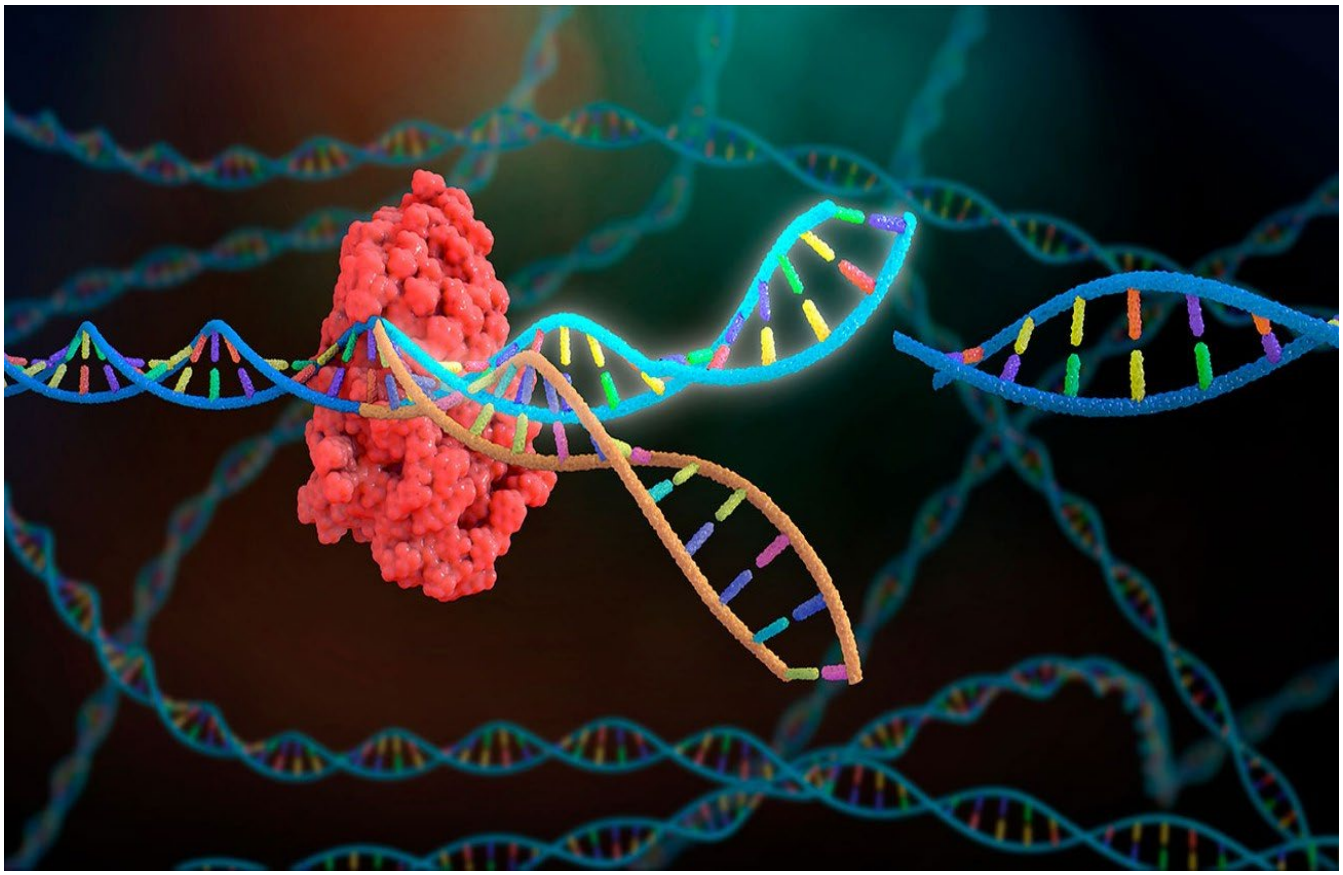
17TH OF FEBRUARY 2023



CRISPR/CAS9

GENE EDITING ON A NEW LEVEL

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1. Preface

Gene technology and particularly gene editing plays a great role in our todays medicine and society. Extensive research brought us to stunning headlines such as: “Maschinen, die lebendige Wesen erschaffen”.[1] This made us question what the CRISPR/Cas9 method is exactly and how it is being used today as well as its potential in future. As a highly promising method, there are many articles and books about it, praising this new way of gene technology. It is however not an uncontroversial method and often a talking point when speaking of possible abuse of gene technology as well as ethics of such methods in general. It is therefore our motivation, to give an overview of this very interesting and innovative method, concerning positive and negative effects as well as potentials, risks, and limits.

To achieve this overview, we concentrate on questions like:

- How exactly does the CRISPR/CAS9 method work?
- Where can it be applied?
- Where are its limits?
- What are potential risks and how can one prevent them?

2. Introduction

2.1 General

In comparison to other gene engineering technologies, the CRISPR/Cas9-Method is a very young and recent discovery. CRISPR stands for “Clustered regularly interspaced short palindromic repeats”. It is used for various genetic modifications, not just in human gene technology but also in agriculture, for example in farming of crops (Zhu et al. 2020) and in large parts for gene editing within animals (Doudna and Sternberg 2017, p.XIX). Because of its development just a brief time ago, the CRISPR method is still in elaboration and has not yet been approved fully for medical use. However, there are high expectations raised for future applications, even to cure AIDS (Prabhune et al. 2022; Doudna and Sternberg 2017, p.XXI).

