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MICE TIMES

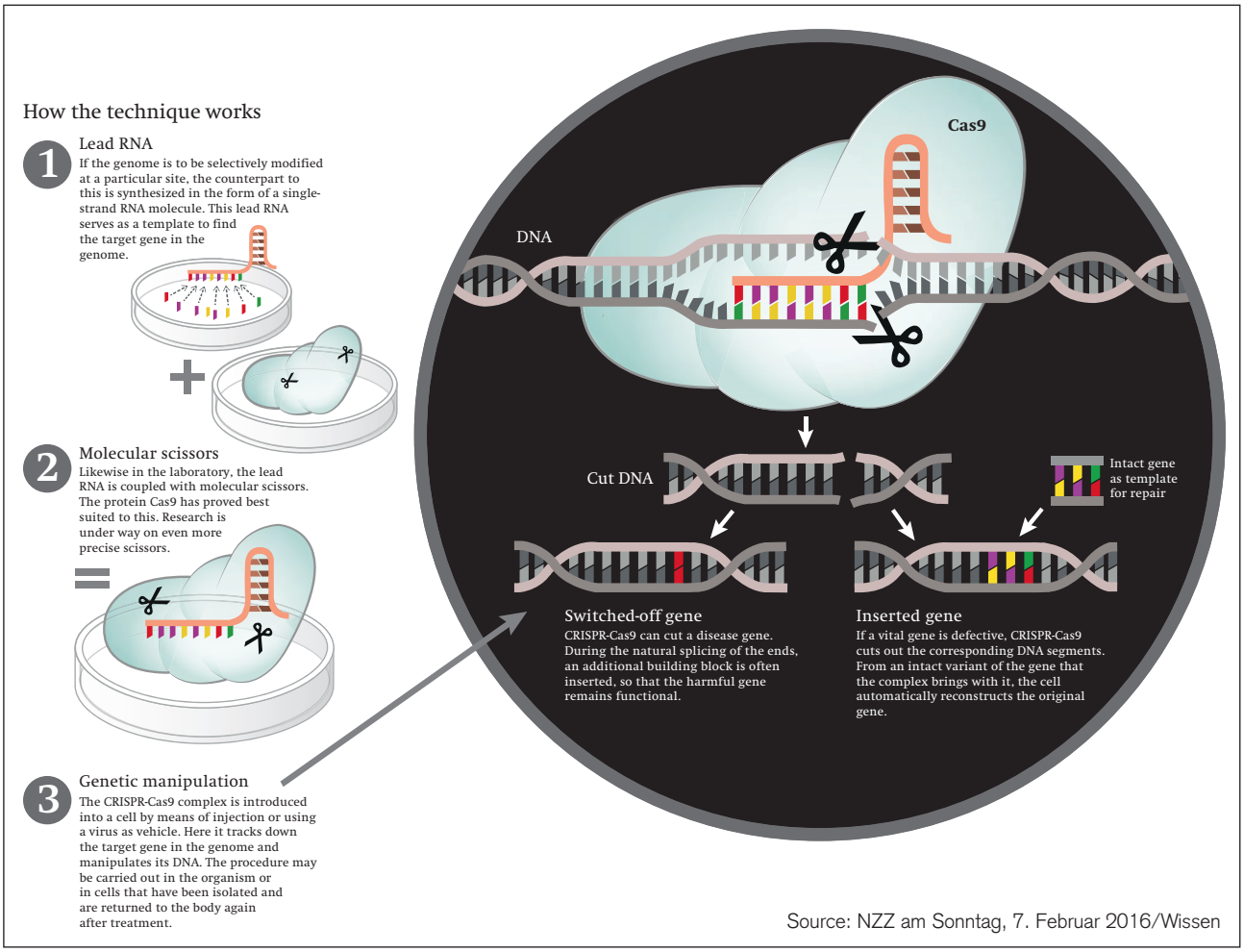
# Gene technology in a new dimension

## Using CRISPR/Cas, it is possible to switch off or replace every gene in every living creature

A new method is conquering laboratories around the world. Using CRISPR/Cas technology, it is possible to modify the genome of every living creature more quickly, more cheaply and more precisely than using any other previous methods. Researchers and journalists are falling over themselves with excitement. The talk is of a "quantum leap", of the "greatest biotech discovery of the century", and the developers of the method, Jennifer Doudna, Emmanuelle Charpentier and Feng Zhang, are already being tipped for the Nobel Prize.

