OUR FUTURE WITH CRISPR: A BRAVE NEW WORLD?

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Imagine a world without malaria, without transplant shortages, and even without genetic diseases. A world where climate change has been halted or reversed. A world where you can design your baby to be the most intelligent, the most creative, and the most courageous. Would you want to live in that world? It may sound like science fiction, but this question is becoming more and more relevant. It is the field of genome engineering that holds all these great promises. Through genome engineering, the DNA of any living organism can be changed to give it the desired characteristics. Mosquitoes that no longer transmit malaria, pigs that grow human-compatible organs, and elephants that resist the cold, just like the woolly mammoth, are all on their way. While genome engineering finds its origins in the previous century, the discovery of CRISPR/Cas9 has enabled rapid progress. But how does this process of genome editing actually work? And, what can and what should we do with this powerful tool?

In 2020, the Nobel Prize in Chemistry was awarded for the 112th time. Yet, it was the first time for the prize to be awarded to two women. Emmanuelle Charpentier and Jennifer Doudna received the prize for discovering "the sharpest tool in gene technology: the CRISPR/Cas9 genetic scissors" [1]. With CRISPR/Cas9, the DNA of animals, plants, and microorganisms can be changed with high precision. In less than ten years from this discovery, CRISPR/Cas9 has become famous for its (promised) applications, ranging from genetically modified foods to the treatment of genetic disease and even to designer babies (Figure 1). To better understand the potential as well as the dangers of CRISPR/Cas9, we will discuss its discovery, mechanism, and (future) applications.

It all started with CRISPR...

The story of CRISPR/Cas9 is a detective story on mysterious microbial defence systems that were developed into the most powerful tool to engineer genetic information. It started in 1987 when studies in *E. coli* bacteria reported remarkable repeats in the DNA, which were short and palindromic [2]. A palindrome is a word or sequence that reads the same backwards as forwards, such as *rotator*, or *ATTA* for a DNA sequence. In certain clusters of the bacterial DNA, the same sequence was repeated over and over, but with variable pieces of DNA, called 'spacers', in between. The same repeats were found in many other bacteria; however, their function remained unknown [3]. Only fifteen years later, the repeats were given the name "CRISPR" for Clustered Regularly Interspaced Short Palindromic Repeats [4]. Yet, it required a change of focus towards the spacers to finally unravel its function. As it turned out, the spacers matched the DNA of bacteria-specific viruses, called bacteriophages. [5]. Upon infection with bacteriophages, the bacteria incorporate a part of the bacteriophage DNA into their own DNA as a variable spacer between the repeats [6]. This process gives the bacteria a memory of their infections as a form of adaptive immunity. Next time, when a bacteriophage infects the bacteria, those that previously acquired the matching spacer DNA will survive the infection.

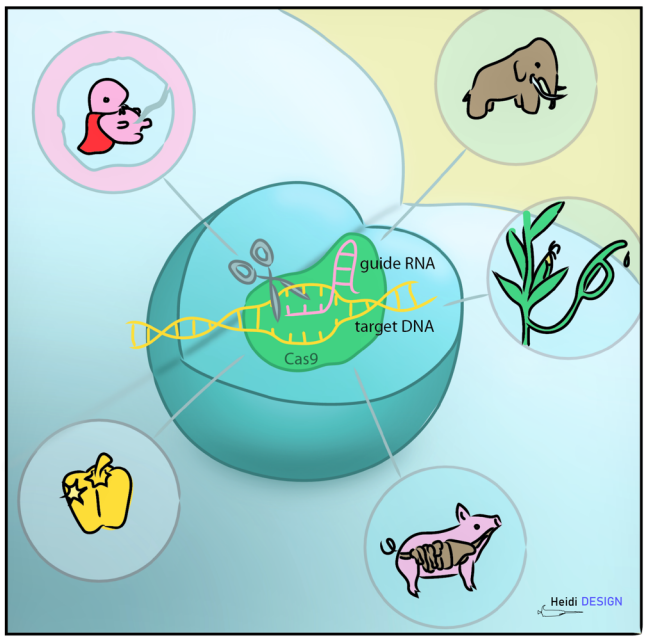

Figure 1: The CRISPR/Cas9 genetic scissors can be used to cut DNA. Through genome editing, superfoods, biofuels, mammoths, xenotransplants, and CRISPR babies can be engineered.

