CRISPR/ Cas9

Opportunity or Risk?

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1. Preface

1.1 Motivation

A couple months before the assignment, a YouTube channel called Kurzgesagt posted a video about CRISPR/Cas9. CRISPR ("clustered regularly interspaced short palindromic repeats ")is a novel cutting edge gene editing techonolgy in which the protein Cas9 ("CRISPR-associated protein 9") plays a vital role. We all found it interesting and the Bio paper assignment was the perfect opportunity to learn more about the topic. It is also a very relevant topic, as we use genetic engineering now more than ever. However, we actually do not know much about our DNA and the impact genetic engineering would have on us.
What is special about CRISPR/Cas9 compared with other methods for gene manipulation is how quickly and precisely it can be used. The material used for it is relatively cheap and easily available. This makes it very attractive to researchers.
An important debate that comes with CRISPR/Cas9 as with any other method for genetic engineering is whether it is ethically appropriate to modify and change any organism, ranging from plants to humans. Yet most methods for genetic engineering such as CRISPR/Cas9 are used in labs for research. Genetically changed plants are already found all over the world even though we do not know what happens to the "natural" plants when they get mixed with the "modified". The methods used for these plants are not yet ready to be used on a much more complex organism such as an animal, or even a human.
Nevertheless, research is fast and CRISPR/Cas9 has brought many new possibilities and opportunities in genetics.
For us it is important to know about these techniques and deal with the questions they give us.

1.2 Our questions

In this paper, we focus on the technical parts of CRISPR/Cas9 as well as the ethical questions that come with genetic engineering. We will discuss how CRISPR/Cas9 was discovered and how it works, where it is already used and where it could be used in the future. The question about the advantages and disadvantages that come with the usage of this method lead to an interesting ethical debate whether CRISPR/Cas9 or any other method for genetic engineering should be allowed to be used or not.

2. Introduction

2.1 What is CRISPR?

CRISPR stands for "clustered regularly interspaced short palindromic repeats ". It is a special region in the DNA of bacteria which consists of nucleotide repeats and nucleotide spacers. The repeated sequences of nucleotides are spread through the CRISPR region. The spacers are sequences of DNA which are placed between this repeats.

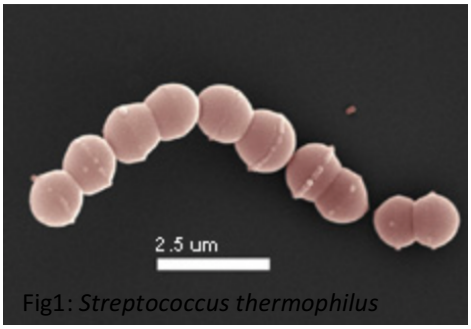

Fig1: *Streptococcus thermophilus*

The function of CRISPR sequences was first discovered in 2007 in bacteria. Researchers observed *Streptococcus thermophilus*, bacteria which is communally found in milk products like yogurt. After the bacteria was confronted with a virus its DNA

changed. In the CRISPR area a new spacer was found. This spacer was part of the DNA of the virus. With this process, the bacteria developed a resistance against the virus. The CRISPR area was like a library. The researchers started to take out the spacers and put them into new ones. With this process the bacteria could be made resistant against nearly every virus. Now, when the bacteria are attacked by the same virus again, the spacer is transcribed into CRISPR RNA (crRNA). The crRNA codes for of a spacer and a nucleotide repeat. The crRNA together with a trans-activating crRNA (tracrRNA) now guides the enzyme Cas9 to the region of the viruses according to the spacer in the crRNA. There Cas9 makes a double- stranded break to turn off the viral genes. To make sure Cas9 cuts at the right place, short DNA sequences called PAMs (protospacer adjacent motifs) sit on the virus' DNA. If the Cas9 does not find a PAM, it will not cut. This way it is ensured, that the Cas9 will not cut the bacteria's DNA.[11]

2.2 Historical background

1987 was the first time that scientists discovered CRISPR sequences in the bacteria *Escherchia coli.* However back then it could not be identified what these sequences are made for. Years later these sequences were found to be common in other microbes. Nevertheless, it took 20 years from the first documentation of CRISPR until scientists found out how bacteria use CRISPR as a defence against viruses. At this point, nobody knew what an impact this discovery was going to have in the future.
Only 5 years after the explanation how CRISPR works it could be used for gene editing. A year later in January 2013 this new method was already used in the cells of mice and humans, and only 3 years ago the first CRISPR gene drive (explained in 3.1) was reported as well as the unsuccessful use of CRISPR on human embryos.[7]