The CRISPR/Cas 9 method is a gene editing tool which makes is possible to change or modify specific sections in the human genome. It was developed by scientists Jennifer Doudna and Emanuelle Charpentier and first published in 2012 (Jinek et al. 2012). For their research, the two scientists were awarded the Noble Prize for Chemistry in 2020 (Nature.com 2020).

2.2 Scientific History

The existence of CRISPR within bacteria was first discovered in 1987 by Japanese scientist Yoshizumi Ishino. He observed that the bacterial DNA was compiled of two major components: The first one being the so-called repeater-spacer-sequence. This sequence consists of short palindromic repeats, segments of non-coding DNA, typically between 23 and 47 base-pairs long and palindromic (mirror-inverted complementary). Those repeats are linked together by spacer DNA segments. This spacer DNA segments are very different to the palindromic repeats because they are not identical to other spacers, unlike the palindromic repeats. The second major component is the so called cas-operon (cas: CRISPR-associated) (Ishino et al. 1987). This operon holds genes (cas-genes) coding for certain proteins necessary for the

[1] Stöcker, Christian. „Crispr, KI und Biotechnologie: Maschinen, die lebendige Wesen erschaffen “. *Der Spiegel*, 11th October 2020

immune reaction within bacteria (Barrangou et al. 2007). Together with the repeater-spacer-sequence, this is called the CRISPR-locus (Ishino et al. 1987).

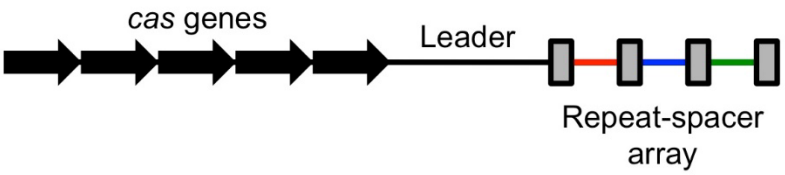

Figure 1: Simplified CRISPR-locus (Wikimedia Commons, n.d.)

2.3 Functionality

Charpentier and Doudna focused on one specific Cas gene, the Cas 9 protein complex. They further discovered how the CRISPR/Cas9 is used in the adaptive immune system of the bacteria: First, the virus injects its viral DNA. The immune system reacts with unwinding a spacer DNA corresponding to the viral DNA. This corresponding spacer is called the crRNA-spacer. Together with the connected repeater sequence (crRNA-repeater), these make the crRNA, which later binds to the Cas-9 protein complex. The Cas-9 is then able to cut the viral DNA, disabling its ability to replicate itself (Jinek et al. 2012).