... then came Cas9

To unravel the mechanisms by which these spacers, also called memory sequences, of the bacteria can provide immunity, researchers turned their attention to the neighbouring DNA. Here, they discovered that the bacteria also have genes for the CRISPR-associated (Cas) enzymes. It turned out that the memory sequences are transcribed from DNA into RNA and that this memory RNA guides the Cas enzymes [7]. This bacterial CRISPR/Cas complex, subsequently, cleaves the matching bacteriophage DNA [7]. Since 2010, we, therefore, know that the Cas9 enzyme is an "RNA-guided DNA nuclease", guided by the memory RNA to destroy the bacteriophage DNA [7].

Genetic scissors

But what brings us from bacteria cleaving their invaders to genome engineering? That is where Emmanuelle Charpentier and Jennifer Doudna come into play. These scientists were the first to understand the Cas9 mechanisms well enough to take them out of the bacteria and put them in a test tube. Charpentier and Doudna showed in these test tubes that the Cas9 activity is programmable; if you give the Cas9 enzyme the right guide RNA (gRNA), which is an artificially designed memory RNA, it can cleave any DNA of interest [8]. Therefore, Charpentier and Doudna could make Cas9 cleave not only bacteriophage DNA but also that of animals [9, 10].

The experiments of Charpentier and Doudna showed that the Cas9 enzyme provides the long-dreamed-of programmable and precise "genetic scissors" [8]. With the right gRNA, any specific piece of DNA, whether human, animal, plant, or microbial, can be cut. The broken DNA will recruit the repair machinery of the cell [11]. Usually, the DNA strand break will be repaired with small changes (non-homologous end-joining) [11]. However, these repair mechanisms can introduce crucial sequence errors, leading to gene inactivation [11]. In some cases, the DNA will be repaired by using the second copy of DNA in the cell as a template (homology-directed repair) [11]. If we provide the cell with a false copy of DNA that looks very similar but contains changes engineered by us, this template will introduce the preferred changes in the genome [11]. Through these options, the Cas9-induced DNA strand break enables us to rewrite the DNA [11]. Wherever the genetic scissors cut, we can add, delete, or replace DNA [11].

Rewriting the code of life

The CRISPR/Cas9 genetic scissors have started a revolution in genome engineering. Now that we can rewrite the DNA code of any form of life, a whole new world of opportunities has opened up. From food production to climate change to public health, many emerging problems could be solved, or at least eased, through genome engineering.

Food production

The genetic engineering of any organism with agricultural or industrial significance could enhance the quality and quantity of our food. Many important candidates can be named, ranging from microbes to plants to animals. For example, microbes in the soil could be engineered to improve the soil quality, thereby increasing the harvest [12]. Agricultural crops could be enhanced such that they are resistant to environmental disturbances or pathogenic infections, or such that the produced food is healthier [13]. For instance, improved rice varieties in which the genes for metal absorption have been edited, are now available [14]. These rice varieties contain lower levels of toxic cadmium and arsenic. Crops that can resist pests offer opportunities to lower the use of pesticides [15].

Climate change

Genetic engineering could also be used in our battle to mitigate or even stop global warming. Bacteria producing the right chemical compounds represent a sustainable source of biofuels [16]. Through genetic engineering, the relevant metabolic pathways may be enhanced towards increased production of the biofuel compounds. A less conventional example is given by the "mammoth project" from Harvard [17]. This group wants to bring the extinct woolly mammoth back to life, or at least they want to bring specific genes, such as those conferring cold-resistance, from the woolly mammoth back into the elephant. Apparently, the woolly mammoths played an important role in limiting methane release (an important greenhouse gas) into the air. If the cold-resistant elephants could do the same, they would contribute to the stabilisation of climate change.

