Alternative Assessment #2

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HEALTH

A Powerful New Way to Edit DNA

By ANDREW POLLACK MARCH 3, 2014

In the late 1980s, scientists at Osaka University in Japan noticed unusual repeated DNA sequences next to a gene they were studying in a common bacterium. They mentioned them in the final paragraph of a paper: "The biological significance of these sequences is not known."

Now their significance 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. 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 as Crispr, is being used to make genetically engineered laboratory animals more easily than could be done before, with changes in multiple genes. Scientists in China recently made monkeys with

changes in two genes.

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, as 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 — like gene therapy, stem cells and 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 ones. 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 of 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 germ line," said Craig C. Mello, a Nobel laureate at the University of Massachusetts Medical School, referring to making genetic changes that could be passed to future generations.

Still, Crispr is moving toward commercial use. Five academic experts recently raised $43 million to start Editas Medicine, a company in Cambridge, Mass., that aims to treat inherited disease. Other start-ups include Crispr Therapeutics, which is being formed 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 generally 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 that were first noticed a decade earlier in Osaka. They were called "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 invader appears. The human adaptive immune system is why people get measles only once and why vaccines work. But it was not imagined that single-cell 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" are excerpts from the sequences of viruses that have attacked the bacterium or its ancestors. 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 Northwestern University professor who helped unravel the mechanism.

And it provides a way to tell two bacterial strains apart, because even two strains from the same species are likely to 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 if 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."

The real frenzy, however, started in 2012, when a team led by Emmanuelle Charpentier, then at Umea University in Sweden, and Jennifer A. Doudna of the University of California, Berkeley, demonstrated a way for researchers to use Crispr to slice up any DNA sequence they choose.

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 corresponding DNA sequence, Cas9 cuts the DNA at that site.

The cell tries to repair the cut but often does so imperfectly, which 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.

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 work in human cells, and those of many other animals and plants, even though these 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."

In the past, making an animal with multiple genetic changes usually required creating separate animals with single changes and then crossbreeding 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 out that work to specialized laboratories or companies. Now, she said, "people are able to make mice in their own labs."

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. The biotechnology company Sangamo BioSciences is already conducting a clinical trial of a treatment for H.I.V. that uses zinc fingers to alter patients' immune cells to make them resistant to the virus.

Both techniques use proteins to guide where the DNA is cut; it is more difficult to develop a protein that binds to a specific DNA sequence than it is to make a piece of RNA with the matching sequence.

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."

Quick is not always accurate, however. 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.

Crispr "may not yet have adequate specificity to completely displace" the older techniques, Dana Carroll, a biochemistry professor at the University of Utah, wrote

in a commentary in Nature Biotechnology in September.

Still, scientists are already figuring out how to make Crispr more specific.

Another obstacle for treating diseases will be the delivery of the genetic changes to all the cells in the body that need it.

For some diseases, it may be possible to extract blood stem cells from the body, alter them using Crispr, and put them back. If that is not possible, the DNA needed to make Cas9, the guide RNA and the corrective patch might be put into a disabled virus. This technique is used for gene therapy, but does not always work well.

It is likely to be a few years before Crispr is tested in people. For now, there is a lot more to learn about it.

Chase L. Beisel at North Carolina State reported that Crispr could be used to kill one strain of bacteria in a mixture of strains, by targeting a sequence unique to that strain. That might one day lead to antibiotics that can kill the bad bugs without also killing the good ones.

David S. Weiss of Emory University found that some bacteria use Cas9 to silence one of their own genes, rather than that of a virus, to help them evade detection by their host's immune system.

The pace of new discoveries and applications is dizzying. "All of this has basically happened in a year," Dr. Weiss said. "It's incredible."

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The Washington Post

Health & Science

Scientists are growing anxious about genome-editing tools

By Meeri Kim May 18, 2015

Every human genome contains the blueprints for building a person, a library of roughly 20,000 genes that encode everything from eye color to cancer risk. Imagine if those genetic instructions could be tweaked at will — with a snip here and a cut there, a gene might be deleted, inserted or replaced by a different piece of DNA.

