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Exploring the Role of CRISPR-Cas9 in Genetic Engineering: Advancements, Applications, and Ethical Issues

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Abstract

Since its discovery in 1987, the emerging genome-modification technology CRISPR-Cas9 has augmented the ever-evolving field of genetic engineering through its advancements in precision and accuracy to simplify efficient genome alteration. This paper introduces the history of CRISPR-Cas9 and explores its underlying mechanisms and advancements. Significant technological advancements have enhanced the precision and efficiency of CRISPR-Cas9 in genetic engineering. Innovations like base and prime editors minimize the unintended off-target effects, improving the accuracy of gene editing. The development of advanced delivery methods, such as magnetic nanoparticles, allows for faster delivery of editing components to their intended destination with greater precision. This complex has a wide range of applications in fields such as medicine, agriculture, and industrial biotechnology. CRISPR-Cas9 has recently grown popular among gene therapy studies for genetic disorders in addition to cancer research for further understanding of cancer cell mechanisms. In agricultural settings, this tool has been used to modify crops to withstand environmental constraints to increase crop yield and alter nutritional content. CRISPR-Cas9's role in industrial biotechnology is also discussed as modifying the metabolic pathways of microorganisms to facilitate higher biofuel production. Ethical considerations related to the technology such as safety, possible human germline misuse, and ecological effects of GMOs have catalyzed social and political restraints with pertinent case studies. Challenges such as off-target effects, generational consequences, and unequal access are mentioned. Nevertheless, ethical questions remain without prominent responses. The future of genetic engineering is in the hands of geneticists working with CRISPR-Cas9 to offer greater treatment options for fatal genetic disorders. This review aims to provide a better understanding of CRISPR-Cas9's significant use and role in genetic engineering.

Keywords: CRISPR-Cas9, gene therapy, genome engineering

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1. Introduction

One of the most fast-paced and promising discoveries of the 21st century, the CRISPR-Cas9 system represents a revolutionary genome-editing tool that has transformed the field of genetic engineering. Representing a more reliable and cost-efficient method of genetic engineering, scientists have harnessed CRISPR-Cas9's profound site-specific gene editing capabilities, enabling precisely targeted alteration in the genome to correct errors and regulate gene expression in cells and living organisms with an unprecedented level of control than ever before.

The technology's potential to edit genes with high accuracy has led to significant advancements in precision and efficiency, including the development of base and prime editors. It has numerous broad applications in medical, agricultural, and industrial sectors, including gene editing, cancer research, agriculture, and biofuel production. However, its transformative capabilities raise concerns, particularly in the possible editing of the human germline, raising critical questions about the moral implications. Similarly, the ecological impact of genetically modified organisms (GMOs) raises concerns about the long-term consequences of such interventions. This paper explores CRISPR-Cas9's role in genetic engineering, examining its technological advancements, applications, and ethical challenges, aiming to provide a better understanding of CRISPR-Cas9's significant role in genetic engineering.

2. Background of CRISPR-Cas9

Clustered regularly interspaced palindromic repeats (CRISPR) were first found in the DNA sequences of the *Escherichia coli* bacteria, discovered in 1987 by Japanese scientist Yoshizumi Ishino from Osaka University (Ishino, Krupovic, & Forterre, 2018). Unable to elucidate the purpose of these repeating palindromic sequences, Ishino and his team concluded the study, and CRISPR remained a mystery.

In 1990, Spanish student Francis Mojica discovered the core mechanism of the gene-editing tool and gave CRISPR its name upon studying other single-celled organisms such as archaea (Gostimskaya, 2022). He did this by extracting each spacer and placing them in a bioinformatics analysis tool: BLAST (Basic Local Alignment Search Tool). Mojica discovered the spacers and matched them with the DNA sequences of viruses. This discovery led Mojica to hypothesize that the spacers encoded instructions for the bacteria's immune system to protect against the designated viruses (Adli, 2018).

The continued discovery of CRISPR took place in 2006 by young scientist Philip Horvath in a yogurt production factory (Barman, Deb, & Chakraborty, 2019). Hortvath and other factory workers struggled with failing bacteria cultures often attacked by viruses. Upon noticing immune bacterial strains resistant to viral attacks, Horvath compared the virus DNA sequence to that of the resistant bacterial strains. The presence of virus DNA in the strains led Horvath to edit virus DNA segments into the CRISPR array of the non-resistant bacterial strains. The experiment results led Horvath to conclude that CRISPR was an adaptive immune system and a precise DNA match between the spacer and the virus (Anik et al., 2021). Another important discovery by Harvath was the gene encoding for a protein enzyme called Cas9. This gene was found in front of the palindromic repeating segments, and it is responsible for cutting virus

DNA and granting immunity (Du et al., 2023). Later that year, in 2006, John Van Der Ooste became the first to create artificial CRISPR arrays for protection against viruses (Cortez, 2015). He created directly programmable CRISPR-based immunity by constructing artificial flu shots for bacteria (Firouzeh Morshedzadeh et al., 2023).