Health

CRISPR/Cas9 also has many applications in biomedicine and public health. To improve our health, genetic engineering can be used directly on human cells or those of organisms that could threaten or ameliorate our health. For example, gene drives with CRISPR/Cas9 could be used to stop the transmission of malaria and other mosquito-transmitted diseases [18, 19]. In such gene driven approaches, genetic modifications are used that either kill mosquitoes or make them unable to transmit the pathogens. As malaria alone kills more than 400,000 individuals per year, this would have great health benefits [20]. Another example is xenotransplantation, where organs from other species, such as pigs, would be transmitted to human patients [21]. This could solve the big problem of transplant waiting times that are a considerable burden on the quality of life and survival chances of patients. The donating animals would be genetically modified to make the xenotransplant organs suitable for transplantation to humans. According to the latest developments, up to 62 pig genes could be edited to increase the immunocompatibility of pig organs [22].

The use of CRISPR/Cas9 in humans offers ample opportunities in the treatment and prevention of disease. Important steps have already been taken in the field of engineered therapeutic cells. For example, chimeric antigen receptor T-cells are T-cells from the patient that are genetically modified *ex vivo* to attack the cancer cells and then infused back into the patient [23]. These CAR T-cells can efficiently recognise the cancer cells through the genetic insertion of the right T-cell receptor sequences. *In vivo* genome engineering is technically more challenging than these *ex vivo* modifications. Yet, if delivery and editing are efficient enough, *in vivo* approaches could offer a life-long cure for certain genetic diseases otherwise requiring life-long medicine intake for symptom alleviation [24]. High up the priority list are monogenic diseases, such as cystic fibrosis and spinal muscular atrophy, where genetic engineering could correct the causative mutation [25, 26]. Over the last years, the first successes have been reported, for example, for patients with sickle cell disease [27].

Designer babies

Probably the most infamous application of genome engineering is that of "designing babies". While engineering of human germline cells or embryos is strictly forbidden in most countries, the upcoming practices of pre-implementation genetics and selection illustrate the wish of some parents to choose for a child without 'detrimental' or even with more 'beneficial' gene variants [28, 29]. Genome engineering might replace this selection in the future, as only one embryo would then be needed to edit genes associated with the demanded characteristics. Next to the correction of disease mutations, such as cystic fibrosis and sickle cell disease, novel, beneficial variants could be edited. For example, mutations in the gene encoding for the C-C chemokine receptor type 5 (CCR5) receptor prevent the human immunodeficiency virus (HIV) from entering cells, thereby offering resistance to this infection [30]. It is exactly this gene that was edited in the first CRISPR babies [31]. When the first reports on these CRISPR babies, two twin girls from China, came out in November 2018, the global debate on the controversial aspects of CRISPR/Cas9 intensified. Not only was this the first time when the genetic code of a future generation was edited, but it also concerned a gene that is somewhat in the rather grey area between disease prevention and human enhancement. Candidate genes for such enhancement include the *myostatin* gene, which is essential for muscle growth and the *basic helix-loop-helix family member e41* gene, for which certain variants reduce the amount of sleep that you need [32, 33]. But who would decide which and how many genes we are allowed to edit? Will only rich people be able to afford this, thereby increasing the gap between the rich and the poor? And will your genetic profile then be screened as a requirement for certain jobs?

