Scientists grapple with rewriting the code of life

BY ANDREW POLLACK

In the late 1980s, scientists at Osaka University in Japan noticed unusual repeating DNA sequences next to a gene they were studying in a common bacterium. They mentioned them in the final paragraph of an otherwise unremarkable paper: "The biological significance of these sequences is unknown." Now the significance of those sequences is known, and it has set off a scientific frenzy.

The sequences, it turns out, are part of a sophisticated immune system that bacteria use to fight viruses. And that system, whose very existence was unknown until about seven years ago, may provide scientists with unprecedented power to rewrite the code of life.

In the past year or so, researchers have discovered that the bacterial system can be harnessed to make precise changes to the DNA of humans, as well as other animals and plants.

This means a genome can be edited, much as a writer might change words or fix spelling errors in a document. It allows "customizing the genome of any cell or any species at will," said Charles Gersbach, an assistant professor of biomedical engineering at Duke University.

Already the molecular system, known by the acronym Crispr (pronounced crisper), is being used to make genetically engineered laboratory animals more easily than could be done before, with changes in multiple genes. Scientists in China have made monkeys with changes in two genes.

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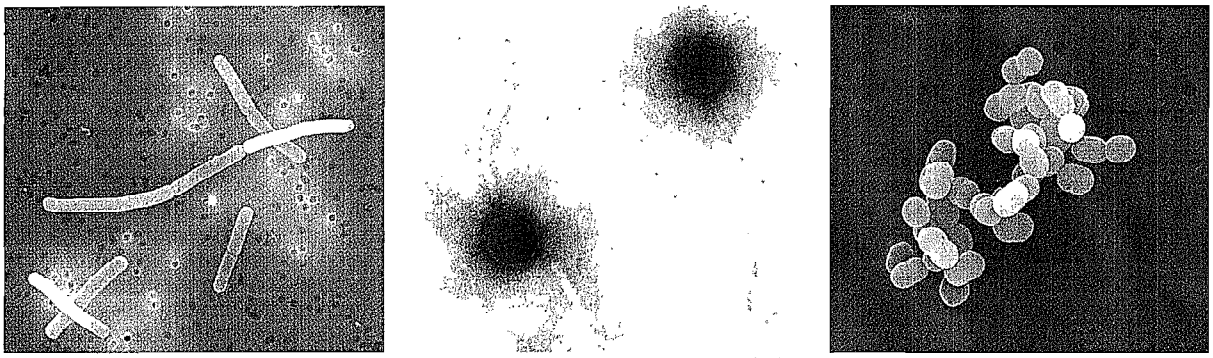

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Three bacterial systems used to make cheese and yogurt can be harnessed to make changes to human DNA. From left, Lactobacillus acidophilus ferments sugars into lactic acid; bacteriophages are viruses that infect bacteria; and lactococcus lactis are used as starter cultures.


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Scientists rewrite the code of life

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Scientists hope Crispr might also be used for genomic surgery, as it were, to correct errant genes that cause disease. Working in a laboratory — not, yet, in actual humans — researchers at the Hubrecht Institute in the Netherlands showed they could fix a mutation that causes cystic fibrosis.

But even as it is stirring excitement, Crispr is raising profound questions. Like other technologies that once wowed scientists — gene therapy, stem cells, RNA interference — it will undoubtedly encounter setbacks before it can be used to help patients.

It is already known, for instance, that Crispr can sometimes change genes other than the intended one. That could lead to unwanted side effects.

The technique is also raising ethical issues. The ease of creating genetically altered monkeys and rodents could lead to more animal experimentation. And the technique used to create these animals — altering genes in their embryos — could conceivably work with human embryos as well, raising the specter of so-called designer babies.

"It does make it easier to genetically engineer the human germline," said Craig C. Mello, a Nobel laureate at the University of Massachusetts Medical School, referring to making genetic changes that can be passed to future generations.

Still, Crispr is moving toward commercial use. Five academic experts have raised $43 million to start Editas, a company in Cambridge, Mass., that aims to treat inherited disease. Other startups include Crispr Therapeutics in London and Caribou Biosciences in Berkeley, Calif.

Agricultural companies might use Crispr to change existing genes in crops to create new traits. That might sidestep the regulations and controversy surrounding genetically engineered crops, which have foreign DNA added.

