CRISPR – Genetic scissors for cutting DNA

by Alexia Grote, April 26, 2016

1) Preface:

Genetic engineering is the general term for the find-and-replace function of a word processor. First a gene is located and then the necessary changes are made. Either deleting a gene or edit a gene. This has gotten very easy because instead of inserting a completely new gene from a different organism one just plays with it and replaces a small part of the DNA from one organism with another organism's DNA and creates new strands from already existing ones. There are many methods already developed and others are still in research, but the one I wrote this paper about seems to be the most promising at the moment.

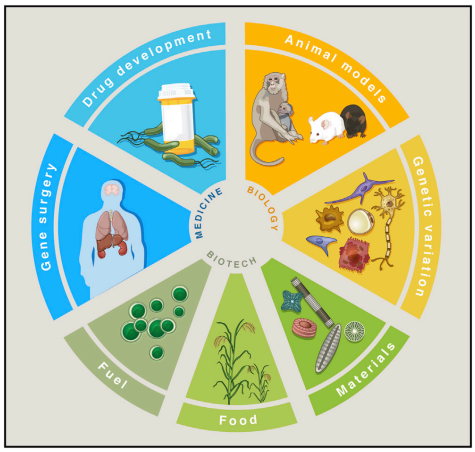

Figure 1: Applications of genome engineering [2]

This paper is about a new method of Genetic Engineering in Biotechnology called CRISPR. CRISPR stands for **C**lustered **R**egularly **I**nterspaced **S**hort **P**alindromic **R**epeats. My motivation to work on this topic was that this new genetic engineering technique enables to cut out any piece of DNA, be it long or short, and replace it by a new piece or completely disable a gene's function. Therefore it becomes possible to modify any piece of a DNA sequence.

It has been going on in research labs since 2007 and has already been used on crops and animals like mice or others. In the future, it may even be possible to heal genetic mutations that lead to disadvantages in life, such as color blindness. The interesting part is that it's a very easy and safe method and it works almost every time.

My main questions are:

- How does CRISPR work exactly?
- Who discovered it and how long did it take to find out how it works?
- Is it easier to use on mammals or plants?
- What are the benefits of using genetic engineering in general and are there any side effects?

2) Introduction:

Context:

In the last thirty years scientists have developed many genetic engineering techniques. They permit to change the DNA of living organisms. The latest and most precise technique is CRISPR. It is a technique that allows to change any part of an organisms DNA anywhere and these

changes will also be inherited by the next generation. It is also very up-to-date. For example there has been a recent event which happened in America. On Sunday the 24th of April it was reported that American regulators have allowed to the cultivation and sale of genetic modified crops. This of course concerns Britain and other parts of Europe because there are lots of doubts about consuming and importing modified crops, but more to that later. (10) Here are some of the main discoveries that led to the discovery of CRISPR – in Figure 2, one can see that the discoveries happened over the entire globe.

Scientific History:

- 1953: The microbiologists James Watson and Francis Crick discover the double-helix structure of the DNA molecule and receive the Nobel Prize.
- 1983: The Biochemist Kary Mullis develops the PCR (Polymerase-Chain-Reaction) procedure. This technique was the basis for sequencing the human genome (1990-2003).
- 1987: Japanese scientists discover the CRISPR- gene sequence in bacteria but don't know its purpose.
- 2007: Researchers of the biotech company Danisco determine that this gene sequence acts as a virus immune system of bacteria.
- 2012: The researchers Jennifer Doudna and Emmanuelle Charpentier were the first to report that genes can easily be manipulated with the Crispr-Cas9 complex.
- 2013: Biologists like Feng Zhang and George Church apply the technique successfully to cells of mice and humans.
- April 2015: Chinese researchers report that they have modified the genetic code of non viable embryos. This results in an ethical debate.
- December 2015: Feng Zhang publishes a more accurate method to target genes even more precisely. In Washington all CRISPR researchers from the entire world meet and decide not to interact with the germline in humans.
- February 2016: British researchers are allowed to manipulate human embryos for research.