Ethical and moral considerations

The sometimes-horrifying consequences that genetic engineering might have are widely presented to us in science-fiction books and movies. Examples include Jurassic Park ("Genetic power is the most awesome force the planet's ever seen, but you wield it like a kid that's found his dad's gun") and genetic discrimination in the movie *Gattaca* ("a new underclass, no longer determined by social status or the color of your skin. No, we now have discrimination down to a science."). Opponents clearly state that gene editing of germline cells and embryos should not be allowed, not under any circumstances [34, 35]. The unpredictable effects on future generations would make such use dangerous and ethically unacceptable. Furthermore, opponents believe that we should prevent a "brave new world", where a kid would be manufactured to

play a specific role in society. Proponents argue the opposite and state that it is not ethical to longer "roll the dice with our kids' lives" [36]. They state that applying CRISPR/Cas9 in adult patients is not enough, as their children will still inherit the disease genes, and that it would be unethical not to put an end to this preventable suffering and death [34].

Conclusion

The story of CRISPR/Cas9, a microbial defence system that turned into powerful genetic scissors, seems to only just have started. By rewriting the code of life, genetic engineering opens up opportunities in food production, climate change stabilisation, and public health. A long road lies ahead, both promising and troublesome. However, while technical challenges continue to be addressed, many ethical challenges remain unsettled. And, while certain CRISPR/Cas9 applications are undoubtedly beneficial, others are alarming. The central question is no longer what we *could*, but rather what we *should* use these powerful genetic scissors for. Now that the future of humanity, and that of all other organisms, lies in our hands, we must act carefully and responsibly. Scientists, ethicists, politicians, and policymakers all must be involved in the debate on regulation and surveillance. Only with societal support and safety requirements can CRISPR/Cas9 indeed change our world for the better.

