TOOLBOX

Refined versions of gene-editing tool increase its versatility

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Four new techniques expand the repertoire of the gene-editing tool CRISPR. The improvements make it easier to check the results, expand the method's potential targets and even ease features of diabetes or other conditions in mice.

One team of researchers developed a version of CRISPR that cuts DNA outside the confines of the cell[1]. This method gives scientists a way to quickly assess the precision of gene editing.

The researchers made their 'cell-free' CRISPR by replacing the main DNA-cutting enzyme, CAS9, with an enzyme called CPF1 that works in solution.

The researchers attached CPF1 to a small strand of RNA that matches a target sequence of DNA. They combined these complexes with DNA from human cells and a gene they wanted to insert. The enzyme cut DNA strands at a specific location and repaired the break by inserting the provided gene.

After 30 minutes, the researchers isolated the DNA and then spliced it into the genome of *Escherichia coli* bacteria. By design, only bacteria with edited DNA were able to grow in the presence of an antibiotic. The researchers then sequenced the genes of the surviving colonies to see exactly how the DNA had been edited.

Adaptable shears:

One major limitation for traditional CRISPR systems is that CAS9 only cuts DNA where it sees a specific sequence. Two new methods address this restriction.

The enzyme typically cuts sites that are within 13 to 17 bases of a DNA triplet that includes two adjacent guanines. Only about 1 in 16 DNA sites has this triad.

Researchers engineered a more flexible enzyme by inserting the CAS9 gene into bacteria and mutating it. They waited 24 days for mutations to accumulate in the dividing bacteria and selected five variants. They let mutations accumulate in those 5 variants for 72 hours and then isolated and tested 14 of them.

One of these variants, they found, can edit genes at more locations than the original: It cuts DNA at more than one in four sites in human cells. It also makes fewer off-target gene edits than other versions of the enzyme[2].

In another study, researchers reported a naturally occurring version of CAS9 that does not require a nearby double-guanine triplet. It also cuts RNA, so it can change gene expression without affecting the DNA template. The enzyme comes from the bacteria *Neisseria meningitidis*, which can cause meningitis[3].

Another group of scientists modified CAS9 to turn on inactive genes rather than edit them[4]. The researchers used their system to treat three genetic conditions in mice.

In the new system, small RNA snippets home in on a genetic target. Then, instead of clipping the DNA, a modified version of CAS9 activates the gene.

The researchers packaged their system into harmless viruses and injected the viruses into mouse models of diabetes, muscular dystrophy and acute kidney disease. In the diabetes mice, for example, they activated a gene in the liver, encouraging liver cells to produce and process insulin more efficiently.

REFERENCES:

1. Sansbury B.M. *et al. CRISPR J.* **1**, 191-202 (2018)