The CRISPR immune system in bacteria works similarly to the human immune system, at least in terms of remembering past viral infections and being able to fight them much quicker and successful if infected by the same virus again. In bacteria, the viral DNA is transcribed to form a crRNA-spacer which is added to the CRISPR-locus. Bacteria can therefore “remember” past infections and activate Cas-proteins to fight future infections. This gives bacteria the ability of an adaptive immune system (Barrangou et al. 2007).

Based on the research achieved by Charpentier and Doudna (2012), this method can be used in various other living organisms apart from bacteria, with introducing CRISPR-genes into the genome.

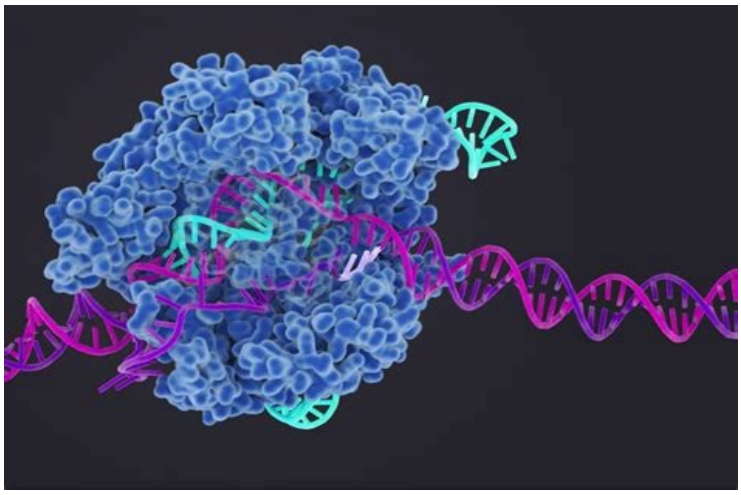

Figure 2: Cas9 Protein attached to DNA-sequence (Biosciences.lbl.gov, 2016)

3. Description

Applying the CRISPR method to humans seems rather easy: If a patient suffers from a gene mutation, the exact place must be determined by sequencing the patient's genome. In a lab, a corresponding RNA-sequence can be synthesized, like the corresponding spacer-RNA for viral DNA in bacteria, and then introduced to a Cas9 protein. This synthesized RNA is called guide RNA (gRNA). Guide RNA is also essential to the CRISPR adaptive immune system in bacteria and was first discovered in 1990 (Blum et al. 1990).

As the name suggests, the gRNA helps guiding the Cas protein to its desired location within the genome. To bind to the mutated gene-sequence of the patient, a second RNA-sequence must be synthesized. These to artificial RNA-sequences combined are called tracrRNA (trans-activating crRNA), which enable the Cas protein to bind and cut the target DNA precisely (Jinek et al. 2012).

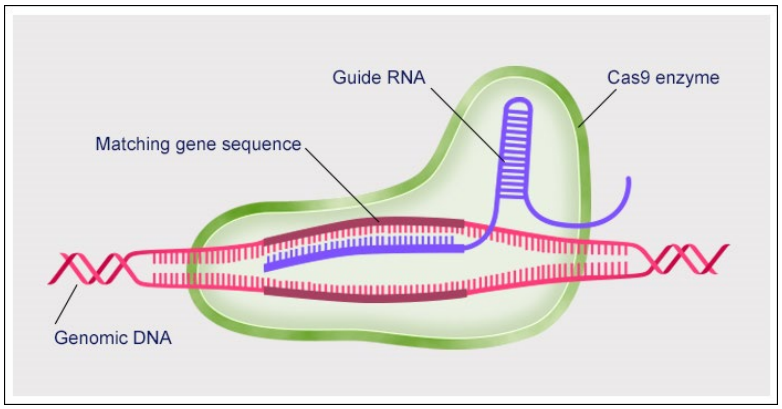

Figure 3: Cas9 matching gene sequence (ABC Radio Adelaide, 2016)

