

CRISPER/CAS: A POTENTIAL TOOL FOR GENOMES EDITING

Sara Rauf¹, Aniqa Shahid², Muhammad Faizan², Muhammad Nouman Khalid^{3*} and Ifrah Amjad³

¹Department of Botany, University of Agriculture Faisalabad, Pakistan

²Department of Biochemistry, University of Agriculture, Faisalabad, Pakistan

³Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, Pakistan

*Corresponding author: noumankhalidpbg@gmail.com

ABSTRACT

This review paper explores the dynamic landscape of genome editing, with a primary focus on the revolutionary CRISPR/Cas technology and its potential to reshape the fields of biology, medicine, agriculture, and biotechnology. The paper is organized into six sections, each delving into essential aspects of CRISPR/Cas technology and its applications. The "Introduction" sets the stage, providing background information on genome editing and the emergence of CRISPR/Cas technology. It also defines the purpose and scope of the review, emphasizing the profound impact of this technology on the scientific community. The "CRISPR/Cas Mechanisms" section dives into the intricate workings of the CRISPR/Cas system, covering key components such as guide RNA, Cas proteins, PAM recognition, and donor DNA. It elucidates the mechanisms of DNA targeting and cleavage, highlighting the precision and versatility that have made CRISPR/Cas a transformative tool in genome editing. "Applications of CRISPR/Cas in Genome Editing" explores the wide-reaching impact of CRISPR/Cas technology, from its use in model organisms for gene function studies to its therapeutic applications in human health and its implications in agriculture and environmental conservation. In "Challenges and Limitations," the review addresses critical issues in CRISPR/Cas technology, including off-target effects and specificity, ethical and regulatory concerns, and technological limitations. It offers insights into ongoing efforts to mitigate these challenges. The "Emerging Techniques and Technologies" section discusses cutting-edge innovations, such as base editing, prime editing, CRISPR screens, functional genomics, multiplex genome editing, and synthetic biology. These emerging technologies expand the genome editing toolkit, paving the way for precise and versatile genetic modifications.

Keywords: CRISPR/Cas technology, Genome editing, Biotechnology

Article History (ABR-23-175) || Received: 17 Oct 2023 || Revised: 15 Nov 2023 || Accepted: 20 Nov 2023 || Published Online: 23 Nov 2023

This is an open-access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. INTRODUCTION

Genome editing, a revolutionary field in biotechnology and molecular biology, has transformed our ability to modify the genetic material of living organisms (Eskandar 2023). It holds immense promise for diverse applications, from treating genetic disorders to engineering crops for food security. Within the landscape of genome editing techniques, the emergence of CRISPR/Cas technology stands out as a pivotal milestone (Khurana et al. 2022). The concept of genome editing is not new; humans have been selectively breeding plants and animals for thousands of years. This process, however, has traditionally been slow and imprecise (Fraczek et al. 2018). It wasn't until the late 20th century that the field of molecular biology began to make significant strides in understanding and manipulating genetic material. In the early days, genome editing relied on techniques such as restriction enzymes and zinc finger nucleases, which were effective but had limitations (Yang and Blenner, 2020). These techniques often lacked the specificity and precision required for complex genetic modifications (Wang et al. 2018). Scientists sought a more efficient, accurate, and versatile method to manipulate DNA. The emergence of genome editing as a prominent field gained traction with the discovery of engineered nucleases, particularly the zinc finger nucleases and transcription activator-like effector nucleases (TALENs) (Dwivedi et al. 2022). These tools allowed researchers to target specific DNA sequences, but their design and assembly were laborious and costly (Brinegar et al. 2017). The breakthrough came with the discovery of the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and the CRISPR-associated (Cas) proteins (Al-Attar et al. 2011). This revolutionary system, derived from the adaptive immune system of bacteria and archaea, allows for precise and programmable editing of DNA (Bao et al. 2021).

