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A Review: The Function of CRISPR/Cas9 and Its Impact on Human Life

Emre ERGENE - Robert College of Istanbul, Turkey

ABSTRACT

CRISPR, which stands for clustered regularly interspaced short palindromic repeats, is an adaptive immune system firstly detected in bacteria. Cas9, a part of this system, is an RNA-guided endonuclease. The process of giving Cas9 a short RNA piece and the endonuclease, Cas9, cutting the specific part of the target DNA with the guidance of the given RNA shows a promising future for genome engineering. The process being simple and achievable results in inventive applications in a broad range of areas, such as biology, biotechnology, and medicine. It is still being discussed if the CRISPR/Cas9 procedure is ethical or not. On one hand, CRISPR/Cas9 is a way for us to cure genetic disorders that are inherited from the parents and the mutations that occur after the person is born, like cancer. On the other hand, it also opens up the way for the horrifying idea of designer babies. So, in this review, it is discussed how
CRISPR/Cas9 works, how it can be used to develop our understanding of genetic disorders, and also how it effects the world as we see it.

Keywords: *CRISPR, Cas9, Genome-Engineering, Genetic Disorder, RNA-Guide*

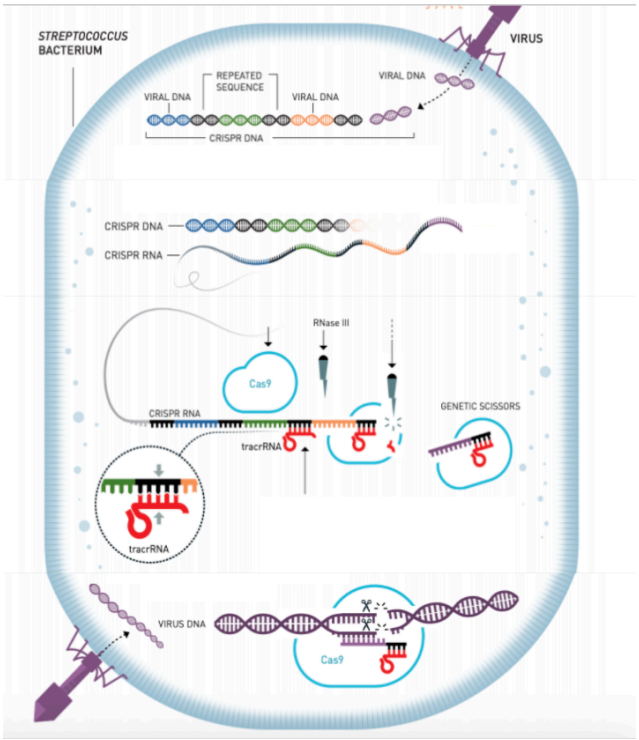
1. Introduction

DNA is the genetic code of life. Sometimes when a specific nucleotide base is replaced with another one because of a mutation, such as in the case of sickle-cell anaemia (a hereditary genetic disorder) in which the sixth amino acid for the beta haemoglobin becomes a valine rather than a glutamic acid (Clancy, 2008), such a little difference in this incredible molecule can change a person's life entirely. CRISPR/Cas9, bringing a whole new perspective to genome engineering, might be a possible solution for genetic diseases like this one (Stein & Kelly, 2019). Naturally, humanity can get much more benefit from this process because it affects humans' life in more than one way. However, the most important point is that where do scientists draw the red line, which diseases should be treated. Lethal genetic disorders like sickle-cell anaemia do exist but also there are non-lethal ones like albinism. Should all genetic
modifications be allowed with the parents' consent? This literature review examines how CRISPR/Cas9 process actually works and tries to find some answers to these ethical questions above. This review discusses the risks and benefits of this incredible discovery and analyses the topic through different aspects such as cancer treatment, biotechnological advancements, and the "designer babies" concept.