2.3 Are there any alternative ways of editing genes?

There are several ways of engineering the gene. One of them are zinc-fingers (ZGNs). Each zinc-finger nuclease (proteins including zinc ions) has two parts or domains, one that binds to DNA bases when it recognizes its unique DNA sequence and other part (the FokI nuclease) which cleaves or splits the DNA strand. The two FokI on the two different strands come together to split the two DNA strands, thus allowing precisely targeted genome edits, such as gene deletions, integrations or modifications. [11]
Another gene editing method is called TALEN, which stands for "Transcription activator-like effector nucleases". Like ZFN it has two domains. One transcripts each DNA base with four other TALE domains, which is a lot easier to bind to a specific DNA sequence than ZFNs. The other domain consists of a FokI too. When two FokI molecules come together from opposite strands it splits the two DNA strands enabling gene editing.[17]
However, none of these methods is as precise and easy to use as CRISPR/Cas9 is.

3. Engineering Technique

3.1 How does CRSIPRS/Cas9 work and how can we use it?

Due to the way that CRISPR/Cas9 works, it could be used on any cell of any organism. Scientists even simplified the procedure to the point, that there is no longer the need for crRNA and a tracrRNA, but only a single guide RNA as well as the enzyme Cas9. To modify any DNA scientists have to insert the guide RNA of the part of the DNA which they want

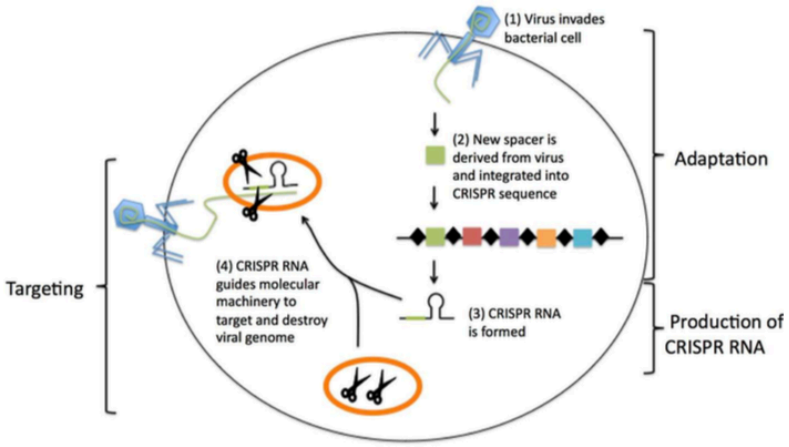

Fig2: A schematic representation of the CRISPR/Cas9 mechanism during a viral infection of bacteria

changed together with the Cas9 enzyme. The RNA reads the DNA till it has found the sequences witch matches. There it attaches to the DNA. Like in viruses the enzyme Cas9 causes a double-stranded break in the DNA as seen in Fig2.
The gap which is so created has to be filled. For this the cell's own natural repair mechanism comes in action. There are two ways of fixing an uncomplete DNA strand. First one is just ligate the loose ends together. Second is to insert a piece of DNA. The process of ligate the ends together often results in mistakes and causes various mutations. To avoid these mistakes the DNA uses another piece of DNA as a template. Scientists can offer the cell a template to change the DNA strand in any way they want. For the DNA to be recognised as a suitable template the two open ends of the DNA piece have to match with the cut ends of the original DNA strand.[7]

Compared to the other genetic engineering methods which were used so far, CRISPR/Cas9 is the cheapest as it only takes an RNA strand and the Cas9 enzyme. It is also very easy to use; it is more efficient and more precise than previous methods. With CRISPR/Cas9 all DNA could be changed. Compared to other gene editing technics CHRISPR was published in many more papers as can be seen in Fig3.

