic circuits that could function as independently as possible from endogenous circuitry or even functionally replace endogenous circuits. For example, early work demonstrated a working bistable switch, as well as self-sustaining oscillations (*8*, *9*). The view was that underlying cellular processes could be used to support the synthetic circuits, for example, by providing gene expression machinery, but that the two layers could function independently.

Recently, a new generation of synthetic biology experiments has moved toward tighter integration between endogenous and synthetic circuitry (Fig. 1). This has been driven both by difficulties in building autonomous synthetic gene circuits—"from scratch"—that behave predictably and by the need to engineer synthetic systems that control central biological processes in the host organism. Here, we discuss results that show how fundamental biological understanding can be obtained at the interface between the natural and the synthetic.

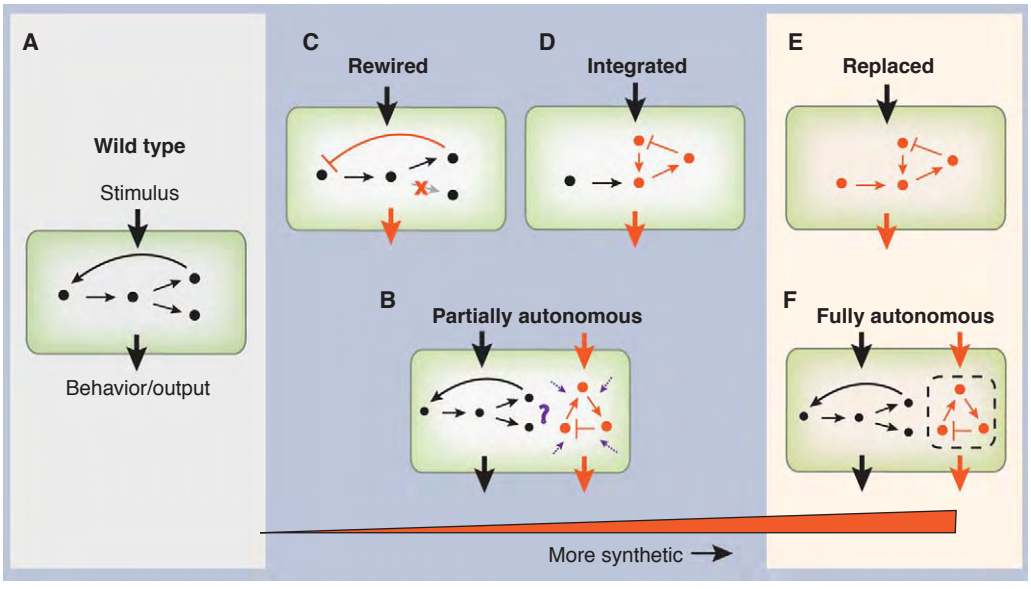

Fig. 1. A continuum of synthetic biology. Wild-type cells (**A**) can be subject to two basic types of synthetic manipulation. (**B**) Autonomous synthetic circuits, consisting of ectopic components, may be introduced into the cell. Such circuits process inputs and implement functions (orange arrows) separate from the endogenous circuitry (black). However, unknown interactions with the host cell may affect their function (purple arrows). (**C**) An alternative is to rewire (orange lines) the endogenous circuits themselves to have new connectivity. (**D**) Extending this line of synthetic manipulation, synthetic circuits could be integrated into appropriately rewired endogenous circuitry to act as sensors and to implement additional functionality. Ultimate goals of this program are to be able to design and construct (**E**) synthetic circuits that can functionally replace endogenous circuits or (**F**) fully autonomous circuits that operate independently of the cellular mileu.

Effects of Cellular Milieu on Synthetic Gene Circuits

Does a synthetic circuit need to operate independently of its host to function reliably? Hasty and co-workers recently constructed a simple transcriptional oscillator that exhibited regular self-sustained oscillations in *Escherichia coli*. Their design, based on previous theoretical work (*10*), consisted of just two genes: an activator and a repressor. Expression of either gene could be enhanced by the activator protein but blocked by the repressor protein, as both were transcription factors. Small molecule inducers could be used to modulate the strength of these two transcription factors, enabling "tuning" of circuit parameters. In individual cell lineages, the oscillations were precise, with sister cells remaining in phase for multiple periods. They were also robust, as they occurred across a broad range of inducer concentrations (*11*).

In fact, the circuit performed almost too well. The model predicted oscillations in a much more limited range of parameters than observed experimentally. Careful analysis showed that this

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apparent discrepancy arose from two unexpected sources: First, time delays inherent in the process of gene expression, although much shorter than the overall period of the oscillator and hence initially ignored, were nevertheless critical for its robust operation. The authors confirmed the importance of these delays by demonstrating that even a one-gene synthetic oscillator based on autorepression could generate oscillations—albeit not as strong, precise, or tunable as those in the two-gene circuit (*11*) [see also (*12*, *13*)].

Second, there was an unintended, but critical, interaction with host cell components: Both the activator and repressor contained identical destabilization sequences that targeted them for proteolysis. High levels of either protein saturated the proteolytic machinery and effectively stabilized both, causing an indirect posttranslational coupling between the activator and repressor. This coupling, a consequence of unintended interactions with the host, helped to reduce phase drifts between the proteins and improved the precision of the oscillator (*14*).

Although such unanticipated interactions are often assumed to be disruptive, it is clear that they may also play more supportive roles in the functioning of synthetic circuits. More generally, this result provokes the questions of how such interactions can be identified and exploited to improve synthetic circuit performance (*15*).

Rewiring Endogenous Gene Circuits

Many important genetic circuits are either incompletely understood or tightly integrated into larger genetic systems that control complex processes. Although replacing or reconstructing such systems synthetically may be impractical, one can in many cases modify ("rewire") parts of these circuits, providing insights into the design principles of the natural circuit architecture.

For example, Çağatay *et al*. recently rewired the *Bacillus subtilis* gene circuit that allows individual cells to sporadically and transiently differentiate into a genetically competent state, where they can take up DNA from the environment (*16*). A core feedback module enables the system to work in an excitable fashion, where fluctuations (noise) stochastically trigger episodes of competence (*17*). The system revolves around the master transcription factor ComK, which is sufficient to initiate and maintain competence but which eventually brings about its own destruction and exit from competence through a negative-feedback loop. In this loop, ComK indirectly represses expression of its stabilizing partner, ComS (Fig. 2A). Exit from competence occurs when ComS decreases to low levels, where it is more susceptible to stochastic fluctuations, which explains the broad distribution of competence durations observed in a wild-type population.

But what if the negative-feedback loop were structured the opposite way—if ComK activated its own inhibitor, MecA (Fig. 2B), rather than inhibiting expression of its activator, ComS (Fig. 2A)? In that case, competence exit would occur at high MecA concentrations and therefore would be less sensitive to noise (Fig. 2B). To test this prediction, the negative-feedback loop was rewired to the alternative feedback architecture. As predicted, the rewired cells exhibited much greater precision in competence durations, while functioning normally in other ways (*16*). Why have cells evolved the inherently more variable design? In this case, variability is functional: At low external DNA concentrations, it allows some cells to stay competent long enough to take up DNA, while ensuring that other cells do not stay in the slow-growing competent state longer than necessary when DNA concentrations are high (*16*, *18*).

Such rewiring can also provide insight into higher organisms, where circuit diagrams are complex and incomplete. For example, Lahav and co-workers recently rewired regulatory circuitry surrounding the mammalian tumor suppressor p53, which plays a central role in cancer and cell cycle regulation. In response to damaging radiation, the endogenous circuit displays sustained oscillations. With the rewired circuit, the authors showed how different features of the circuit, especially its feedbacks, tune the amplitude, frequency, and damping of p53 responses (*19*).

Rewiring Signal Transduction

A set of core signaling pathways allows cells to send, receive, and process information from the environment and other cells. Signaling pathways have undergone considerable diversification during evolution. Several synthetic experiments have exploited the evolutionary plasticity of signaling pathways to gain basic insights into their structure and mode of diversification and to elucidate their signal-processing capabilities.

Modifying signaling specificity. Mitogen-activated protein kinase (MAPK) pathways integrate information from a wide range of growth factors and other pathways and activate specific classes of targets. These input-output connections have changed over evolution. How is specificity encoded in these proteins, and can we learn to reprogram it?

Pioneering work from Lim and co-workers demonstrated that specificity could be rewired by modifying the scaffold proteins (illustrated in Fig. 3B) that bring together multiple MAPK components (*20*). Even with scaffolds, however, MAP kinases still require correct molecular recognition for specific phosphorylation of substrates. To understand and reprogram these specificities, Mody *et al*. analyzed MAPKs from four different families, including orthologs of the yeast MAPKs Hog1 (high osmolarity) and Fus3 (mating), across diverse eukaryotic species (*21*). They identified distinct patches on the protein surface that had residues that were variable across families, but conserved within the same family, and that may determine interaction specificity. They reasoned, for example, that a residue in Hog1 that is conserved in its orthologs is likely to be important for the functioning of the pathway but can only be responsible for the interaction specificity of Hog1 if it differs in other families, such as Fus3.

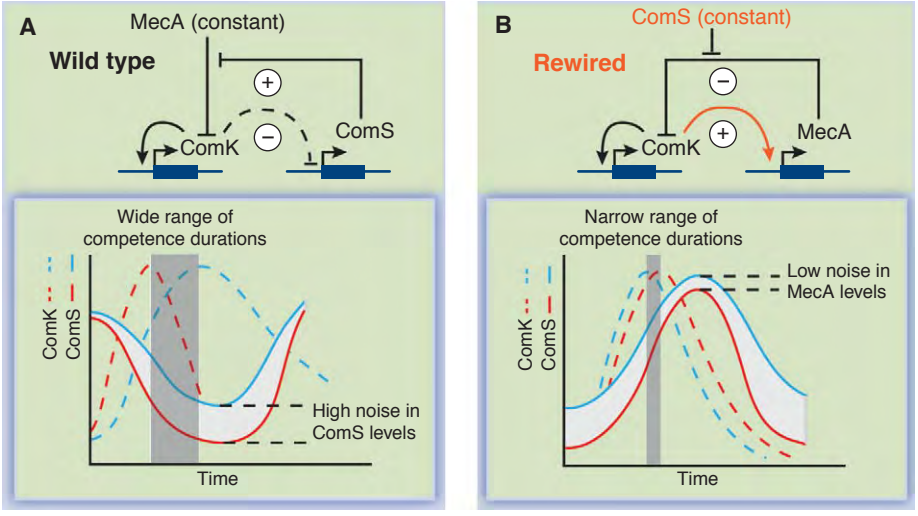

Fig. 2. Rewiring an endogenous gene circuit. (**A**) (Top) Part of the natural competence circuit from *B. subtilis*. The MecA protease adaptor (assumed to be constant) degrades ComK; ComS inhibits this degradation and thus is an indirect activator. ComK indirectly represses ComS. (Bottom) Exit from competence depends on returning to low ComS levels. Noise in ComS (white region between red and blue curves, representing the extremes of the distribution of ComS profiles in a population) is significant at such low levels. The resulting distribution in ComK curves (red dashed and blue dashed)—and thus competence durations (vertical gray bar)—is wide. (**B**) (Top) The rewired competence circuit: Here the activation and repression loops have been switched. Competence exit occurs when MecA levels reach a high threshold. (Bottom) The resulting distribution of competence curves is narrow because variability in MecA is relatively low at high MecA concentrations.

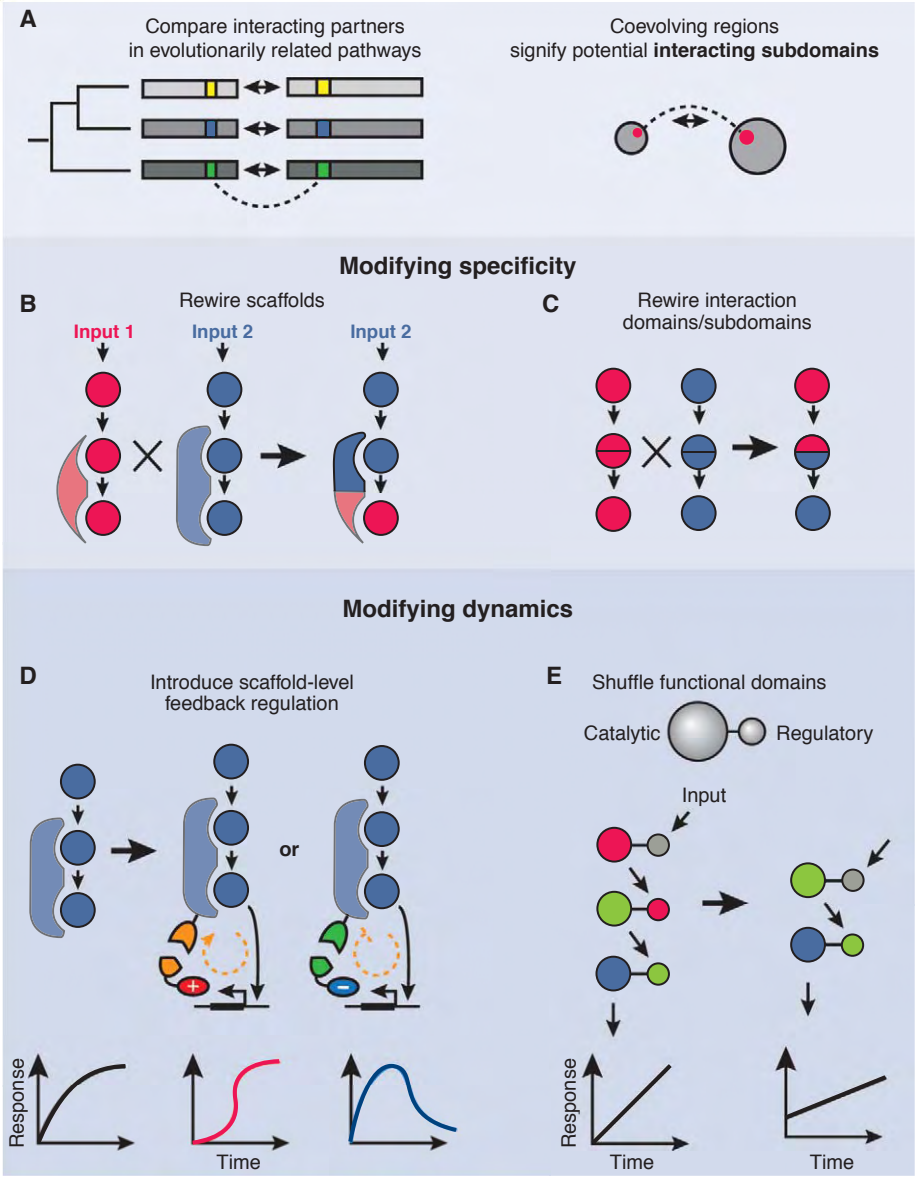

Fig. 3. Diversifying signaling pathways through rewiring. (**A**) Sequence correlations among proteins from a large family (left, SCA, also see text) can be used to identify interacting subdomains (right). Interaction specificity can be altered by rewiring scaffolds (**B**) or by shuffling specificity-determining domains or subdomains (**C**). The dynamic behavior of a pathway can be modified by (**D**) introducing new autoregulatory connections or by (**E**) altering regulation directly at the protein level by generating new combinations of catalytic and regulatory protein domains.