The components of the new molecular toolkit occur naturally. CRISPR/Cas was discovered in the genome of bacteria almost 30 years ago. But it was only recently established that bacteria use it to protect themselves from invading viruses. The immune defence system of bacteria consists of two components: a cellular scalpel that can cleave DNA (Cas) and a short RNA segment that guides the scalpel to the site that is to be cleaved.

But these tools do not only work in bacteria. In the laboratory the scalpel also cleaves isolated DNA and the genetic information in cells of plants, animals and humans. Depending on the programming, it is possible then either just to cut out or destroy a gene using CRISPR/Cas or, in the case of a defective muta-




Source: NZZ am Sonntag, 7. Februar 2016/Wissen

tion, replace the gene by incorporating an intact base sequence. The method works in principle like the search-and-replace function in a computer. The researcher decides what is to be searched and replaced through the added lead RNA, which can be produced in the laboratory to match each site in the genome.

The technique is still in its infancy, but it will change both humans and the environment. Two years ago, Chinese scientists removed all three copies of a wheat gene and created a variety of wheat that is resistant to mildew. Japanese researchers had already successfully used the technique to prolong the life of tomatoes.

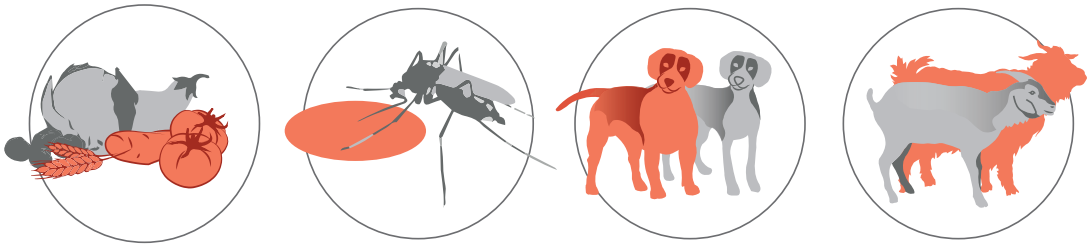
Dirk Becker from the Biozentrum Klein Flottbek in Hamburg sets great store by CRISPR/Cas: "The great potential of this method lies in the ability to completely switch a gene off. Using other methods in the past, the shut-down was not so complete." An example of its use can be seen in potato plants, which contain a special kind of starch. "For this, CRISPR/Cas is used to switch off a gene in the synthesis pathway of starch", explains Becker. As a result, the starch in these potatoes does not consist of amylose and amylopectin, as is normally the case, but only of amylopectin. The producers of paper and adhesive are interested in this, because they need starch without amylose for their production processes.

In Europe, the question of whether plants modified using CRISPR/Cas have to be declared as GM plants is currently a hotly debated issue. In the USA, it has been decided they do not have to be. If the method is used only to switch off the function of a gene, then CRISPR/Cas is simply another method in the category of classical plant breeding. This view is also shared by Dirk Becker: "Only if a transgene is added should the plant fall under gene technology legislation."

It is already hard to imagine basic biomedical research in cell cultures or animal models without CRISPR/Cas. "What I like most about CRISPR is that you can switch off any gene you like in any cancer cell line and look for where die Achilles' heel of the tumour lies", says Eric Lander, Director of the Broad Institute in Cambridge, Massachusetts, and one of the leading figures behind the Human Genome Project. It is even possible to switch off several genes at the same time and thus allowing diseases with a complex genetic background such as diabetes, Alzheimer's, autism or cancer to be better understood.

