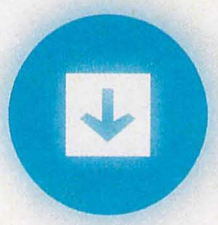
MEDICINE

THE GENE GENIE

A DNA-editing technique based on bacterial "memories" could revolutionize medicine. But some worry it could get out of control

By Margaret Knox



THE AGE OF GENETIC ENGINEERING began in the 1970s, when Paul Berg spliced DNA from a bacterial virus into a monkey virus and Herbert W. Boyer and Stanley N. Cohen created organisms in which introduced genes remained active for generations. By the late 1970s Boyer's company, Genentech, was churning out insulin for diabetics using *Escherichia coli* modified to contain a synthetic human gene. And in laboratories around the country, researchers were using transgenic mice to study disease.

These triumphs changed the course of medicine. But the early methods had two big limitations: they were imprecise and hard to scale. Researchers overcame the first limit in the 1990s by designing proteins that could snip specific locations of DNA, a big improvement over inserting DNA into cells at random and hoping for a useful mutation. Yet they still had to devise a new protein tailored to every sequence of DNA that they wanted to target—and that was slow, painstaking work.

Then, two years ago, a small group of researchers working in the labs of Emmanuelle Charpentier at Umeå University in Sweden and of Jennifer Doudna at the University of California, Berkeley, reported the discovery of a genetic mechanism in cells that allows scientists to edit genomes with unprecedented speed and ease. Shortly thereafter, a team of scientists at Harvard University and the Massachusetts Institute of Technology showed that the technique could be used to make multiple changes in a cell's genome, with great precision, all at once.

Already the advance has accelerated the genetic-modification industry in ways that are almost certain to have profound and beneficial effects on the field of genetics and medicine. Scientists can now engineer custom transgenic lab animals in a matter of weeks—saving about a year's worth of work. Researchers are using the technique to explore therapies for diseases as diverse as HIV, Alzheimer's disease and schizophrenia. Yet the technique makes genetic modification so easy and inexpensive that some ethicists are anticipating possible negative consequences.

IN BRIEF

Scientists have known how to alter the genomes of living organisms since the 1970s, but the tools available to them have long been imprecise and hard to scale. As a result, many experiments remained too difficult or costly to conduct.

Now a new method called CRISPR could foment the genome-editing revolution. Based on the immune defenses of bacteria, it is faster, cheaper and easier than older techniques. Money is pouring into companies seeking to commercialize CRISPR applications.

Researchers are already exploring CRISPR-based treatments for diseases, including HIV and schizophrenia. Yet CRISPR makes it so easy to alter the genomes of living organisms that ethicists worry about negative ramifications.

The technology is called CRISPR, after *c*lustered, *r*egularly *i*nterspaced, *s*hort *p*alindromic *r*epeats—the genetic mug shots that bacteria use to remember viruses that have attacked them. Scientists have been studying these odd genetic sequences since Japanese researchers discovered them in the late 1980s. But CRISPR's promise as a gene-editing tool did not become clear until Doudna's and Charpentier's teams figured out how to use a protein called Cas9.

THE POWER OF RNA

DOUDNA AND CHARPENTIER MET IN 2011, at a scientific conference in San Juan, Puerto Rico. They had a lot in common. Both man-

aged research groups that studied how bacteria defend themselves against viruses. Both had done work confirming that a bacterium identifies attacking viruses by using "memories" of past invaders' DNA to spot those enemies when they reappear.

Shortly after the meeting, Charpentier and Doudna decided to join forces. Charpentier's lab in Umeå was picking up clues that *Streptococcus* bacteria used a single protein, Cas9, as a kind of sword to chop up viruses that breached their cell walls. Doudna put her Berkeley lab on the job of figuring out how Cas9 worked.

By one of those quirks of fate that underpin many scientific discoveries, it turned out that Krzysztof Chylinski, a researcher in Charpentier's group, and Martin Jinek, then in Doudna's, had grown up in neighboring towns and spoke the same Polish dialect. "They started speaking by Skype, hit it off, and started to share data and discuss ideas for experiments," Doudna says. "The project really took off from there."

Scientists in both labs realized that Cas9 might be useful for genome editing, a type of genetic engineering that uses enzymes as molecular pruning shears. The enzymes, called nucleases, create breaks at specific sites in the double-stranded DNA helix; a cell then repairs the break, sometimes incorporating new genetic material that a scientist has placed in the nucleus. When Doudna and Charpentier began collaborating, the most advanced method available for disabling or altering a gene was to customize an enzyme that could find and cut the desired DNA target. In other words, for every genetic modification, scientists had to tailor a new protein targeted to the right DNA sequence.