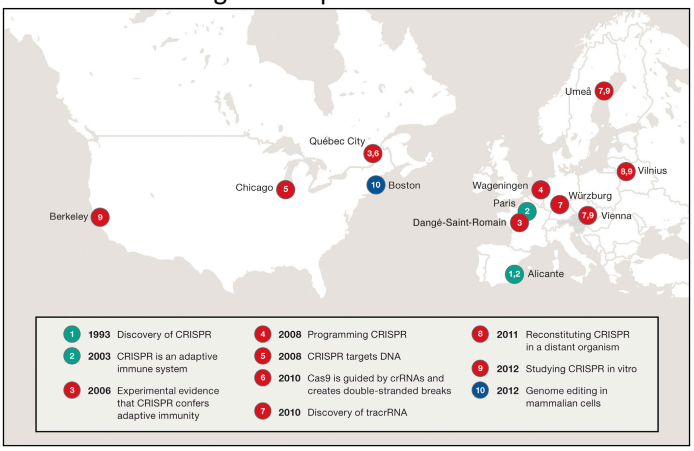

Figure 2: The story of CRISPR [3]

Where and why is CRISPR used?

Curently CRISPR is used in research all over the world, in Sweden, in China, in Great Britain, in Switzerland and other countries too. It has been used on crops, plants and animals. Scientists are still trying to develop more therapies and uses on humans because it is much cheaper than the techniques known before and because it is very precise and a lot of things can be done with it. CRISPR enables to modify the exact spot targeted on the DNA [4]. It can be applied outside the organism and then reintroduced into the new DNA and these changes will be inherited by the next generation.

An alternative technique to modify genes is called ''Glybera''. It is a treatment for lipoprotein lipase deficiency (LPLD), a rare genetic disorder affecting about 150-200 people in Europe. LPLD causes fat to build-up in the blood which leads to cardiovascular disease (heart problems which can lead to heart attacks), diabetes, and life-threatening, recurrent periods of pancreatitis. The only treatment options that have been possible so far were dietary changes and symptomatic treatment of pancreatitis. Glybera, the gene therapy developed by the Dutch biotech firm ''UniQure'' and brought to market by its partner Italian firm ''Chiesi'' in the European Union, has been released as an official drug in the US and Germany in the early 2015. It is independent of CRISPR, very expensive and not as reliable, since it works with a system based on viruses. [12]

Figure 3 The treatment Glybera [14]

3) Description of engineering technique

Explanation step-by-step [1]:

1. To determine the spot where the DNA should be modified one creates a single stranded RNA molecule with opposite bases. This RNA is used as a template to find the gene in the DNA and is called the „Guide RNA“

2. In the lab the created RNA strand is put together with molecular ''scissors''. The best protein to represent these scissors was found out to be Cas9, but research on even more precise scissors is currently performed.

3. The CRISPR Cas9 complex in Figure 3 is transported into a cell with help of a virus or a different kind of injection. Once inside the cell, the RNA molecule complex searches for its targeted strand in the gene and manipulates its DNA. In Figure 3 you can see two possible results. You can either delete a gene by transplating a Stopp codon or add a new sequence to repair a gene. This process can take place in an organism itself or in isolated cells in the lab that can later be reinjected into the organism.

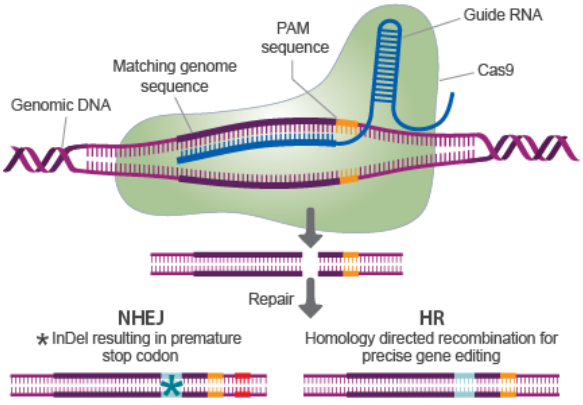

Figure 4: CRISPR-Cas9 complex [6]

4.) Visit of Prof. Mihaela Zavolan's lab at the Biozentrum, University of Basel

Interview with Prof. Mihaela Zavolan (Feb. 11, 2016)

Figure 5: Prof. Dr. Mihaela Zavolan [5]

Me: Hello Mrs. Zavolan. Thank you for your time and for this interview. I have some questions I would like to ask you about CRISPR. First, is CRISPR really as cheap and easy as I have read about it?

Zavolan: Sure. You must know that for CRISPR you design an RNA strand that is supposed to be the correct gene and because of the so-called RNA pairing rule it's very easy to design something specific. You can synthesize the correct order of the bricks of the RNA and then design it. It sure is easier and cheaper than creating a whole new protein.

Me: So it's easier to make RNA than a protein?

Zavolan: Yes, it's not that easy and for every different region of the DNA you want to correct, you would need a whole new protein but to create RNA, you just change the order of the bricks in the RNA which is so much easier and faster.