2. A detailed look at CRISPR/Cas9

CRISPR systems are mainly divided into six groups and each of the six groups have a unique set of Cas9 proteins. The most commonly known, also highly preferred by the researchers, is the type II CRISPR systems (Jiang & Doudna, 2017). CRISPR (clustered regularly interspaced short palindromic repeats) and the endonuclease that is employed by the type II CRISPR systems, Cas9, together are a system of bacterial adaptive immunity. Its main job is to defend the bacteria against a bacteriophage infection. When a bacteriophage infiltrates a bacterium, it gives off its DNA (or RNA in some cases). If the bacterium survives the infection, it takes a part of DNA that is derived from the bacteriophage's DNA and adds it into its CRISPR-DNA, between the repetitive sequences. Then a CRISPR-RNA is transcribed from that CRISPR-DNA. tracrRNAs (trans-activating CRISPR RNAs) bind to the repetitive sequences. This is essential to form a dual guide, in order to achieve target recognition (Faure, 2019). After that, endonucleases called Cas9 sticks to the mechanism with the help of the RNase III proteins. Each Cas9 protein takes up the
genetic code of a single virus. The genetic code of the virus, one repetitive sequence, and a tracrRNA, all together, act as a wanted poster. When the bacterium is invaded by the same bacteriophage again, Cas9 scissor proteins recognise the virus's DNA with the help of this wanted poster and disarm it by cutting it. This entire process is represented in Figure 1.

Figure 1. A representation of the functioning of the bacterial adaptive immune system, CRISPR Cas9.

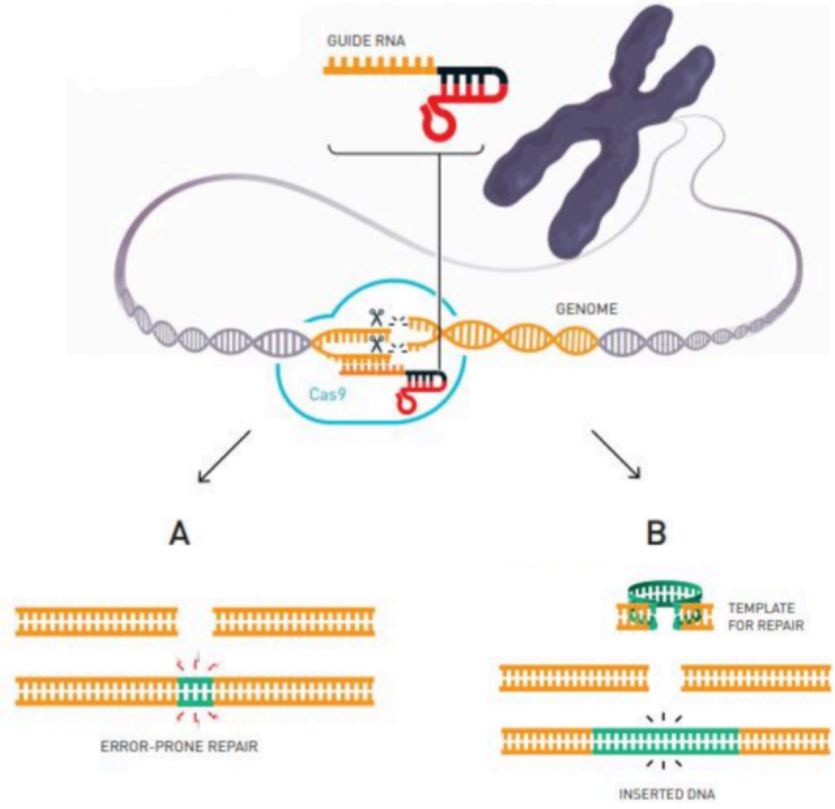


Source: Nobel Media AB 2021. (n.d.). Popular information. NobelPrize.org. Retrieved April 30, 2021, from https://www.nobelprize.org/prizes/chemistry/2020/popular-information/