Emmanuelle Charpentier later accidentally discovered the last essential component of the CRISPR-Cas9 in 2008: tracrRNA (trans-activating CRISPR RNA) (Adli, 2018). She discovered tracrRNA when studying the bacterium *Streptococcus pyogenes*. In 2011, Charpentier and her team confirmed the role of tracrRNA in processing pre-cRNA into mature cRNA (Barman, Deb, & Chakraborty, 2019). They demonstrated the precise biochemical steps required for the system to function, which helped scientists understand how to design synthetic guide RNAs to direct Cas9 to specific DNA sequences (Adli, 2018). In 2012, Emmanuelle Charpentier and Jennifer Doudna discovered that the CRISPR-Cas9 complex could be programmed with synthetic RNA to cleave specific DNA sequences in vitro (Asmamaw & Zawdie, 2021). This was a significant step, as previous studies were limited to in vivo testing. Charpentier was later awarded the Nobel Prize in 2020 for her contribution to gene editing (Ng, 2023).

In 2013, Feng Zhang and George Church accomplished gene editing in a mammalian cell using CRISPR efficiently and accurately (Anzalone et al., 2019). This was a pivotal discovery as, until then, the CRISPR-Cas9 system seemed to be limited to prokaryotic organisms. Zhang and Church optimized the guide RNA design and delivery methods, enhancing the practicality of the CRISPR-Cas9 (Adli, 2018).

Furthermore, the applications and implications of CRISPR-Cas9 expanded significantly from 2015 to 2016. These include, but are not limited to, applications in medicine, agriculture, and biotechnology (Anik et al., 2021). Unfortunately, with the rapid rise of these applications, ethical concerns have increased (National Human Genome Research Institute, 2017).

Mechanisms

There are three essential components of the CRISPR complex. The first is the CRISPR array, which is a segment of DNA in a bacterial genome containing repeated palindromic sequences and unique sequences derived from viruses (spacers) (Adli, 2018). The second component is the Cas9 protein, which stands for CRISPR-associated nuclease protein. Nucleases cleave DNA at specific nucleotide linkages. Cas9 is one of the nucleases found in Streptococcus pyogenes, and it has two active sites responsible for cutting the two DNA strands (Barman, Deb, & Chakraborty, 2019). The last component is the guide RNA, otherwise known as gRNA. The gRNA has a specific RNA sequence that recognizes the target DNA region of interest and directs the Cas9 nuclease for editing (Asmamaw & Zawdie, 2021). The gRNA is composed of a crRNA that is fused to the tracrRNA (Firouzeh Morshedzadeh et al., 2023).

The CRISPR-Cas9 complex goes through three steps to perform genetic editing. The first step is to identify the specific DNA sequence in the genome that needs to be edited. This target sequence must be located in a short DNA sequence known as the protospacer adjacent motif (PAM). This is because the Cas9 protein requires the presence of the PAM to bind to the target DNA, preventing it from cutting the bacteria's genome (Adli, 2018). Once the DNA sequence is identified, the guide RNA is synthesized to match the target sequence. As mentioned before, the gRNA includes CRISPR RNA (a 20-nucleotide sequence

complementary to the target DNA) and Trans-activating CRISPR RNA (a sequence that binds to the Cas9, providing structural support) (Ansori et al., 2023). In many cases, the crRNA and tracrRNA are combined into a single guide RNA (sgRNA) (Adli, 2018).

The second step is the preparation of the CRISPR-Cas9 components. The Cas9 enzyme, synthesized or commercially purchased, will be used to create the double-strand breaks in DNA (Adli, 2018). The components of CRISPR can be delivered through plasmid DNA, Ribonucleoprotein Complex, and viral vectors. Plasmid DNA can form the active complex for gene editing as it can be transcribed and translated to produce the Cas9 and the sgRNA. Ribonucleoprotein (RNP) can directly deliver the Cas9-sgRNA complex into the cell (Du et al., 2023). Lastly, viral vectors can express the Cas9 and sgRNA within the cells by infecting target cells and integrating the CRISPR-Cas9 components into the host genome (Firouzeh Morshedzadeh et al., 2023).

The third step is the actual delivery of the CRISPR-Cas9 into cells. The first method is electroporation, where an electrical field is used to increase cell membrane permeability temporarily, allowing CRISPR-Cas9 components to enter with ease (Du et al., 2023). The second method is the utilization of lipid nanoparticles, which encapsulate the CRISPR-Cas9 components, fuse with the cell membrane, and release the components into the cell (Movahedi et al., 2023). The last method is viral transduction, utilizing viral vectors as mentioned previously (Du et al., 2023).

