Enzyme tweak boosts precision of CRISPR genome edits

Engineered enzyme drives genome-editing errors below detection limit.

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Article toolsA powerful technique for editing genomes is now more precise. By tweaking an enzyme, researchers have reduced the error rate for the technique, known as CRISPR–Cas9 — in some cases to undetectable levels, they report on 6 January in *Nature*[1].

Researchers use CRISPR–Cas9 to make precise changes to genomes that remove or edit a faulty gene. It has worked on nearly every creature on which they have tested it, including human embryos.

The technique relies on an enzyme called Cas9, that uses a 'guide RNA' molecule to home in on its target DNA. Cas9 cuts the DNA at that site, and the cell's natural DNA repair machinery then takes over to mend the cut — deleting a short fragment of DNA or stitching in a new sequence in the process.

But the technology is not infallible: sometimes the Cas9 enzyme creates unwanted mutations. As CRISPR inches out of the laboratory and towards the clinic — with debates raging overwhether it should be deployed in embryos — researchers havepushed to reduce the error rate.

The latest study moves the field closer to that goal, says lead author Keith Joung, a pathologist at Massachusetts General Hospital in Boston. “This is a significant move forward,” he says. “We can very much reduce the probability of off-targets.”

Some researchers argue that the error rate does not have to be zero for CRISPR to be clinically useful. “At some point everyone needs to decide how specific is specific enough,” says Charles Gersbach, a bioengineer at Duke University in Durham, North Carolina. “The idea that you would make a tool that has absolutely no off-target effects is a little too utopian.”

Safety first

Previous work has shown that using a shorter strand of ‘guide RNA’ to direct the Cas9 enzyme to the targeted DNA could cut down on some errors[2]. And in December, synthetic biologist Feng Zhang of the Broad Institute of MIT and Harvard in Cambridge, Massachusetts, and his colleagues announced that they had engineered Cas9 to make it less error-prone[3].

For the latest study, Joung and his colleagues tackled a different region of the Cas9 enzyme, altering the part of the protein that makes contact with the DNA target. The team also used a more sensitive method for detecting errors.

They tested their high-fidelity enzyme, called SpCas9-HF1, with eight different guide RNAs. The engineered enzyme cut its target DNA nearly as well as the unaltered form, and made only one mistake with one of the guide RNAs. The unaltered Cas9 enzyme, by contrast, made mistakes when guided by

seven of the eight RNAs.

Cas9's mistakes have been a focus in many discussions about genome editing — including in thedebate over using the technique in human embryos. But that focus may be misplaced, says George Church, a geneticist at the Wyss Institute in Boston. With careful design of the guide RNA, Church says that researchers could already avoid most off-target cuts.

And although the work is important given the speed with which CRISPR–Cas9 is moving into therapeutics, says Gersbach, the system will need extra safety checks before it is deemed safe for use in humans.

Pursuit of perfection

In December, Gersbach and his colleagues announced that they had used CRISPR–Cas9 to repair the genetic mutation that causes Duchenne muscular dystrophy in mice4. To do so, his team used a virus to carry Cas9 into muscle cells. That virus can continue to express the enzyme for much longer than it was in Joung's experiments, leaving more opportunity for off-target cuts.

The US Food and Drug Administration has not outlined its requirements for approving a CRISPR–Cas9 clinical trial, but Sangamo BioSciences of Richmond, California, has already used another genome-editing tool, called zinc finger nucleases, in clinical trials in more than 80 patients.

For those trials, regulators wanted safety data on how well the modified cells performed, in addition to information about off-target mutations, says Fyodor Urnov, a senior scientist at the Sangamo. The company was required to show that altered immune cells called T cells still behaved like normal T cells, for example, or that edited liver cells continued to function without showing signs of toxicity.

"This study is a solid advance for the Cas9 field," says Urnov. "But when you think about deploying editing in the clinical space, we have a healthy sense of how long the road ahead is."

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References

1. Kleinstiver, B. P. *et al*. *Nature* http://dx.doi.org/10.1038/nature16526 (2015).
   Show context
2. Yu, F. *et al*. *Nature Biotechnol.* **32**, 279–284 (2014).
   - ArticlePubMedChemPort
3. Show context
4. Slaymaker, I. M. *et al*. *Science* http://dx.doi.org/10.1126/science.aad5227 (2015).
   Show context
5. Nelson, C. E. *et al*. *Science* http://dx.doi.org/10.1126/science.aad5143 (2015).
   Show context