Me: So CRISPR is cheaper because you don't create proteins for the system all the time?

Zavolan: Exactly. Always when a new protein is designed tests have to be done and then money accumulates, especially if you make many new proteins.

Me: Interesting, which leads me to my next question: Is CRISPR easier to perform on plants or mammals?

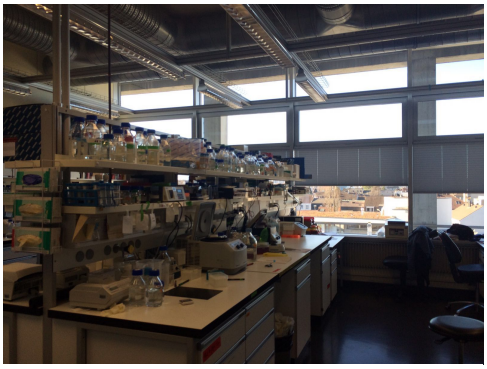

Figure 6: Prof. Zavolan's lab

Zavolan: That wasn't actually a big problem. The problem was at the beginning that the CRISPR molecule Cas-9 comes from bacteria and exists in the bacterial cells. We weren't sure that it would work in the eukaryotic cells as well, because the structures of these cells are so different. Fortunately that was solved in 2012. But it's basically the same principle to use CRISPR in animal or plant cells.

Me: That makes sense. Now a completely different question: I have read something about Glybera - another type of gene technology. Have you ever heard of it and do you think it could become a competitor to CRISPR?

Zavolan: No, I haven't heard of it. [She looks it up in the internet]. Here it says that it's already been going on before CRISPR, because it's been available since 2015. To develop this method for will take decades. It works with viruses and is mainly used to heal diseases connected to excessive levels of Cholesterol in the liver.

Me: Is it easier to use than CRISPR?

Zavolan: Not really. The problem with viruses is that we cannot really control them. The gene in the virus could go somewhere completely different than planned. But CRISPR is easy to target and the RNA goes precissely to the spot where it matches because of the base pairing rule.

Me: I see. Now that leads me to my next question: In a lot of pictures you see scissors that cut the DNA. What exactly are these `scissors'' made off?

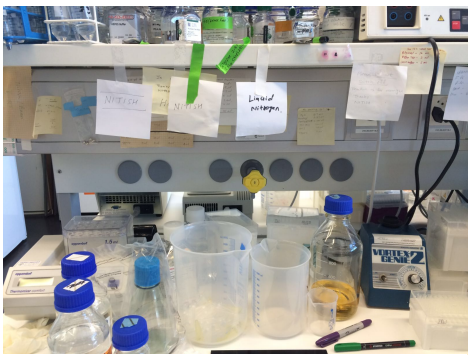

Figure 6: lab desk

Zavolan: This ist the Cas-9 protein which is coupled with the designed RNA and then used on cells. There are different parts of the protein called „protein domains“. They work together as one compound. They place themselves around the DANN. Because of their chemical properties, they just cut through the nucleotides which hold together the DNA.

Me: Now I understand. But in which parts of the world is CRISPR mostly used? Is it allowed everywhere?

Zavolan: It's used everywhere in science. You can use it and experiment with it everywhere. The problems are the ethical aspects. The questions are: how far can you go with it? Are you allowed to use it on humans, and so on? And some countries are further in these aspects than others. For example, Britain I think is very far ahead and uses it more than Germany, which is very cautious and avoids using it on humans, except for special reasons. There is some discussion about who gets the rights for the whole system, because for decades many small steps were discovered in different parts of the world.

Me: Sure, because everybody wants to profit from it. But are there any instances when CRISPR didn't work, because of a mistake or something else, or is it always 100% secure?

Zavolan: No, it actually always works with the right RNA sequence. If you have the wrong one it doesn't work anymore. But what can happen are off-target effects. That happens when the RNA matches to several or maybe entire DNA sequences, then it goes somewhere else where it maybe matches less, but this has already been looked at. Most of the time it works perfectly fine.

Figure 7: fridge with stem cells

Me: That's good, so it can really be used properly. When did you get to work on CRISPR here in Basel and whose idea was it?

Zavolan: Here in Basel we don't really develop or work on CRISPR. We work on another principle with micro- RNAs. These micro-RNAs don't target DNAs but other RNAs. They then react with each other and prevent the production of proteins for the RNA which lets them decay faster. There are a lot of targets for these micro-RNAs and we are trying to find out the exact consequences of these interactions and we use CRISPR to manipulate one binding side of the RNA so the micro-RNAs don't attach to that target anymore and the RNA doesn't decay that fast.