After the delivery of the CRISPR-Cas9 into cells, the target DNA binds, and cleavage occurs. Inside the cell, the Cas9 binds to the sgRNA, forming the RNP complex. Then, the sgRNA guides the Cas9 to the complementary DNA sequence (Adli, 2018). The Cas9 then induces the double-strand break (DSB) at the target site, forming blunt ends. Once the DNA is cleaved, DNA repair mechanisms take place. The first mechanism is non-homologous end joining (NHEJ). Although this method quickly rejoins the DNA ends, it is prone to error and may result in insertions or deletions (Cortez, 2015). The second, more accurate mechanism is known as homology-directed repair (HDR). This process utilizes a homologous sequence as a template for repair, naturally making it more accurate (Adli, 2018).

Screening and validation follow the repair mechanisms. Cells may be screened to identify those that underwent the desired genetic modification. This could include the utilization of fluorescent markers to identify cells with fluorescent tags used in HDR, or antibiotic selection by using a selectable marker in the donor template (Adli, 2018). Edited cells can then be sequenced to confirm the presence of the desired genetic modification and later assayed to ensure the desired phenotype is achieved (Barman, Deb, & Chakraborty, 2019). Finally, once the edited cells are complete, they can be used in vitro (outside of an organism) or in vivo (inside of an organism) (Firouzeh Morshedzadeh et al., 2023).

3. Advancements of CRISPR- Cas9

CRISPR-Cas9's off-target effects have posed significant challenges in genetic engineering due to unintended alterations to the genome. Off-target genome editing is a type of unintentional and non-specific genetic modification that occurs when a molecular scissors alters the wrong section of the target genome, resulting in a non-specific, unwanted and even adverse alteration to the genome (Asmamaw and et al, 2021). When these errors occur, the CRISPR-Cas9

system produces genetic mutations rather than fixing them, limiting the technology's ability to treat genetic disorders. However, technological innovations like Base Editors and Prime Editors have significantly reduced these potential off-target effects, enhancing the precision and efficiency of gene editing.

Base and Prime editing

Base editors can directly install point mutations in cellular DNA without inducing a double-strand DNA break (DSB), making them suitable for gene editing using the CRISPR-Cas9 system (Kantor,Ariel, et al, 2020). Particularly, two DNA base editors, cytosine base editors (CBEs) and adenine base editors (ABEs), allow for direct point alterations without needing DSBs or subsequent repair mechanisms. These base editors have shown the ability to significantly improve gene editing efficiency while minimizing off-target effects (Du, Yimin, et al, 2023) DNA base editors consist of two key components: a Cas9 enzyme for programmable DNA binding and a single-stranded DNA modifying enzyme for targeted nucleotide alteration. The CRISPR-Cas9 Base Editors allow for the installation of all four transition mutations (C→T, T→C, A→G, and G→A), (Du, Yimin, et al, 2023). Adenine and cytosine base editors can facilitate targeted changes for all four transition mutations, respectively, which can potentially treat most human disease-associated single-nucleotide polymorphisms, making them suitable for treating genetic disorders within the CRISPR-Cas9 system.

Prime Editors are capable of introducing all twelve potential transition and transversion mutations, as well as small insertion or deletion mutations, without the need for double-strand breaks or donor DNA templates (Anzalone, Andrew V., et al, 2019). This revolutionary genome editing technology utilizes a catalytically impaired Cas9 endonuclease linked with a reverse transcriptase and a prime editing guide RNA (pegRNA) to specify the target site and inscribe new genetic information, with the ability to significantly reduce off-target effects, improving efficiency and accuracy in CRISPR-Cas9 gene editing (Kantor, Ariel, et al., 2020). With the potential to correct up to 89% of known disease-causing gene mutations, it has been utilized in human cells to treat sickle cell disease and Tay-Sachs disease (Kantor, Ariel, et al., 2020).This genome editing tool represents a significant advancement in CRISPR-Cas9 technology, significantly expanding its genome editing capabilities.

Base and Prime editors simplicity and precision have been proven as the most effective and versatile tools for reducing off-target effects. These advancements have significantly improved the accuracy of gene editing, aiding in the treatment of genetic disorders and further advancing the field of genetic engineering.

Magnetic Nanoparticles

Safe and efficient delivery methods of these editing systems to their target cells are the most crucial and challenging part for their success in reducing off-target effects in gene editing, as the non-viral delivery of CRISPR-Cas9 components have the potential to significantly improve future therapeutic delivery (Anik, Muzahidul I., et al., 2021).