They synthesized a library of 64 Fus3-Hog1 hybrid proteins by dividing the primary sequence of each protein into six putative specificity-determining segments, with each synthetic protein incorporating one of the two variants for each segment. The library was highly enriched for functional proteins capable of rerouting signaling specificity (*21*) (Fig. 3C). For example, some variants activated the same pathway in response to either input; others activated both pathways in response to one input.

Similar questions occur in prokaryotes, which rely heavily on two-component systems for signaling. In the canonical two-component system, a sensor histidine kinase (HK) phosphorylates a corresponding response regulator (RR). Tens or even hundreds of such systems can occur in a single genome, and interactions are highly specific, with one HK almost always signaling to one RR (*22*). It had long remained unclear whether and how it would be possible to rationally and systematically reengineer their specificity.

To address these issues, Skerker *et al.* used statistical coupling analysis (SCA). SCA quantitatively examines evolutionary correlations between amino acid positions, by assuming that pairs of amino acids that functionally interact with each other are more likely to covary during evolution (*23*). Applying SCA to an alignment of many HK-RR sequence pairs and taking advantage of existing structural data, they identified potential specificity-determining residues in covarying patches on the interacting protein surfaces (Fig. 3A). By systematically mutating these residues in one HK to the corresponding amino acids in another HK, they created a new highly specific HK-RR pair (*23*). Evidently, these proteins have evolved an economical structure, where specificity determinants are concentrated into relatively compact regions of the proteins, facilitating functional diversification through minimal sequence evolution. More generally, evolutionary information is proving to be a powerful tool both for addressing fundamental questions in structural biology, such as the mechanisms of allostery (*24*), and for engineering new protein components for synthetic biology.

Programming signaling dynamics. Signaling pathways are characterized not just by their molecular interactions but also by their response dynamics. Recently, Peisajovich *et al.* showed that new signaling responses could be efficiently generated by systematically shuffling regulatory and catalytic domains within the yeast mating pathway (*25*). For example, in a 66-protein library containing domains from 11 yeast mating-pathway proteins, they observed several variants that exhibited qualitative changes in the mating pathway response dynamics (Fig. 3E). Remarkably, 6 out of the top 10 variant strains created by domain recombination mated more efficiently than wild type (*25*).

Feedback loops strongly modulate response dynamics in several pathways. In order to understand the role of feedback, Bashor *et al.* (*26*) rewired the yeast MAPK pathway by genetically modifying the scaffold protein to recruit positive or negative modulators. By expressing these modulators under the control of the pathway itself, they were able to create a variety of feedback structures, whose strength could be controlled with competing "decoy" proteins. The scaffold thus became a versatile synthetic "signal hub" that integrated regulatory information from multiple sources. A slew of nonnative dynamic responses, from adaptation to ultrasensitivity, could be generated by modulating the strength, timing, and sign of these synthetic feedbacks (Fig. 3D) (*26*).

Deciphering signal encoding. Feedback, crosstalk, and the induction of dramatic cellular changes like differentiation make signaling difficult to study in natural contexts. To circumvent this problem, researchers have begun to transplant signaling pathways from one organism to another and to divert the outputs of signaling pathways away from their native targets to reporter genes that permit quantitative readouts.

For instance, MAPK pathways display diverse behaviors, ranging from graded responses

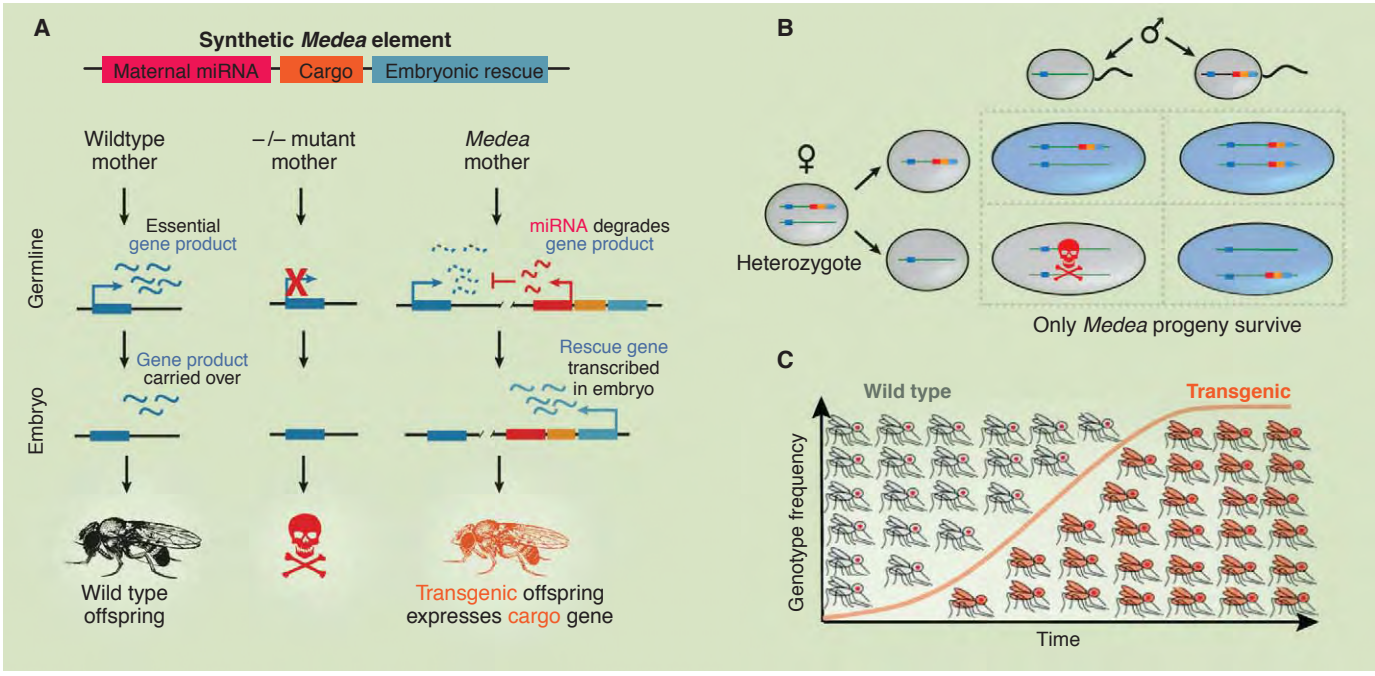

Fig. 4. An integrated synthetic circuit controls development and population dynamics. (**A**) In *Drosophila*, the synthetic *Medea* element (top) maternally expresses an miRNA (red) that silences a maternally expressed gene whose product is essential for embryogenesis (left column). Eggs from female flies mutant for this gene do not hatch (middle column). The *Medea* element also contains a rescue gene that is expressed only in the early embryo. The *Medea* element may also accommodate a cargo gene that is expressed in *Medea* progeny (right column). (**B**) Progeny of female *Medea*-positive flies will only survive if they receive the *Medea* element from either parent. (**C**) This super-Mendelian inheritance pattern can efficiently drive *Medea* into populations.

(*25*) to ultrasensitivity (*27*), in different contexts. To better understand this physiological plasticity, O'Shaughnessy *et al*. ported into yeast the core mammalian cascade, comprising the three kinases Raf, MAPK kinase, and ERK (extracellular signal–regulated kinase, a type of MAPK) (*28*). They also modified the upstream kinase Raf to make it directly controllable by β-estradiol. This system showed that ultrasensitivity was an inherent feature of the cascade and that the sharpness and amplitude of the ultrasensitive response could be independently controlled simply by varying the relative concentrations of kinase components. Thus, the core MAPK cascade acts as a tunable amplifier, whose behavior can be modulated by the cell to generate diverse responses (*28*).

In the Notch pathway, which is normally under elaborate regulation, diverting signaling has provided qualitative insights that would have been difficult or impossible to obtain in native systems. The membrane-bound Notch receptor and its ligand Delta together enable direct communication between neighboring cells in developmental patterning processes (*29*). Previous work showed that Delta inhibits Notch in its own cell, but activates Notch on neighboring cells (*30*). Activation involves the release of the cleaved Notch intracellular domain, which translocates to the nucleus to activate target genes (*31*).

To understand how Notch output depends on ligand levels in its own cell and neighboring cells, Sprinzak *et al*. sought to reconstruct the signaling pathway from the bottom up (*32*). They incorporated Notch-Gal4 hybrid receptors, which activate engineered nonnative target genes in response to signals (*31*), and analyzed their activation in individual cells. These studies revealed that, because of inhibitory interactions between Notch and Delta in the same cell, the pathway acts like a "walkie-talkie," allowing cells to send or receive signals but not both at the same time. This property could facilitate many Notch-dependent developmental patterning processes, by helping to enforce sharp distinctions between neighboring cells (*33*).

Toward Functional Replacement

Could complicated endogenous circuits eventually be replaced by controllable synthetic counterparts that have altered functionality (Fig. 1E)? Coudreuse and Nurse took a step in this direction by replacing much of the eukaryotic cell cycle control network with a single gene (*34*).

Although much is known about regulation of cell cycle progression, the specific roles of many components, as well as aspects of the overall logic of the system, remain unclear. Working in fission yeast, Coudreuse and Nurse began by systematically deleting many of the regulatory components associated with the cell cycle, including the single cyclin-dependent kinase (CDK) and all the cyclins known to interact with it. In this background, they expressed a cyclin-CDK fusion protein, under the control of the endogenous cyclin promoter. This minimal replacement was sufficient to drive the cell cycle, from G_1 through S, G_2 and M, with no observable differences from the wild type under laboratory conditions.

To understand how the cell executes different cell cycle phases with a single cyclin-CDK fusion, the authors used an engineered inhibitor of the CDK. They found that different inhibitor concentrations were required for impairment, depending on the stage of the cell cycle. For instance, more inhibitor was required to delay the G_2/M phase progression than the G_1/M. This suggested that different checkpoints in the cell cycle were navigated simply by varying the concentration of active CDK. Moreover, the cycle could be reset and controlled arbitrarily by inhibiting the cyclin-CDK fusion at different points and to different extents. The cell cycle is one of the foundations of life; successfully replacing it with a synthetic module represents a fundamental advance in synthetic biology.

Integrated Synthetic Circuits

Sensing cell state. Several new "plug-and-play" synthetic devices can interface with cells as sensors (*35*, *36*) to monitor dynamic changes in cell state. Burrill and Silver recently created a synthetic memory circuit that can remain on for several generations after an activating event, and they interfaced it with the natural DNA damage response in yeast (*37*). Using this system, they learned that DNA damage response was heterogeneous in the population and led to heritable

pleiotropic effects in progeny. Cleverly designed sensors like these may prove to be useful in studies of cell differentiation and decision-making, where cells are thought to progress through a continuum of poorly understood cellular states.

Controlling multicellular development and genetic inheritance. Recent work in *Drosophila* has shown that synthetic circuits can fundamentally alter the development and life cycle of a multicellular organism in a controlled way. Chen *et al*. created a synthetic selfish genetic element, named *Medea*, capable of spreading through a population (*38*). The synthetic *Medea* element (Fig. 4A) maternally expresses a microRNA (miRNA) that blocks expression of an essential protein normally produced by the mother and deposited in the egg. The element also expresses an "antidote" to this toxic miRNA, which consists of a second copy of the gene (with different codons) expressed by the embryo rather than the mother. Replacing the maternally expressed gene with its zygotically expressed *Medea*-based counterpart maintained normal development in offspring. *Medea*-positive mothers always express the toxic maternal miRNA. Thus, progeny of such mothers only survive if they inherit *Medea* from either or both parents—a dramatically non-Mendelian inheritance pattern.

A key consequence is that *Medea* is capable of invading populations. When *Medea*-positive flies are introduced into a wild-type laboratory population, the *Medea* element rapidly takes over the whole population (*38*). A similar synthetic system in mosquitos could in principle be engineered to carry a "cargo" gene that would diminish the ability of malarial parasites to survive in the mosquito or to be transmitted to human hosts (Fig. 4C).