The CRISPR/Cas system is made up of two main components: guide RNA (gRNA) and Cas proteins. The gRNA is designed to complement the target DNA sequence, guiding the Cas protein to the desired location in the

Citation: Rauf S, Shahid A, Faizan M, Khalid MN and Amjad I, 2024. CRISPER/CAS: A potential tool for genomes editing. *Agrobiological Records* 15: 13-23. <https://doi.org/10.47278/journal.abr/2023.044>

genome (Bao et al. 2021). Once the Cas protein binds to the DNA, it can cut the DNA at that specific site. This simple yet powerful system enables researchers to insert, delete, or replace specific DNA sequences with high precision. What makes CRISPR/Cas technology even more remarkable is its flexibility. Unlike its predecessors, it is relatively easy and cost-effective to design gRNAs for various DNA targets (McGinn and Marraffini 2019). This flexibility, coupled with high precision, has revolutionized the field of genome editing. The CRISPR/Cas system was first harnessed for genome editing in 2012, when Jennifer Doudna, Emmanuelle Charpentier, and their teams demonstrated its ability to cut DNA *in vitro*. The potential of this technology was immediately recognized, and researchers worldwide began to explore its applications. Since then, the CRISPR/Cas system has been continuously refined, leading to various adaptations and innovations, such as CRISPR/Cas9, CRISPR/Cas12, and CRISPR/Cas13, each with specific advantages and applications (Bondy-Denomy et al. 2015; Nussenzweig and Marraffini 2020).

The purpose of this review is to provide a comprehensive overview of the CRISPR/Cas technology's role as a powerful tool in genome editing. We aim to explore its origins, mechanisms, and its broad range of applications, covering diverse fields such as medicine, agriculture, and biotechnology. In this paper, we will delve into the intricate mechanisms of the CRISPR/Cas system, explaining how it precisely targets and edits DNA. We will also discuss the various CRISPR/Cas variants and their specific applications. Understanding the underlying science is crucial for appreciating the potential and limitations of this technology. Furthermore, we will explore the diverse applications of CRISPR/Cas technology. This includes its use in model organisms for understanding gene function and developing potential therapies for genetic diseases (Razaq et al. 2023; Kamal et al. 2019a; Kamal et al. 2019b). We will also discuss its application in agriculture, where it can be employed to engineer crops for increased yields, improved nutritional content, and resistance to pests and diseases. While the emergence of CRISPR/Cas technology has opened exciting possibilities, it has also raised important ethical, regulatory, and safety concerns. In this review we have addressed these challenges and the ongoing efforts to ensure responsible use of this powerful tool (Ali et al. 2023).

In addition, we will discuss emerging techniques and technologies related to genome editing, such as base editing, prime editing, CRISPR screens, and multiplex genome editing. These innovations build upon the CRISPR/Cas system and expand its capabilities, offering new avenues for research and applications. This review aims to provide a comprehensive and up-to-date understanding of CRISPR/Cas technology's potential, limitations, and future prospects in the realm of genome editing. By the end of this exploration, readers will have a thorough grasp of the groundbreaking impact of CRISPR/Cas technology and its continued evolution.

1.1. CRISPR/Cas Mechanisms

The CRISPR/Cas system is a groundbreaking tool in genome editing, revolutionizing our ability to precisely modify DNA sequences in various organisms (Hille and Charpentier 2016). To understand the full potential of this technology, it is essential to explore the intricate mechanisms that underlie it, from its key components to the methods by which it targets and cleaves DNA. This section provides an in-depth examination of the CRISPR/Cas mechanisms, offering insights into the system's versatility and its expanding range of applications (Rath et al. 2015).

The CRISPR/Cas system is composed of several essential components that work in harmony to facilitate precise genome editing. These components include Guide RNA (gRNA): The gRNA is a critical element of the CRISPR/Cas system. It is a short RNA molecule that has been designed to complement a specific target DNA sequence (Ali et al. 2023). This gRNA serves as a molecular beacon, guiding the Cas protein to the precise location in the genome that requires editing. The customizable nature of gRNA is a significant advantage, allowing researchers to target virtually any DNA sequence of interest (Waddington et al. 2016).

1.1.1. Cas Protein: The Cas protein, which stands for CRISPR-associated protein, is responsible for cutting the DNA at the target site. Different Cas proteins are associated with various CRISPR systems, and they exhibit varying cleavage capabilities (Haroon et al. 2023). Cas9 and Cas12 are two of the most commonly used Cas proteins, known for their precision in cleaving DNA at the target site. Once guided to the target by the gRNA, the Cas protein initiates a break in the DNA, initiating the process of DNA repair and modification (Xu and Li 2020).