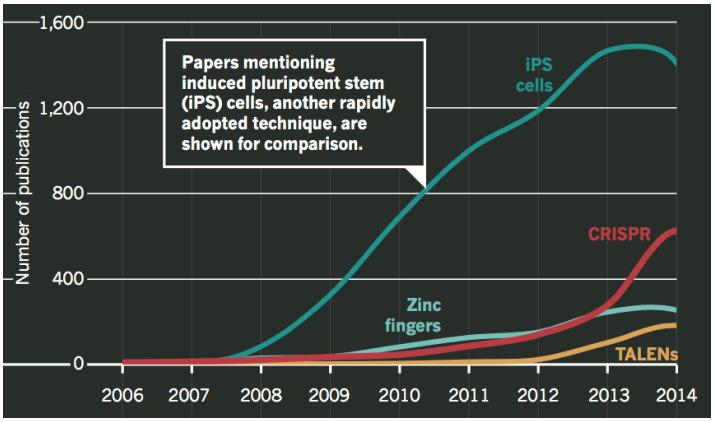

Fig3: The Graph shows the number of paper published talking about a method for gene editing.

Studies have already shown that CRISPR/Cas9 can be effective in correcting mutations in the human or animal genome such as cystic fibrosis. It has already been used to modify crops or other foods to make them resistant against specific viruses.
It can also be used for increasing gene drives. We talk about a gene drive when a genetically engineered gene is passed to the offspring. Such a gene drive could eliminate a mutation in a population for example malaria carrying mosquitos. Once an individual gene is modified, the chance of the offspring inheriting it only about 50%, as both parents pass only one chromosome to their offspring. However, if the gene modified in a way, that CRISPR/Cas9 is on the modified chromosome in the newly formed diploid cell, it could then transfer to the second chromosome as well and so both chromosomes are modified. As soon as the two genes come together the crRNA is send out to modify the wild DNA. So, gene drives would make a specific trait more likely to be passed on to the offspring. Such gene drives could

eventually help us fight against malaria or erase other mutations which are passed to the offspring. How such a gene drive works is shown in Fig 4.

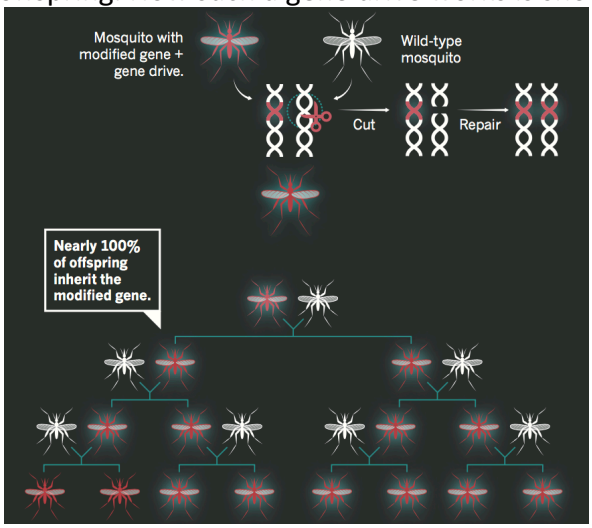

Fig4: A with CRISPR modified mosquito passes the modified gene to its offspring. In the offspring's genome CRISPR replicates itself onto the wild type chromosome, effectively remaining the germ line for all generations.

As it can be used in any cell and organism we could even use CRISPR/Cas9 to change our physical features like our hair, eye colour or even our intelligence. If used on human embryos this technic could produce designer babies.
Speaking of humans, CRISPR/Cas9 could also be used therapeutically to fight disease such as cystic fibrosis or sickle cell disease. For this treatment, the sick cells or the cells with the mutation in their DNA are taken out of the body. The RNA of the DNA sequence with the mutation on it is inserted in the cells together with the Cas9 enzyme and a corrected sequence of the DNA. The wrong templates will be corrected and the modified cells are put back into the human's body. This could also be done by inserting the RNA with the enzyme directly into the affected part of the body. After it enters the body the CRISPR/Cas9 will start to cut out all the mutated parts of the DNA of other cells. This leads to the elimination of the affected gene.[7]