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References

1. The Nobel Prize in Chemistry 2020 [internet]. NobelPrize.org. 2020 [cited: Dec 23, 2020]; available from: https://www.nobelprize.org/prizes/chemistry/2020/press-release/
2. Ishino, Y., *et al.* Nucleotide sequence of the iap gene, responsible for alkaline phosphatase isozyme conversion in Escherichia coli, and identification of the gene product. *J Bacteriol* **169**, 5429-5433 (1987).
3. Mojica, F.J.M., *et al.* Biological significance of a family of regularly spaced repeats in the genomes of Archaea, Bacteria and mitochondria. *Molecular Microbiology* **36**, 244-246 (2000).
4. Jansen, R., *et al.* Identification of genes that are associated with DNA repeats in prokaryotes. *Mol Microbiol* **43**, 1565-1575 (2002).
5. Mojica, F.J.M., *et al.* Intervening Sequences of Regularly Spaced Prokaryotic Repeats Derive from Foreign Genetic Elements. *Journal of Molecular Evolution* **60**, 174-182 (2005).
6. Barrangou, R., *et al.* CRISPR Provides Acquired Resistance Against Viruses in Prokaryotes. *Science* **315**, 1709-1712 (2007).
7. Garneau, J.E., *et al.* The CRISPR/Cas bacterial immune system cleaves bacteriophage and plasmid DNA. *Nature* **468**, 67-71 (2010).
8. Jinek, M., *et al.* A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science* **337**, 816-821 (2012).
9. Mali, P., *et al.* RNA-Guided Human Genome Engineering via Cas9. *Science* **339**, 823-826 (2013).
10. Cong, L., *et al.* Multiplex genome engineering using CRISPR/Cas systems. *Science* **339**, 819-823 (2013).
11. Brinkman, E.K., *et al.* Kinetics and Fidelity of the Repair of Cas9-Induced Double-Strand DNA Breaks. *Molecular cell* **70**, 801-813. e806 (2018).
12. Liu, C., *et al.* Ambient nitrogen reduction cycle using a hybrid inorganic–biological system. Proceedings of the National Academy of Sciences, 201706371 (2017).
13. Bortesi, L. & Fischer, R. The CRISPR/Cas9 system for plant genome editing and beyond. *Biotechnology Advances* **33**, 41-52 (2015).
14. Tang, L., *et al.* Knockout of OsNramp5 using the CRISPR/Cas9 system produces low Cd-accumulating indica rice without compromising yield. *Scientific Reports* **7**, 14438 (2017).
15. Courtier-Orgogozo, V., *et al.* Agricultural pest control with CRISPR-based gene drive: time for public debate: Should we use gene drive for pest control? *EMBO Rep* **18**, 878-880 (2017).
16. Jagadevan, S., *et al.* Recent developments in synthetic biology and metabolic engineering in microalgae towards biofuel production. *Biotechnology for biofuels* **11**, 185 (2018).
17. Woolly Mammoth Revival [internet]. ReviveRestore.org. 2020. [cited: Dec 23, 2020]; available from: https://reviverestore.org/projects/woolly-mammoth/
18. Hammond, A., *et al.* A CRISPR-Cas9 gene drive system targeting female reproduction in the malaria mosquito vector Anopheles gambiae. *Nature Biotechnology* **34**, 78-83 (2016).
19. Achee, N.L., *et al.* Alternative strategies for mosquito-borne arbovirus control. *PLOS Neglected Tropical Diseases* **13**, e0006822 (2019).
20. Organization, W.H. World malaria report 2015, (World Health Organization, 2016).
21. Naeimi Kararoudi, M., *et al.* Clustered Regularly Interspaced Short Palindromic Repeats/Cas9 Gene Editing Technique in Xenotransplantation. *Front Immunol* **9**, 1711-1711 (2018).
22. Yang, L., *et al.* Genome-wide inactivation of porcine endogenous retroviruses (PERVs). *Science* **350**, 1101-1104 (2015).
23. June, C.H., *et al.* CAR T cell immunotherapy for human cancer. *Science* **359**, 1361-1365 (2018).
24. Xiong, X., *et al.* CRISPR/Cas9 for Human Genome Engineering and Disease Research. *Annual Review of Genomics and Human Genetics* **17**, 131-154 (2016).
25. Maule, G., *et al.* Gene Therapy for Cystic Fibrosis: Progress and Challenges of Genome Editing. *International journal of molecular sciences* **21** (2020).
26. Lattanzi, A., *et al.* 131. Targeted Genome Editing in Spinal Muscular Atrophy. *Molecular Therapy* **23**, S53-S54 (2015).
27. Frangoul, H., et al. CRISPR-Cas9 Gene Editing for Sickle Cell Disease and β-Thalassemia. *New England Journal of Medicine* **384** (2020).
28. Baltimore, D., *et al.* A prudent path forward for genomic engineering and germline gene modification. *Science* **348**, 36-38 (2015).
29. Robertson, J.A. Extending preimplantation genetic diagnosis: the ethical debate: Ethical issues in new uses of preimplantation genetic diagnosis. *Human Reproduction* **18**, 465-471 (2003).
30. Liu, R., *et al.* Homozygous Defect in HIV-1 Coreceptor Accounts for Resistance of Some Multiply-Exposed Individuals to HIV-1 Infection. *Cell* **86**, 367-377 (1996).
31. Greely, H.T. CRISPR'd babies: human germline genome editing in the 'He Jiankui affair'. *J Law Biosci* **6**, 111-183 (2019).
32. Moro, L.N., *et al.* Generation of myostatin edited horse embryos using CRISPR/Cas9 technology and somatic cell nuclear transfer. *Scientific Reports* **10**, 15587 (2020).
33. Hirano, A., *et al.* DEC2 modulates orexin expression and regulates sleep. *Proceedings of the National Academy of Sciences* **115**, 3434-3439 (2018).
34. Brokowski, C. & Adli, M. CRISPR Ethics: Moral Considerations for Applications of a Powerful Tool. *Journal of Molecular Biology* **431**, 88-101 (2019).
35. Brokowski, C. Do CRISPR Germline Ethics Statements Cut It? *The CRISPR Journal* **1**, 115-125 (2018).
36. Stephen Hsu in Human Nature [documentary]. Written and directed by Adam Bolt, The Wonder Collaborative (2019).