Low delivery efficiencies of genome editing agents, including base and prime editors to target cells, limit the actual efficiency of the genome-editing process, and the precision with which the CRISPR-Cas system operates (Anik, Muzahidul I., et al., 2021). Magnetic Nanoparticles have emerged to facilitate the safe and efficient delivery of CRISPR-Cas9 genome editing

agents, which significantly improves the transfection efficiency, biocompatibility, and genome-editing accuracy of this technology. Magnetic Nanoparticle various advantageous characteristics, such as their large surface area and magnetic properties, allow for this precise on-set targeted gene delivery (Hryhorowicz, Magdalena, et al., 2018). MNP's large surface area enables them to carry a substantial load of gene-editing tools, such as CRISPR-Cas9 components, base, and prime editors. They are allowing for the simultaneous delivery of multiple components needed for successful genome editing, increasing the concentration of these agents at their target site and thereby enhancing the efficiency of the editing process. MNP's magnetic properties allow scientists to precisely direct MNPs to the exact location where gene editing is required, minimizing the risk of off-target effects (Hryhorowicz, Magdalena, et al., 2018). Their low toxicity and stability make them ideal for gene editing tools like CRISPR-Cas9, enhancing the efficiency and effectiveness of target gene alteration. Their ability to control the movement and connection of base and prime editors provides a level of accuracy challenging to achieve with other delivery methods making MNPs useful for the precise targeted gene delivery of genome editing agents.

4. Applications of CRISPR-Cas9

Medicine

The CRISPR-Cas9 system is a highly effective gene-editing tool for disease prevention and treatment, leveraging its mechanism as an immune system in bacteria (Asmamaw & Zawdie, 2021). In 2013, Wu et al. published a groundbreaking study using CRISPR-Cas9 to treat cataracts in mice. The team coinjected mRNA encoding Cas9 and sgRNA into mouse eggs destined to develop cataracts. Of the ten mice with the mutant allele, six exhibited NHEJ-mediated insertions and deletions, while four underwent HDR-mediated repairs. Notably, the four HDR-treated mice were cured of cataracts, as were two of the NHEJ-treated mice. This study was significant as it demonstrated CRISPR-Cas9's potential in genetic editing for disease treatment (Adli, 2018).

CRISPR-Cas9 also has significant applications in editing viral and bacterial genomes to understand their biology and identify vaccine targets. For example, a study published in Cell used CRISPR-Cas9 to create a modified strain of the influenza virus, allowing researchers to study its immune evasion mechanisms and identify new vaccine targets (Donohoue, Barrangou, & May, 2018). CRISPR-Cas9 has also been applied to stem cell research, leading to promising advances in cell therapies for disorders like blood diseases and degenerative conditions (Du et al., 2023). A notable example is using CRISPR-Cas9 to edit stem cells from sickle cell patients, correcting the defective hemoglobin gene. The edited cells were then reintroduced into the patients, demonstrating CRISPR-Cas9's potential for personalized genetic therapies (Firouzeh Morshedzadeh et al., 2023).

Beyond treating genetic disorders, CRISPR-Cas9 has proven valuable in cancer research. The technology has been used to knock out specific genes in cancer cells to study their functions, allowing scientists to identify genes critical to cancer development (Anik et al., 2021). CRISPR-Cas9 also enables the creation of mutations similar to those found in human tumors, facilitating the study of these mutations in controlled environments. This has led to the development of novel cancer treatments, including enhancing CAR-T cell therapy by

knocking out genes that cause T-cell exhaustion (Ravichandran & Maddalo, 2023). Additionally, CRISPR-Cas9 is used to mimic chemotherapy resistance in cancer cells, helping researchers devise strategies to overcome these challenges (Du et al., 2023).

Agriculture

CRISPR-Cas9's influence extends beyond medicine into agriculture, where it holds transformative potential (Liu et al., 2021). One of the most pressing issues in agriculture today is the need for crop resilience in the face of unpredictable climate change. Researchers have used CRISPR-Cas9 to identify and modify genes responsible for stress responses, making plants more resistant to droughts and extreme temperatures (Barman, Deb, & Chakraborty, 2019). This system has also been employed to genetically modify plants for resistance to pests and diseases. For instance, CRISPR-Cas9 was used to develop gluten-free wheat, a significant advancement for individuals with celiac disease (Movahedi et al., 2023).