Depending on the specific application, the cut DNA can be repaired in numerous ways, leading to different outcomes. In our example with a mutated gene of a patient, a correction of the mutation is the target. However, there is also the option to introduce another piece of DNA and replace the cut DNA-sequence with it, changing the genome in the process and opening the possibility to introduce new mutations or make changes to the genome. It is possible as well to simply disregard the cut DNA and not replace or correct it and therefore deleting a portion of the genome. These multiple options allow to use the CRISPR/Cas9 method to an unimaginable extend, being able to change the genome freely almost anywhere (Doudna and Sternberg 2017).

4. Interview with Prof. Dr. Rolf Zeller

Prof. Dr. Zeller currently holds a professorship for anatomy and embryology at the department of biomedicine at the University of Basel and is a research group leader of the Zeller/Zuniga lab. Prof. Dr. Zellers research focuses on signal interactions and gene networks controlling the development of organs in vertebrates. With this interview, we discover the focus on the research to the given topic by the Zeller/Zuniga lab at the University of Basel.

Interviewer: ***What are the main focuses or points of interest when it comes to the research of CRISPR today?***

Prof. Dr. Zeller: With today's knowledge we have about CRISPR, we mainly focus on somatic cells. Somatic cells are an overall term for body cells such as muscle tissue, organs and tissue cells or neuron cells. They all have special properties and are derivatives of stem cells. Very lucrative for research are the red blood cells….

Why exactly red blood cells?

Red blood cells are easily accessible by taking out blood from the body and extracting the cells. With CRISPR and red blood cells, we mainly work on genetic disorders such as beta thalassemia and the sickle cell anemia. Both are caused by a malformation of a protein leading to anemia. With CRISPR, we can modify the genome to correct this mutation. In difference to stem cell research, our method with CRISPR is much faster. Nevertheless, there are still many difficulties we must overcome. An important thing to notice is that there is still no absolute safety with this method. That means that if we're cutting the genome, it is still possible that the result carries an undesired mutation which could potentially lead to blood cancer.

You talked about the fact that CRISPR could be able to trigger blood cancer by an unintended mutation. Is it, however, also possible to cure cancer with this technique?

It is possible, yes. For this purpose, researchers try to modify T-helper cells of our immune system that fight against cancer cells.

Are you using CRISPR to modify plant genomes as well?

We (our institution) only worked with human cells. But there are other institutions that focus on plants. Labs applying CRISPR in plants try to optimize the tolerance of cultivated plants, which means the same yield and growth results despite of changing environmental conditions. Higher tolerance can also involve growing on less fertile soils or resistance against invading insects or bacteria or better growth results despite of less water and salty soils. The main idea behind all of this is to minimize the input but maximize the output in terms of yield.

Cas proteins and Cas genes play a key role when it comes to gene editing. What other Cas genes are currently being used?

The most common Cas gene is of course the Cas 9 complex. But there are many attempts on modifying Cas genes. A successful modification of a Cas gene different to the Cas 9 complex could lead to a bigger versatility concerning gene editing. This could enable us to use a broader range of possibilities when it comes to gene editing.

One example is a recently elaborated Cas gene which attaches to a mutating region. As a result, this specific area was not expressed anymore. This means that the Cas protein acted as a silencer gene and therefore inhibited gene expression.

CRISPR is often said to be the relief for » in the past incurable diseases ». When can we expect CRISPR to be applied for medical purposes?

In the past I participated in multiple conferences and meetings where we discussed approving CRISPR for medical use and many ethical issues that come along the use of the CRISPR method. The focus here is safety. It is important that CRISPR should only be applied to cure diseases that have a strong negative impact in the everyday life of the patient.

