THE KAVLI PRIZE

NANOSCIENCE PRIZE 2018
EXPLANATORY NOTES

A pair of scissors for genes

Understanding and controlling matter at the atomic and molecular scale, with the goal of creating new materials and functionalities at the macro scale, are at the very heart of nanoscience and nanotechnology. DNA, the double-helix molecule that contains the information on the development and growth of living organisms, can be seen as the ultimate nanostructure. Manipulating DNA at the molecular level can have enormous implications for all living species.

In making their award, the Kavli Prize in Nanoscience committee has selected three scientists who developed a convenient tool, generally referred to as CRISPR-Cas9, that allows the editing of DNA by cutting out specific segments very selectively. This form of gene editing can not only be used as a powerful tool to understand how genes work, but could have wide applications in many fields, such as in the cure of a range of diseases and in agriculture.

The development of the CRISPR-Cas9 gene-editing tool built on 25 years of research on the DNA in bacteria and archaea. Back in 1987, a group of Japanese scientists observed a DNA sequence in *Escherichia coli* bacteria that consisted of identical short segments separated by spacers. The repeated segments were palindromic, that is, the base sequences read exactly the same, independently of the direction in which they were observed. For that reason, the sequence is now known as 'clustered regularly interspaced short palindromic repeats' or more simply by the acronym CRISPR.

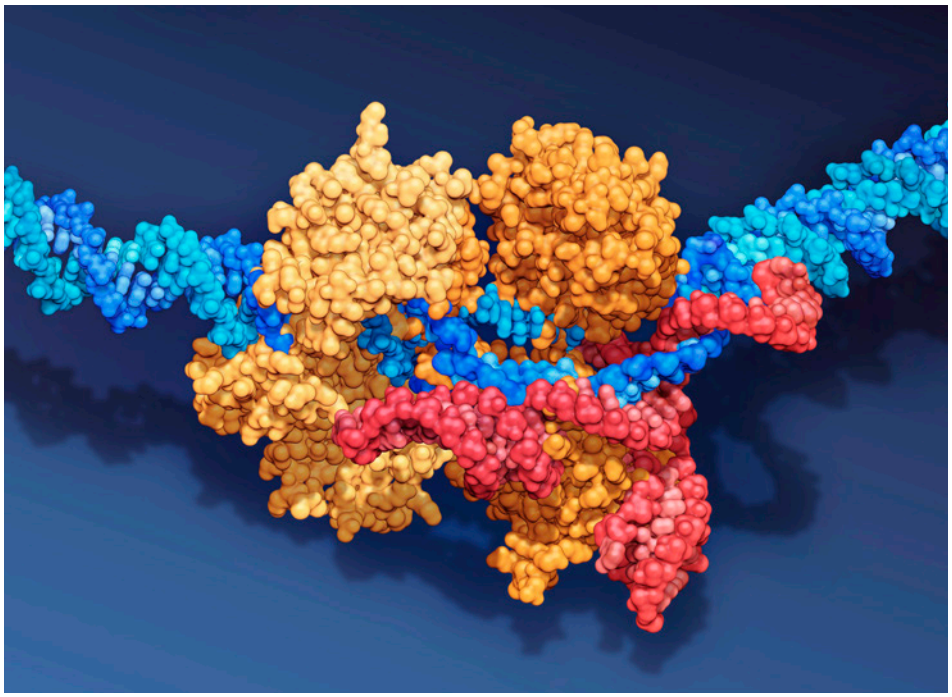

Figure 1: A schematic representation of the CRISPR-Cas9 system. The Cas9 enzyme (orange) cuts the DNA (blue) in the location selected by the RNA (red). Image courtesy of CARLOS CLARIVAN/SCIENCE PHOTO LIBRARY/NTB Scanpix

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.

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

Figure 2: Change in appearance of a butterfly due to gene mutation induced by CRISPR-Cas9. The left panel shows the wild type, while the mutated version is on the right. From PNAS 114, 10701–10706 (2017).

Charpentier, then at Umeå in Sweden. The other was led by Virginijus Šikšnys based in Vilnius, Lithuania. Both groups reported the isolation of Cas9–RNA complexes from bacteria and the demonstration *in vitro* that Cas9 could be used to cut out a segment of an external double strand DNA sequence that matched the sequence of the RNA (Figure 1). Effectively the results showed that the Cas9 enzyme can be used as a pair of nanoscale scissors to selectively cut out the pieces selected by the RNA.

In general, once DNA is broken, the cell will repair it, either by stitching together the loose ends, or by inserting a new segment. This process forms the basis of gene editing. Before the results found by Doudna, Charpentier and Šikšnys, gene editing had been approached with complicated systems involving binding between DNA proteins, primarily zinc-finger nucleases and transcription activator-like effector nuclease. 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 mosquitos 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

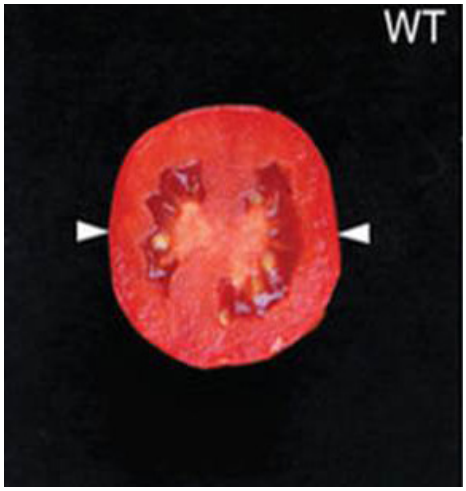

Figure 3: Effect of two gene mutations induced by CRISPR-Cas9 on tomatoes. The upper panel shows the wild type with only two locules; the centre and lower panels show the results of two different gene mutations, producing 6 and 12 locules respectively. From Nature Genetics 47, 784–792 (2015). Image courtesy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

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".

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