A striking aspect of the *Medea* system is that it works across multiple levels: At the circuit level, it rewires expression of a critical gene to alter the timing and genetic source of expression (Fig. 4A). At the developmental level, this leads to a selective killing of embryos that lack the *Medea* element (Fig. 4B). Finally, at the population level, this gives *Medea* transgenic organisms the ability to efficiently spread through a population (Fig. 4C). Although many challenges remain, this system and others [see (*39*, *40*)] demonstrate the power of integrating synthetic biology approaches into the circuitry of a complex organism.

Conclusion: Exploring the Biology That Could Be

Synthetic biology opens up the possibility of creating circuits that would not survive in the natural world and studying their behaviors in living cells, expanding our notion of biology (*41*). The last decade has shown how even our first steps toward building and analyzing synthetic circuits can identify fundamental biological design principles and can produce useful new understanding. Future progress will require work across a range of synthetic levels (Fig. 1), from rewiring to building autonomous and integrated circuits de novo. Going forward, we anticipate that synthetic biology will become one of the primary tools we use to understand, control, imagine, and create biological systems.

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REVIEW

Synthetic Biology Moving into the Clinic

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Synthetic biology is an emerging field focused on engineering biomolecular systems and cellular capabilities for a variety of applications. Substantial progress began a little over a decade ago with the creation of synthetic gene networks inspired by electrical engineering. Since then, the field has designed and built increasingly complex circuits and constructs and begun to use these systems in a variety of settings, including the clinic. These efforts include the development of synthetic biology therapies for the treatment of infectious diseases and cancer, as well as approaches in vaccine development, microbiome engineering, cell therapy, and regenerative medicine. Here, we highlight advances in the biomedical application of synthetic biology and discuss the field's clinical potential.

A little over a decade ago, the development of two engineered gene networks—a toggle switch (*1*) and an oscillator (*2*)—set in motion the rapid emergence of synthetic biology as a field. In the years following, increasingly sophisticated synthetic gene circuits have been designed and constructed. Inspired by electrical circuits as well as natural biomolecular networks, these devices include timers, counters, clocks, logic processors, pattern detectors, and intercellular communication modules (*3*–*9*). These DNA-encoded synthetic circuits are typically uploaded into cells, with their programmable abilities allowing for the precise control of cellular behavior and phenotype.

Meanwhile, there is a growing need for the development of new, important medical treatments. Bacteria, for example, are becoming resistant to antibiotics faster than we can develop effective replacements (*10*). Additionally, surgery remains a common cancer treatment, and when

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radiation and chemotherapy do work, patients suffer off-target effects. Customized therapies that can be designed to interact with a patient's physiology in prescribed ways are needed.

The field of synthetic biology is beginning to use its methods and platforms to bring engineering approaches into biomedicine. Effective synthetic biology therapies are being rationally designed and implemented as researchers build constructs (e.g., engineered biomolecules, synthetic gene networks, and programmable organisms) to alter mechanisms underlying disease and related biological processes (Fig. 1). Here, we highlight synthetic biology strategies that have been developed to target infectious diseases and cancer, as well as approaches in vaccine development, microbiome engineering, cell therapy, and regenerative medicine. We conclude by discussing how future work in synthetic biology could affect biomedicine and by describing the challenges that need to be overcome for the field to translate its promise into practice.

Treatment and Prevention of Infections

In addressing the need to develop strategies to enhance our antimicrobial arsenal, synthetic biology constructs have been developed to treat bacterial infections, as well as improve the efficacy of existing antibiotics. For example, bacteriophage—viruses that only infect specific bacteria— have been engineered to attack or weaken resistant bacterial strains by disrupting antibiotic defense mechanisms.

In an initial study, enzymatic bacteriophage were engineered to degrade bacterial biofilms and kill off bacterial cells in the biofilm (*11*). Biofilms, which play a critical role in the pathogenesis of many persistent infections, are surface-associated bacterial communities encapsulated in an extracellular polymeric matrix that shields bacteria from attack by host immune defenses and antibiotics. Lytic T7 phage were engineered to express the biofilm-matrix–degrading enzyme dispersin B (DspB) as well as rapidly replicate during infection. In a two-pronged attack, bacterial lysis induced by the engineered phage killed the infected bacterial cells in the biofilm and dispersed DspB along with the newly produced phage. The released DspB degraded the biofilm matrix, which exposed newly unprotected bacteria to the released phage, resulting in a cyclic process that eventually removed 99.997% of bacterial cells in treated biofilms.

In a second study, synthetic adjuvants were designed by engineering bacteriophage to enhance the killing efficacy of existing antibiotics (*12*). This approach focused on disrupting bacterial networks that regulate antibiotic defense mechanisms. All bactericidal antibiotics induce DNA damage, resulting in the activation of the SOS response network (*13*). Nonlytic M13 phage, chosen to minimize activation of bacterial adaptation mechanisms, were engineered to inhibit the damage response by overexpressing *lexA3*, a repressor of the SOS network (Fig. 2A). Phage treatment resulted in significantly enhanced killing of bacterial strains by three major classes of antibiotics, that is, quinolones, β-lactams, and aminoglycosides. For example, in vitro treatment with engineered phage and the quinolone ofloxacin resulted in a 5000-fold increase in the killing of resistant bacteria compared to treatment with the antibiotic alone. In an animal study, treatment with engineered phage and ofloxacin resulted in an 80% survival rate in *Escherichia coli*–infected mice, compared to 20% with antibiotic treatment alone.

Synthetic constructs can also be designed to limit the spread of infection by targeting disease vectors. Along these lines, Crisanti and colleagues recently attempted to reduce malaria transmission by rationally modifying the disease's mosquito vector using a synthetic biology approach. Specifically, they built a synthetic construct that could, in principle, enable a laboratory mosquito population to rapidly disseminate a genetic modification (e.g., disruption of genes encoding malaria vector capability) to a field population (*14*).

This transgenically introduced construct—a synthetic, homing endonuclease-based gene (HEG) drive—consisted of mosquito regulatory regions and a homing endonuclease gene, *I-SceI* (fig. S1). The gene drive first used endonuclease to induce double-strand DNA breaks that activated the recombinational DNA repair system in mosquito cells. The homologous chromosome, carrying the HEG (and potentially any other synthetic or endogenous gene), was then used as a template for repair, resulting in both chromosomes carrying the synthetic drive. The HEG drive rapidly spread in transgenic cage populations that carried corresponding endonuclease recognition sites, matching analytical model predictions, and molecular analyses showed high rates of chromosomal cleavage and conversion. For the eventual deployment of this system in the wild, the synthetic HEG drive will require, among other things, identifying or engineering a homing endonuclease with recognition sites in the native vector genome. Of note, homing endonucleases have been designed to target specific DNA sequences for potential genome engineering and gene therapy (*15*, *16*). Alternatively, in addition to targeted disruptions, new genes could be distributed to suppress malaria vector capacity. In a review, Nandagopal and Elowitz (*17*) describe a synthetic *Medea* system inspired by natural gene drives (*18*), which quickly distributed genetic cargo to wild *Drosophila* populations.

Cancer Treatment

Despite the success of modern cancer therapies, the three major therapeutic interventions—surgery, radiation, and chemotherapy—still typically result in considerable damage to healthy tissue. We need new cancer treatments that precisely distinguish between diseased and healthy cells. To this end, synthetic biologists have engineered bacteria to target and invade cancer cells. In one study, the invasion was designed to occur only in specific tumor-related environments, whereas in another, the bacterial invaders were engineered to knock down a specific, endogenous cancer-related gene network.

In the first study, Voigt and colleagues engineered bacteria to invade cancer cells only in the hypoxic environment often indicative of tumor tissue (*19*). Cell-invasion ability was enabled in *E. coli* by engineering them to express the invasin (*inv*) adhesion protein from *Yersinia pseudotuberculosis*, which tightly binds mammalian β1 integrin receptors, inducing uptake. Invasin expression was placed under the control of an anaerobically induced formate dehydrogenase promoter, resulting in bacteria that only invaded mammalian cell cultures in hypoxic environments. Tissue is typically hypoxic, however, when it has no access to blood, which could limit the efficacy of intravenously delivered,

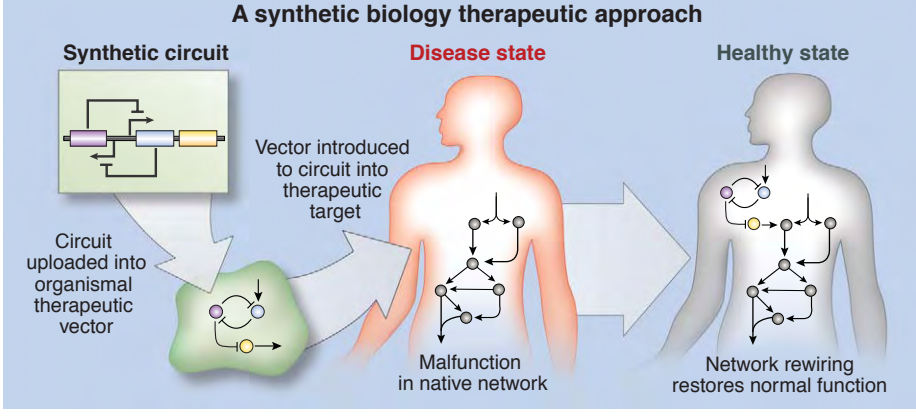

Fig. 1. Synthetic circuit development for the treatment of disease. Synthetic gene networks are uploaded into cells to therapeutically target the body's endogenous networks, causing a transition from disease to healthy state. Here, the uploaded network is a bistable toggle switch, which enables cellular memory with a network of two mutually repressible modules.

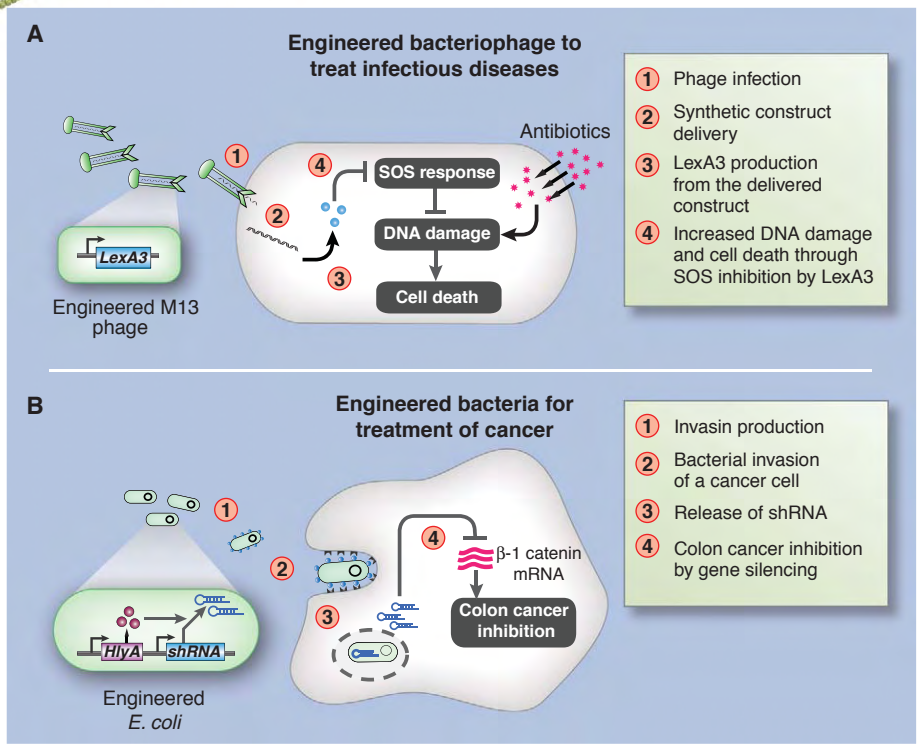

Fig. 2. Synthetic biology approaches for treatment of bacterial infection and cancer. (**A**) Engineered bacteriophage boosted antibiotic killing efficacy by disrupting repair of antibiotic-induced damage. (**B**) Engineered bacteria invaded cancer cells and knocked down a cancer gene using RNAi.

cancer-targeting bacteria. Also, given the dynamics of blood flow, the bacteria would need to be engineered to quickly express invasin and enter cells.

In the second study, Li and colleagues were able to intravenously deliver engineered, cancer-invading bacteria to target a specific tumorigenic pathway in vivo (*20*). Using RNA interference (RNAi), bacterial invaders were designed to knock down expression of *CTNNB1* (encoding β-1 catenin), a gene that initiates many colon cancers upon its overexpression or oncogenic mutation (Fig. 2B). The engineered bacteria accomplished gene knockdown by generating short hairpin RNA (shRNA) segments that bound to *CTNNB1* mRNA transcripts and induced mRNA cleavage. Along with the shRNA and invasin, the synthetic system produced lysteriolysin O (encoded by the *hlyA* gene), which enables molecular transport out of vesicles in a process believed to involve entry vesicle disruption.

Bacteria cells uploaded with the synthetic circuitry robustly invaded colon cancer cells in vitro and knocked down *CTNNB1* expression. Intravenous administration of the engineered *E. coli* into immune-deficient mice with subcutaneously xenografted human colon cancer cells resulted in significant knockdown of the gene in tumor cells, showing that bacterial invaders could be directed to distal cancer targets. In the future, the two synthetic constructs described above could be coupled, potentially producing programmable bacteria that invade cancer cells under specific in vivo conditions and, once inside, target specific cancer-related pathways.