3. Genome editing and CRISPR/Cas9

As Emmanuelle Charpentier and Jennifer A. Doudna have discovered, CRISPR/Cas9 can also be activated in a lab condition (Nobel Media AB 2021, n.d.). It was concluded that the Cas9 scissor protein could be used for genome editing. For this to occur, an RNA-guide that matches a specific part of the target DNA can be constructed. The Cas9 protein binds to that RNA-guide, which is a combination of tracrRNA and CRISPR-RNA, forming a complex mechanism. Then, it finds the particular gene that needs to be cut. After that there are two options. The cell can repair the cut by itself, which usually results in the gene's affects being turned off or the loss of the gene completely. In order to insert, repair, or edit a gene; scientists prefer the second option in which a DNA template is given to the cell. This template is used to repair the cut made by Cas9. This procedure is described in Figure 2. The process being faster, cheaper, more accurate, and more efficient than other existing genome editing
methods makes it much more feasible for genome editing and creating innovative solutions for genetic disorders (What Are Genome, 2020).

Figure 2. The Cas9 protein at work while cutting a specific part of the DNA with the help of the RNA-guide.



Source: Nobel Media AB 2021. (n.d.). Popular information. NobelPrize.org. Retrieved April 30, 2021, from https://www.nobelprize.org/prizes/chemistry/2020/popular-information/

4. Application areas of CRISPR/Cas9

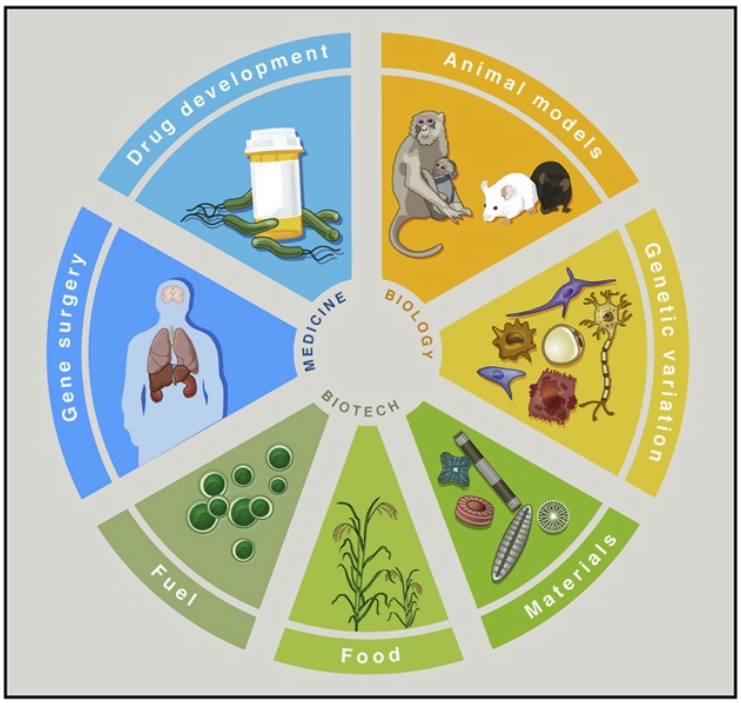
CRISPR/Cas9 opens the path for a broad range of possibilities. As shown in Figure 3, CRISPR/Cas9 mediated applications can be narrowed down to three main groups: medicine, biology, and biotechnology. Researchers started using this new genetic scissor in labs and it seems to have increased the range of molecular tools available to them (Barangou & Doudna, 2016).

Firstly, new kinds of drugs can be developed, such as more specific antibiotics that target only disease-causing bacterial strains while sparing beneficial bacteria (Harvey, 2014) and eliminating the risk of antibiotics-resistant bacteria. In addition to that CRISPR/Cas9 screening of proteins revealed that with this process, therapeutic targets in diseases like cancer can be discovered (Shi et al., 2015). This makes it possible for in vivo correction of genetic disorders resulting in a permanent solution for a particular genetic disease, which scientists call a gene surgery.