Moreover, CRISPR-Cas9 has been utilized to enhance crop yields by making multiple mutations that improve plant growth and stress responses (Asmamaw & Zawdie, 2021). By genetically altering crops to require less water, pesticides, and fertilizers, CRISPR-Cas9 contributes to more sustainable and environmentally friendly farming practices (Zhu, Li, & Gao, 2020). Additionally, scientists have applied CRISPR-Cas9 to enhance the taste and nutritional content of crops. For example, researchers in Brazil and Ireland are using CRISPR-Cas9 to create the first naturally spicy tomatoes, addressing the challenge of declining chili pepper growth (Wenham, 2023). In the UK, Tropic Biosciences developed a gene-edited variety of decaffeinated coffee beans, offering a healthier alternative while preserving the coffee's natural taste (Liu et al., 2021). Other advancements include using CRISPR-Cas9 to remove allergenic genes from foods like milk, eggs, and peanuts (Adli, 2018). Notably, the first CRISPR-edited organism approved by the US government was a mushroom engineered to resist browning. Yinong Yang, a plant pathologist at Penn State, achieved this by targeting and knocking out a gene responsible for the enzyme polyphenol oxidase, reducing its activity by 30% (Donohoue, Barrangou, & May, 2018). This development exemplifies CRISPR's potential to improve farm productivity, food security, and population health (Zhu, Li, & Gao, 2020).

CRISPR-Cas9's applications also extend to livestock, where it has been used to optimize traits like milk and meat productivity, disease resistance, and environmental adaptability (Movahedi et al., 2023). In 2022, the FDA approved beef from gene-edited cattle designed to grow shorter hair, enabling better heat tolerance and maximizing efficiency throughout the seasons (Ravichandran & Maddalo, 2023). Additionally, researchers have inserted an alligator gene into catfish to enhance their disease resistance, leveraging alligators' robust immune systems (Ng, 2023). A notable example of CRISPR's impact on livestock was the approval of two gene-edited fish for commercial sale in Japan in 2021: a beefed-up red sea bream and a heavier tiger puffer fish (Zhu, Li, & Gao, 2020). Despite these advancements, concerns about decreasing biodiversity among livestock persist, prompting scientists to carefully consider the ecological implications while optimizing CRISPR-Cas9's use (Friends of the Earth, 2018).

Industrial Applications

This gene-editing technology also has profound effects on the production of biofuel (Javed et al., 2019). With the rising demand for fuel due to its high consumption in transportation,

energy, and industrial settings, researchers have been looking to maximize fuel production using efficient gene editing tools (Adli, 2018). Biofuels produced from biomass have received attention from many biotechnology companies as they are environmentally sustainable and cost-efficient, unlike fossil fuels (Adli, 2018). Biodiesel, a biofuel obtained from lipids and fats of soya beans, canola seeds, algae, and other crops, has gained popularity (Javed et al., 2019). CRISPR-Cas9 can be used to modify the metabolic pathways of microorganisms such as bacteria and yeast, enhancing the production of biofuels (Barman, Deb, & Chakraborty, 2019). The company Synthetic Genomics has created strains of algae that produce twice as fast by utilizing CRISPR-Cas9 to remove genes that limit fat production (Donohoue, Barrangou, & May, 2018). Then, the algae fat is used to produce biodiesel (Javed et al., 2019). Furthermore, microorganisms can be engineered to tolerate various feedstocks, even those deemed toxic, broadening the range of materials that can be used for biofuel production (Adli, 2018). This system could also be utilized to engineer microorganisms to be more robust and capable of surviving extreme industrial conditions such as high temperatures or fluctuating pH levels, increasing the scale of biofuel production (Javed et al., 2019).

5. Ethical Issues and Considerations

Human Germline Editing

As genetic editing technologies, such as CRISPR-Cas9, gained efficiency and have become prevalent in various major countries worldwide, many ethical concerns have arisen regarding human germline genome editing. However, due to popular opinions regarding the ethics of human germline editing, it has yet to become clinically available.

One primary ethical concern is the safety of editing human embryos using CRISPR-Cas9. While this gene-editing technology is more advanced and innovative than similar technologies, it has not been classified as safe enough for reproductive application, with the possibility of causing a genetic malfunction that could be inherited for generations. Researchers and ethicists at the International Summit on Human Gene Editing recommend further research to guarantee the safety of germline editing before allowing it to be offered clinically. Other researchers state that it may never be safe enough for such approval (Schmerker, 2024c). Furthermore, debates surrounding consent from the affected individual also raise concerns about the ethicality of genome editing. Seeing that genome editing alters the genetics of a single-celled embryo, as well as its future progeny, informed consent is not obtainable (Savulescu et al., 2015c). Many refute this argument, stating that parents already make similar decisions, such as IVF (Schmerker, 2024c).

