Researchers greatly increase precision of new genome editing tool

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CRISPR-Cas9 is a powerful new tool for editing the genome. For researchers around the world, the CRISPR-Cas9 technique is an exciting innovation because it is faster and cheaper than previous methods. Now, using a molecular trick, Dr. Van Trung Chu and Professor Klaus Rajewsky of the Max Delbrück Center for Molecular Medicine (MDC) Berlin-Buch and Dr. Ralf Kühn, MDC and Berlin Institute of Health (BIH), have found a solution to considerably increase the efficiency of precise genetic modifications by up to eightfold.

"What we used to do in years, we can now achieve in months," said gene researcher and immunologist Klaus Rajewsky, indicating the power of this new genome-editing technology. CRISPR-Cas9 not only speeds up research considerably - at the same time it is much more efficient, cheaper and also easier to handle than the methods used so far.

The CRISPR-Cas9 technology allows researchers to transiently introduce DNA double-strand breaks into the genome of cells or model organisms at genes of choice. In these artificially produced strand breaks, they can insert or cut out genes and change the genetic coding according to their needs.

Mammalian cells are able to repair DNA damage in their cells using two different repair mechanisms. The homology-directed repair (HDR) pathway enables the insertion of preplanned genetic modifications using engineered DNA molecules that share identical sequence regions with the targeted gene and which are recognized as a repair template. Thus,

HDR repair is very precise but occurs only at low frequency in mammalian cells.

The other repair system, called non-homologous end-joining (NHEJ) is more efficient in nature but less precise, since it readily reconnects free DNA ends without repair template, thereby frequently deleting short sequences from the genome. Therefore, NHEJ repair can only be used to create short genomic deletions, but does not support precise gene modification or the insertion and replacement of gene segments.

Many researchers, including Van Trung Chu, Klaus Rajewsky and Ralf Kühn, are seeking to promote the HDR repair pathway to make gene modification in the laboratory more precise in order to avoid editing errors and to increase efficiency. The MDC researchers succeeded in increasing the efficiency of the more precisely working HDR repair system by temporarily inhibiting the most dominant repair protein of NHEJ, the enzyme DNA Ligase IV. In their approach they used various inhibitors such as proteins and small molecules.

"But we also used a trick of nature and blocked Ligase IV with the proteins of adeno viruses. Thus we were able to increase the efficiency of the CRISPR-Cas9 technology up to eightfold," Ralf Kühn explained. For example, they succeeded in inserting a gene into a predefined position in the genome (knock-in) in more than 60 per cent of all manipulated mouse cells. Kühn has just recently joined the MDC and is head of the research group for "iPS cell based disease modeling". Before coming to the MDC, he was on the research staff of Helmholtz Zentrum München. "The expertise of Ralf Kühn is very important for gene research at MDC and especially for my research group," Klaus Rajewsky said.

Concurrent with the publication of the article by the MDC researchers, *Nature Biotechnology* published another, related paper on CRISPR-Cas9

technology. It comes from the laboratory of Hidde Ploegh of the Whitehead Institute in Cambridge, MA, USA.

Somatic gene therapy with CRISPR-Cas9 is a goal

The new CRISPR-Cas9 technology, developed in 2012, is already used in the laboratory to correct genetic defects in mice. Researchers also plan to modify the genetic set up of induced pluripotent stem cells (iPS), which can be differentiated into specialized cell types or tissues. That is, researchers are able to use the new tool to introduce patient-derived mutations into the genome of iPS cells for studying the onset of human diseases. "Another future goal, however, is to use CRISPR-Cas9 for somatic gene therapy in humans with severe diseases," Klaus Rajewsky pointed out.

Klaus Rajewsky: "One of the hottest topics in biomedicine and an innovative field"

"The CRISPR-Cas9 technology is one of the hottest topics in biomedical research and an innovative field," said Klaus Rajewsky. He pointed out that the new capabilities to precisely edit the genome has sparked off an intense debate in the USA and elsewhere, since the new precision tools could also be applied to modifying the genome in human germ cells or embryos. Although manipulation of the human germline is prohibited by law in many countries, including Germany, a global ban is not in effect. The MDC researchers are fascinated by the new opportunities the CRISPR-Cas9 system offers for biomedical research, but strictly reject genetic modification of the human germline.

More information: Increasing the efficiency of homology-directed repair for CRISPR/Cas9-induced precise gene editing in mammalian cells, *Nature Biotechnology*, www.nature.com/nbt/journal/vao …

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