For as long as we are not able to clearly verify the safe use of CRISPR, this gene editing tool will remain in the lab and won't be applied for patients. But I think I heard that there are aspirations to approve CRISPR in a certain way already this year. (2023)

You mentioned various ethical issues with the use of CRISPR. Can you tell us about a case in which someone tried to abuse CRISPR?

Three years ago, a Chinese scientist modified two germ cells applying the CRISPR method resulting in two genetically modified embryos. The genetical modification of embryos is currently prohibited and the scientist faced some time in prison for his unlawful research.

Let's assume that someone modified an embryo. How can this be verified?

That's in fact quite easy! A procedure like that is quite difficult to perform and thus requires more than one single person. The probability that such an attempt will be detected is quite high. The important thing is that if you want to modify an unborn human being during embryonal stages, you need special tools and equipment. These are only accessible for big companies. Small institutions that don't draw as much attention usually don't have access to these kinds of tools.

Should CRISPR also be used for beauty purposes in your opinion?

No! Science only considers CRISPR to cure diseases and to use it in gene therapies, but certainly not for own purposes such as aesthetical motivations. CRISPR is although not always suitable for purposes other than in a medical context. For example, to increase the muscle production, anabolic would be much safer and cheaper to use.

Last question: We heard that CRISPR compared to other gene editing tools is rather cheap (Doudna and Sternberg 2017). Is that correct?

Well, what we can say is that the final application of it is cheap but the procedure of elaborating and developing a reliable and safe editing tool is very costly and extensive. My answer is therefore yes and no.

5. Discussion

CRISPR/Cas9 is a groundbreaking gene editing tool, opening great possibility which were unreachable before. However, these possibilities come with great responsibility and bring up many ethical questions and issues.

5.1 Possibilities and applications

As stated by Prof. Zeller, the CRISPR/Cas9 method makes us dream of curing cancer, which would be an enormous achievement for the human species. Given the flexibility of the CRISPR-method to change genome sequences at virtually any place within the whole genome (Doudna and Sternberg 2017), it is possible to imagine that almost every disease linked to a genetical mutation can be cured using CRISPR/Cas9, making it the "superweapon of the future" concerning healthcare.

If we extend our view to beyond just the Cas9-complex, other Cas proteins might extend the use of CRISPR drastically and even further (Zeller 2023). The CRISPR method itself, whether combined with Cas9 or other Cas proteins, can therefore not only cure disease present, but

also actively change genomes with the given possibilities stated in chapter 3, p. 3 (Doudna and Sternberg 2017).

All the previous applications named are limited to the human species. However, CRISPR is not limited to a specific species and can technically be applied to any genome, no matter if it's prokaryotic or eucaryotic, for example also plants, making it possible to optimize harvest (Zeller 2023; Zhu et al. 2020). Even mushrooms can be genetically edited using CRISPR/Cas9 and are further allowed to be sold without any restrictions in the United States of America (Waltz 2016).

The CRISPR-method can thus not only be used to correct mutations and cure diseases, but also in a preventative sense. One example is the so-called Gene Drive. With the CRISPR/Cas9 method, the genome of a living organism is cut at a specific place, introducing a new DNA-sequence, a part of which coding for the CRISPR itself. The changed genome can now copy itself into new chromosomes, spreading exponentially through a whole population (Doudna and Sternberg 2017). In 2015, scientists managed to introduce a Gene Drive to gnats, making them resistant towards the parasite causing millions of malaria infections every year (Gantz et al. 2015). This makes CRISPR even more powerful, allowing to exterminate diseases before they can even cause the application of the CRISPR-method to cure them.