Vaccine Development

The development of new vaccines is limited by several drawbacks, including risks associated with the use of attenuated pathogens, along with difficulties altering vaccine target specificity. To address these issues, Mastrobattista and co-workers used liposomes—synthetic vesicles consisting of a lipid bilayer—to encapsulate a combination of a reconstituted bacterial transcription-and-translation network and DNA encoding a model antigen (β-galactosidase) (*21*). The system (fig. S2) produced functional antigen protein in vitro. In live mice, antigen-expressing liposomes generated a higher humoral immune response compared with control vaccines (liposomes encapsulating only the antigen, the transcription-and-control network, or the DNA template, respectively). This system can be easily altered for other antigens by simply changing the DNA template and carries no risk of infection by attenuated pathogens.

Additional progress in the field may come from combining synthetic circuits with recent genomic engineering advances in vaccine development. For example, Wimmer and colleagues attenuated poliovirus by exploiting species-specific bias for codon pairs (*22*). Although DNA codons are synonymous (several different codons can encode a single amino acid), every species has a bias for the adjacent codon pairs it can translate efficiently into protein.

To exploit this bias, hundreds of synonymous codon pairs were switched in the gene sequence encoding the poliovirus capsid protein, resulting in decreased translational efficiency. The resulting inefficient, attenuated virus was sufficient to provide protective immunity after challenge. However, in this case, the DNA encoding the capsid protein was altered through de novo synthesis and reinserted into living cells. If a synthetic circuit could be designed to automatically swap synonymous codons in the genome of infected cells, a completely cell-based system for virus attenuation would be possible.

Microbiome Engineering

The human microbiome—the microorganisms associated with the human body—is a complex ecosystem increasingly implicated as a regulator of host physiology. It numbers over 1000 species and outnumbers human cells by a factor of 10 to 100 (*23*). As microbiome constituents are typically well-tolerated, naturally commensal microorganisms, they are potentially excellent vectors for deploying synthetic gene circuits to fight disease and correct aberrant conditions. Social interactions within and between species also play a critical role in microbiome communities (*24*, *25*) and could be harnessed.

Along these lines, Duan and March recently used *E. coli* to prevent cholera infection by engineering a synthetic interaction between gut microbes (*26*). During cholera infection, *Vibrio cholerae* secrete virulence factors, such as cholera toxin (CT), only at low population density. To assess its own density, *V. cholerae* uses quorum sensing, a process in which autoinducer signaling molecules are both secreted and detected by members of a population. *V. cholerae* detects levels of cholera autoinducer 1 (CAI-1) and autoinducer 2 (AI-2), and when both are high, ceases expression of virulence factors. Duan and March took advantage of this mechanism and engineered *E. coli* that produce AI-2 to also secrete CAI-1 (Fig. 3). When infant mice ingested the engineered *E. coli* 8 hours before *V. cholerae* ingestion, their survival rate increased dramatically and cholera toxin intestinal binding was reduced by 80%.

Alternatively, a patient's microbiome could be engineered to deliver therapeutic molecules directly to the body. For example, commensal bacteria strains have been engineered to secrete key molecules for potential disease treatment, including insulinotropic proteins for diabetes (*27*), an HIV fusion inhibitor peptide for prevention of HIV infection (*28*), and interleukin-2 for immunotherapy (*29*). Although these studies showed effective expression of therapeutically relevant molecules, each would benefit from the development and use of synthetic circuits. By placing, for example, the expression of therapeutic molecules under the control of cell-based sensors that detect

aberrant, pathological conditions, gene expression could be turned on and tuned accordingly only when the prescribed molecular interventions are needed, reducing metabolic load on the bacteria and increasing their ability to assimilate into the microbiome.

Cell Therapy and Regenerative Medicine

Cell therapy—the introduction of prescribed cells into the body to treat disease—is promising, yet challenges remain due to an inability to control post-implantation cell behavior and phenotype. One solution could involve uploading synthetic circuits into cells before implantation, thus endowing them with sophisticated control systems. Unfortunately, the great majority of synthetic gene circuits designed thus far have been limited to microbes. The recent extension of synthetic circuits to mammalian cells, however, has opened the door to new and enhanced cell therapies.

Tight control of specific genes is critical for effective cell therapies. To address this problem, we recently developed a tunable, modular mammalian genetic switch (*30*). This entailed creating a synthetic gene network that couples repressor proteins with an RNAi design involving shRNA. Gene expression is turned on by adding an inducer, which controls the repressor elements at the transcriptional level, while simultaneously turning off the RNAi component to allow the transcript to be retained and translated (fig. S3). The switch offers >99% repression, as well as the ability to tune the expression of the gene of interest. Modular capabilities of the system allow for the regulation of any gene, as well as the potential for tissue-specific use (its genetic elements can be controlled by tissue-specific promoters). The switch was validated in mouse and human cells. This tight, tunable, and reversible control of mammalian gene expression could be used in cell therapy applications, as well as to determine whether a disease phenotype is the result of a threshold response to changes in gene expression.

Fussenegger and colleagues recently designed a synthetic mammalian gene circuit to regulate uric acid homeostasis in vivo, the disturbance of which is associated with tumor lysis syndrome and gout (*31*). This synthetic device sensed uric acid using an engineered repressor that could be induced (i.e., derepressed) by uric acid. Upon derepression, the network expressed an engineered urate oxidase that eliminated uric acid (Fig. 4A). Circuit-expressing cells implanted in urate oxidase–deficient, transgenic mice decreased urate concentrations to subpathological levels and reduced uric acid crystal deposits in the kidneys.

Shifting from transcriptional control to translational control, Smolke and colleagues constructed a synthetic device using a drug-responsive-RNA module for gene regulation in mammalian cells (*32*). In mice, the RNA device controlled T cell proliferation by linking a drug-responsive ribozyme to growth cytokine expression. Programming cells to execute sophisticated processes upon implantation could eventually allow synthetic gene circuits to be customized for individual patients.

The tailoring of engineered cells to a patient's physiology will also be critical in the field of regenerative medicine, where the eventual gold standard therapy likely will involve tissues created from a patient's own stem cells. Although the adult body maintains clinically accessible niches for some stem cell lineages (e.g., hematopoetic and adipogenic), many others are difficult to access. With the development of induced pluripotent stem cells (iPSCs), adult-derived stem cells that, in principle, could be differentiated into any cell type are now available. iPSCs can be created from an adult patient's cells upon the insertion and expression of only four genes [e.g., KLF4, c-MYC, OCT4, and SOX2 (KMOS)] (*33*), a breakthrough methodology that nonetheless comes with concerns and drawbacks (*34*). For example, virally introduced extra copies of all four genes must be inserted permanently into the cellular genome, which can make such cells prone to forming tumors.

Rossi and colleagues recently addressed this problem by adopting a synthetic biology approach and chemically transfecting cells with synthetic, modified RNA molecules that function as mRNA transcripts for the four key genes (*35*). Once inside cells, the transcripts are translated into proteins that induce pluripotency without the integration of extra genes into the genomes. Using this method, investigators were able to create iPSCs faster and with a greater yield than viral delivery (Fig. 4B). The team also used this method to create RNA-iPSCs (RiPSCs) from multiple human cell types and further showed that the same technology can efficiently direct RiPSCs to terminally differentiate into myogenic cells. In the future, it will be exciting to see whether synthetic biology approaches can create constructs that enable targeting and reprogramming of injured, diseased, or aged tissue in vivo.

Outlook

Although synthetic biology is in its infancy as a field, its practitioners are taking initial steps toward developing new biomedical therapies. The field initially arose from the combined efforts and insights of a small band of engineers, physicists, and computer scientists whose backgrounds dictated the early directions of synthetic biology. For the field to reach its full clinical potential, it must become better integrated with clinicians.

Clinical applications will surely necessitate increasingly complex circuits and constructs. Up to this point, the field has developed circuits using, more or less, the same collection of basic regulatory components. However, in order to build more complicated, clinically applicable circuits, it will be necessary to identify entirely new modules and components from endogenous networks as well as to synthesize and characterize diverse component libraries. Additionally, although most synthetic systems have been transcriptional, post-transcriptional systems, particularly protein-based systems, will be needed to enable faster responses. Along these lines, Voigt and colleagues have engineered protein-based light sensors and used them to activate mammalian cell signaling (*36*). We also will need more effective computational tools to fast-track synthetic biology, both for identifying new components and predicting the behavior of complicated synthetic systems.

Moreover, there exists a critical need to move synthetic biology increasingly toward mammalian systems. Most synthetic constructs have been deployed in microbes; however, many clinical problems will require mammalian circuits, components, and constructs. An expanded mammalian toolbox would enable synthetic biology to address a broader range of applications in

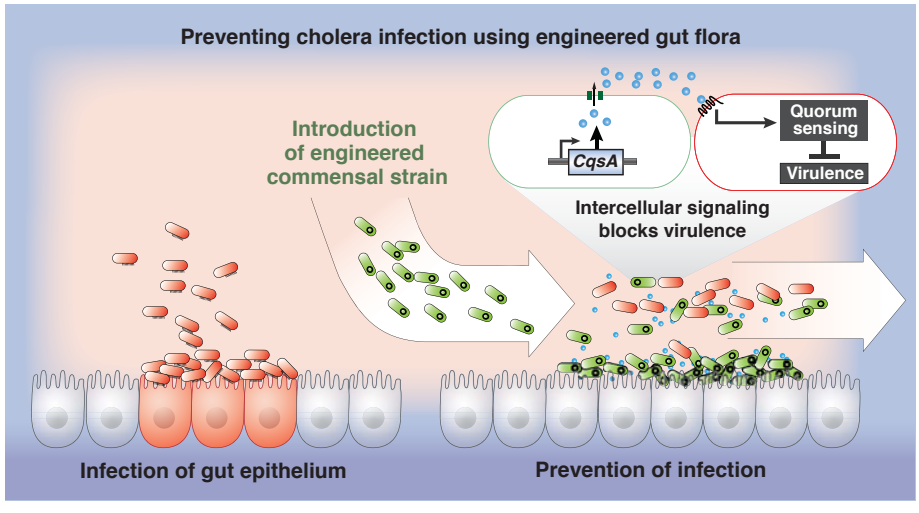

Fig. 3. Synthetic biology approach for microbiome engineering. Native commensal bacteria were engineered to secrete the molecular signal cholera autoinducer (CAI-1), which leads to inhibition of *V. cholerae* virulence.

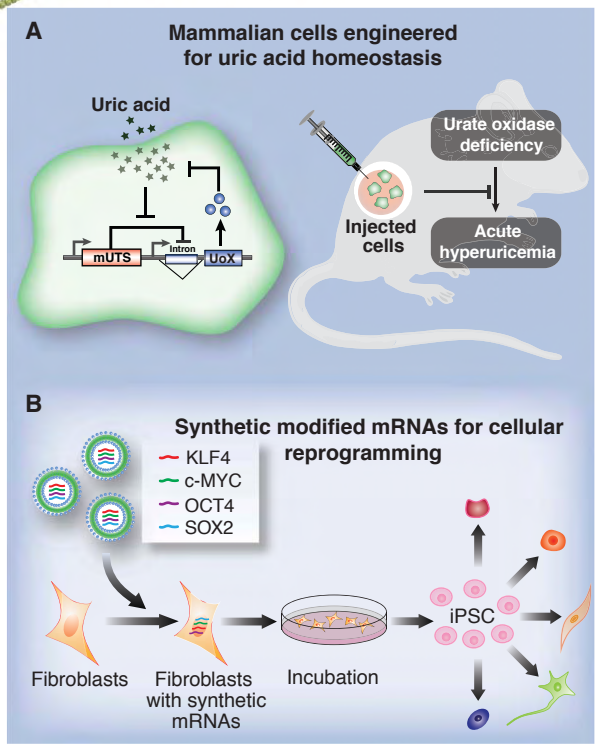

Fig. 4. Diseases can be targeted with new synthetic biology methods for cell therapy and regenerative medicine. **(A)** Urate homeostasis was restored in vivo by the delivery of cells with a synthetic circuit. Uric acid induced the derepression of an engineered urate oxidase, which then lowered uric acid levels in mice. **(B)** Synthetic modified RNAs encoding the KMOS transcription factors were delivered to mammalian fibroblasts to induce pluripotency upon translation. The RNA-induced pluripotent stem cells could be driven down numerous cell lineages.

translational medicine. These and related thrusts will benefit from emerging efforts to integrate synthetic biology with systems biology (*37*, *38*).

These developments will aid in the understanding of potential immune responses to synthetic constructs in the body and help identify approaches to ameliorate such responses. These efforts will be critical for developing safe and effective synthetic biology therapies.

Ultimately, we envision synthetic constructs that can sense and seek out aberrant conditions, remediate clinical insult, and restore function. Clearly, there is much to do before synthetic biology can realize its full clinical potential, but the examples discussed here provide insight into the field's exciting potential for helping to prevent and treat disease.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/333/6047/1248/DC1
Figs. S1 to S3

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PERSPECTIVE

Bottom-Up Synthetic Biology: Engineering in a Tinkerer's World

Petra Schwille*

How synthetic can "synthetic biology" be? A literal interpretation of the name of this new life science discipline invokes expectations of the systematic construction of biological systems with cells being built module by module—from the bottom up. But can this possibly be achieved, taking into account the enormous complexity and redundancy of living systems, which distinguish them quite remarkably from design features that characterize human inventions? There are several recent developments in biology, in tight conjunction with quantitative disciplines, that may bring this literal perspective into the realm of the possible. However, such bottom-up engineering requires tools that were originally designed by nature's greatest tinkerer: evolution.