3.2 What is it already used for?

All of this works in theory – however in practice it is a bit more difficult. Most features are polygenetic, meaning one sequence of DNA contains the code for several features and some features, like our hair colour, are based on several sequences. For example, changing your hair colour to red can lead to something harmless such as having freckles on your face, or to something deadly like increasing the chance of skin cancer. This makes it difficult to use CRISPR/Cas9 as a method to modify our body or to create perfect designer babies.
However, the technique is used in research as the material needed only costs about 30$ and it works way more efficiently and precisely compared to older methods. Therefore, it is an excellent tool for scientist to learn more about our DNA.
CRISPR/Cas9 has already been used to modify certain plants and make them more resistant against different viruses or climates. It has been used in cells of mice and humans to edit their DNA. In China, it was even tried to change a human embryo's DNA with it but this ended without any success.
Nevertheless, CRISPR/Cas9 is already used for therapeutics. The company "CRISPR therapeutic" uses the technology to find a way to correct gene mutations like sickle cell disease or CTX001 in somatic cells. However as far as we know they have not yet found a fully successful way to do so.[10]

3.3 What will it be used for?

As CRISPR's technology develops, in the far future, it may be possible to change the human genome in a way that gives us special features we admire. This could be done in a human embryo to create a designer baby who is very beautiful or intelligent or even "super soldiers

“, who do not need much food or sleep. But once this has been done, we may have opened a door that can no longer be closed.
However, it might be more likely that CRISPR will be used to eliminate genetic mutations and to cure illnesses like diabetes or sickle cell disease.

4. Interview

Fig5: Dr. Priya Satalkar

Fortunately, we had the opportunity to talk to Priya Satalkar. She is a postdoc at the Institute for Biomedical Ethics at the University of Basel. As her research focuses on new medical technology such as CRISPR/Cas9 and what their sociocultural context in our society is. We could ask her about her thought whether genetic engineering especially with CRISPR/Cas9 is ethically acceptable or not.

1. Should humans be allowed to use CRISPR/ Cas9 on their embryos?

- 2 aspects: technology and notion/moral status of the embryo, which is very diverse (some believe that a zygote has a moral status so you can't manipulate/change it.)
- Religion has an aspect as well; after 14d the soul enters the "mass of cells"/body
- (All of this is reflected in the regulations around the world)
- --> Working with embryotic stem cells/embryos has its own moral questions proving difficult to reconcile the different opinions/perspectives.
- Interviewee's personal opinion:
- [Edited]: "No. Embryos are a mass of cells, so there are no ethical limitations for me. What concerns me however is that the technology hasn't advanced far enough. We could cause some changes in gene manipulation that we cannot predict yet; how far they could go and how imprecise they could be. We don't have enough safety data from animal studies to begin with humans. Thus I stand against the usage of CRISPR on human embryos today. (But I would have a different opinion as more evidence on safety and specificity becomes available and as technology develops over time.)"

2. Should CRISPR be available for the general public, buying on the Internet, doing it at home? If not, why? If yes, what should the limitations be? (For example: money, social status etc.)

- Bio hacker publicly injected himself with CRISPR - he regrets it because multiple people started doing it as well
- People often aren't properly informed about the consequences, but since a trend was created, people started trying it out themselves, like the ice bucket challenge (in which you pour a bucket of ice-cold water on top of yourself.)
- For correcting genetic diseases has been made public to the health system/medical profession
- No, it's too early to make it public (and definitely too early if through the Internet). Bio hackers might be knowledgeable but the common man might not be. If it

randomly becomes accessible through the Internet, we will have difficulties to deal with the consequences. Though there are consumer genetic tests - with some websites and companies like "23andMe" - you can order kits in which you send DNA from your oral cavity back to them. It has defined terms and conditions, which most of the people do not read or pay attention to.
- At this stage it must only be used in a safe and regulated lab setting.

3. At the moment CRISPR/ Cas9 is mostly used in research on somatic cells, although it has already been used on human embryos. Should CRIPSR be used for anything else than research, for example engineering foods, fighting disease or even to improve humans?

- Do we even need CRISPR? We have other methods, for example to prevent genetic diseases we have screening methods. Screen the population and prevent the effected people with (homozygous) traits of the disease to mate, which is an easy method to prevent genetic diseases.
- If it's polygenetic, we don't know how with CRISPR one gene could affect the others
- *"I don't have a good insight to plant breeding."* In the USA, plant breeding isn't considered a genetically modified organism because you don't introduce a new DNA into the plant DNA. In EU we banned GMO (genetically modified organism).
- Super humans and designer babies - Difference between treatment and enhancement is very narrow
- CRISPR isn't the first to "improve humans". Nano technology is already being used to enhance soldiers; not changing their bodies, but with the Nano-sized drones with high sensitivity cameras, which you could send on the field to inform the soldiers if they should go in or not. Facilitating someone with technology externally is different than changing them. With Nano technology, there was this fear of changing something in the soldier's body so they could live with less food, water and sleep.
- (Glasses with thermal vision enhances a soldier's abilities externally)
- Do we change the dignity of a person? (Who are we to manipulate these basic characteristics?)
- Super soldiers and designer babies are still far into the future, yet we need to be aware of it because technology develops and many funders support military research. Scientists might develop it from a different perspective but if the scientists aren't aware and aren't engaged in political and social debates, someone else with more power and money could take that research and use it for something else. Thus this is also a public consensus/agreement in which we have to discuss what we want to use this technology for and what we don't want to use it for.