1.1.2. Protospacer Adjacent Motif (PAM): The PAM is a small, specific DNA sequence adjacent to the target site that the Cas protein recognizes. The presence of a PAM sequence is crucial for the CRISPR/Cas system to initiate DNA cleavage. PAM sequences vary depending on the Cas protein used, contributing to the system's specificity (Gleditsch et al. 2019).

1.1.3. Donor DNA (optional): In some applications of CRISPR/Cas, researchers introduce donor DNA to facilitate precise genetic modifications. This donor DNA carries the desired genetic information and is integrated into the target site during the DNA repair process (Seruggia and Montolieu 2014).

Understanding these key components of the CRISPR/Cas system is essential for appreciating how the technology achieves precise genome editing. The customizable nature of the gRNA, the specificity of the Cas protein, and the role of PAM sequences collectively enable researchers to edit DNA with unprecedented precision (Zafar et al. 2022a).

1.2. Mechanisms of DNA Targeting and Cleavage

The mechanisms of DNA targeting and cleavage by the CRISPR/Cas system as presented in Fig. 1 are a testament to its elegance and precision (Wang et al. 2020). The process begins with the design of a gRNA that precisely matches the target DNA sequence. This step is highly customizable, allowing researchers to direct the Cas protein to virtually any location in the genome (Zafar et al. 2022b). Once the gRNA is designed, it binds to the Cas protein. The gRNA is designed to complement the target DNA sequence, serving as a guide. Simultaneously, the Cas protein is ready to cleave the DNA. The Cas protein, guided by the gRNA, searches for the presence of a PAM sequence adjacent to the target site. The PAM sequence acts as a recognition signal for the Cas protein. When the PAM is identified, the Cas protein binds to it. With the gRNA in place, the Cas protein guided to the PAM, and the target DNA sequence properly positioned, the Cas protein initiates a precise break in the DNA strand. This break triggers the cell's DNA repair machinery (Ren et al. 2019; Zhang et al. 2022). Cells have inherent DNA repair mechanisms that can be harnessed to introduce specific genetic modifications. Depending on the desired outcome, researchers can manipulate these repair mechanisms to insert, delete, or replace genetic material. The ability to guide the Cas protein to the target site with such precision is a hallmark of the CRISPR/Cas system (Dong et al. 2021). It allows researchers to carry out a wide range of genetic modifications with a high degree of accuracy, from correcting disease-causing mutations to engineering beneficial traits in crops (Knott and Doudna 2018). The success of CRISPR/Cas technology has led to the development of various CRISPR variants (Zafar et al. 2020; (Koonin and Makarova 2022), each with unique features and applications as presented in Table 1.

Table 1: Various CRISPR variants along with their applications

CRISPR Variants	Applications
CRISPR/Cas9	Perhaps the most well-known variant, CRISPR/Cas9 is versatile and widely used. It has applications in gene therapy, functional genomics, and synthetic biology. CRISPR/Cas9 is known for its precision in DNA cleavage and has been used in a myriad of research and clinical applications (Concordet and Haeussler 2018).
CRISPR/Cas12	This variant, also known as Cpf1, offers advantages in terms of smaller gRNA size and distinctive PAM recognition, expanding the range of targetable DNA sequences. It has applications in gene editing and diagnostic technologies (Broughton et al. 2020).
CRISPR/Cas13	Unlike Cas9 and Cas12, Cas13 does not cleave DNA but targets RNA, making it particularly valuable in RNA-focused research and diagnostic applications, such as detecting viral RNA (Abudayyeh et al. 2017).

1.2.1. Base Editing and Prime Editing: These advanced techniques, built upon the CRISPR/Cas system, enable the precise modification of individual DNA bases without causing double-strand breaks. Base editing and prime editing offer greater control over genetic alterations. The expanding family of CRISPR variants continues to diversify and enhance the capabilities of genome editing. Researchers are continually fine-tuning these variants and exploring their potential in a wide range of applications, from treating genetic diseases to advancing biotechnology and agriculture (Kantor et al. 2020). In conclusion, the CRISPR/Cas system's mechanisms, including its components, DNA targeting, and cleavage processes, are central to understanding the technology's profound impact on genome editing. With a firm grasp of these mechanisms, researchers can harness the versatility of the system and explore the expanding array of CRISPR variants, each with its unique applications and potential to shape the future of genetic modification (Zafar et al. 2020).