An important feature of "synthetic biology" is that it draws on expertise from diverse disciplines; however, these disciplines have not converged on what the new field encompasses. Biotechnologists view it mainly as a new way to organize and structure the art of genetic engineering. To them, synthetic biology enforces the traditional engineering concepts of modularity and standardization and adapts logical operator structures from information processing (*1*). Nevertheless, the assembly of new biological systems for a variety of applications is still carried out in an existing organism; for clinical examples, see the review by Ruder *et al*. [see (*2*)]. Perhaps a more daring view comes from chemists and physicists who take the words literally and focus on the construction of biological systems from the bottom up. They suggest that synthetic biology could follow the tracks of synthetic organic chemistry and open up a new understanding of biology (*3*). This is not to suggest that something as complex as a eukaryotic or even a prokaryotic cell—end

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products of billions of years of evolution—could be constructed from scratch. Our current knowledge of genes and gene products, of networks and feedback systems, make us all too aware of the daunting complexity that is often compared with interlinked hyper-systems like the World Wide Web. On the other hand, biology has long surpassed its mainly descriptive stage, and the questions now asked are increasingly amenable to experimental approaches and theoretical concepts taken from the physical and engineering sciences. This in turn has directed quantitative scientists and engineers toward studying biological phenomena, bringing with them their successful "divide and conquer" rigorous reductionist approach.

From such a perspective, the underlying question of synthetic biology would not be how a biological system actually functions, but rather, how it could in principle function with a minimal set of elements. Unequivocally testing hypotheses, and quantitatively predicting experimental outcomes, is only possible if all parameters of interest can be independently regulated and determined—a formidable task for living systems. However, a quantitative molecular-level understanding has been achieved for reconstituted minimal systems that were stripped of their cell-inherent complexity, such as in the investigation of motor-filament assemblies at the single-molecule level (*4*) or the study of protein-controlled membrane transformations (*5*, *6*), to name but two. Clearly, understanding the biochemical or mechanical features of limited subsystems—although being an important prerequisite for a quantitative understanding of the cell—does not suffice for addressing cellular complexity, let alone the organization and function of whole organisms. But perhaps a reductionist approach can take biological sciences back to their roots: What is life? How did it originate? And how could its very simplest representation, the minimal cell, be envisioned (*7*)?

Many biologists reasonably argue that biology and biological systems can only be understood in the light of evolution and that speculations about how life could be simulated in a minimal chain of causes and effects are pointless. Indeed, it is tempting to suggest that the very features of biological systems that so discourage quantitative approaches—compositional complexity, low hierarchy, and large redundancy of regulatory processes—at the same time impart unparalleled adaptability and resilience. The critical engineer, rather than marveling at the beauty and design of biological systems, may consider some of them suboptimal and inefficient in terms of material usage and energy consumption. Francois Jacob, in his seminal article of 1977 (*8*), compared evolution with a tinkerer rather than an engineer. Rather than designing a tool from scratch, specifically tailored for a certain task, a tinkerer takes what he finds around him and adapts it to his use. The complicated biological solutions to seemingly simple tasks, such as identifying the middle of a cell or budding vesicles from membranes, might call to mind Rube Goldberg's famous Professor Lucifer Gorgonzola Butts (the godfather of all tinkerers) rather than a brilliant engineer. That biology still functions, and functions robustly, may indeed thwart any hopes of arriving at a set of minimal functional elements sufficient to reconstitute a living system.

On the other hand, important human inventions, such as the airplane, have been sparked by biological phenomena without the end products being even close to their sources of inspiration. In this vein, engineering biological molecules and using these as functional units—although its potential for truly understanding living systems may be limited—could yield miniaturized functional elements for sustainable and resource-efficient nanotechnology. Perhaps this is where the disciplines meet; chemists and physicists arriving here from their bottom-up approach come face to face with the biotechnologists who always had cell engineering as their goal. Here, all can agree that synthetic biology goes far beyond the insertion or deletion of single genes. Those practicing synthetic biology are aware that every module added or changed in an already well-evolved system has to be considered in the context of cellular metabolism and growth while also taking into account the host cells' ability to deal with (usually hostile) alien DNA and gene products. Therefore, engineering biology implies the design of whole systems and circuits, along with the standardization and shuffling of protein modules tailored to specific functional tasks. Thus, to be successful, synthetic biology of any kind will have to join forces with systems biology.

What are realistic goals for bottom-up synthetic biology in the next five years, and how may it converge with cellular-level engineering? There are some exciting developments that have raised expectations: Several genetic circuits have been successfully constructed in vivo [see the Review by Nandagopal and Elowitz see (*9*)], and solution- and membrane-based protein oscillators (*10*, *11*) have been realized in cell-free minimal systems, pointing the way to the molecular origin of polarization and pattern formation, two important phenomena in understanding the emergence of order from self-organized systems (*12*). Researchers are engaged in the bottom-up assembly of protein-based functional toolboxes for building self-organizing systems (Fig. 1). Ideally, these toolboxes will contain motifs for cooperativity and nonlinearity, feedback loops, and energy-dependent conformational toggle switches for activity and large-scale localization (*13*). Beyond this, it is possible to insert in vivo functional switches that can be addressed by temporally and spatially well-controlled physical rather than biochemical signals, such as light-activated protein modules (*14*) in optogenetic approaches.

From a physicist's perspective, although the ultimate goal may be the design of a minimal cell, the primary goal is to characterize the interactions between hybrid systems of nucleic acids, lipids, and proteins under well-defined conditions. Here, fundamental physical concepts, which are usually rather neglected in complex biosystems (such as surface and line tension in membrane transformations or electrostatic forces between charged residues) can be precisely addressed and compared with activation energy barriers and free energies of key regulatory processes. Since the advent of sophisticated single-molecule methods, tiny quantities such as femtonewton forces or single units of thermal energy are no longer inaccessible. Once these interactions are quantitatively understood, the next task is to assemble a set of key motifs and functions in biomolecules so as to construct minimal analogies of specific

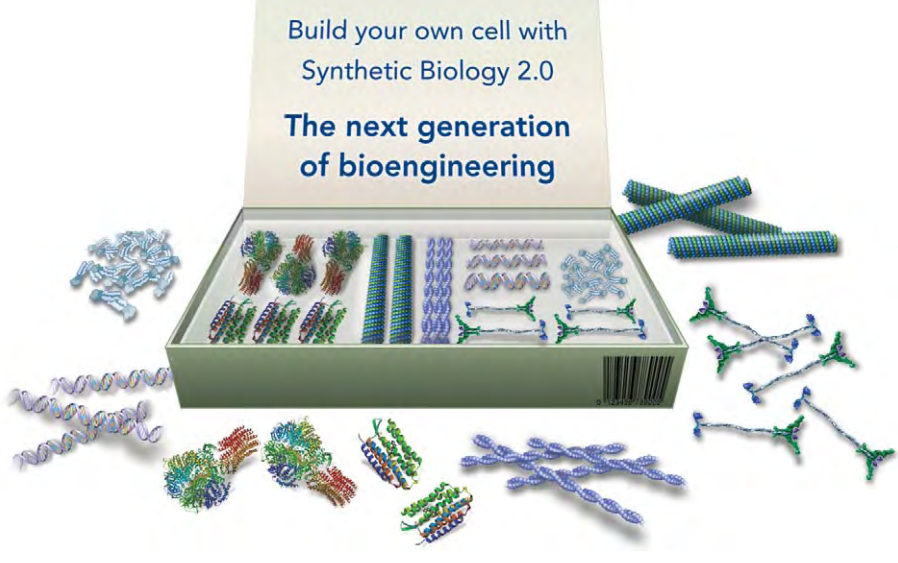

Fig. 1. The biological toolkit: Will it become reality? [Image source courtesy of Jakob Schweizer, BIOTEC/TU Dresden]

cellular tasks and phenomena. In such bottom-up approaches to biological function, there is no need to be constrained to bio-derived molecules. If a synthetic polymer or a piece of DNA origami can do a specific task as well as a lipid or protein module, why not construct bottom-up systems as a molecular "Borg," with biological, bioderived, and nonbiological elements combined for higher efficiency and robustness? Polymersomes made of block copolymers have already been shown to support protein activity in adenosine triphosphate–producing "artificial organelles" (*15*). And, multidimensional RNA structures were successfully designed as scaffolds in vivo to engineer the spatial organization of bacterial metabolism (*16*).

Synthetic biology is benefiting from and contributing to an increasing understanding of biology. The fascination is no longer limited to life scientists but has drawn in polymer chemists, physicists, and lately also engineers. In this exciting time, crossing traditional disciplines may lead us to new bioderived technology and an even deeper admiration of the power of living systems.

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POLICY FORUM

Synthetic Biology: Regulating Industry Uses of New Biotechnologies

Brent Erickson, Rina Singh,* Paul Winters

In our view, synthetic biology is an extension of the continuum of genetic science that has been used safely for more than 40 years by the biotechnology industry in the development of commercial products. Examples of synthetic biology use by biotechnology companies illustrate the potential to substantially reduce research and development time and to increase speed to market. Improvements in the speed and cost of DNA synthesis are enabling scientists to design modified bacterial chromosomes that can be used in the production of renewable chemicals, biofuels, bioproducts, renewable specialty chemicals, pharmaceutical intermediates, fine chemicals, food ingredients, and health care products. Regulatory options should support innovation and commercial development of new products while protecting the public from potential harms.

The emergence of synthetic biology into the public's perception has raised some concerns analogous to those expressed at the introduction of genetic engineering in the 1970s, particularly focusing on the potential for developing biological weapons, possible unforeseen negative impacts on human health, the morality of creating artificial life forms, and any potential environmental impact (*1*). Although some nongovernmental organizations have called for "an immediate moratorium on the release and commercial use of all synthetic organisms" or for regulation of the tools used in synthetic biology research, the President's Bioethics Commission "found no reason to endorse additional federal regulations or a moratorium on work in this field at this time" (*2*–*4*). The biotechnology community recognizes that synthetic biology, like other areas of biotechnology, can have both positive uses and negative impacts, and it has responded with guidelines for ethical, self-regulated research (*5*). Beyond that, the current framework for regulation of laboratory research and development of commercial biotechnology products can serve as a basis for regulation of synthetic biology.

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What Is Synthetic Biology?

In our view, synthetic biology is an extension of the continuum of genetic science that has been used safely for more than 40 years by the biotechnology industry in development of commercial products (Fig. 1). For instance, gains in the speed and efficiency of DNA synthesis, sequencing, and recombinant DNA technology combined with cataloging of genomic data permit advanced methods for predictable biological production of commercial proteins and chemicals. Gene shuffling and directed evolution, based on the rapid iteration and sequencing of recombinant proteins, are other outgrowths of the increased efficiency of standard biotechnology techniques and have been safely used for many years. Metabolic engineering—the optimization of microbial fermentation pathways, cellular processes and enzymatic activity for biochemical production—is an outgrowth of the increased knowledge of genomics.

Synthetic biology encompasses a set of emerging tools, including applied protein and genome design, the standardization of genomic "parts" or oligonucleotides, and synthesis of full genomes, that are important to the continued evolution of biotechnology. The continued refinement and capability of metabolic engineering techniques, combined with digitized proteomic and genomic data, are expected to enable increasingly complex, multistep fermentation of organic chemicals and longer gene synthesis. Novel proteins and biological functions are envisioned as tools for advanced metabolic engineering. The BioBricks Foundation is creating a catalog of oligonucleotides that they believe can be certified to perform standardized biological functions when inserted into a microbial system (*6*). Similarly, the Massachusetts Institute of Technology has established a Registry of Standard Biological Parts (http://partsregistry.org/) and the International Genetically Engineered Machine (iGEM) competition (http://igem.org). The J. Craig Venter Institute has achieved initial steps in the design and construction of a simplified genome for a natural, self-replicating bacterium (*7*, *8*).

As often occurs with the introduction of new technology, metaphors that exploit effective, yet still imperfect, similarities in more familiar technologies are used to help illustrate the potential offered in the new field. The BioBricks Foundation, for instance, has consciously sought to leverage "time-honored engineering principles of abstraction and standardization" "to reduce the complexity and cost of producing synthetic living organisms" (*9*). The foundation has established four standards—for assembly, measurement, compatibility and exchange of data—taken directly from the field of mechanical engineering, as requirements for BioBricks listed in its catalog. Metaphors utilized for synthetic biology have often been based on electronic toolkits—i.e., systems that are modular and open to reconfiguration. However, these metaphors can mislead public perception of biotechnology because living organisms are not directly analogous to modular electronics, and therefore, law, policy, and research and development in synthetic biology probably should not be modeled after law, policy, and research and development in the fields of computer science and electronics.

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Another popular metaphor is the development of computers. Much as earlier developments in somatic cell nuclear transfer were described with terms such as "reprogramming," synthetic biology has been described with terms such as "booting up" of genetic code. J. Craig Venter, in announcing achievement of the first self-replicating cell from a chemically synthesized genome, stated, "This is the first self-replicating cell we've had on the planet whose parent is a computer" (*10*). In looking at the rate of productivity of DNA sequencing and synthesizing technologies to project the potential economic impact, one report notes that productivity is doubling every 24 months and invokes Moore's law (*11*). A recent academic paper described a method for massive parallel replacement of codons within a genome as treating "the chromosome as both an editable and an evolvable template" and was in turn described in the popular press as a method to "seize control of the microbe's genetic code and reprogram it" (*12*, *13*).