CRISPR/Cas is suited not only to research, but also to the treatment of diseases. There is a wealth of ideas for the use of such "genetic surgery". For example, certain virus infections could be treated. Some viruses, such as the hepatitis B virus or HIV, incorporate their genetic information in the genome of the infected host cells. In this way, they can remain dormant in the body for life, despite medication, with the risk that the viruses will "come back to life" and actively replicate again. Researchers from the University College of Medicine in Taipei (Taiwan) have tried curing infection with hepatitis B virus in mice using CRISPR/Cas. Although the "gene scissors" removed some of the virus DNA, it has not (yet) been sufficient to stop the infection completely.

**Uses in the animal and plant world**



Optimized crops
Wheat that is resistant to powdery mildew, tomatoes that ripen more slowly and last longer, or soybeans that are more nutritious: CRISPR-Cas9 has already produced optimized crops.

Gene drive in mosquitoes
Mosquitoes that carry the malaria parasite can be genetically modified so that they have fewer offspring and die out over time. Using gene drive, a special application of CRISPR-Cas9, this trait is spread more quickly in a population.

Customized dogs
Beagles are popular as model organisms in research. Using CRISPR-Cas9, Chinese scientists have produced two muscular specimens. Other traits that dog owners might want to see in their dogs would also be conceivable.

More productive goats
Chinese researchers have modified the genome of Kashmir goats so that they have longer hair and grow more muscle – thus delivering a greater yield. Other scientists are working on cows without horns.

Source: NZZ am Sonntag, 7. Februar 2016/Wissen

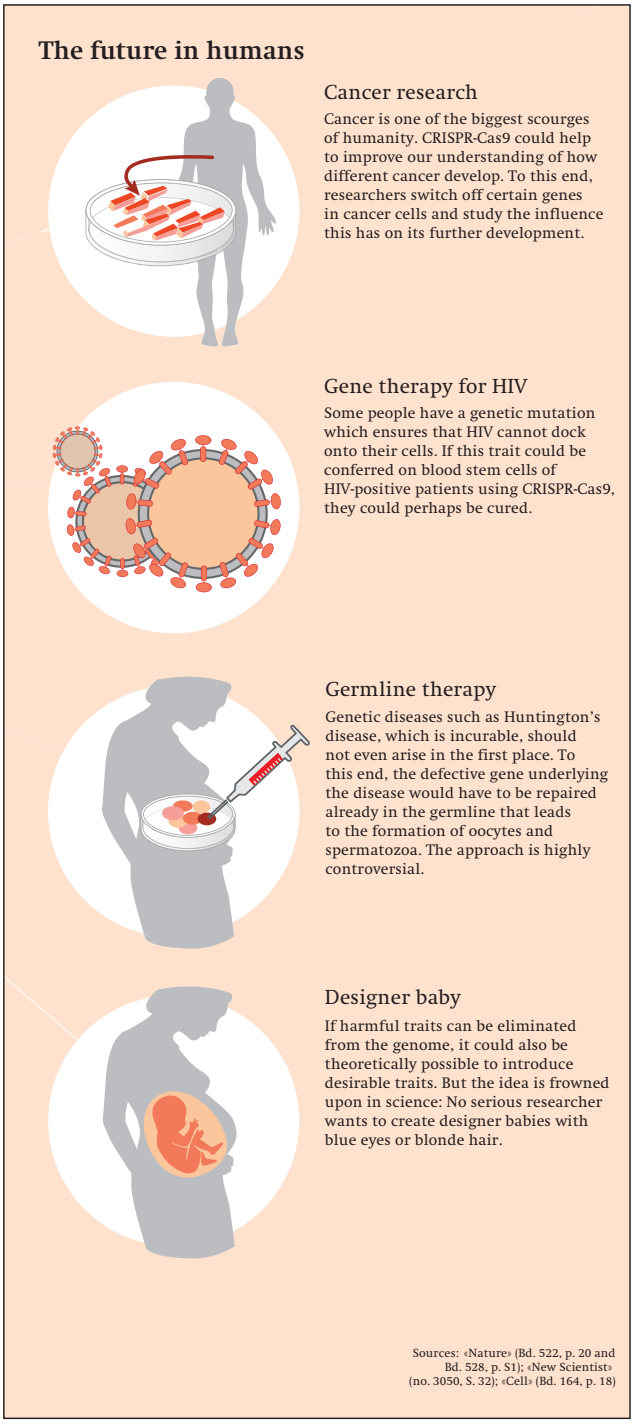
Before CRISPR/Cas can be used for therapeutic purposes, not only efficacy but a number of other obstacles also have to be overcome. For example, how do you get the "gene scissors" to that part of the body where they are supposed to work? And is the technique really so precise? Is the genome really cut only at the intended site or might it also be cut at an unknown site by chance – with incalculable consequences? According to studies on the precision of CRISP/Cas, the method varies between no off-targets in the genome and 150 off-targets. But if a cut is made where a cut should not be made, for example at a site of the gene that regulates cell division, this could trigger unchecked cell proliferation – in other words cancer.