Another primary ethical concern regarding human germline editing is the ability to differentiate between therapeutic (medical) and non-therapeutic (enhancement) use. If germline editing were to become publicly available, many might try to utilize it to strengthen favored genes, such as height, in the embryo to obtain a designer baby. By allowing the use of CRISPR-Cas9 to treat embryos with various genetic disorders clinically, it may soon become challenging to distinguish therapeutic from non-therapeutic treatment, as anyone with the corresponding funds would claim that their genetic alteration is "necessary" for the embryo. This capability raises concerns surrounding equity in germline editing. Regarding this indifference, in March 2019, the WHO Director-general stated that "it would be irresponsible

at this time for anyone to proceed with clinical applications of human germline genome editing" (World Health Organization, 2023). This quote demonstrates the importance of addressing these ethical concerns before CRISPR-Cas9 germline editing becomes available.

Ecological Impact of GMOs

With new discoveries on the application of CRISPR-Cas9 technology on various organisms and species, researchers and scientists have identified substantial benefits of genetically modified organisms (GMOs). Of these benefits include: pest-resistant crops, enhanced food security, and disease-resistant animals (Movahedi et al., 2023b). For instance, the rise of drought-resistant and more nutritious food has been able to put populations in rigid climates at ease. In fact, GMOs have even been used to prevent the spread of viruses. For example, in Hawaii, the Rainbow Papaya, which is a genetically modified crop, helped to prevent the spread of the ringspot virus (Nutrition, 2020c). Despite the apparent upsides to GMOs, there are several potential ecological consequences of releasing genetically edited organisms. As with the use of CRISPR-Cas9 technology on human cells, the genetic modification of plant and animal cells can result in unintended mutations caused by off-target manipulation, causing disruptions of proteins, and resulting in harmful organisms that can negatively impact the ecosystem (CBAN, 2023b).

Regulatory and Social Implications

The rise and spread of CRISPR-Cas9 gene-editing technology across the world has led to deliberation regarding regulations on the technology. With many countries concerned about the safety of utilizing this technology, not all are open to permitting the continuation of trials and testing. Internationally depicted guidelines are yet to be put in place, causing countries to enforce their own regulations. In the United States, FDA guidelines prohibit government funding for research on human germline gene editing. Such federal laws are put in place to prevent misuse of germline editing, averting the rise of designer babies within the country. Privately funded research may be conducted as there are no restrictions on the matter (Genetic Literacy Project, 2019b). China has comparably more lenient restrictions, as they only allow human embryos to be developed for up to 14 days. Distinct approaches have been taken to enforcing guidelines for crops that have been genetically modified. Two significant paths include regulations enforced according to the process taken to produce the product and those according to the final product itself. Countries under the European Union analyze the process of production to determine whether the crop is permissible for commercial consumption. On the other hand, countries such as the US and Canada prefer to evaluate the final genetically edited crop to classify it as admissible (*Updates on Global Regulatory Landscape for Gene-Edited Crops*, n.d.-b). The majority of countries emphasize the analysis of genetically edited crops before distribution. Meanwhile, Japan has enabled the sale of such produce without the need for safety evaluations (Schreiber, 2019c). New laws and regulations continue to be authorized as greater discoveries are made regarding gene-editing technologies and their impact on the human body post-consumption.

In addition to government-level debates and ordinances created to regulate the release and usage of gene-editing technology, potential social implications have raised concern among various populations. With common trends of new medical advancements being out-of-reach for the average income in the United States, many individuals have expressed unease at the possibility of yet another treatment option that would only be available to the upper class. The

possibility of only increasing the economic inequality that is present today leads populations to gain the desire to reject an opportunity that would otherwise be helpful to them. Another social setback of technology is the possibility of designing babies. The birth of the world's first CRISPR-Cas9 genetically modified baby in 2018 has left people wondering how genome modification will go (Rose & Brown, 2019c). What may start off as the prevention of deadly disorders from reaching the next generation could quickly turn into the rise of parents selecting their preferred traits for their future fetuses. With this in mind, many are debating against the approval of CRISPR-Cas9 technology for clinical use. CRISPR-Cas9's ability to prevent life-threatening disorders and cure fatal diseases is facing a roadblock with the social implications it would likely create.

Ethical Case Study

Ethical concerns surrounding CRISPR-Cas9 gene-editing technology have been prominent since the advancement first rose to the public eye. These opinions only intensified when Chinese scientist He Jiankui revealed the birth of genetically edited twins. He managed to edit the genomes to acquire resistance to HIV. This illegal medical case raised many concerns regarding the use of CRISPR-Cas9 technology to treat genetic disorders (*Chinese Scientist Who Gene-Edited Babies Is Back in Lab after Jail Time*, n.d.-b). Controversy concerning the safety and long-term effects of genome editing stirred a commotion between many scientists and researchers. His impermissible approach to experimentation on human genomes led him to serve three years in prison. Though this new development opens many doors for disorder prevention between generations, His premature trial led to more apprehension rather than praise.

