

GENE EDITING

CRISPR alternative doubted

Reports of irreproducibility multiply, but author stands by his NgAgo gene-editing system.

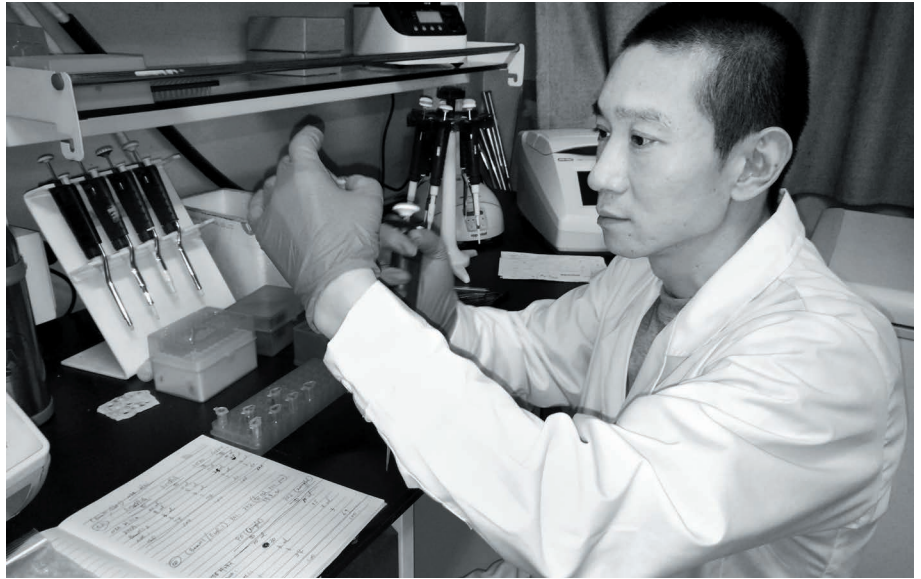
BY DAVID CYRANOSKI, SHIJIAZHUANG, CHINA

Controversy is escalating over whether a gene-editing technique proposed as an alternative to the popular CRISPR–Cas9 system actually works.

Three months ago, Han Chunyu, a biologist at Hebei University of Science and Technology in Shijiazhuang, reported that the enzyme NgAgo can be used to edit mammalian genes. But scientists are increasingly complaining that they cannot replicate the results — although one researcher has told *Nature* that he can. *Nature Biotechnology*, which published the research, is investigating the matter.

Han says he receives dozens of harassing calls and texts each day, mocking him and telling him that his career is over — but he is convinced that the technique is sound. On 8 August, he submitted a protocol to the online genetic-information repository Addgene. He hopes that this will help efforts to reproduce his work, but other scientists say it does not clear things up.

The stakes are high. Over the past few years, the CRISPR–Cas9 system has transformed biology. But it has also made scientists hungry to expand the gene-editing toolkit (see ‘A guide to the many other ways to edit a genome’). NgAgo is one of several methods that have emerged. “A lot of us are really cheerleading and hoping that



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Han Chunyu maintains that the NgAgo enzyme can edit genes.

it works,” says geneticist George Church of Harvard Medical School in Boston, Massachusetts.

CRISPR–Cas9 uses small genetic sequences to guide an enzyme to cut DNA in a particular location. In the *Nature Biotechnology* paper, Han’s team reports using a wide variety of genetic sequences to guide NgAgo — which

belongs to the Argonaute (Ago) family of proteins that others had flagged as potential gene editors — to edit eight different genes in human cells and to insert genes at specific points on chromosomes (F. Gao *et al. Nature Biotechnol.* **34**, 768–773; 2016).

NgAgo cuts only the target genes, says Han,

BEYOND CRISPR

A guide to the many other ways to edit a genome

The CRISPR–Cas9 tool enables scientists to alter genomes practically at will. It has blazed through labs around the world, finding new applications in medicine and basic research.

But the zeal with which researchers jumped on a possible new system called NgAgo earlier this year reveals an undercurrent of frustration with CRISPR–Cas9 — and a drive to find alternatives. Some are variations on the CRISPR theme; others offer new ways to edit genomes (see go.nature.com/2bbgxbw for more).