But Doudna and Charpentier realized that Cas9, an enzyme that the strep bacterium used in its immunological defense, employed RNA to guide it to the DNA target. Probing for the target, the Cas9-RNA complex would bounce off the DNA, seemingly at random, until it found a promising site. The bouncing turned out to be the Cas9 enzyme searching, each time, for the same short "signal" sequence of DNA; Cas9 would attach to that sequence, pry open the double helix of the adjacent DNA and see if it matched the RNA guide. Cas9 would make the cut only when the RNA matched the DNA molecules. If that natural RNA-guided system could be harnessed, researchers would not have to construct a new enzyme to reach every target on the genome. Editing might become simpler, cheaper and more efficient.

After months of studying Cas9 together, the transatlantic team had a breakthrough. Doudna recalls the moment vividly: Jinek, then a postdoctoral researcher, had been running tests on Cas9 in the lab, which sits across from the Greek Theatre on a leafy hillside at the edge of the Berkeley campus. He showed up in Doudna's office one day to discuss results, and they mused about something that he had been discussing with Chylinski: in nature—in *Streptococcus* bacteria—Cas9 used not one but two RNA guides to target the right spot in the double helix of an invader's DNA. What if they could streamline those two guides into a single, artificially produced RNA strand without harming its effectiveness as a guide? With only one RNA sequence to modify, the engineering might be sped up tremendously. An RNA guide would be much easier to construct than the binding agents of the old customized enzymes, with their elaborate coding schemes.

"It was one of those moments when you see data, and something clicks," Doudna says. "We realized that we could design those RNA molecules into a single guide. A single protein and a single guide would be a powerful tool. I had chills running down my spine and realized, 'Oh, my gosh, run, don't walk, to the lab. If this works....'"

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And work it did, with implications that Doudna, for all her excitement, could never have imagined. When Doudna and Charpentier published the results of their CRISPR-Cas9 research on August 17, 2012, scientists in the field immediately recognized its transformative potential—and a global race was on to test the applications.

RUSH TO COMMERCIALIZATION

BY LAST YEAR RESEARCHERS were getting CRISPR-Cas9 to work in the cells of plants and animals much more complex than bacteria, and they were speculating about applications as fantastical as bringing back Neandertals and woolly mammoths. At Harvard, a team led by geneticist George Church used CRISPR to alter genes in human cells, opening up a whole new world of therapeutic possibilities.

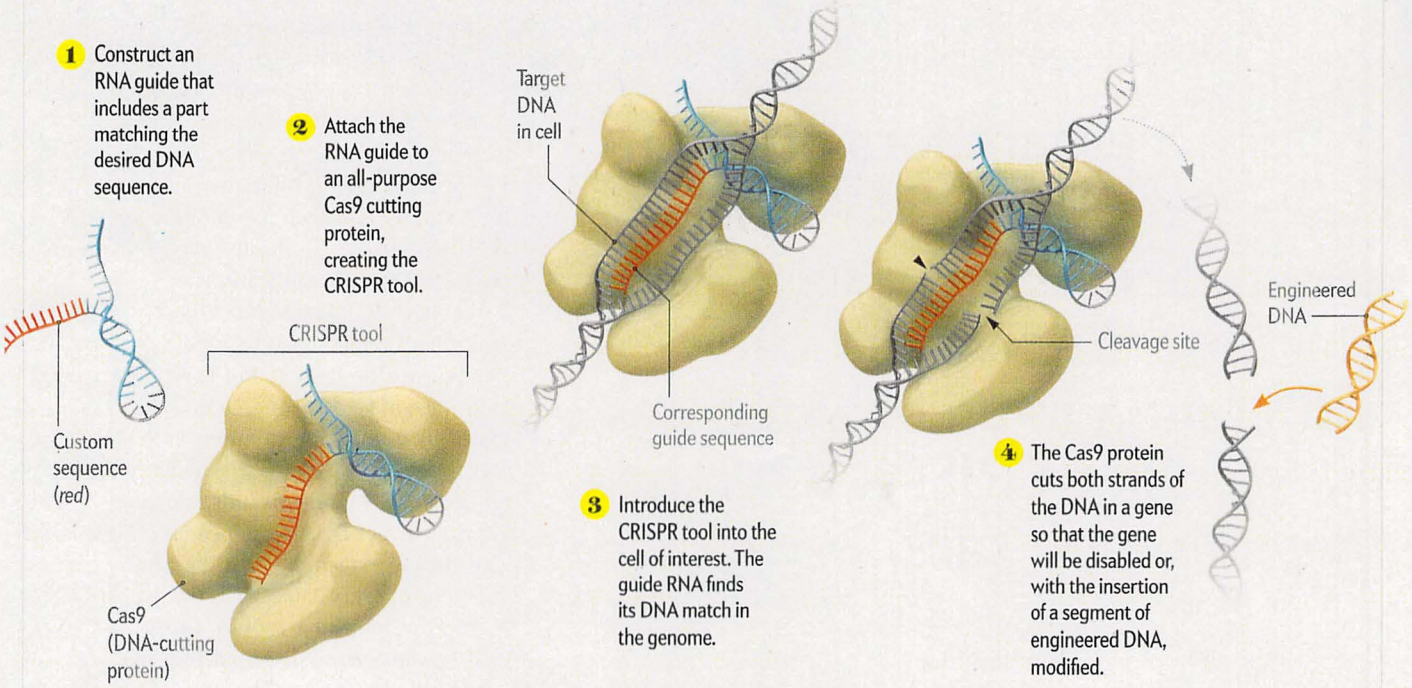
Not surprisingly, money soon began to pour into CRISPR-Cas9 work. A little more than a year ago Doudna teamed up with Church, Feng Zhang of M.I.T. and other researchers to launch Editas Medicine, with $43 million in venture capital and the goal of developing a new class of drugs based on CRISPR. (The company is not yet talking about which diseases it will target first.) In April, CRISPR Therapeutics launched in Basel and London, with investments of $25 million and a similar goal. Therapies from companies like CRISPR Therapeutics and Editas Medicine are still years away. But lab-supply firms are already shipping ready-to-inject CRISPR and made-to-order, CRISPR-altered mice, rats and rabbits to customers around the world.