The biotechnology industry has used the metaphor of husbandry and hybridization to contextualize its history of technology developments. Breeding genetic traits in animals and plants that are conducive to human interests should be familiar to individuals and societies around the world, even as modern breeding techniques incorporate precise screening, analysis, and long-distance shipment of genetic material—and even reproductive cloning. Use of microbes for production of useful foods and chemicals—such as beer, wine, bread, and yogurt—also has a long history among many cultures around the globe. Biotechnology, the direct manipulation of the genes of microbes, plants, and animals, therefore can be understood as a more precise, predictable, and speedy method for "breeding" useful traits for the benefit of mankind. Synthetic biology, based on the increased speed and precision of standard biotechnology tools, can be understood as a new set of laboratory tools and techniques that now enable biotech researchers and product developers to more rapidly design and build microbial systems, rather than finding and extracting them from nature and modifying their genomes or metabolic pathways.

Innovations from Biotechnology

Innovation for any industry is based on increased speed, efficiency, performance, and cost-effectiveness within product development. The addition of synthetic biology tools to the field of metabolic engineering can enable further innovation in biotech product development in the chemical, pharmaceutical, and food industries (*14*). For example, polyhydroxyalkanoates (PHAs), a broad family of biopolymers, are produced naturally in many microorganisms. However, the cost and range of PHA compositions required for commercial polymers and plastics dictated that PHA pathways had to be assembled in a robust organism that does not naturally produce the product. Metabolic pathway engineering was used to accomplish this task, including DNA sequencing and synthetic construction of genes encoding the same amino acid sequence as the donor strain, but optimized for expression in the engineered industrial host. These technologies provided rapid development and optimization of robust industrial production strains that would not have been feasible by using classical techniques relying on isolation and transfer of DNA from one species to the other.

More than 200 U.S. firms and universities are engaged in synthetic biology research, development, and product commercialization (*15*). Although synthetic biology research is an emerging science that has yet to reach its full potential, there are several products based on synthesized genetic sequences and computer-aided design of metabolic pathways that are at a precommercial stage, with a few already on the market. One of the pioneers of synthetic biology is the life sciences and materials company DSM. The company utilized synthetic biology to improve an existing process for the commercial production of cephalexin, a synthetic antibiotic. Starting with a penicillin-producing microbial strain, DSM introduced and optimized two heterologous genes encoding acyl transferase and expandase, respectively, for a one-step direct fermentation of a dipoyl-7-aminodesacetoxycephalosporanic acid (dipoyl-7-ADCA). This product was then converted into cephalexin via two enzymatic steps, which replaced a process requiring 13 chemical steps. The new process resulted in significant cost and energy savings. DSM has gone on to build a business in antibiotics, vitamins, enzymes, organic acids, and performance materials (*14*).

Several biotechnology companies have used synthetic biology techniques to speed research and development cycles for biological production of specific chemicals. Adipic acid, a building block chemical for Spandex and other polymers with an annual market of ~$5.2 billion, is not naturally produced. Verdezyne used synthetic gene libraries to design a recombinant yeast microorganism capable of expressing a precursor to adipic acid. The bio-based production method could reduce cost by 20% or more compared with petrochemical methods (*16*). Sitagliptin, a dipeptidyl peptidase-4 inhibitor, is a treatment for type II diabetes that also is not naturally produced. Codexis developed a highly active, stable transaminase enzyme capable of producing this substance with a higher degree of selectivity for the specific therapeutic enantiomer than an existing process using metal catalysts (*17*). OPXBIO comprehensively redesigned a natural microbe to optimize its metabolism for low-cost production of acrylic acid from renewable resources. OPXBIO is already producing BioAcrylic at pilot scale and is now in joint development with Dow Chemical.

Isoprene is an important commodity chemical used in a variety of applications, including the production of synthetic rubber. Isoprene is naturally produced by nearly all living things (including humans, plants, and bacteria), but the gene encoding isoprene synthase has only been identified in plants such as rubber trees. Although plant enzymes can be expressed in microorganisms through gene transfer, it is a long and cumbersome process. Genencor, a Danisco Division, has used synthetic biology to construct a gene that encodes the same amino acid sequence as the plant enzyme but is optimized for expression in an engineered *Escherichia coli*. This microorganism is capable of channeling carbon through the mevalonic acid biosynthetic pathway to deliver isoprene at titers exceeding 60 g/liter. Unlike other bio-based systems to produce renewable chemicals, BioIsoprene is produced as a gas-phase product that is released as soon as it is produced into the vapor phase of the reactor. Polymer-grade BioIsoprene is recovered from the integrated process. The production of BioIsoprene from renewable raw material is under development by Genencor and the Goodyear Tire & Rubber Company, and it is considered a major achievement for industrial biotechnology because it has the potential to enable a low-cost monomer as a large-volume

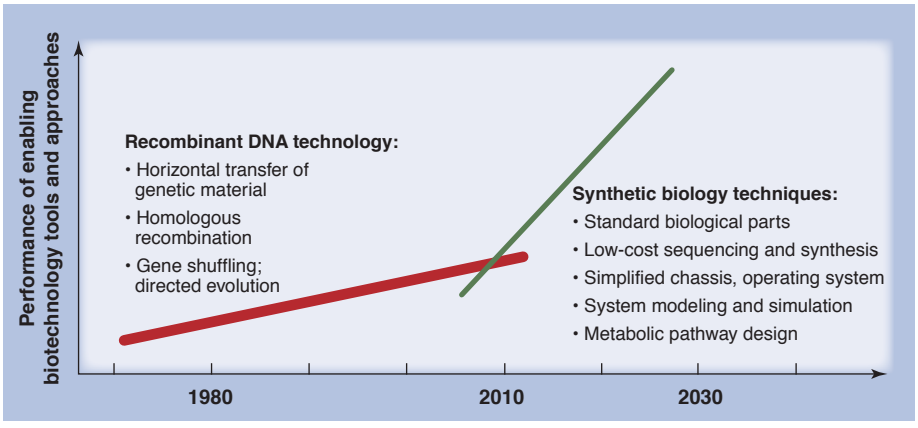

Fig. 1. Evolution of innovation. [Modified from (*11*), ©2007 by Bio Economic Research Associates, www.bio-era.net]

alternative to *Hevea* natural rubber and petroleum-derived isoprene (*18*).

In response to recent oil spills where large volumes of toxic chemical dispersants were used, Modular Genetics, Inc., of Cambridge, Mass., used a computer library of genetic code to generate iterations of a previously engineered microorganism, each producing a different biodispersant for testing. Modular Genetics's work was part of a consortium with three universities working under a National Science Foundation RAPID Response Grant to develop less toxic biodispersants (http://nsf.gov/awardsearch/showAward.do?AwardNumber=1059174).

Options for Governance

Regulatory options should support innovation and commercial development of new products while protecting the public from potential harms. One of the key needs for regulation identified by the biotechnology community is to inculcate the biomedical culture of safety in engineers, chemists, material scientists, computer modelers, and others drawn into synthetic biology by its interdisciplinary nature (*3*, *4*). The community also recognizes that synthetic biology has dual-use implications, in that the speed in creation of novel genetically engineered organisms and the sharing of this information via computer or mail order apply equally to beneficial uses and nefarious purposes.

Because synthetic biology is not constrained to use readily available genetic material, the directed synthesis of polynucleotides has great potential to generate novel organisms or to regenerate ones that no longer exist, including pathogens. To reduce the risk that individuals with ill intent may exploit nucleic acid synthesis technology to access genetic material derived from or encoding select agents or toxins, the U.S. government has developed recommendations for a framework for synthetic nucleic acid screening (*19*). This document for voluntary use is intended to provide guidance and to encourage best practices among producers of synthetic genomic products so that they screen and fill orders in compliance with current U.S. regulations. Voluntary guidelines for sharing synthesized genetic sequences should help providers meet their responsibilities of knowing who is receiving their product and if the sequence they are providing contains "in part or in whole" a "sequence of concern." In light of public concern, NIH established guidelines in 1976 that are mandatory for investigators at institutions that receive NIH funds doing research involving recombinant DNA (*20*). The guidelines encompass synthetic biology and are followed voluntarily by scientists and organizations, both public and private.

At the dawn of the era of recombinant DNA technology, researchers in the field agreed to develop similar guidelines to ensure the safe practice of the technology. The Asilomar Conference on Recombinant DNA Molecules held in 1975 proposed the outlines for a system of regulating biotechnology research, commercial development, and commercial production in which levels of containment of biohazards were balanced against potential risks. As the biotechnology industry grew and spread to other countries, the culture of safety that prompted the Asilomar Conference strengthened.

The President's Bioethics Commission, charged with reviewing the field of synthetic biology and identifying appropriate ethical boundaries, in response to the announced creation of a self-replicating cell from a chemically synthesized genome, put forward 18 recommendations not only for regulating the science, but also for educating the public and regulators about the science. The key five principles established by the commission were public beneficence, responsible stewardship, intellectual freedom and responsibility, democratic deliberation, and justice and fairness. The report advocates prudent vigilance—which balances responsible stewardship of the technology with intellectual freedom for continued investigation—and regulatory parsimony—establishing only as much oversight as is necessary to ensure public safety and public benefits from the technology. A key recommendation is to ensure regulators have adequate information to conduct risk analysis and harmonization of regulatory standards.

Many groups worldwide, including government organizations, nonprofits, academia, and the amateur synthetic biology community have been discussing the implications of synthetic biology, and a complete listing is beyond the scope of this article. There have been meetings of members of the U.S. National Academies, U.K. Royal Academy, and Chinese Academy of Sciences and Engineering (*21*), and there are ongoing conversations in many countries. Synthetic biology has also been included as a topic in the Science and Technology assessments prepared by the U.S. National Academies and the Chinese Academy of Sciences for the Seventh Review Conference of the Biological Weapons Convention to be held at the United Nations Office in Geneva later this year (*22*). Industry groups have also proposed codes of conduct. Through the International Association Synthetic Biology, the International Consortium for Polynucleotide Synthetics published a potential oversight framework for the development and implementation of sequence screening tools and mechanisms for reporting and resolving concerns about orders of potentially dangerous sequences (*23*).

At this early stage of development, synthetic biology does not pose novel threats that are fundamentally different from those faced by the current biotechnology industry. The regulatory framework that has been shaping continually evolving recombinant DNA technology for the past 40 years is generally applicable and relevant, and we recommend that academic researchers and industry continue to develop synthetic biology technology and derive products under the framework. In the future, as the technology matures and if scientific consensus warrants it, the need may exist to develop a regulatory framework as overarching federal policy, based on the existing voluntary regulatory guidelines.

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Entrainment of a Population of Synthetic Genetic Oscillators

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Biological clocks are self-sustained oscillators that adjust their phase to the daily environmental cycles in a process known as entrainment. Molecular dissection and mathematical modeling of biological oscillators have progressed quite far, but quantitative insights on the entrainment of clocks are relatively sparse. We simultaneously tracked the phases of hundreds of synthetic genetic oscillators relative to a common external stimulus to map the entrainment regions predicted by a detailed model of the clock. Synthetic oscillators were frequency-locked in wide intervals of the external period and showed higher-order resonance. Computational simulations indicated that natural oscillators may contain a positive-feedback loop to robustly adapt to environmental cycles.

One focus of synthetic biology is the genome-scale synthesis of DNA for the creation of novel cell types (*1*). This approach could lead to cells with highly reduced genomic complexity, as genes that govern the ability to adapt to multiple environments are eliminated to construct specialized organisms for biotechnology and basic research. Another branch of synthetic biology involves the engineering of gene circuits, in which mathematical tools are developed to systematically design and construct circuits from a standardized list of biological parts (*2*–*11*). The engineering approach allows the construction of circuits that mimic natural networks to understand the design principles that underlie a given network motif (*12*, *13*). In this context, molecular clocks are a natural application of synthetic biology, and recent efforts have led to a deeper understanding of the robustness and reliability of time-keeping at the intracellular level (*3*, *7*, *8*, *10*).