The usage of animal models and cells dramatically enhanced biological research in general. For example, CRISPR/Cas9 was able to genetically manipulate the patterns on a butterfly wing by cutting a gene called Distal-less (Hesman Saey, 2016). CRISPR technology also proved quite useful in curing a rare and genetic liver disorder that occurred in adult mice (Harvey, 2014), thus showing that this kind of treatment could also be possible for humans. In terms of biotechnology, manipulating biological circuits could provide useful synthetic materials. Additionally, with much more precise genetic engineering for food, health risks could be reduced drastically. Specific edits algae or corn genome could result in ethanol production that would later be turned into biofuel (Hsu et al., 2014).

CRISPR/Cas9 shows promise in curing a lot of diseases that genetics play a role in. It can also be utilized to target human pathogenic viruses as an antiviral (Soppe & Lebbink, 2017). For instance, an in vivo treatment with CRISPR/Cas9 was developed for the hepatitis B virus (Kayesh et al., 2020). Pharmaceutical companies had been working on a CRISPR-mediated treatment for a congenital eye disease known as LCA10 and they have injected the editing tool into a human patient for the first time in 2021. This treatment aimed to restore the vision of the patient by turning off the mutated gene and eliminating the factor that prevented the CEP290 production, which is critical for the eye to function properly (Scharping, 2021). While its long-term effects are unknown, this marks a milestone for humanity. In addition, CRISPR accelerated cancer research and is thought useful in possible treatments of cancer. It provides a solution to dissect mechanisms, genes, of tumorigenesis (Zhan et al., 2018). Finally, CRISPR also promoted advancements in neuroscience (Heidenreich & Zhang, 2016) and could be used to treat neurological genetic disorders, such as Alzheimer's disease (Page, 2020). Just the idea of being able to cure the untreatable diseases pushes researchers to focus on CRISPR-mediated treatments.

Figure 3. Different application areas for CRISPR/Cas9.



Source: Hsu, P. D., Lander, E. S., & Zhang, F. (2014, June 5). Development and applications of CRISPR-Cas9 for genome engineering. ScienceDirect. Retrieved March 13, 2021, from https://www.sciencedirect.com/science/article/pii/S0092867414006047#fig1

5. Ethics of CRISPR/Cas9

Such a powerful gene-editing tool brings unanswered questions with its advantages. There seems to be an abundant number of benefits that we can get from CRISPR/Cas9. However, there are also ethical concerns about the risks that affect individuals and society as a whole (Rath, 2018). The main question is: when to use it? When the main focus is on the public good, it isn't much of a problem (Mulvihill et al., 2017). On the other hand, things start to get
complicated when people seek individual profit. Some genetic disorders are fatal, such as hemophilia which causes deficiency in blood clotting. Some are not fatal, such as autism or deafness. Which of these diseases should be treated, ultimately which of those people who have these diseases get to live like a "normal" person? Even though "designer babies" are not the most urgent concern; being able to genetically modify an embryo, choosing the characteristics of our offspring raises bothersome questions (Kung, 2017). CRISPR/Cas9 is definitely the beginning of a new era for humanity. It also affects military and politics. Eugenics and advanced human beings also become a risk with this process. Strict and international regulations are needed to prohibit these from happening. All clinical research should be supervised by the authorities in order to prevent unethical or immoral experiments. Because of the unknown long-term effects; serious injury, disability, or death to research participants and offspring might be possible (Brokowski & Adli, 2019).

6. Conclusion

In conclusion, CRISPR/Cas9 has brought a new perspective to genome editing and it opens up the doors to a whole new world where some of the common and fatal diseases like cancer might not be a problem anymore. As expected, the question of the process being ethical or not emerges. However, there isn't any new ethical issues, compared with genetic engineering in general (Mulvihill et al., 2017). Of course, the fact that every technological development in human history has sparked new ethical questions in people's minds should not be forgotten. Scientific community should gather and discuss the ethical aspects of CRISPR/Cas9. The possible risks cannot be ignored and these debates are a part of this process. It seems that the benefits we can get from CRISPR/Cas9 are of greater amplitude than the risks that it might bring so, a lot of research being done are focused on CRISPR/Cas9. The scientific community is excited and it's quite understandable since it is the beginning of a new era and it will change human life as we know it.

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