Me: That sounds very interesting and I would like to know more about it but I still have some questions about CRISPR. Do you think it will become a common thing that everyone will have access to, some day in the near future?

Zavolan: I think it's really tempting to use it for human diseases. There will be big pressure to work this out properly and ethically. It sure is a powerful technology and it's going to be very hard to prevent people from trying to use it.

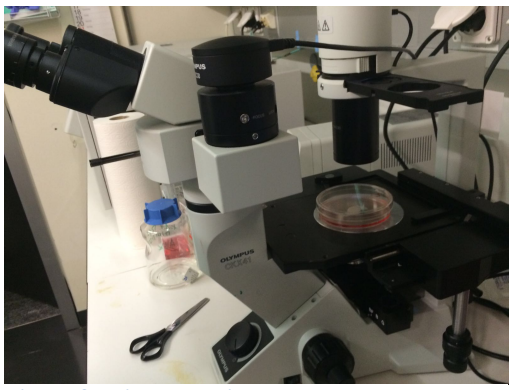

Figure 8: microscope in the lab

Me: Could one even imagine CRISPR as a pill, where everything is included and you could just take it and all happens by itself?

Zavolan: The question is the delivery system such that it will reach the right place in the right cell. What I could imagine is putting the whole system into a nanorobot with receptors on it and let it find its target cell. Ideally, one would be using it on cancer cells, but you don't recognize cancer in the early stage

from the surface, only in the later stages when it's too late to stop it and one uses chemotherapy. But for other things I could imagine using it, why not?

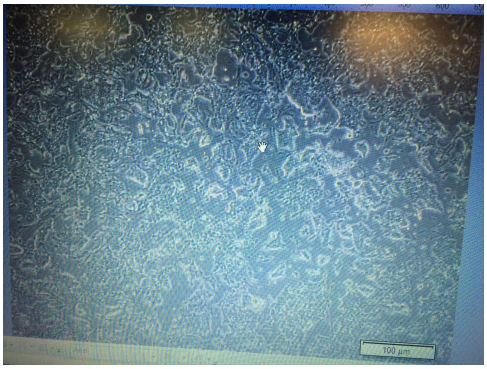

Figure 9: stem cells under microscope

Me: Yes, why not? Actually that sounds like a good idea. Thank you very much for your time. It has been very interesting and I have learned many new things. Thank you and good luck with your research on CRISPR and the micro-RNAs. Good-bye it was a pleasure to meet you.

5) Discussion

Much progress was made with CRISPR. One can now manipulate DNA at any particular spot. It is much faster and cheaper but also more precise than any previous technique. So far scientists have manipulated crops, such as in China, where they have developped a special crop resistant against the disease *Mehltau.* Scientists have also genetically modified goats so they would produce more meat and wool, and also dogs so they would have twice as much muscle. Since the technique works on plants and animals, why not use it on humans?

In the future scientists want to use CRISPR on human cells to heal people from certain diseases. For example, they plan to use CRISPR-Cas9 on cancer cells to delete particular genes and observe the impact on their development. Then CRISPR could help understanding the formation of different cancer types. CRISPR could also help to heal people infected with AIDS. Since some people have a genetic mutation which stops the HI virus from docking to their cells, this feature could be transplated to blood-stem cells of infected people, who then could maybe be healed. In fact, this list goes on because there are many future possibilities for CRISPR. One idea that I find really fascinating is to use genetic engineering on humans so they could better survive in outer space [8]. Of course, many ethical aspects and questions remain, which is why people are afraid of applying CRISPR on humans or harvesting too many modified crops. Here are some pros and cons about why CRISPR should be used and where it really could get dangerous [7].

Pros: There are many benefits but only if we let scientists further research genetic engineering:

1. Tackling and Defeating Diseases
There are lots of diseases and genetic mutations that humans suffer from and with the help of genetic engineering you could end and wipe out all of them. One can also genetically engineer the next generation to withstand these problems.

2. Getting Rid of All Illnesses in Young and Unborn Children
There are many problems that can be detected before a child is born. For example Down's syndrome. But with genetic engineering you can cure unborn children of these diseases or mutations and all children would be born healthy and strong. Even if a disease or mutation doesn't show at birth, the child could be a carrier of it. Thanks to genetic engineering one could not only help the children live healthy lives but also let them have healthy children of their own.

