During the following years, the CRISPR sequence was observed in other types of prokaryotic system. It became clear that the spacers were in fact copies of viral DNA that the cell could use to detect and eliminate viruses. Importantly, it was demonstrated that the number of spacers increases when bacteria were exposed to viruses. In other words, the CRISPR sequence provides the organism with an adaptive immune system.

The way in which the cell uses CRISPR sequences as a defence mechanism involves an enzyme known as Cas — short for CRISPR-associated — linked to a strand of RNA molecule generated as a copy of one of the spacers in the CRISPR DNA. Once a virus with a specific DNA sequence enters the cell this is matched by the RNA and deactivated by the Cas enzyme.

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Research up to 2012 had provided essential information about the way CRISPR works. In that year however, two independent research groups showed how to harness and control the CRISPR-Cas system — more specifically, the version using a specific type of Cas enzyme known as Cas9.

One team was led by Jennifer Doudna from Berkeley and Emmanuelle
CRISPR-Cas9 approach is much simpler than earlier techniques which involved binding between DNA proteins, particularly zinc-finger nucleases and transcription activator-like effector nucleases. Being based only on DNA–RNA linking, the CRISPR-Cas9 approach is much simpler. Already at the beginning of 2013, a few reports confirmed that through CRISPR-Cas9 it was possible to affect human and plant DNA, providing further evidence of the potential of the technique in gene editing.

Given the simplicity of CRISPR-Cas9, many teams around the world have been exploring its potential in a variety of biomedical applications. Gene editing has the potential to treat genetic diseases by removing the mutation that causes the disease from a patient’s DNA. Initial demonstrations in vitro were followed by in vivo experiments in which CRISPR-Cas9 was injected in animal models. One of the early examples was the treatment of Duchenne muscular dystrophy in mice. Other studies have focused on cystic fibrosis. Applications in certain types of cancer have also been explored, which has already led to one clinical study approved by the National Institutes of Health in the US, and several ongoing ones, primarily in China.

The anti-viral origin of CRISPR-Cas9 inspired studies exploring its use in fighting viral infections such as papillomavirus and hepatitis B. Encouraging results have also been obtained for the HIV virus, which was shown to regress in a number of animal models.

Aside from therapeutic applications, we can imagine that gene editing can be used on animals and plants for other purposes. By modifying or simply eliminating a gene responsible for a certain trait in a species it is possible to eliminate that trait, whether it is the appearance (Figure 2), or, more interestingly, the ability to carry a virus. Experiments have already demonstrated, for example, the possibility of using CRISPR-Cas9 to mutate Anopheles mosquitoes and make them resistant to the malaria parasite. Most importantly, the mutation was carried out in such a way that it could be transferred to the offspring of every insect. If pursued, this type of research could have enormous consequences on the spread of diseases carried by insects.

The possibility of mutating traits also has interesting potential for breeding livestock, for example by making animals stronger or more resistant to viruses. The latter has already been shown in pigs. In a similar way, by mutating genes in plants it is possible to improve agriculture. Experimental work has already shown the generation of crops with improved resistance to pests or to adverse weather conditions such as drought. More generally, even the quality of fruit and vegetables, in terms of size, colour or taste can be affected.

It should be mentioned that the great potential of the CRISPR-Cas9 gene editing tool also calls for responsibility in its use. The benefits could be enormous, especially because of the relative simplicity of the technique that can be applied by a large number of scientists and indeed commercial enterprises. However, there are serious concerns about its potential misuse and negative effects. Just imagine the ethical dilemmas that could be generated by the modification of genes in embryos to change specific traits in people, or by the introduction of mutations that can be inherited by offspring.

Even without going that far, changing animals or plants, even if for potentially beneficial purposes, could lead to catastrophic effects on entire ecosystems. Research is ongoing to understand potential unwanted effects of introducing gene mutations in any type of species. The situation is complicated by the fact that the views of the public and of policymakers on gene editing are different in different countries, and it is difficult to establish a unique regulatory system. The good news is that scientists are all too aware of these issues and are...
working with regulators to ensure the mitigation of negative effects.

Beyond real world applications, CRISPR-Cas9 represents a powerful tool for scientific research. Just to name a couple of examples, in order to study the function of a gene, scientists can use CRISPR-Cas9 to mutate or even silence it, thus observing the effect it has on an organism. Alternatively, the DNA of an animal model could be modified with humanized mutations to perform studies that cannot be carried out on humans because it is too dangerous or unethical.

In less than six years since the work by Doudna, Charpentier and Šikšnys, CRISPR-Cas9 has developed into one of the most powerful tools in genetics. As Professor Arne Brataas, of the Norwegian University of Science and Technology, and chairman of the Kavli Prize in Nanoscience Committee said “CRISPR-Cas9 is a breakthrough nanotool that will considerably enhance our understanding of genetic mechanisms. This great invention confers to society enormous capabilities for positive innovations”.

By Fabio Pulizzi, science writer

Figure 3: Effect of two gene mutations induced by CRISPR-Cas9 on tomatoes. The upper panel shows the wild type with only two locules; the centre and lower panels show the results of two different gene mutations, producing 6 and 12 locules respectively. From Nature Genetics 47, 784–792 (2015). Image courtesy of Macmillan Publishers Ltd