The development of the new tool is an example of the unanticipated benefits of basic research. About 15 years ago, after it became possible to sequence the entire genomes of bacteria, scientists noticed that many species had those repeated DNA sequences first noticed a decade earlier in Osaka. They dubbed them "clustered regularly interspaced short palindromic repeats" — Crispr, for short.

But what was their purpose? In 2007, researchers at Danisco, a company that supplies bacterial cultures used in making cheese and yogurt, confirmed hypotheses that Crispr protects bacteria from viruses. It is part of an adaptive immune system — one that remembers a pathogen so it is ready the next time that same germ appears. The human adaptive immune system is why people get measles only once and why vaccines work. But it was not imagined that single-celled organisms like bacteria had such systems.

Here is how it works. The repeated DNA sequences in the bacterial genome are separated from one another by other sequences. These spacers, as they are called, are excerpts from the sequences of viruses that have attacked the bacterium. They are like genetic mug shots, telling the bacterium which bad guys to watch for. The Crispr defense system will slice up any DNA with that same sequence, so if the same virus invades again, it will be destroyed.

If a previously unseen virus attacks, a new spacer, a new mug shot, is made and put at the end of the chain.

That means the Crispr region "is like a tape recording of exposure to prior invaders," said Erik J. Sontheimer, a professor of molecular biosciences at Northwestern University who helped unravel the mechanism.

And it provides a way to tell two bacterial strains apart, because even two strains from the same species will probably have encountered different viruses. This is already being used to identify sources of food-poisoning outbreaks.

Cheese and yogurt companies can examine Crispr regions to see whether their bacterial cultures are immunized against particular viruses that could slow production.

"Now you can extend the shelf life of that great strain," said Rodolphe Barrangou of North Carolina State University, who previously worked at Danisco and was the lead author on the 2007 paper. "That has changed the game quite a bit for the dairy industry."

But the real frenzy started in 2012, when a team led by Emmanuelle Charpentier, then at Umea University in Sweden, and Jennifer A. Doudna at the University of California at Berkeley demonstrated a way for researchers to use Crispr to slice up any DNA sequence they chose. Scientists must synthesize a strand of DNA's chemical cousin RNA, part of which matches the DNA sequence to be sliced. This "guide RNA" is attached to a bacterial enzyme called Cas9. When the guide RNA binds to the matching DNA sequence, Cas9 cuts the DNA at that site.

Would this work in organisms besides bacteria? "I knew it was like firing a starting gun in a race," Dr. Doudna said, but sure enough, by early 2013 scientists had shown it would in human cells, and those of many other animals and plants, even though those species are not known to have Crispr-based immune systems.

"I don't know any species of plant or animal where it has been tried and it failed," said George Church, a professor of genetics at Harvard Medical School. "It allows you to do genome engineering on organisms that are very hard to do otherwise."

Cas9 creates a break in both strands of the DNA double helix. That is enough to disable, or knock out, a gene. To change a gene, scientists usually insert a patch — a bit of DNA similar to where the break occurred but containing the desired change. That patch is sometimes incorporated into the DNA when the cell repairs the break.

In the past, making an animal with multiple genetic changes usually required creating separate animals with

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single changes and then cross-breeding them to produce offspring with multiple changes. With Crispr, multiple genetic changes can be made in one step, by putting multiple guide RNAs into the cell.

"It just completely changes the landscape," Dr. Doudna said. Berkeley scientists used to farm that work out to specialty companies. Now, she said, "people are able to make mice in their own labs."

To be sure, there are other techniques that can do what Crispr does, though Crispr is "the easiest by far," Dr. Church said. RNA interference, for instance, can silence particular genes. It is similar to Crispr in that it also uses RNA that matches the gene to be silenced.

But RNA interference works by inhibiting messenger RNA, which translates a gene into a protein. That usually provides only a partial and temporary disabling of the gene, because the cell can make new messenger RNA. Crispr disables the gene itself, potentially a more complete and permanent inactivation.

There are also already ways to change genes, namely zinc finger nucleases and transcription activator-like effector nucleases, or Talens. With zinc fingers "it might take you months or years to get something to work well for one gene," said Dr. Gersbach at Duke. With Crispr "it takes days to weeks."

But quick is not always accurate. While Crispr is generally precise, it can have off-target effects, cutting DNA at places where the sequence is similar but not identical to that of the guide RNA. Still, scientists are already figuring out how to make Crispr more specific.

It will probably be a few years before Crispr is tested in people. But the pace of new discoveries and applications is dizzying. "All of this has basically happened in a year," said David S. Weiss of Emory University. "It's incredible."