1.3. Applications of CRISPR/Cas in Genome

The advent of CRISPR/Cas technology has not only revolutionized the field of molecular biology but has also opened the door to a myriad of applications that impact various domains of science and industry. In this section, we explore the diverse applications of CRISPR/Cas in genome editing, including its use in model organisms for understanding gene function, its therapeutic potential in human health, and its far-reaching implications in agriculture and the environment (Bao et al. 2021).

1.4. Genome Editing in Model Organisms

Model organisms have long been critical in advancing our understanding of genetics, developmental biology, and disease mechanisms. CRISPR/Cas technology has significantly enhanced the precision and efficiency of genome editing in these organisms, allowing researchers to investigate gene function, model human diseases and

develop potential therapies. *Drosophila melanogaster* (Fruit Flies): Fruit flies have been a fundamental model organism in genetics research for decades. CRISPR/Cas has enabled researchers to create precise genetic modifications in fruit flies, facilitating studies on a wide range of biological processes, including embryonic development, circadian rhythms, and neurological disorders. *Caenorhabditis elegans* (Roundworms): Roundworms have provided valuable insights into the genetics of development and aging. With CRISPR/Cas technology, scientists can create specific mutations in roundworms, elucidating the roles of various genes and pathways in these fundamental processes (Ma and Liu 2015).

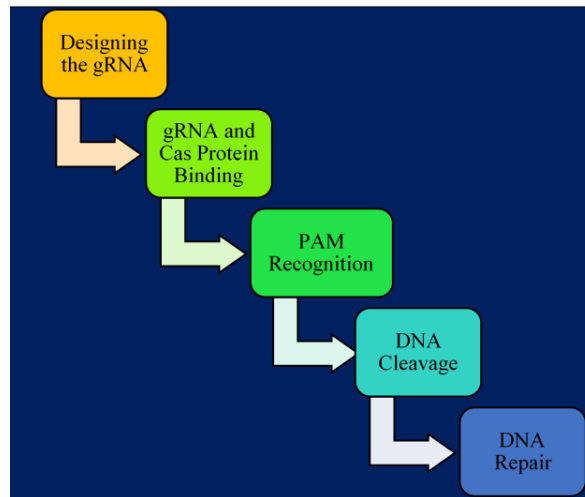


Fig. 1: An overview of DNA targeting and cleavage.

Zebrafish share genetic similarities with humans, making them an excellent model for studying vertebrate development and diseases. CRISPR/Cas has accelerated gene targeting in zebrafish, allowing researchers to generate precise genetic modifications and explore the underlying genetic mechanisms of various conditions, including cancer and cardiovascular diseases. The mouse model is a cornerstone in biomedical research. CRISPR/Cas technology has transformed the creation of genetically modified mice, allowing for the study of human diseases, the testing of potential therapies, and the development of mouse models that closely mimic human conditions. Beyond the mentioned models, CRISPR/Cas technology has been adapted for use in numerous other organisms, including yeast, bacteria, and plants. The ability to edit genes in these models expedites research in various fields, such as biotechnology, microbiology, and agriculture (Ali et al. 2023).

1.5. Therapeutic Applications in Human Health

One of the most promising and widely recognized applications of CRISPR/Cas technology is in the field of human health (Fig. 2). It has the potential to revolutionize the diagnosis and treatment of genetic diseases, offering hope for individuals suffering from a wide range of conditions (Liu et al. 2017).

1.6. Agricultural and Environmental Implications

CRISPR/Cas technology has substantial implications for agriculture and environmental conservation. It offers new tools for enhancing crop traits, mitigating environmental challenges, and promoting sustainability (Piergentili et al. 2021).