Almost all organisms use molecular clocks to keep their physiology and behavior in synchrony

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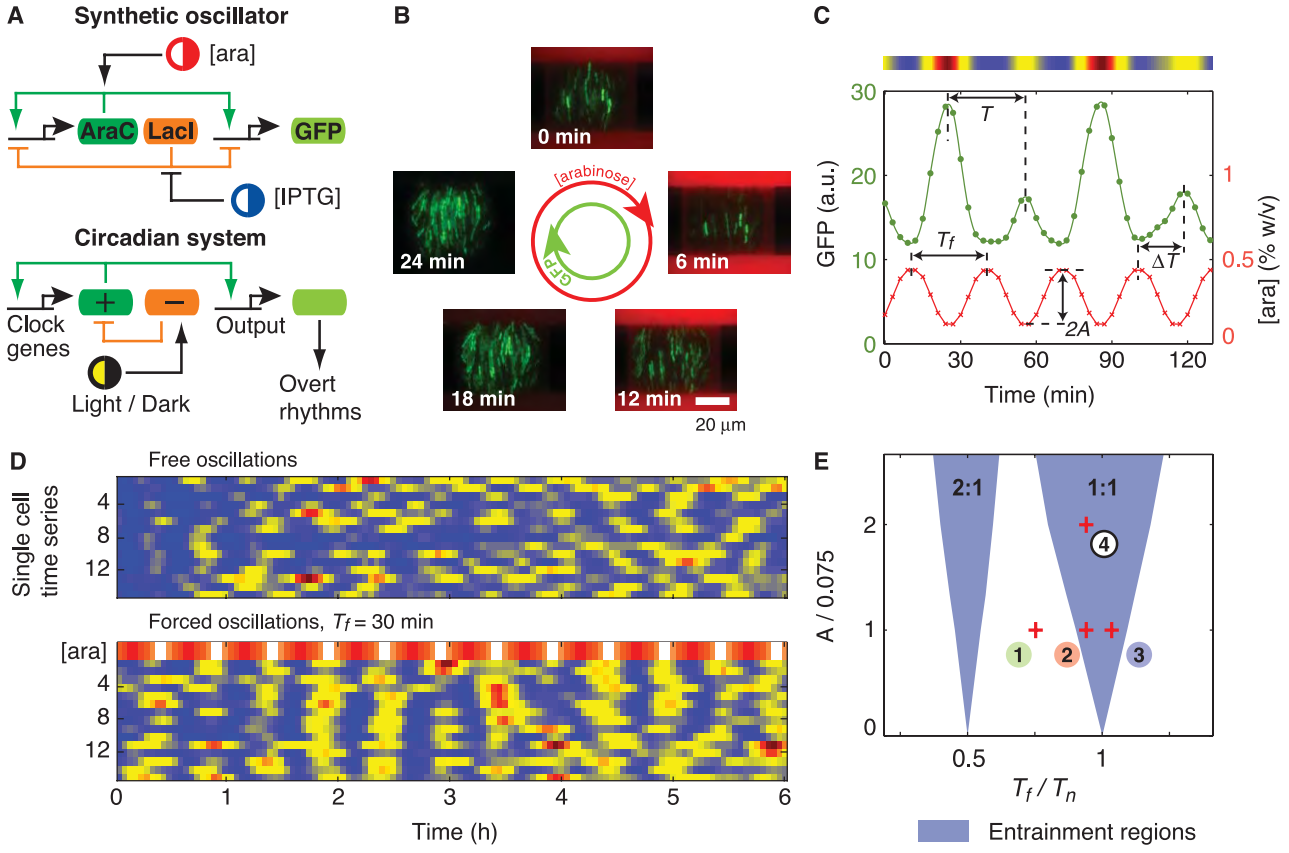

Fig. 1. We use single-cell data from time-lapse fluorescence experiments to investigate the entrainment of a synthetic oscillator. (**A**) Architectures of eukaryotic circadian clocks and bacterial synthetic oscillators contain positive- and negative-feedback loops that are sensitive to external stimuli. (**B**) Fluorescence images from a time-lapse experiment show coherent GFP oscillations (green) in a colony of single-cell oscillators subject to a 30-min cycle of arabinose (red) (movie S1). (**C**) Fluorescence time series of a single-cell oscillator (green). The concentration of arabinose (red) changes sinusoidally according to $[ara](t) = 0.3 + A\sin(2\pi t/T_f)$ [percent weight/volume (% w/v)], with A = 0.15% and T_f = 30 min. The intensity plot above the graph corresponds to the cell trace. a.u., arbitrary units. (**D**) Fluorescence intensity plots of free-running and forced oscillators. Each row in the two panels represents a single-cell trace. The top row of the forced set represents the modulated concentration of arabinose (A = 0.15%). (**E**) Entrainment regions indicate which forcing periods (T_f) and amplitudes (A) result in locking of the oscillator according to a deterministic model (SOM text). Entrainment of order 2:1 means that two oscillation peaks are observed for one peak of arabinose. T_n is the natural period of the oscillator. Images and cell traces shown in (B), (C) and [(D), forced oscillations] correspond to point 4. Points 1 to 3 signal some parameter values explored experimentally.

with their surroundings (*14*). Such coordination is mediated by entrainment, whereby a population of intracellular clocks oscillate in unison guided by a common external signal (*14*, *15*). Quantitative descriptions of entrainment that arise from the tight coupling of computational modeling and experimentation are challenging to develop because of the complexity of the underlying gene-regulatory networks, in which dozens of genes are involved in the core clocks and hundreds more act as their modifiers (*16*). Moreover, a quantitative description of inherently stochastic circadian clocks requires abundant long-term single-cell data, which are technically challenging to obtain (*17*–*19*). We combined synthetic biology, microfluidic technology (*20*), and computational modeling to investigate the fundamental process of entrainment at the genetic level.

We used a synthetic oscillator that has coupled positive- and negative-feedback loops that are characteristic of many circadian gene-regulatory networks (Fig. 1A) (*7*). The green fluorescent protein (GFP) was used as a readout of the transcriptional activation state of the promoter that drives the expression of the oscillator genes. We stimulated the expression of the oscillator genes (*araC* and *lacI*) by periodically modulating the concentration of the transcriptional inducer arabinose, which acts on the positive-feedback loop. Such stimulation is referred to as the forcing of the oscillator. To generate long-term single-cell data for comparison with computational modeling, we constructed microfluidic devices in which bacterial colonies can grow exponentially for at least 150 generations (fig. S1) (*21*). For each experimental run, we tracked the phase of the oscillations with respect to the arabinose signal in ~1600 cells (Fig. 1B and movies S1 and S6) (*21*). The period of oscillations T was measured as the peak-to-peak interval in the GFP fluorescence time series. The phase difference between an oscillator and the arabinose signal was calculated as $\Delta\phi = 2\pi\Delta T/T_f$, where T_f is the period of the forcing signal and ΔT is the measured time interval between a crest of arabinose and the immediate following peak of GFP fluorescence (Fig. 1C). Entrainment of the intracellular oscillations to the chemical signal was readily identified from color density maps of the fluorescence trajectories (Fig. 1D); by taking crests of GFP fluorescence as a marker of the phase, one can see that whereas in the autonomous set single cells are not always in phase with respect to each other, maxima in the forced colony occur almost simultaneously during most of the run.

The entrainment of any self-sustained oscillator can be characterized by comparing its natural period (T_n) and phase (ϕ) to those of the external signal. When T_f is sufficiently close to the natural period of the oscillator, the oscillator can be entrained. In the entrainment regime, the period of the oscillator T is equal to T_f, and the phase difference $\Delta\phi$ between the oscillator and the forcing signal is fixed. In the plane defined by the period and amplitude of the external signal (T_f, A), a triangular region near $T_f/T_n = 1$ indicates where the oscillator is entrained [Fig. 1E and supporting online material (SOM)

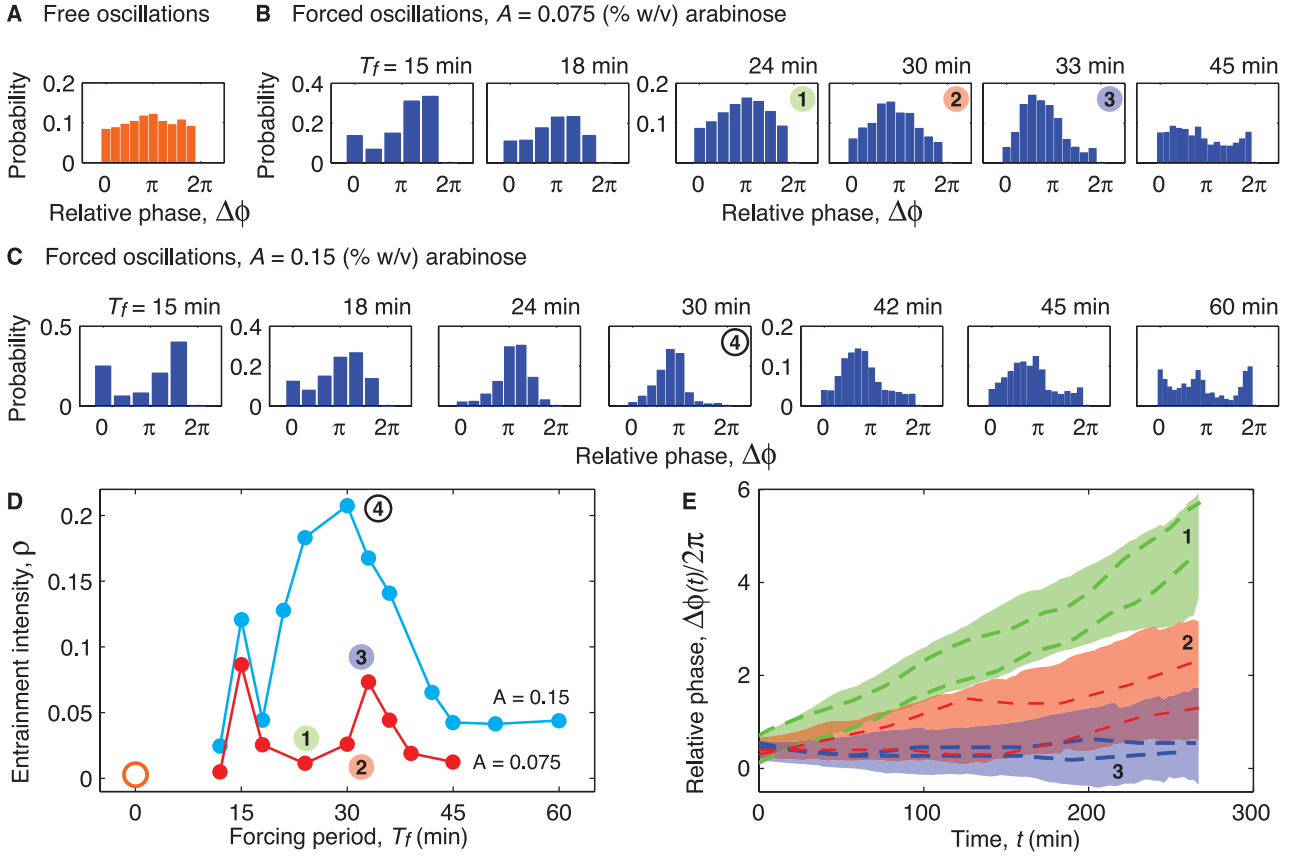

Fig. 2. Probability distributions of the relative phase of oscillators with respect to the external signal allow the detection of entrainment. (**A**) Probability distribution of the relative phase of free-running oscillators in several colonies with respect to a virtual sinusoidal signal of period T_f = 30 min. Constant concentrations of inducers were used ([IPTG] = 2 mM, [ara] = 0.3%). (**B**) Probability distributions of the relative phase for multiple forcing periods with amplitude A = 0.075% (w/v). In the presence of the external stimulus, distributions acquired a preferred phase that depends on the forcing period T_f. (**C**) Same as (B) for stronger forcing with A = 0.15%. Increased amplitude sharpens the peaks of the relative phase distributions with respect to those for A = 0.075% as in T_f = 15, 30, and 45 min. (**D**) Intensity of entrainment as a function of the forcing period for the two values of the forcing amplitude. In each curve, two peaks centered near $T_f \sim T_n$ = 31.8 min and $T_f \sim T_n/2$ = 15.9 min reveal the intervals of T_f where the phase is locked to the arabinose input. For free-running oscillators, ρ is nearly zero (orange open circle). (**E**) Relative phase as a function of time for three experiments shown in (B). Colored regions correspond to ±SD around the mean phase drift. Dashed lines indicate representative single-cell traces.

text]. Entrainment may also occur near other rational values of T_f/T_n. Collectively, these regions are known as Arnold tongues. The order of locking in each region is indicated by the ratio *n*:*m*, which denotes that *m* oscillations of the clock correspond to *n* oscillations of the arabinose signal. We computed the tongues for entrainment of order 1:1 and 2:1 with a deterministic model of the synthetic oscillator (*7*), in which we periodically modulated the arabinose concentration.

To experimentally map the entrainment regions, we first determined the natural period of the oscillator by tracking the expression of GFP of cells at constant inducer concentrations (movie S2). Because the oscillators are not synchronized with respect to each other, their phases are uniformly distributed between 0 and 2π (Fig. 2A). Similar to its naturally occurring counterparts (*17*, *18*, *22*), the synthetic oscillator shows considerable fluctuations (Fig. 3A). Given the natural period of ~32 min, we varied the period of the arabinose concentration from 6 to 60 min for two values of the amplitude. Coherent oscillations emerged over a range of periods that bounded the natural period (movie S3). Phase-locking was characterized by a narrow peak in the phase distribution (*23*), which became difficult to discern as the period of the signal diverged from the natural period but reappeared as the forcing period approached half of the natural period (Fig. 2B and movie S4). An increase in the forcing amplitude by a factor of 2 led to sharper distributions of the relative phase (Fig. 2C and movies S1 and S5). To quantify the degree of phase-locking, we used an entropy-based index (ρ) to characterize the width of the distributions (*23*); wider distributions imply less phase-locking and lead to smaller values of ρ (SOM text). Accordingly, maxima of the entrainment index appeared at both the natural period and half of the natural period (Fig. 2D).

The flattening of phase distributions and the decay of the phase-locking index around T_f/T_n = 1/2,1 indicates the breaking of entrainment. To investigate this transition in more detail, we examined the dynamics of the oscillation phase relative to the forcing signal in single cells. We chose three values of the forcing period that cross the left boundary of the computed main Arnold tongue (Fig. 1E). We used peak positions to determine the phases of the arabinose signal $\phi_{ara}(t)$ and single-cell oscillations $\phi_c(t)$, and we calculated their difference $\Delta\phi(t) = \phi_{ara}(t) - \phi_c(t)$ (Fig. 2E). Near the center of the entrainment region (T_f = 33 min), $\Delta\phi$ for most oscillators was nearly constant (Fig. 2E, blue shaded region and curves). Toward the left boundary of the tongue (T_f = 30 min), there is a slow mean phase drift with a broad distribution (Fig. 2E, red shaded area and curves); some cells exhibit phase drift (with an evidence of occasional phase slips), whereas other cells are still phase-locked. Finally, between the two Arnold tongues (T_f = 24 min), the rate of phase drift was even faster and almost uniform because the phases of most oscillators did not lock to the arabinose signal (Fig. 2E, green shaded area and curves). The continuous phase drift indicates quasi-periodic behavior outside entrainment regions, which is observed in the computation of Arnold tongues (Fig. 1E and SOM text).