Tools that can do this already exist, but one in particular, called CRISPR/Cas 9, has leapt ahead of other genome-editing tools because it's cost-effective and simple. Researchers say that human clinical trials to fix genetic diseases — for example, sickle cell anemia — are only a few years away.

Last month, a team of Chinese researchers reported that, in a first-of-its-kind event, they had edited the genomes of human embryos. (The embryos, obtained from local fertility clinics, would not have resulted in live births because they contained an extra set of chromosomes.) The scientists' aim was to modify the gene responsible for a common blood disorder using CRISPR.

The majority of embryos developed unintended mutations, and only a small fraction of them had the blood-disorder gene correctly modified — not what you'd call a success.

Many experts believe it was too soon for such work to be carried out — both because the technology is not yet perfected and because the ethical, medical and legal implications of the research have not been hashed out. Soon after the results were published — by the open-access journal Protein & Cell — the National Institutes of Health reaffirmed its ban on gene editing of human embryos.

In a statement released April 29, NIH Director Francis S. Collins said that genome editing in human embryos is "a line that should not be crossed" due to safety and ethical issues "presented by altering the germline in a way that affects the next generation without their consent, and a current lack of compelling medical applications justifying the use of CRISPR/Cas9 in embryos."

Genome editing of a human embryo would affect every cell in the embryo's resulting fetus, as opposed to altering the DNA of a select type of cells — such as the stem cells that produce blood cells.

'A molecular scalpel'

"You can think about it as a molecular scalpel," said biochemist Jennifer Doudna of the University of California at Berkeley. "It's a way for making a really precise change in an organism, such as changing a disease-causing mutation into one that is harmless."

The CRISPR system is a collection of molecules that work together to edit an organism's DNA. These molecules can be packed into an empty virus and then injected along with a molecule to jump-start the process once the virus encounters a cell. Another approach is to remove cells with undesirable mutations from the body, edit them in a petri dish and insert them back into

the body.

Such an easy method of genome manipulation has prompted tremendous excitement in the biological sciences community, as a flood of academic labs and start-up companies scramble to take advantage of CRISPR's capabilities. But with that buzz have come big ethical questions.

"No one should be doing anything right now until we figure out what the hell is going on with this technique in animals," said bioethicist Arthur Caplan of New York University. "That's how we perfected in vitro fertilization, and that's how you establish safety — you do it in animals first."

But amid the fears of mad scientists creating designer babies and real-life Jurassic Parks, experts agree on one thing: It is no longer a matter of if but rather of when CRISPR-based genomic surgery will come to a hospital near you. Genomic sequencing is readily and cheaply available, and genetic testing and disease screening have long been underway in clinics across the country.

"As much as we worry about premature or ethical use, using these technologies to get rid of genetic disease would be amazing: Tay-Sachs, cystic fibrosis, sickle cell disease, hemophilia," Caplan said. "We've never really wiped out a disease except for smallpox, and these technologies might actually add to the list."

Rumors of the experiment by scientists based at Sun Yat-sen University in Guangdong spurred others, including Doudna, to meet in January to discuss the future of the technology and its responsible use in humans. A particular focus was germline editing, which refers to changing the DNA of fertilized eggs or embryos. Such editing affects all cells in the developing organism and passes those changes down to future generations.

The scientists, in a summary of their meeting published by the journal Science in April, strongly discourage their colleagues from attempting any

germline editing in humans. However, they remain supportive of basic CRISPR research on animals and non-embryonic human cells to see if human germline gene therapy might be helpful in the future to fix genetic mutations.

Doudna is planning to convene a larger, international meeting later this year.

“We really need to be getting in front of this conversation,” she said. “The field has just taken off in an unbelievable way, with many hundreds of papers being published in the scientific literature now, not just in human health but other areas as well.”

While Doudna and French microbiologist Emmanuelle Charpentier are commonly cited as the inventors of CRISPR/Cas9, we can actually thank modest, single-celled bacteria for this revolutionary genome editing system.

“CRISPR” stands for “clustered regularly interspaced palindromic repeats,” first described in 1987 by Japanese researchers who found chunks of oddly repeating DNA sequences in the genome of *E. coli*.