6. Conclusion

Previous experiments and trials of various genetically modified organisms have given insights into the future of CRISPR-Cas9. The gene-editing technology's ability to alter the human genome to not only cure but prevent a broad spectrum of disorders and diseases has encouraged more research to take place surrounding the innovation. CRISPR-Cas9's greater accuracy and precision compared to previous genetic modification inventions gives scientists and researchers hope regarding the future of gene editing benefits. From providing more food to economically challenged communities by modifying crops to create a larger output to possibly being the essential tool to finding the cure to cancer, CRISPR-Cas9 continues to play a prevalent role across numerous industries. Ethical concerns continue to rise alongside these positive outlooks, causing tension and hesitance to expand the technology to be available for clinical use. Whether the balance between scientific advancement and ethical responsibility is soon to be achieved is a question that still remains. It is now in the hands of prospective researchers to considerately apply this influential advancement to proper use while respecting relative ethical considerations. The future of CRISPR-Cas9 is the future of the genetic-editing industry as a whole.

References

Adli, M. (2018). The CRISPR tool kit for genome editing and beyond. *Nature Communications*, *9*(1). https://doi.org/10.1038/s41467-018-04252-2

Anik, Muzahidul I., et al. "Biomedical Applications of Magnetic Nanoparticles." *Elsevier eBooks*, 2021, pp. 463–97. https://doi.org/10.1016/b978-0-12-823688-8.00002-8.

Ansori, A. N., and et al (2023). Application of CRISPR-Cas9 genome editing technology in various fields: A review. *Narra J*, *3*(2), e184–e184. https://doi.org/10.52225/narra.v3i2.184

Asmamaw, M., & Zawdie, B. (2021). Mechanism and Applications of CRISPR/Cas-9-Mediated Genome Editing. *Biologics : Targets & Therapy*, *15*(1), 353–361. https://doi.org/10.2147/BTT.S326422

Anzalone, Andrew V., et al. "Search-and-replace Genome Editing Without Double-strand Breaks or Donor DNA." *Nature*, vol. 576, no. 7785, Oct. 2019, pp. 149–57. https://doi.org/10.1038/s41586-019-1711-4.

Barman, A., Deb, B., & Chakraborty, S. (2019). A Glance at Genome Editing with CRISPR–Cas9 Technology. *Current Genetics*, *66*. https://doi.org/10.1007/s00294-019-01040-3

CBAN. (2023). *Environmental Impacts*. Canadian Biotechnology Action Network. https://cban.ca/gmos/issues/environmental-impacts/#:~:text=Biodiversity%20Loss%3A%20The%20use%20of

Chinese Scientist Who Gene-Edited Babies Is Back In Lab After Jail Time. (n.d.-a). NDTV.com. https://www.ndtv.com/world-news/chinese-scientist-who-gene-edited-babies-is-back-in-lab-after-jail-time-5369252#:

Cortez, C. (2015, March 12). *CRISPR 101: Homology Directed Repair*. Blog.addgene.org. https://blog.addgene.org/crispr-101-homology-directed-repair

Donohoue, P. D., Barrangou, R., & May, A. P. (2018). Advances in Industrial Biotechnology Using CRISPR-Cas Systems. *Trends in Biotechnology*, *36*(2), 134–146. https://doi.org/10.1016/j.tibtech.2017.07.007

Du, Yimin, et al. "CRISPR/Cas9 Systems: Delivery Technologies and Biomedical Applications." *Asian Journal of Pharmaceutical Sciences*, vol. 18, no. 6, Nov. 2023, p. 100854. https://doi.org/10.1016/j.ajps.2023.100854.

Firouzeh Morshedzadeh, et al.(2023). An Update on the Application of CRISPR Technology in Clinical Practice. *Molecular Biotechnology*, *66*. https://doi.org/10.1007/s12033-023-00724-z

Friends of the Earth. (2018, September 12). *NEW REPORT: Gene Editing in Agriculture Poses New Risks to Health, Environment*. Friends of the Earth. https://foe.org/news/gene-editing-risks-health-environment/

Genetic Literacy Project. (2019b, July 23). *United States: Germline / Embryonic*. Global Gene Editing Regulation Tracker. https://crispr-gene-editing-regs-tracker.geneticliteracyproject.org/united-states-embryonic-germline-gene-editing/

Gostimskaya, I. (2022). CRISPR–Cas9: A History of Its Discovery and Ethical Considerations of Its Use in Genome Editing. *Biochemistry (Moscow)*, *87*(8), 777–788. https://doi.org/10.1134/s0006297922080090

Hryhorowicz, Magdalena, et al. "Improved Delivery of CRISPR/Cas9 System Using Magnetic Nanoparticles Into Porcine Fibroblast." *Molecular Biotechnology*, vol. 61, no. 3, Dec. 2018, pp. 173–80. https://doi.org/10.1007/s12033-018-0145-9.