4. What kind of social effects would a "CRISPR-ized" human have on society?

- GMO with CRISPR is available generally for the developed countries first, and then the prices drop - like smartphones
- A concern for justice - how do we distribute the benefits of our technology? For the society with more power and money? - Should it first be available respectively to their economic status or first to a few who are weaker to bring them at the same level to the majority so that they can compete or reach their highest potential (-> health conditions?).

- --> Do we prioritize those economically or those with health conditions? The same problem occurs with organ donations.
- Topics about super humans/CRISPR-ized humans tend to lose the focus of near future.

5. Could you imagine using CRISPR/ Cas9 on your body or even on your own child?

- Own body - No. I don't want to go to any extreme/to use CRISPR to fix broken things in my body. For example I don't want to be kept alive with machines; I have signed the "do not resuscitate (revive) form". No immortality.
- Child - As of today technology is not yet refined, so no. Even if my child had a genetic disease, Huntington's chorea, where I know he's going to die, I'd rather help him have a painless, healthy and normal life, than exposing my child to these early experimental interventions/CRISPR.
- The conflict that I had was that my child could say in the future that I, as a mother, could've prevented this/his suffering; you had a chance to save my life, yet you didn't take it. I wouldn't know how to give my child a satisfying answer. Also when you modify something in the germ line, the modifications will be descended to future generations. (Professionally I would have my arguments but personally I would struggle to find my arguments.)

6. To what extent should we allow CRISPR/ Cas9 to be used on human embryos? For example, only changing their eye, hair colour or to an even more drastic extent like modifying them to have above average strength or intelligence.

- Hair colour would be easy, suppressing certain genes/pathways
- Though if you manipulate something it affects something else as well; for example red hair increases the chance of the child to have Melanoma/skin cancer.
- At the moment we aren't as far as to increase intelligence; but in certain places around the globe, knowledge/intelligence can pull you out of poverty thus parents prioritize education for their child.
- As parents, we try to create and environment around the child to help him achieve his highest potential, but with CRISPR we change their very being at cellular level. (Personally I think, whatever the motive, it's selfish for the parents to change something in the child without the child's consent)
- To conclude, I wouldn't use CRISPR to focus on designer babies but to treat genetic diseases, creating a better life for the client, the answer might be yes.

7. Humans have been selectively breeding crops and animals for millennia, for example different wheat, dogs, horses, pigs etc. To what extent is selective breeding more ethical than modern gene manipulation (GMO) and/ or CRISPR/ Cas9 in particular?

- If we manipulate something the effects don't become visible right away
- If we believe that we understand biology so much that we start tinkering with it, we would be a bit overconfident.
- In the future it could be possible; regulations will follow/adapt to the progress and the technology. (Thus the regulations have to be more flexible and must take into account the new evidence that is available.) Thus if it truly becomes safe and precise

with no unwanted side effects that we can't deal with, then we would have to allow human experiments.