We also used period distributions to characterize the response of the oscillator (Fig. 3). Forcing periods close to both the natural period and half of the natural period reduce the spread of the period distribution in a manner similar to that observed with light pulses resetting peripheral clocks (*22*). For a lower amplitude of the arabinose signal, oscillators were entrained over an interval of periods that was consistent with the width of the 1:1 phase-locking regime determined with the use of the entropy-based measure (Figs. 2D and 3D). For a larger forcing amplitude, the 1:1 plateau extended over a larger interval of periods, and a 2:1 plateau indicated the presence of the higher-order resonance. Some of these distributions displayed two modes, which presumably indicated simultaneous occurrence of 1:1 and 2:1 frequency-locking.

Direct comparison between our experimental results and the computed Arnold tongues indicated that the locations of the experimental entrainment plateaux correspond closely to the regions where frequency-locking is predicted (Fig. 4A). The width of the plateau increased

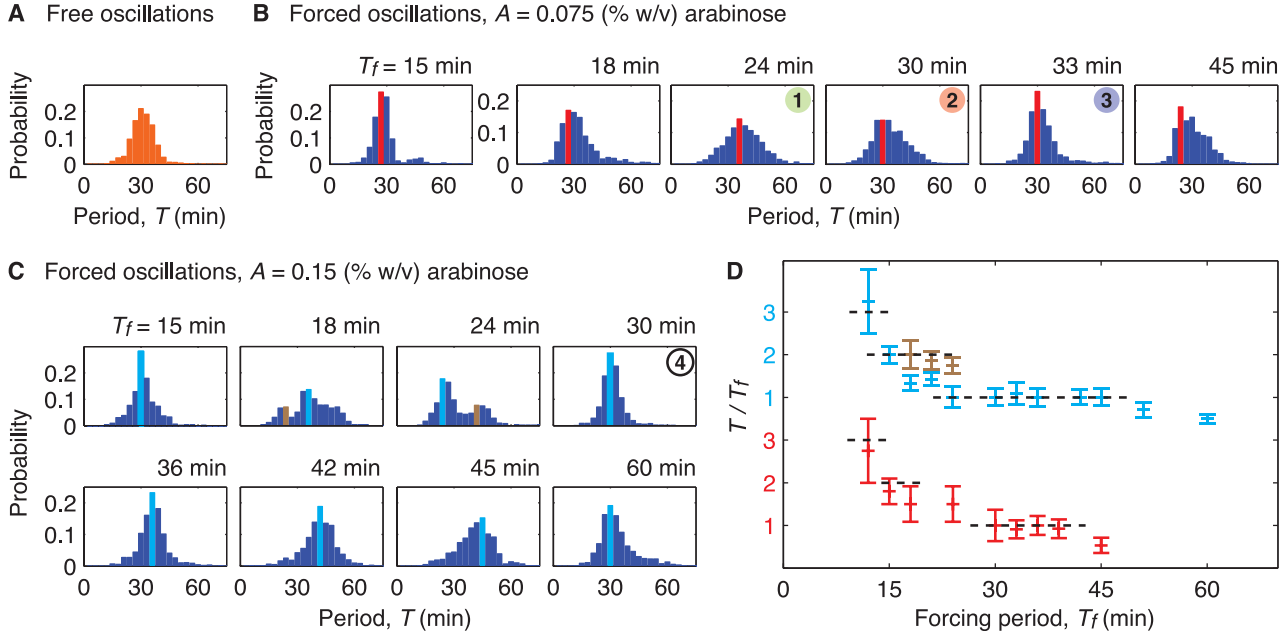

Fig. 3. Probability distributions of the period of oscillations allow us to find the forcing periods that lead to frequency-locking. (**A**) Probability distribution for the period of free-running oscillators in constant concentrations of inducers ($[\overline{IPTG}]$ = 2 mM, $[\overline{ara}]$ = 0.3%). In forcing experiments, the concentration of arabinose oscillates sinusoidally around $[\overline{ara}]$ = 0.3%. We defined the natural period as the mean period of free oscillations, T_n = 31.8 min with standard deviation δT = 5.7 min. (**B**) Probability distributions of the period for multiple values of the T_f with A = 0.075%. For T_f near T_n = 31.8 min or $T_n/2$ = 15.9 min, the dispersion of the period is the least. Red bars indicate the mode of the distributions. (**C**) Probability distributions of the period for multiple values of the forcing period with A = 0.15%. Period distributions for this higher amplitude can contain two modes (light blue and brown bars). (**D**) The ratio T/T_f as a function of T_f for the two forcing amplitudes, where T is the mode(s) of the period distributions. The intervals of the forcing period where $T/T_f \sim 1,2$ provided evidence for entrainment of order 1:1 and 2:1, respectively.

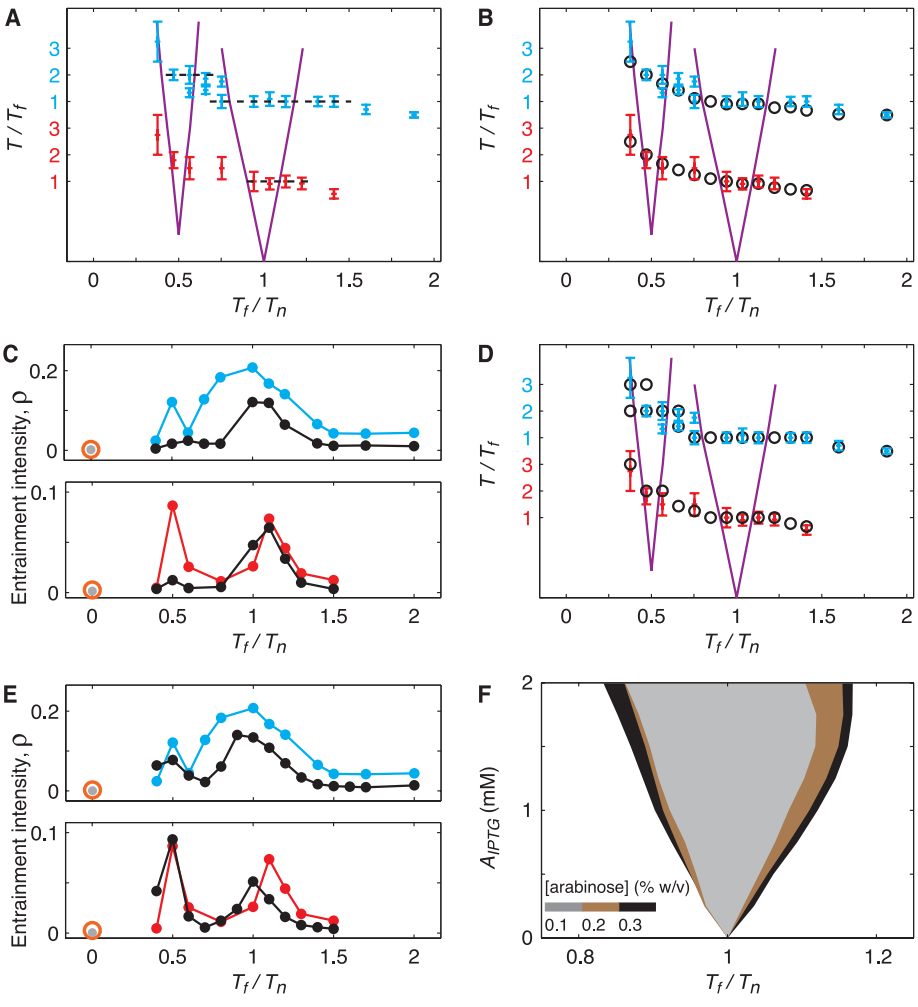

Fig. 4. Computational modeling shows that extrinsic sources are the dominant contribution to variability. Blue and red data points indicate experimental data for A = 0.075% and A = 0.15%, respectively. Error bars represent ±SD. (**A**) Experimental values of T/T_f alongside computed entrainment regions (purple lines), which are shifted with respect to each other to account for the gap between the T/T_f = 1 and T/T_f = 2. Entrainment was observed for T_f outside the computed entrainment areas. (**B** and **D**) Same as (A), along with the prediction for the ratio T/T_f (open circles) from a stochastic model (B) and from a deterministic model with distributed parameters in a set of 550 oscillators (D). Unlike the oscillator subject to intrinsic noise (B), the oscillator with distributed kinetic parameters became phase-locked outside computed entrainment regions (D). The ratio T/T_f diverges from 1 or 2 outside Arnold tongues (B). (**C** and **E**) Experimental values of the intensity of entrainment ρ alongside the prediction (black circles) from a stochastic model (C) and a deterministic model with distributed parameters in a set of 550 oscillators (E). Intrinsic variability destroys the resonance around T_f/T_n = 0.5 (C), whereas the model with distributed parameters captures it (E). (**F**) Main entrainment region for forcing with a sinusoidal IPTG signal of amplitude A_{IPTG} for three concentrations of arabinose from a deterministic model (SOM text). When the oscillator is forced through its negative-feedback loop (Fig. 1A), the range of entraining frequencies increases with the constant arabinose concentration (strength of positive-feedback loop).

with the amplitude of the forcing signal, as follows from classical theory (*24*). However, the experimental entrainment regions were consistently wider than the computed Arnold tongues. The major discrepancy between the naive model and experiment is that the model assumes that all oscillators are identical and have the same natural period, whereas the bacterial colony exhibits a broad distribution of periods (Fig. 3A).

The observed variability of the oscillatory dynamics can be attributed to both intrinsic and extrinsic origins (*25*). We incorporated both sources of variability into our model because it is difficult to ascertain which one dominates. We used a Gillespie algorithm (*26*) to simulate the stochastic model of the oscillator network with intrinsic noise only (the kinetic parameters of all oscillators were set to be identical). Although the simulated distributions appeared similar to experimental data (figs. S2 to S5), the stochastic model did not account for the higher-order (2:1) resonance entrainment, the period bimodality, or the wider entrainment regions (Fig. 4, B and C, black circles, and figs. S2 to S5). We therefore modeled extrinsic variability by varying the kinetic parameters of the deterministic model across a population of 550 cells. In particular, we assumed that the rates of transcription, translation, enzymatic degradation by proteases, and plasmid copy numbers were normally distributed around their nominal values. Using a coefficient of variation (CV) of 0.15, close to CV = 0.18 of the experimental probability distribution of the free-running period, we obtained good agreement between the modes of simulated and experimental period distributions (Fig. 4D, black circles, and figs. S7 and S9). Accordingly, the distributions of the relative phase and the peaks in the curves for the intensity of phase-locking were comparable (Fig. 4E and figs. S6 and S8). Deterministic simulations with randomized parameters accounted for the width of both the 1:1 and 2:1 entrainment regions. Simulations also reproduced peaks in some bimodal period distributions (figs. S7 and S9).

These results can be readily understood in the context of the phase dynamics. For fixed concentrations of arabinose and isopropyl-β-D-thiogalactopyranoside (IPTG), the natural period of the oscillator T_n is a function of the parameters of the model—for instance, the rates of transcription, translation, enzymatic degradation, and of the ratio of activator to repressor plasmids. Therefore, variability in these parameters will lead to the observed variability in the periodicity of free-running oscillations (Fig. 3A). Each individual oscillator will respond differently to the forcing arabinose signal, and depending on its natural frequency, it may or may not entrain. If the natural-frequency distribution occupies an interval of a given width, the entrainment interval will broaden by the same amount (SOM text). Moreover, the broad distribution of natural frequencies of oscillators explains the occurrence of bimodal period distributions. If the forcing frequency is shifted with respect to the peak of the free-running frequency distribution, both the entrainment peak at T_f and the "free" peak at T_n may coexist.

Because circadian oscillators can be entrained by stimuli that act on different components (*27*), we explored the entrainment of the oscillator through the periodic modulation of the concentration of IPTG (Fig. 1A). We did this through deterministic simulations of the model, in which arabinose was kept constant and the concentration of IPTG oscillated sinusoidally (SOM text). We found a similar behavior to forcing with arabinose, with a main entrainment region that widened with the amplitude of change in IPTG concentration. Because the concentration of arab-

inose defines the strength of the positive feedback through the AraC-DNA binding rate, we used different values to explore how entrainment depends on the strength of positive feedback. Lower concentrations of arabinose yielded narrower Arnold tongues (Fig. 4F). In other words, a weaker positive loop makes the oscillator less entrainable.

We have shown how the coupling of synthetic biology, microfluidic technology, and computational modeling can be used to explore the complex process of entraining molecular clocks. Our results indicated that the positive-feedback loop widens the entrainment region for single cells, providing insight into the possible role of positive feedback in the robust adaptation of variable clocks to complex environments (*28*). The observation of higher-order entrainment and the wider entrainment regions allowed us to discriminate intrinsic sources in favor of extrinsic noise as the main contribution to stochastic variability in computational modeling of the clock. Other manifestations of strong cell-cell variability in gene networks have been quantified (*29*). Although cell-cell variability may be deleterious to biological function, variable entrainment properties across a population may provide increased flexibility to the various signals that reset clocks. This may be relevant in the context of multicellular circadian systems where uncoupled peripheral oscillators display variability and are exposed to multiple signals (*17*, *18*, *22*, *30*). Other properties at the cell and tissue level have been found to contribute to the flexibility of circadian clocks; recent work found an effect of the strength of coupling between cell clocks on the range of entrainment in mammalian circadian clocks (*19*).

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Supporting Online Material

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