BIOMEDICINE

Mini enzyme moves gene editing closer to the clinic

Discovery expands potential CRISPR toolbox for treating genetic diseases in humans.

BY HEIDI LEDFORD

A tweak to a technique that edits DNA with pinpoint precision has boosted its ability to correct defective genes in people. Called CRISPR, the method is already used in the lab to insert and remove genome defects in animal embryos. But the genetic instructions for the machinery on which CRISPR relies — a gene-editing enzyme called Cas9 and RNA molecules that guide it to its target — are simply too large to be efficiently ferried into most of the human body's cells.

This week, researchers report a possible way around that obstacle: a Cas9 enzyme that is encoded by a gene about three-quarters the size of the one currently used. The finding, published on 1 April in *Nature*, could open the door to new treatments for a host of genetic maladies (F. A. Ran *et al. Nature* http://dx.doi.org/10.1038/nature14299; 2015).

"There are thousands of diseases in humans associated with specific genetic changes," says David Liu, a chemical biologist at Harvard University in Cambridge, Massachusetts, who was not involved in the latest study. "A fairly large fraction of those have the potential to be addressed using genome editing."

Genome editing has generated controversy, with unconfirmed reports of its use in human embryos. Some scientists have expressed concern that the technique might be used by fertility doctors to edit the genes of human embryos before its safety is established (see also E. Lanphier *et al. Nature* **519,** 410–411; 2015). That concern is exacerbated by the fact that changes made by the procedure in embryos would be passed to all subsequent generations without giving anyone affected the opportunity to consent (see *Nature* **519,** 272; 2015). But in the non-reproductive cells of children and adults, where intergenerational issues are not a concern, researchers and companies are already racing to develop CRISPR as a clinical tool.

The ethics of that pursuit may be more straightforward, but its execution can be harder than using CRISPR in embryos. An embryo consists of a small number of cells that give rise to a human. To edit the genome at that stage is simply a matter of injecting the necessary CRISPR components into a few cells. An adult human, however, is a mix of trillions of cells assembled into many different tissues. Researchers fret over how to target the CRISPR machinery to the specific cells where defective genes are disrupting physiological processes.

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"You can have the most optimal gene-editing system in the world, but if you can't deliver it to the proper cell type, it's irrelevant," says Nessan Bermingham, chief executive of Intellia Therapeutics in Cambridge, Massachusetts, which aims to bring genome editing to the clinic. "We're spending a tremendous amount of time working on it."

SNUG FIT

Gene-therapy researchers often harness a virus called AAV to shuttle foreign genes into mature human cells. However, most laboratories use a gene encoding the Cas9 protein that is too large to fit in the snug confines of the AAV genome alongside the extra sequences necessary for Cas9 function.

Feng Zhang of the Broad Institute of MIT and Harvard in Cambridge, Massachusetts, and his colleagues decided to raid bacterial genomes for a solution, because the CRISPR system is derived from a process that bacteria use to snip unwanted DNA sequences out of their genomes. Zhang's team analysed genes encoding more than 600 Cas9 enzymes from hundreds of bacteria in search of a smaller version that could be packaged in AAV and delivered to mature cells.

The gene encoding Cas9 in *Staphylococcus aureus* — a bacterium best known for causing skin infections and food poisoning — was more than 1,000 DNA letters smaller than the one for the commonly used Cas9. The researchers packed it into AAV along with RNAs that would target the enzyme to modify a cholesterol regulatory gene in the liver. Within a week of injecting mice with the modified virus, the team found that more than 40% of liver cells contained the modified gene.

"It's a terrific addition to the set of tools that genome engineers have at their disposal," says Liu. He has been developing ways to transport the larger Cas9 protein, bound to its guide RNAs, into cells without relying on a virus. Bermingham says that he expects labs to develop multiple delivery mechanisms that are tailored to individual tissues.

For now, biomedical engineer Charles Gersbach of Duke University in Durham, North Carolina, is eager to use the smaller Cas9 enzyme in mice to try to correct mutations associated with Duchenne muscular dystrophy, a devastating human disease that strikes 1 in 3,500 boys worldwide. Perhaps this will be the method that carries CRISPR into the clinic, he says, but it is too soon to tell. "It's a rapidly developing field," he says. "There are a lot of things that just haven't been tried yet." ■

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CORRECTIONS

The News Feature 'Eyes on the ocean' (*Nature* **519,** 280–282; 2015) used an earlier version of the name of the Pew Charitable Trusts. And the News Feature 'Biotech boot camp' (*Nature* **519,** 402–405; 2015) should have said that Steve Blank was involved in eight technology companies — he didn't launch all eight.

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