Ishino, Y., Krupovic, M., & Forterre, P. (2018). History of CRISPR-Cas from Encounter with a Mysterious Repeated Sequence to Genome Editing Technology. *Journal of Bacteriology*, *200*(7), e00580-17. https://doi.org/10.1128/JB.00580-17

Javed, M. R., et al. (2019). Current situation of biofuel production and its enhancement by CRISPR/Cas9-mediated genome engineering of microbial cells. *Microbiological Research*, *219*, 1–11. https://doi.org/10.1016/j.micres.2018.10.010

Kantor, Ariel, et al. "CRISPR-Cas9 DNA Base-Editing and Prime-Editing." *International Journal of Molecular Sciences*, vol. 21, no. 17, Aug. 2020, p. 6240. https://doi.org/10.3390/ijms21176240.

Liu, Q., et al. (2021). Application of CRISPR/Cas9 in Crop Quality Improvement. *International Journal of Molecular Sciences*, *22*(8), 4206. https://doi.org/10.3390/ijms22084206

McCarthy, S. (2023, July 3). *Controversial Chinese scientist He Jiankui proposes new gene editing research*. CNN. https://edition.cnn.com/2023/07/03/china/he-jiankui-gene-editing-proposal-china-intl

Movahedi, A., Aghaei-Dargiri, S., Li, H., Zhuge, Q., & Sun, W. (2023). CRISPR Variants for Gene Editing in Plants: Biosafety Risks and Future Directions. *International Journal of Molecular Sciences*, *24*(22), 16241. https://doi.org/10.3390/ijms242216241

National Human Genome Research Institute. (2017, August 3). *What are the Ethical Concerns of Genome Editing?* National Human Genome Research Institute; National Institutes of Health. https://www.genome.gov/about-genomics/policy-issues/Genome-Editing/ethical-concr

Ng, D. (2023, November 10). *A Brief History of CRISPR-Cas9 Genome-Editing Tools*. Bitesize Bio. https://bitesizebio.com/47927/history-crispr/

Nutrition, C. for F. S. and A. (2020b). How GMO Crops Impact Our World. *FDA*. https://www.fda.gov/food/agricultural-biotechnology/how-gmo-crops-impact-our-worl

PhD, A. A. S. (2023, April 3). *CRISPR-Made Greener Products and Processes in Industrial Biotechnology*. GEN - Genetic Engineering and Biotechnology News. https://www.genengnews.com/resources/crispr-made-greener-products-and-processes-in-industrial-biotechnology/

Ravichandran, M., & Maddalo, D. (2023). Applications of CRISPR-Cas9 for advancing precision medicine in oncology: from target discovery to disease modeling. *Frontiers in Genetics*, *14*. https://doi.org/10.3389/fgene.2023.1273994

Ronald, P., & Kliegman, M. (2023). *CRISPR in Agriculture*. Innovative Genomics Institute (IGI). https://innovativegenomics.org/crisprpedia/crispr-in-agriculture/

Rose, B. I., & Brown, S. (2019). Genetically Modified Babies and a First Application of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR-Cas9). *Obstetrics & Gynecology*, *134*(1), 1. https://doi.org/10.1097/aog.0000000000003327

Savulescu, J., Pugh, J., Douglas, T., & Gyngell, C. (2015c). The moral imperative to continue gene editing research on human embryos. *Protein & Cell*, *6*(7), 476–479. https://doi.org/10.1007/s13238-015-0184-y

Schmerker, J. (2024b, April 15). *CRISPR-Cas9: 10 pros and 7 cons*. Integrated DNA Technologies. https://sg.idtdna.com/pages/community/blog/post/crispr-cas9-what-are-the-10-pros-and-7-cons

Schreiber, K. (2019b, December 12). *India: Crops / Food*. Global Gene Editing Regulation Tracker. https://crispr-gene-editing-regs-tracker.geneticliteracyproject.org/india-crops-food/

Updates on Global Regulatory Landscape for Gene-Edited Crops. (n.d.). Science Speaks. https://www.isaaa.org/blog/entry/default.asp?BlogDate=1/24/2024

Wenham, L. (2023, September 6). *CRISPR in agriculture: Applications, benefits & risks*. Automata. https://automata.tech/blog/crispr-agriculture/

World Health Organization. (2023). *Human genome editing*. World Health Organization. https://www.who.int/health-topics/human-genome-editing#tab=tab

Zhu, H., Li, C., & Gao, C. (2020). Applications of CRISPR–Cas in agriculture and plant biotechnology. *Nature Reviews Molecular Cell Biology*, *21*(11), 661–677. https://doi.org/10.1038/s41580-020-00288-9