On a steamy day this past summer I visited SAGE Labs in St. Louis, the first company to license Doudna's CRISPR technology for altering rodents, so I could see for myself how CRISPR works. SAGE ships to about 20 of the top pharmaceuticals companies, along with lots of universities, biotech institutes and foundations. (Horizon Discovery Group, a biotechnology company based in Cambridge, England, which was already barreling into CRISPR production of its own, bought SAGE for $48 million in September.) At SAGE, a set of low office buildings on a cul-de-sac in an industrial complex, scientists receive an online order from a lab in, say, Sacramento, Calif., for 20 *Pink1* knockout rats for research on Parkinson's disease. In a new, $2-million wing of the building, rats with this modification, as well as other CRISPR-modified rodents, live in superclean, climate-controlled cages that are neatly stacked from floor to ceiling. Filling the order is as easy as selecting 20 of the right rats, packing them gently into

BASICS

How CRISPR Works

Bacteria use a weapon called CRISPR to julienne invading viruses. Scientists can hijack this process to chop up sequences of DNA they would like to modify instead. Unlike previous genome-editing methods, the CRISPR system uses a single, all-purpose enzyme, called Cas9, to do the slicing. All the researcher has to do is create an RNA "guide" to steer it there; RNA is vastly easier to synthesize than enzymes.



boxes and airfreighting them to California. The same goes for animals ready-made for research on ills ranging from schizophrenia to pain control.

If a customer needs a rat or mouse that is not in stock, however, the process is different. A SAGE customer who wants to study a link between Parkinson's and a newly suspect gene—or even a specific mutation within a gene—has several options. SAGE scientists can use CRISPR to turn off the targeted gene, to introduce a mutation, or to turn off the gene and insert a human gene in its place. Many diseases, from Parkinson's to cystic fibrosis to AIDS, are affected by multiple genetic variants, and it used to take up to a year to create the complex, sequential mutations in animals that were needed to study such illnesses. Unlike previous genome-editing techniques, CRISPR allows researchers to make multiple genetic changes to a cell quickly and simultaneously, reducing the time it takes to produce a modified animal to a matter of weeks.

The SAGE employees start this process by making customized DNA from a chemical kit—and RNA to match the DNA. In a petri dish, they mix the RNA and Cas9, which combine into a chemical substance with gene-editing powers: the CRISPR tool. Then they spend about a week testing that tool on animal cells, using what looks like a desktop scanner to run electric currents that shock the CRISPR into the cells. The CRISPR goes to work, cutting the DNA and causing small insertions or deletions. Because CRISPR is not 100 percent efficient, it makes cuts and creates mutations in some cells but not in others. To see how well the CRISPR has performed, the scientists collect the DNA from the cells, pool it, and make copies of the region around the site of the supposed mutation. After processing and analyzing that pooled DNA, they look at the results on a computer monitor. Cut, mutated DNA shows up as a dim band—and the more DNA the CRISPR has cut, the brighter that dim band will be.

Next the process moves to the animal wing, where scientists use CRISPR to churn out genetically modified embryos and create mutant rodents. In one of those labs, I watched biologist Andrew Brown work the magic of CRISPR. Swaddled in surgical gloves and blue paper clothing—robe, overshoes and puffy bonnet—he hunched over a dissecting microscope, sucking at the end of a glass pipette to bring up a rat embryo. He then trundled the embryo across the room to a bigger microscope, flanked by robotic arms, released it into a drop of liquid on a slide and settled onto a stool. With his right hand, he commanded a joystick that moved a hollow glass needle into place against the wall of the embryo.