- CRISPR/Cas technology enables precise genome editing in crops. This means that scientists can create crops with desirable traits, such as improved yield, resistance to pests and diseases, and enhanced nutritional content. CRISPR-edited crops have the potential to address food security challenges and reduce the need for chemical pesticides.
- The precision of CRISPR/Cas technology is valuable in environmental conservation efforts. It can be used to modify organisms to combat invasive species, restore ecosystems, and protect endangered species.
- CRISPR/Cas technology can be applied to environmental bioremediation. This approach uses genetically engineered microorganisms to remove pollutants and contaminants from the environment, such as oil spills and toxic waste.

- CRISPR/Cas can be used to control disease vectors, such as mosquitoes that transmit malaria and other diseases. By editing the genes of these vectors, it is possible to reduce their ability to transmit diseases, ultimately benefiting human health and the environment (Chaudhry et al. 2023).

Genetic Diseases	Cancer Therapy	Infectious Diseases	Regenerative Medicine	Immunotherapy
<ul style="list-style-type: none"> • Conditions such as sickle cell anemia, cystic fibrosis, and muscular dystrophy are among the many genetic diseases that may benefit from CRISPR-based therapies (Zhang, 2021) 	<ul style="list-style-type: none"> • The precision of CRISPR/Cas editing allows for targeted cancer therapies. Researchers are developing strategies to edit cancer cells, making them more susceptible to treatment or even directly targeting the genetic mutations that drive cancer. This approach offers the potential for more effective and less invasive cancer treatments (Awwad et al., 2023) 	<ul style="list-style-type: none"> • CRISPR/Cas technology is being explored for its ability to combat infectious diseases. It has the potential to modify human cells to resist infections, offering a novel approach to prevent diseases caused by viruses, bacteria, and parasites 	<ul style="list-style-type: none"> • The ability to edit genes in stem cells and other regenerative tissues opens doors to regenerative medicine. This technology may be used to repair damaged organs, treat degenerative diseases, and develop personalized regenerative therapies. 	<ul style="list-style-type: none"> • CRISPR/Cas is integral to the development of immunotherapies. Researchers are using the technology to engineer immune cells, enhancing their ability to target and eliminate cancer cells and other disease-causing agents.

Fig. 2: Therapeutic applications in human health.

The applications of CRISPR/Cas technology in agriculture and the environment offer solutions to some of the most pressing challenges of our time, from feeding a growing global population to addressing climate change and biodiversity loss. In summary, the applications of CRISPR/Cas technology in genome editing are extensive and continually expanding. From its role in studying model organisms to its promise in therapeutic applications for human health and its far-reaching implications in agriculture and the environment, CRISPR/Cas technology represents a transformative tool that has the potential to reshape numerous aspects of science, medicine, and industry. As research and innovation in this field progress, the full impact of CRISPR/Cas on our world is yet to be fully realized, promising a future of exciting possibilities and breakthroughs (Khanna et al. 2023).

1.7. Challenges and Limitations

CRISPR/Cas technology has undoubtedly transformed the landscape of genetic engineering and genome editing, offering immense potential in various fields. However, alongside its remarkable capabilities, there are important challenges and limitations that need to be addressed. This section delves into the complexities of CRISPR/Cas technology, including off-target effects and specificity issues, ethical and regulatory concerns, and the current technological limitations while exploring the exciting future prospects (Bao et al. 2021).

1.8. Off-Target Effects and Specificity

One of the most significant challenges in CRISPR/Cas technology is off-target effects, which refer to unintended modifications to the genome. These off-target effects can occur when the CRISPR/Cas system, guided by the gRNA, inadvertently binds to and cleaves DNA sequences that closely resemble the target sequence. Such off-target alterations can have adverse consequences, potentially leading to genetic mutations and unwanted changes (Naeem et al. 2020). Researchers have taken several measures to enhance the specificity of CRISPR/Cas technology:

The design of the gRNA is a crucial factor in minimizing off-target effects. Advances in gRNA design software and algorithms aim to predict and reduce potential off-target sites, enhancing the accuracy of genome editing. Cas proteins with improved specificity have been developed, such as "high-fidelity" Cas9 variants (Zafar et al. 2020). These proteins exhibit a lower propensity for off-target cleavage, increasing the precision of genome editing. Various techniques, including high-throughput sequencing, have been employed to identify and characterize off-target effects. This allows researchers to assess the accuracy and safety of CRISPR/Cas-mediated edits more thoroughly. While significant progress has been made in minimizing off-target effects, the potential for unintended modifications remains a concern, particularly in clinical and therapeutic applications. Continued research and

development are necessary to enhance the system's specificity and reduce the risk of off-target effects further (Giordano 2023).