3. Potential to Live Longer
Even though humans already live longer on Earth because of huge progress in Medicine, genetic engineering could extend our life time even more. Many illnesses come at later age and kill people earlier than necessary (such as cancer, Alsheimer). Genetic engineering could also help us to adapt faster to our changing environment such as global warming. Evolution itself takes thousands of years but with the help of genetic engineering, we could adapt more quickly and perhaps even better.

4. Produce New Foods
Genetic engineering can also be used on plants. With genetic engineering we can design food producing crops that better withstand harsh extreme temperatures. We could also design food to contain more nutrients that we need. It is also possible, and has already been done, is modifying crops to make them more resistant to insects or parasites.

Cons: There are also some disadvantages which currently prevent scientists from using and developing the techniques on humans or crops:

1. Is it 'Right'?
Many religious people are against using genetic engineering on humans because of their belief that doing so would be playing God. Besides these religious objections, there are also ethical objections. For instance, these horrible diseases are there for a reason and have always been present in the history of mankind. Without those diseases the Earth would soon become overpopulated. People living longer is already causing social problems and if people lived even longer, new problems might come up we don't even know yet.

2. May Lead to Genetic Defects
Another problem is that nobody actually knows what happens if you change organisms at the cellular level, in particular, which impact could it have on babies that are still embryos. It could lead to complications like premature birth or stillbirth. If we can really succeed in wiping out a disease, maybe something completely new and more dangerous will appear. Scientists need to know all the affects their actions can have but they just can't account for everything and there will always be cases where things do not go as planned.

3. Limits Genetic Diversity
If we genetically engineer our race, it will have an effect on our diversity. There are scientists and researchers which try to heal and eliminate diseases with the help of genetic engineering and who

have good intentions to do so. But what if some people take the research too far and start demanding designer babies whose hair, eye color, height and intelligence we can decide and dictate? It would at some point also be possible to decide the sex of the babies, which could probably be used in some parts of the world where parents prefer to have a boy. In the end, all people might look alike and special features, like red hair or blue eyes, wouldn't be special anymore.

4. Not naturally occurring genes in nature

The problem with genetic modified plants is that once set into the environment, they will interbreed with other plants which are not genetically modified and could lead to their extinction. If one makes plants resistant to pesticides and herbicides, the green cycle will be interrupted, which can have other effects on nature we don't know yet. Finally, once these genetically modified plants have been released in nature, one cannot remove them anymore [9].

6) Summary

Working on CRISPR was very interesting for me and it was much fun. To conclude I want to answer the questions I listed at the beginning. I now know that CRISPR works with a protein called Cas-9, which comes from the bacterial immune system. It was first not sure if it would work on humans or any organism in general. Scientists found the CRISPR gene sequence in bacteria but had no idea what to do with it. It took many years to develop a mechanism one can actually use on crops. And this development, as I now know, didn't just happen in one place but all over the world. There are obviously many benefits that can be achieved with CRISPR like better crops, better human development and healing humans from terrible diseases but one should not ignore the possible side effects. We don't know yet what happens with our environment if we start to modify parts of it. It will probably take longer than another ten years until society agrees on how to use genetic engineering on naturally developping organisms and also for scientists to develop even more precise procedures to calculate all the impacts on the environment. I'm very much looking forward to seing how society, including humans, animals and agriculture will develop.

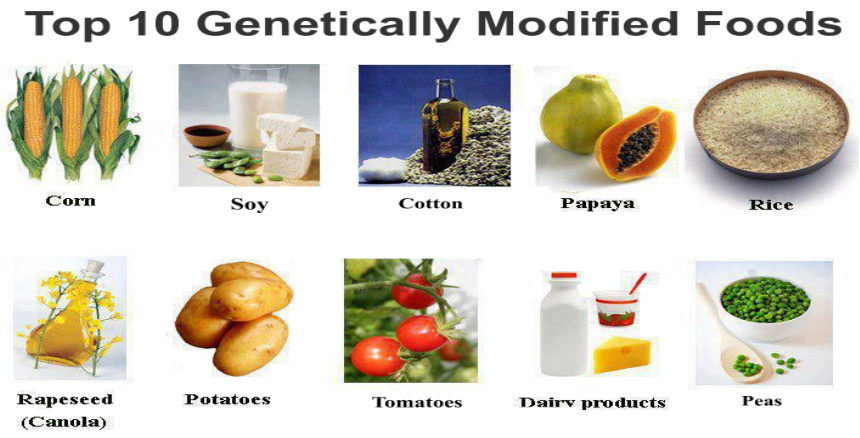

Figure 10: Foods that have already been genetically modified [13]

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