It wasn’t until about 15 years later that scientists realized the purpose of CRISPRs: They are a key part of the bacterium’s immune system. Each CRISPR is followed by unique DNA segments of viruses that the bacterium previously encountered: If that type of virus tries to attack the cell again, a CRISPR can recognize and defend against it. CRISPRs have been found in 45 percent of sequenced bacteria genomes and in 84 percent of archaea, a type of single-cell microorganism.

Cas9, a scissors-like enzyme, uses the viral DNA segments as an identification system to check for foreign invaders. If the DNA of an incoming suspicious entity is a match, Cas9 proceeds to chop up the intruder’s genetic material by severing both strands of the double helix molecule.

In 2012, Doudna and Charpentier published a study in which they adapted parts of the bacterium's immune system to create a simple two-part genome editing tool: The Cas9 enzyme and the guide sequence — a small piece of single-stranded RNA 20 nucleotides, or "letters," long — could be used to target any spot in the genome for cutting.

From that point on, CRISPR/Cas9 technology exploded. The next year, it was used to edit the genomes of human and mouse cells, followed by frogs, monkeys and such crop plants as rice and wheat.

"The range of possible applications is really quite broad, but translating from this very experimental science into real therapeutics — there is a lot of work to be done," said Katrine Bosley, chief executive of Editas Medicine, a start-up in Cambridge, Mass., that is trying to develop CRISPR/Cas9-based drugs to treat genetic mutations.

Perhaps the biggest issue left to solve involves off-target cuts, which occur when Cas9 gets a bit snip-happy and chops the genome in unintended places. These mistakes can cause big problems, including cell toxicity and cancer.

"Sometimes Cas9 can recognize sequences that are very similar but not identical to the target sequence," said biological engineer Feng Zhang of the Massachusetts Institute of Technology, a co-founder of Editas and head of a lab at the Broad Institute, a Harvard-MIT biomedical research collaborative. "Depending on the specific sequence, the off-target effect may be more severe or less of a problem."

Biomedical engineer Gang Bao of Rice University said that scientists cannot answer yet whether, even with some off-target effects, it can be used for humans.

In his lab, Bao is analyzing off-target effects for his treatment of the genetic mutation that causes sickle cell disease. The idea is to take blood-cell-

producing stem cells from a patient, use CRISPR to correct the mutation and then reinsert the stem cells back into the bone marrow. Every step of the process requires precision, from minimizing off-target cuts to getting the modified stem cells to survive and transform into blood cells.

Stem cell trials

After having initial success using CRISPR therapy with mice, he has moved on to inserting human stem cells into monkeys for his experiments. He hopes to initiate the first human clinical trials in 2018.

Editas is taking a different approach, working to neatly package CRISPR/Cas9 into a virus for direct delivery into the patient's body and letting the genome editing happen there. For certain diseases, such as cystic fibrosis, which affects the lungs and other organs, Editas researchers must figure out how to get the viruses into the right cells while avoiding others.

"From the patient's perspective, [the therapeutic] would be an injection of some sort — maybe an IV, maybe a local injection," Bosley said. "If you're working on a genetic disease of the eye, you might do a direct injection into the eye."

Bao and Bosley say they aren't interested in germline editing and don't believe that it is necessary for the medical applications they are working on. Their work, which involves modifying somatic, or non-germline, cells, might have unexpected consequences down the line as well. The technology is so new that no one really knows.

Kim is a freelance science journalist in Philadelphia.

1. Imagine you've been offered a deal from a genomics company. You can get a free genome sequence – an analysis of all of your DNA that includes a report of your ancestry, traits and a medical profile. The medical profile tells you about diseases for which you have a low risk of getting, and also those you have a high risk of getting. Are you interested? Use information from the articles to explain why or why not.

2. For the first 100 volunteers, the company is offering to "correct" several of the disease-related genes found by the analysis. Imagine this were a very new procedure approved by the government for safety, but without a great deal of long term study. Would you volunteer for this added service? Use information from the articles to explain why or why not. (Note: This service is not currently available and will not be in the near future, so use your imagination.)