"Genetic surgery" is much more reliable when it is used outside the body. "If your treat cells in the lab, you can only return those cells to the body in which the repair or the switching-off of a gene has actually worked", says Gerald Schwank from the Institute of Molecular Health Sciences at the Federal Institute of Technology (ETH) Zurich. Schwank's team has isolated stem cells from the intestinal tissue of patients with the genetic disorder cystic fibrosis. "With the aid of CRISPR/Cas we managed to repair the defective gene in these cells, and the previously defective channel for the outflow of chloride ions functioned again", explains Schwank.

For this procedure to be of any benefit to patients, the repaired cells would have to be returned to the patient's body and become established in the intestine. But here, too, it is not yet known how this can be achieved. In the experiment in mice, only a very small proportion of the modified cells became established in the intestine. A more receptive organ for the integration of repaired cells that are incorporated from outside appears to be the liver. "Urea cycle defect, a metabolic disorder of the liver, could become the first disease to be cured in this way using CRISPR/Cas", says Gerald Schwank. The technique offers huge promise here to make gene therapy a part of routine clinical practice in the near future.

Attempts to correct genetic defects also in germ cells or embryos using CRISPR/Cas have been making considerable waves. In mice, this has already succeeded with genes whose mutation causes cataracts and degenerative muscle disorders. In April last year, Chinese researchers gave rise to some excitement, when they tried to repair the gene responsible for the blood disorder known as thalassaemia in 86 fertilized, human oocytes. The researchers used the (triploid) egg cells that arise in in vitro fertilization and are bot viable.

But most cells in which an intact gene was incorporated in place of the defective one did not integrate it permanently into their genome. Moreover, the molecular scalpel cleaved the DNA at unwanted

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sites more often than expected. The consequences of these defects are incalculable, and the method is still too risky. “The researchers have done good work with the study because they were able to show that CRISP/Cas is not yet ready for such use”, says Dieter Egli, stem cell researcher from Columbia University in New York. For Klaus Ammann, it is clear that genome editing will one day be used in humans. The emeritus professor of botany and ecology from the University of Basel has compiled a monograph on more than 1000 publications concerning CRISPR/Cas. “The euphoria is laid on too thick and too uncritically to my mind”, says Ammann. As with any scientific advance, you have to look closely and weigh the evidence.

It would be ideal if we could understand the complicated mechanisms of a body without stressful animal experiment. Unfortunately that is not yet possible today. But the dilemma will remain for a long time to come: basic research without experiments in animals would mean abandoning any medical progress. Mice Times aims to explain why and therefore reports on medical success stories that were only possible thanks to animal experiments.

IMPRESSUM

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