1.9. Ethical and Regulatory Concerns

The remarkable potential of CRISPR/Cas technology has ignited a series of ethical and regulatory discussions. Some of the primary ethical and regulatory concerns include: The ability to modify the human germline, including eggs and sperm, raises profound ethical questions. Germline editing has the potential to introduce genetic changes that will be inherited by future generations, and the consequences of such edits are uncertain. There are concerns about how CRISPR/Cas technology might exacerbate existing social and economic disparities. Access to gene therapies and enhancements may be unequal, potentially leading to genetic privilege (Valdés and Lecaros 2023). The release of genetically modified organisms into the environment raises ecological concerns. Unintended consequences of gene-edited organisms escaping into the wild could have far-reaching ecological implications. Ethical considerations also encompass the diversity and inclusivity of CRISPR/Cas research. Ensuring equitable representation and participation in research and access to potential therapies is crucial. CRISPR/Cas technology has dual-use potential, meaning it can be used for both beneficial and harmful purposes. Regulatory frameworks must address and mitigate potential risks associated with misuse, such as bioterrorism (DiEuliis and Giordano 2018). To address these ethical and regulatory concerns, there have been calls for international guidelines and cooperation in the responsible use of CRISPR/Cas technology. Many countries have established regulations governing the use of genome editing, particularly in clinical and human applications. These regulations aim to strike a balance between harnessing the technology's potential and ensuring ethical and responsible use (Fig. 3).

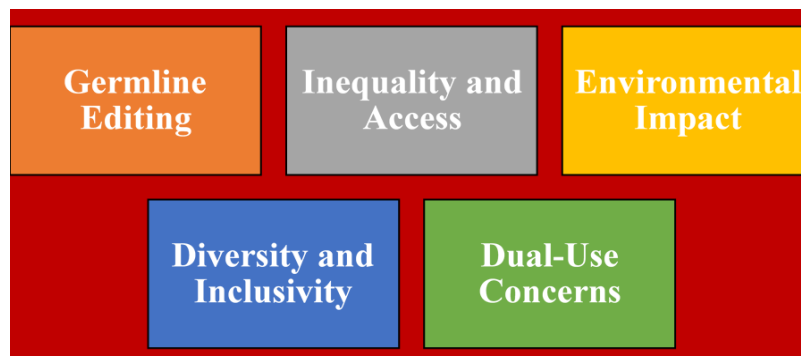


Fig. 3: Ethical and regulatory concerns.

1.10. Technological Limitations and Future Prospects

While CRISPR/Cas technology has rapidly advanced, it still faces certain technological limitations that researchers are actively addressing. Key technological challenges include efficient delivery of the CRISPR/Cas system into target cells remains a challenge. Developing safe and effective delivery methods for different tissues and cell types is an ongoing area of research. Inserting large DNA sequences with high precision is technically challenging. Many applications require the insertion of sizable genetic constructs, which necessitates further improvements in CRISPR/Cas technology. Currently, CRISPR/Cas technology primarily focuses on DNA editing. Enabling precise RNA editing could open up new avenues for the treatment of diseases that involve RNA, such as certain genetic disorders. The ability to make specific epigenetic modifications, such as DNA methylation changes, remains a challenge. Epigenetic editing is an emerging area with significant potential. In vivo genome editing, where the technology is applied directly inside a living organism, is still being refined. Improving the efficiency and safety of in vivo editing is crucial for medical applications. Despite these limitations, the future of CRISPR/Cas technology is highly promising. Ongoing research and innovation continue to advance the field, and researchers are exploring exciting prospects for the technology: CRISPR/Cas technology is likely to play a pivotal role in the development of personalized medicine. Tailored therapies for individuals based on their genetic profiles hold significant potential for treating a wide range of diseases. The ability to correct genetic defects and deliver therapeutic genes to target cells offers tremendous promise for the field of gene therapy. CRISPR/Cas is being harnessed to develop treatments for a variety of genetic disorders. CRISPR/Cas technology is poised to accelerate agricultural improvements. The development of crops with increased yield, nutritional content, and resistance to environmental stressors will be essential for addressing global food security (Seruggia and Montoliu 2014). CRISPR/Cas technology is revolutionizing biotechnology and industrial processes. It is being used to optimize biofuel production, biopharmaceuticals, and various other applications (Zhang, 2021). CRISPR/Cas technology is a