A MINI-ME

CRISPR–Cas9 may one day be used to rewrite the genes responsible for genetic diseases. But the components of the system — an enzyme called Cas9 and a strand of RNA that directs the enzyme to the desired sequence — are too large to stuff into the genome of the virus most

commonly used in gene therapy to shuttle foreign genetic material into human cells.

A solution comes in the form of a mini-Cas9, which was plucked from the bacterium *Staphylococcus aureus*. It’s small enough to squeeze into the virus used in one of the gene therapies currently on the market. Two groups have now used the mini-Cas9 in mice to correct the gene responsible for Duchenne muscular dystrophy.

EXPANDED REACH

Cas9 will not cut everywhere it’s directed to — a certain DNA sequence must be nearby for that to happen. This demand is easily met in many genomes, but can be a painful limitation for some experiments. Researchers are looking to microbes to supply enzymes that have different sequence requirements to expand the number of sequences they can modify.

One such enzyme, called Cpf1, may become an attractive alternative. Smaller than Cas9, it has different sequence requirements and is highly specific.

Another enzyme, called C2c2, targets RNA rather than DNA — a feature that holds potential for studying RNA and combating viruses with RNA genomes.

TRUE EDITORS

Many labs use CRISPR–Cas9 only to delete sections in genes, thereby abolishing their function. “People want to declare victory like that’s editing,” says George Church, a geneticist at Harvard Medical School in Boston, Massachusetts. “But burning a page of the book is not editing the book.”

Those who want to swap one sequence with another face a more difficult task. When Cas9 cuts DNA, the cell often makes mistakes as it

whereas CRISPR–Cas9 sometimes edits the wrong genes. And CRISPR–Cas9 requires a certain genetic sequence to be near the cutting site to initiate its activity, but NgAgo does not, which could broaden its applications, adds Han.

The initial reaction to the work in China was laudatory, including a visit to the lab by China Central Television. It was overwhelming, says Han. He doesn't like to travel and has never left China: a trip to visit a collaborator in Hangzhou in March was the first time the 42-year-old had boarded a plane. Before his paper came out, "I was completely unknown", says Han, who spoke to *Nature* at his laboratory and at a restaurant.

Doubts about the research first surfaced at the beginning of July, when Fang Shimin, a former biochemist who has become famous for exposing fraudulent scientists, wrote on his website New Threads (xys.org) that he had heard reports of failed reproduction efforts, and alleged that Han's paper was irreproducible. Criticism grew on various Chinese sites.

On 29 July, Gaetan Burgio, a geneticist at the Australian National University in Canberra, posted thorough details of his failed attempts to replicate the experiment on his blog. Normally, his posts get a few dozen hits, but this one spiked to more than 5,000.

On the same day, geneticist Lluís Montoliu at the Spanish National Centre for Biotechnology in Madrid e-mailed his colleagues at the International Society for Transgenic Technologies to recommend "abandoning any project involving the use of NgAgo". The e-mail was leaked and posted on Fang's website.

An online survey by molecular biologist Pooran Dewari of the MRC Centre of Regenerative Medicine in Edinburgh, UK, has found only 9 researchers who say that NgAgo works — and

97 who say that it doesn't. And two researchers who initially reported success with NgAgo in an online chat group now say they were mistaken.

Debojyoti Chakraborty, a molecular biologist at the CSIR-Institute of Genomics and Integrative Biology in New Delhi, says that he repeated a section of Han's paper that described using NgAgo to knock out a gene for a fluorescent protein. The glow was reduced in his cells, so Chakraborty assumed that NgAgo had disabled the gene. But DNA sequencing revealed no evidence of gene editing. Jan Winter, a PhD student in genomics at the German Cancer Research Center in Heidelberg, describes a similar experience.

Han has only got the system to work on cells cultured in his laboratory. It failed in cells that he purchased, which he later found to be contaminated with *Mycoplasma* bacteria. Others might be having the same problem, he says, and some graduate students might not be being careful with reagents. Winter disagrees: "I do not think it is a problem of the scientists doing something wrong."

One researcher in China who doesn't want his name to be entangled in the controversy told *Nature* that he had tested NgAgo in a few kinds of cell and found that it was able to induce genetic mutations at the desired sites — a finding that he verified by sequencing. He

adds that the process was less efficient than CRISPR–Cas9, "but, in short, it worked".