- Regulation options:
- Outright banning CRISPR will never work. It will create underground/hidden researches - not regulated by the government - becoming even more difficult to track/follow on what's going on. (In comparison today would be medical marihuana; ban or regulate it?)
- Self-regulations/moratorium - which most scientists agreed on for CRISPR - that we as scientists at this stage take responsibility to not go into human germ line - this was a good starting point, but we are diverse and have different opinions. Thus self-regulations wouldn't work on a global scale
- Laissez-faire - "let it be as it is" - keeping track if a breakthrough comes. If you laissez-faire, you wouldn't know what people could do. (For example, there was a huge concern about Chinese scientists working with human embryos - In China and India in that matter there is no prohibitions that are legally bound. But we have guidelines, which are very soft regulations, that when disobeyed nobody could punish/stop you.
- So no regulations or soft regulations such as a guideline are a no-go. There will always be crazed scientists, which for example - there was a case in India - try to inject stem cells to prevent a patient's death. - The Indian council of medical research couldn't do anything because all they had was a guideline. -
- In my opinion, regulations that are transparent, open and fair are always a better deal than out rightly banning something. But it may prove problematic if in some parts of the world the regulations are highly open and in some they are highly closed. (For example, in some countries you can't have embryo donations, so people go to other countries - like to Spain where it's allowed.) So for regulations more or less, the key partners in research have to come together, agree and make it consistent and compatible. - You can build walls around your country but you can't build walls for other countries. 31:35
- Whether we allow gene manipulation of human embryos is diverse around the world in terms of regulations.

8. About 100 years ago things like *in vitro* fertilization was not common in our society. Today this process is completely normal and not disputed. Do you think the same could happen with "CRISPR-ized" humans? - Could CRISPR-izing anything at some point become a norm in our society?

- Yes it might over time. With the conditions that these processes will be heavily monitored - like in the UK. As more data and evidence becomes available, it gives us confidence to declare it being safe.
- Editing a germ line leads to a change for multiple future generations. - Most likely in our lifetime, we won't be able to see what happens, so there must be a continued surveillance over generations/centuries. (So that could be a possibility, but not in my lifetime.)

9. How far are ethical regulations limiting CRISPR/ Cas9 research and development in Switzerland compared to other countries?

- At the moment, Switzerland does not allow modification of human germ line and embryos. It probably will change in the future because of the rapid progress of science that would allow a change in their opinion

10. Does in your opinion CRISPR/ Cas9 have higher opportunities than risks and why?

- At this stage ,there are more risks than opportunities with a number of reasons:
- Off target effects
- Would it be consistent?
- Cells could have other unwanted changes
- How does it carry on? - How does it look like for future generations?
- CRISPR has huge potential. I would definitely want to see more research going into the field. (But the risk and opportunity balance is not good enough to try it in human beings.)

5. Discussion

5.1 Advantages

In comparison with other gene engineering methods, CRISPR is cheap, precise, needs little time to conduct experiments and applicable at cellular level; in other words: it is usable on basically every organism and pretty much anyone with a lab can do it. It offers us several new opportunities we have never had before. Especially in fighting certain genetic diseases which we have not had a chance of curing before.
As we use more resources than we have it can also help in developing plants which can live under certain circumstances.

5.2 Risks

When we would try to cure a disease, it could have unforeseen consequences. For instance, an unwanted template could enter the DNA, which could further lead to a or multiple new mutations ore even diseases; and after we would try to cure them, we could accidentally get more and more. That could lead to a disastrous domino effect, where we would engineer ourselves into a corner and we would end up in a worse position, than the one we started in.
Gene drives are also a double-edged sword. While they can be very useful, they could also be devastating for a species. If we were to engineer a DNA sequence for a specific area (for instance only for mosquitos in Africa), there is a risk of a spread of this particular gene and its side effects to other areas, where it could lead to a decline in genetic diversity or, in worst case, extinction.
After it has become more common a risk of home-made CRISPR/Cas9 therapeutics could appear, where people try to edit their genes without the knowledge needed. In the US it is already possible to order CRISPR-kits to engineer your own glowing pet. This could lead people to start inject themselves CRISPR to change their genome like a Biohacker already did as Mrs Satalkar told us in the interwiev.

5.3 The ethical question

There is hardly a debate as big as the one about genetic engineering. Are we allowed to play god and change the genes of plants, animals or even humans, especially in human embryos, as we like?

At this point of research, we know a lot about our DNA, however we might do not know enough. Most DNA sequences do not code for one single trait so as most traits are not based on a single DNA sequence - they're polygenetic. This means, when we change one template we don't know all the side-effects this change could have on the organism. So by now it is not ethical to change an organism especially a human embryo without knowing what comes out.
What differentiates CRISPR/Cas9 from most genetic engineering methods as well as from selective breeding, is the fact that we change the genes to obtain a specific and precise change. Most other methods so as selective breeding get new mutations by chance.
CRISPR/Cas9 is a powerful tool to change DNA and erase genetic mistakes. But as stated before, we do not yet know what impact these changes and corrections may have on the whole organism. We could accidentally worsen an organism's state instead of improving it. Especially when it comes to embryonic engineering, this danger is very high.
Another opposition of genetic engineering is religion. Most religions prohibit any kind of gene editing as such would violate god's work.
In our opinion we are not yet ready to use CRISPR/Cas9 on individuals or even on human embryos. When the technology has developed the burden of whether they want to be modified or not will lie in the hands of the person itself or the parents.