versatile tool for scientific exploration. It will continue to facilitate groundbreaking discoveries in fields such as genetics, developmental biology, and microbiology (Xu and Li 2020).

In conclusion, while CRISPR/Cas technology has opened new horizons in genome editing and genetic engineering, it is not without challenges and limitations. Addressing off-target effects and ensuring specificity, navigating ethical and regulatory concerns, and overcoming technological constraints are crucial for the responsible and effective application of this technology. Nevertheless, the future prospects for CRISPR/Cas remain extraordinarily promising, with potential applications spanning medicine, agriculture, biotechnology, and scientific discovery. As research and innovation continue, CRISPR/Cas technology is poised to transform how we approach genetic modifications and their impact on our world.

1.11. Emerging Techniques and Technologies

In the ever-evolving landscape of genome editing, emerging techniques and technologies continually expand the toolkit available to scientists and researchers. This section explores three cutting-edge innovations: base editing and prime editing, CRISPR screens and functional genomics, and multiplex genome editing combined with synthetic biology, offering insights into their potential, applications, and the exciting possibilities they bring to the field.

1.12. Base Editing and Prime Editing

While traditional CRISPR/Cas technology offers remarkable precision in genome editing, it primarily focuses on introducing double strand breaks in the DNA, which can result in random insertions and deletions during the repair process. Base editing and prime editing represent revolutionary advances that allow for more precise and controlled genetic modifications (Kantor et al. 2020).

1.12.1. Base Editing: Base editing techniques enable the direct conversion of one DNA base pair into another without causing double-strand breaks. This precise modification of individual base pairs is achieved through the fusion of Cas proteins with enzymes that can chemically alter the DNA base, effectively rewriting genetic information. Base editing is a powerful tool for correcting point mutations responsible for various genetic diseases.

1.12.2. Prime Editing: Prime editing takes precision to the next level by allowing researchers to directly write, or "edit," new genetic information into the DNA. This innovative technique combines Cas proteins with a prime editing guide RNA (pegRNA) that includes both the desired sequence to be inserted and a template for the edit. Prime editing offers the potential to correct a wide range of genetic mutations with high precision and minimal off-target effects.

Base editing and prime editing are groundbreaking because they expand the scope of genome editing, enabling the correction of specific mutations and the addition of new genetic information with unmatched precision. These techniques hold immense promise for the development of therapies for genetic diseases and the advancement of genetic research.

1.13. CRISPR Screens and Functional Genomics

CRISPR screens and functional genomics provide powerful tools for understanding gene function, gene interactions, and the roles of specific genes in various biological processes. These techniques are instrumental in identifying potential therapeutic targets and advancing our knowledge of genetics and biology (Hartenian and Doench 2015).

1.13.1. CRISPR Screens: CRISPR screens are high-throughput experiments that use CRISPR/Cas technology to systematically target and manipulate specific genes across an entire genome. By assessing the effects of gene knockouts or alterations on cellular or organismal phenotypes, researchers can identify genes that are essential for particular processes, such as cell growth, immune response, or cancer development.

1.13.2. Functional Genomics: Functional genomics aims to decipher the functions of genes and their interactions within biological systems. It involves a combination of techniques, including CRISPR screens, RNA interference (RNAi), and high-throughput sequencing. These methods collectively provide insights into the genetic basis of diseases, cellular processes, and regulatory networks.

The applications of CRISPR screens and functional genomics are broad, ranging from identifying potential drug targets to understanding complex diseases and biological pathways. These techniques empower researchers to conduct large-scale studies that would have been prohibitively labor-intensive and time-consuming using traditional methods.