Two more Chinese scientists, who also asked not to be named, say they have initial results showing that NgAgo works but they still need to confirm with sequencing.

"It might, might work," says Burgio, "but if so, it's so challenging that it's not worth pursuing. It won't surpass CRISPR, not by a long shot."

He says there is little that is new in the revised protocol on Addgene. There is a warning to maintain levels of magnesium in cells, "but that doesn't make any sense to me", he says. It also warns against *Mycoplasma* contamination. But Montoliu, who might now give NgAgo one more chance in September, doubts that this could account for all the reported problems.

The failure of NgAgo "would be disappointing", says microbiologist John van der Oost of Wageningen University in the Netherlands, a co-author of the 2014 analysis of Argonaute proteins that laid the groundwork for their use in gene editing (D. C. Swarts *et al. Nature* **507**, 258–261; 2014). "But then there is work for us left to do to see whether other Argonaute systems can get it to work somehow."

Last week, *Nature Biotechnology* sent a statement to *Nature's* news team, which is editorially independent, saying that "several researchers" have contacted the journal to report that they cannot reproduce the results, and that "the journal is following established process to investigate the issues".

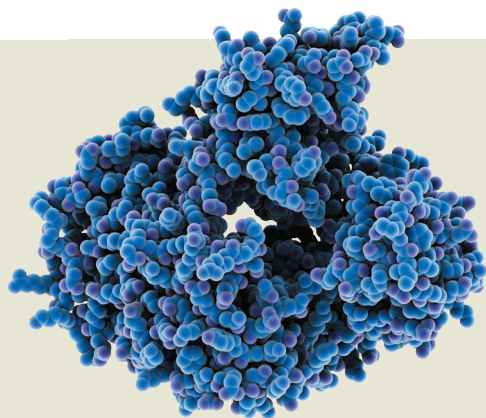
Hebei University says that it will ask Han to repeat the experiment so that it can be verified by an independent party within a month, according to Chinese state media. ■

Additional reporting by Heidi Ledford.



"It's not worth pursuing. It won't surpass CRISPR, not by a long shot."

Gaetan Burgio



An argonaute protein is one of many alternatives to the CRISPR–Cas9 gene-editing system.

stitches together the broken ends. This creates the deletions that many researchers desire. But researchers who want to rewrite a DNA sequence rely on a different repair pathway that can insert a new sequence — a process that occurs at a much lower frequency than the error-prone stitching. That low efficiency poses a problem in many organisms, including some

plants. "Everyone says the future is editing many genes at a time, and I think: 'We can't even do one now with reasonable efficiency,'" says plant scientist Daniel Voytas at the University of Minnesota in St Paul.

But developments in the past few months have given Voytas hope. Two groups of researchers have come up with techniques that disable Cas9 then tether it to an enzyme that converts one DNA letter to another. Voytas and others are hopeful that tethering other enzymes to the disabled Cas9 will allow different sequence changes.

PURSuing ARGONAUTES

When researchers claimed in May that they could use a protein from the Argonaute family called NgAgo to slice DNA at a predetermined site without needing a guide RNA or a specific neighbouring genome sequence (F. Gao *et al. Nature Biotechnol.* **34**, 768–773; 2016), they kicked off a wave of excitement. But laboratories have so far failed to reproduce the results. Even so, there is still hope that other Argonaute

proteins could provide a way forward, says genome engineer Jin-Soo Kim at the Institute for Basic Science in Seoul.

PROGRAMMING ENZYMES

Other gene-editing systems are also in the pipeline, although some have lingered there for years. For an extensive bacterial project, Church's lab did not reach for CRISPR at all. Instead, the team relied heavily on a system called lambda Red, which can be programmed to alter DNA sequences without the need for a guide RNA. But despite being studied for 13 years in Church's lab, lambda Red works only in bacteria.

Church and Feng Zhang, a bioengineer at the Broad Institute of MIT and Harvard in Cambridge, Massachusetts, say that their labs are also working on developing enzymes called integrases and recombinases for use as gene editors. "By exploring the diversity of enzymes, we can make the genome-editing toolbox even more powerful," says Zhang. "We have to continue to explore the unknown." **Heidi Ledford**