Through the eyepiece of the microscope, the embryo's two pronuclei, one from each rat parent, looked like little craters on the surface of the moon; Brown nudged the cell until a pronucleus spun close to the tip of the needle. He clicked the button of a computer mouse, and the needle squirted a tiny drop of liquid containing CRISPR through the plasma membrane of the cell. The pronucleus swelled like a flower blooming in fast motion.

Illustration by AXS Biomedical Animation Studio

With luck, Brown had created a mutant cell. SAGE's three technicians repeat the task as many as 300 times a day, four days a week.

When Brown finished injecting his rat embryo, he sucked it into a pipette, deposited it in a petri dish and stored it in a cupboard heated to body temperature. He would eventually inject the modified embryo—and some 30 to 40 others—into a surrogate rat mother. Twenty days later the rat would bear five to 20 pups, and when the pups were 10 days old, SAGE scientists would take tissue samples to see which ones had the modified gene.

In June, researchers at M.I.T. reported curing adult mice of tyrosinemia—a rare liver disorder caused by a mutation in an enzyme—by injecting CRISPR directly through their tails.

"That's the exciting part," Brown said. "It might be just one of 20 that have the modification. That's what we call our founder animal. When we get to that point, everybody celebrates." Watching the SAGE scientists making RNA or injecting embryos, it all looked easy—and the same processes are turning out genetically engineered animals at many labs. It is, as SAGE CEO David Smoller put it, gene editing "for the masses."

PROMISE AND MAYBE A LITTLE PERIL

AS CRISPR CHARGES AHEAD into commercial use, researchers and entrepreneurs keep imagining new applications for the technology, and some can come across as hubristic. It might be possible to tweak the chromosomal abnormality associated with Down syndrome early in a pregnancy, for example, or to reintroduce susceptibility to herbicides in resistant weeds, or to bring back animal species that have gone extinct. Not surprisingly, some people find it scary. Startled commentators have warned that in our rush to rid the world of malarial mosquitoes, cure Huntington's disease or design better babies, we could create a *Jurassic Park*-ful of harmful new genes.

Consider the idea of using CRISPR to eliminate malarial mosquitoes. It is one thing to vanquish the malarial parasite but quite another to annihilate its vector, says Todd Kuiken, a biosecurity analyst at the Woodrow Wilson International Center for Scholars in Washington, D.C. If the goal is eradicating malaria—which infects 200 million people a year and kills 600,000—Kuiken says that we have to be careful not to cause 10 other problems. "We've got to have an opportunity to ask, 'Do we really want to do this?' And if the answer is 'yes,' what kinds of systems do we have in place, what kinds of safeguards?"

To their credit, scientists are moving quickly to envision the most realistic dangers of CRISPR technology and to develop responses. In July, when a Harvard team published a paper on CRISPR-powered mosquito elimination, the scientists called for a public discussion and began to suggest technological and regulatory fixes for altered genes gone wild. "CRISPR is happening so incredibly fast," observes Jeantine Lunshof, a bioethicist on the team. "Many people have not heard of it, but people are using it. That is a new dynamic." Within Berkeley's Innovative Genomics Initiative, Doudna has been assembling a group designed specifically to discuss the ethical implications of CRISPR applications.

It is hard to imagine ethics concerns smothering the excitement over CRISPR. In June, for example, researchers at M.I.T. reported curing adult mice of tyrosinemia—a rare liver disorder caused by a mutation in an enzyme—by injecting CRISPR directly through their tails. Delivering three RNA guide strands, along with Cas9 and the correct DNA sequence for the mutated gene, they managed to insert the correct gene in about one of every 250 cells in the livers of mice. During the following month, the healthy liver cells thrived, eventually replacing a third of the bad cells, enough to rid the mice of the disease. And in August virologist Kamel Khalili of Temple University and his colleagues reported having used CRISPR to slice the HIV virus, which causes AIDS, out of several human cell lines.

For Khalili, who has labored in the trenches of HIV/AIDS since the dark days of the 1980s, CRISPR is nothing short of revolutionary. Despite huge strides in AIDS treatment, today's medications only control the virus—they do not eradicate it. But by using CRISPR, Khalili's team completely excised the integrated copy of HIV, converting infected cells to uninfected cells. Besides eliminating the virus from an infected cell, CRISPR can also protect an uninfected cell, Khalili says, immunizing it by incorporating a sequence from the attacking virus, just as Doudna and her team observed primitive bacteria doing. You could call it a genetic vaccine. "If you'd asked me two years ago, 'Can you precisely excise the HIV from a human cell?' I would have said that's a tall order. Now we've done it," Khalili says. "That is the ultimate cure." SA

MORE TO EXPLORE

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FROM OUR ARCHIVES

The RNA Revolution. Christine Gorman and Dina Fine Maron; April 2014.