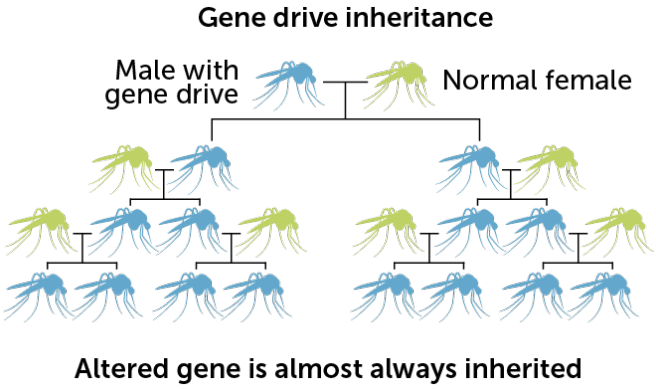

Figure 4: Gene Drive inheritance in gnats (E. Otwell, M. Telfer, 2022)

5.2 Risks and ethical issues

When it comes to gene technology, one of the most critical issues are negative side effects. These can occur with CRISPR as well. Even though the Cas 9 method is more reliable and precise than many other gene technologies, it still comes with a small risk of changing the genome in a unplanned or unwanted way. The consequences of mutations like these are unpredictable and can cause new diseases. It is therefore important to carefully consider this risk before applying CRISPR to a genome (Zeller 2023).

Together with the possibility of editing genomes, the inheritance of changed genomes are possible. Therefore, applying this method can affect many future generations. It is thus also possible, that the appliance of the CRISPR method generates mutations which do not occur in the first generation, but rather in subsequent generations. This poses an ethical problem: Is it ethically justifiable to actively intervene into the evolution of the human species, possibly massively affecting future generations without their consent on the way? This is one of the main questions French Anderson, considered the "father of gene therapy", posed upon himself and the society. Already in 1990, he questioned the ability of scientists to use their new gained force of gene therapy responsibly, a question which is even more important with the CRISPR method (Anderson 1990).

Another important question is who will have access to CRISPR. Not every institution or not every person has the same intentions on editing genes. There is the possibility to abuse the CRISPR method, for example in sports. There have already been studies and attempts with animals where certain deletions of regulatory genes triggered an increased production of muscle tissue like the myostatin gene (Qian 2015). This procedure could possibly apply to humans as well. Especially because it can't be detected in doping tests, it would be a very lucrative way to improve performances in competitions. However, different strategies to maximize performance such as anabolic are cheaper and safer (Zeller 2023).

Especially embryonal research is a highly controversial and emotional subject, applying to CRISPR as well. The possibility to "design" a human being to ones one wishes is fascinating and frightening at the same time. Linked to ethical and scientific questions of inheritance and access, using CRISPR to unnecessarily edit a human is highly controversial and unlawful as to present laws. Despite the controversy and law, the CRISPR method can still be abused, as named by Zeller concerning Chinese embryonal research (Cyranoski 2015).

6. Summary

CRISPR/Cas9 is a powerful gene editing tool, opening a new era of gene therapy. Using a technique discovered in bacteria to fight viral infections, the CRISPR method allows to change genomes freely, using Cas proteins which can cut and repair DNA-sequences. Cas proteins can be introduced to somatic cells and other living organisms, together with a synthesized "guide", to achieve a high level of precision and efficiency in gene therapy. Thanks to its universal combability and relatively easy usage, CRISPR is unlike anything gene therapy has seen before and has a massive impact on gene studies today.

"Although we learned a lot about CRISPR, about the technique, the impacts on human being and the advantages of applying it, we also had to admit that CRISPR brings not only relief for us as single individuals and the broad mass regarding to cure diseases or to increase yield in farming. There are still many disputed questions and missing links, ethical and medical ones. The major problem there is not the CRISPR method itself. The burden relies more on our genes and their versatility as well as their complexity, making the prediction of the outcome of such inventions verry difficult.

The best thing in our opinion is that the principle of CRISPR is easy to apply in the lab despite the impact of CRISPR being extremely powerful and effective. We come to the conclusion, that CRISPR is a huge breakthrough in science and can really change the world, even though the risks of abuse and ethical issues coming along. According to us, restrictions and legal requirements must be introduced to limit and regulate the access to this powerful tool, making sure it is a tool staying true to its original focus: Changing the world for the better.

We would like to especially express our gratitude towards Prof. Dr. Rolf Zeller, answering our questions and helping us in the process.

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