5.4 Regulations

There are different regulations about gene editing especially in human embryos all over the world that can be separated in four categories. Gene editing could be out banned. This could lead to underground and hidden research which would be unregulated. Another way is self-regulations where scientists take responsibility not to go into human germ line. Laissez-faire – "let it be as it is" – means to keep track if a breakthrough comes. This would allow people to be free to choose what they do with CRISPR; in other words, there are no legally bound prohibitions, thus, when disobeyed, nobody could stop or punish you. Regulations, which are transparent, open and fair are the last one. If they are not the same all over the world it could lead people to go to another country with different regulations
To conclude, the key partners in research should come together and agree to suitable conditions and regulations, making it consistent and compatible.

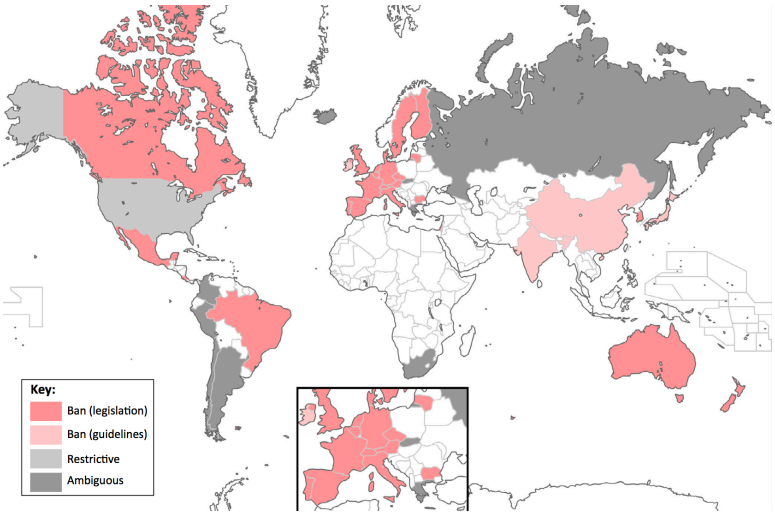

Fig6: A map of the world showing the regulations in power on gene editing in human embryos as of 2015.

5.5 What future research steps are there?

As CRISPR's technology develops, in the far future, it may be possible to cure many diseases. Before this we need to learn much more about our DNA and to improve the CHRISPR/Cas9 method so there is no possibility of a mistake. This is only possible if we allow CRISPR/Cas9 to be used for research.

6. Summary

CRISPR is a newly discovered method of gene manipulation that can be used on any organism. It is based on the defence mechanism used by bacteria to fend off virus infections. With the help of the enzyme Cas9, scientists can now selectively cut out strands of DNA, which in turn can be replaced by another DNA sequence.
CRISPR/Cas9 is mostly used in research and for plant modification. It has also been used to manipulate mice cells and human cells, not on a whole human body, but on one particular area. In China some scientists have even used it on human embryos but without success.
Due to our knowledge of DNA being limited, we cannot precisely determine what effects changing a strand of DNA will have on our body.
Because CRISPR is so cheap, precise and easy to use it offers many opportunities. It could be used to cure gene mutations like sickle cell disease or cystic fibrosis.
However, it also comes with many risks. Because of our knowledge of DNA being incomplete, we could cause mutations that could possibly worsen the subject's state with just one small change.
In the far future, it could theoretically become possible to alter particular attributes and abilities in humans, to create Super humans with superhuman strength, above average intelligence, or something as absurd as eternal youth.
An important matter to consider is whether it is ethically appropriate to edit an organism's genes or not. Is it correct for us to determine who or what will be our test subject without their consent?
Some religions prohibit the usage of CRISPR or any other gene editing method on organism for various reasons. Some might say it is forbidden to alter what has been created by god or it will deny science.
Regulations around CRISPR differ greatly around the world. While western countries have legislation, some eastern countries offer only guidelines. But most of the world has no regulations whatsoever.

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