1.14. Multiplex Genome Editing and Synthetic Biology

Multiplex genome editing and synthetic biology represent the fusion of CRISPR/Cas technology with the principles of genetic engineering, enabling the simultaneous modification of multiple genes and the creation of entirely new biological systems (Cobb et al. 2015).

1.14.1. Multiplex Genome Editing: Multiplex genome editing allows researchers to target and modify multiple genes within the same cell or organism simultaneously. This technique has a broad range of applications, including the development of complex genetic pathways, enhancing the efficiency of metabolic engineering, and accelerating synthetic biology projects. It is particularly valuable in biotechnology and industrial processes.

1.14.2. Synthetic Biology: Synthetic biology involves the creation of artificial biological systems or the redesign of existing biological components for specific purposes. CRISPR/Cas technology has significantly advanced synthetic biology by offering precise control over genetic modifications. Researchers can use CRISPR-based techniques to design and engineer genetic circuits, organisms, and biological products with a wide range of applications, from biofuel production to medical research. The integration of multiplex genome editing, and synthetic biology is revolutionizing biotechnology, providing the means to engineer organisms and systems for various industrial, medical, and environmental applications. Researchers can construct customized biological platforms tailored to specific needs, paving the way for innovative solutions in a wide array of fields. In summary, emerging techniques and technologies in genome editing are reshaping the landscape of genetics and biology. Base editing and prime editing offer unprecedented precision in genetic modifications, CRISPR screens and functional genomics provide insights into gene function and regulation on a genome-wide scale, and multiplex genome editing combined with synthetic biology are driving innovations in biotechnology and beyond. As these technologies continue to advance, they promise to unlock new possibilities for research, therapy, and industrial applications, reshaping our understanding of the genetic code and its potential applications.

1.15. Conclusion and Future Directions

The revolutionary CRISPR/Cas technology has undeniably transformed the field of genetic engineering and genome editing, offering immense potential across a spectrum of applications. As we conclude this review, we'll summarize the exceptional potential of CRISPR/Cas, assess the current state of genome editing, and explore the exciting future prospects and areas for further research in this rapidly evolving field.

1.16. Summary of CRISPR/Cas Potential

The potential of CRISPR/Cas technology is multifaceted and profound. This innovative system has enabled precise and customizable genome editing, ushering in a new era of biotechnology and genetic engineering. Some of the key highlights of CRISPR/Cas potential include:

CRISPR/Cas technology allows for precise modifications to the genetic code, whether it's to correct disease-causing mutations, enhance crop traits, or engineer novel biological systems. Its versatility in targeting and editing genes is a hallmark of its potential (Dwivedi et al. 2022). CRISPR/Cas holds enormous promise in the development of gene therapies, where it can be used to treat a wide range of genetic disorders, including those that were previously considered untreatable (Dwivedi et al. 2022). Technology's role in agriculture is transformational. It enables the development of crops with increased yield, resistance to pests and diseases, and enhanced nutritional content, addressing global food security challenges. CRISPR/Cas technology is catalyzing biotechnological advances in areas such as biofuel production, biopharmaceuticals, and industrial processes, offering solutions for sustainable practices. In the realm of scientific research, CRISPR/Cas technology facilitates groundbreaking discoveries in genetics, developmental biology, microbiology, and more. It empowers researchers to explore new frontiers in our understanding of the genetic code.

1.17. Current State of Genome Editing

The current state of genome editing is characterized by remarkable achievements, growing understanding of CRISPR/Cas technology, and ongoing research efforts to address its challenges and limitations. Some key developments include: CRISPR-based therapies are advancing through clinical trials, offering hope to patients with genetic diseases (Metzger et al. 2023). The first-ever clinical trials involving CRISPR/Cas technology have demonstrated safety and efficacy in specific applications. Genome editing has made significant strides in agriculture. CRISPR-edited crops are being developed to address the world's growing population and changing environmental conditions (Mahler et al. 2023). These crops have the potential to enhance food security and sustainability. Countries around the world are establishing regulatory frameworks to govern the use of genome editing, particularly in the context of clinical applications and agriculture. These regulations aim to balance the benefits of technology with ethical and safety considerations. Efforts to improve the precision of CRISPR/Cas

technology are ongoing. Researchers are working to minimize off-target effects and enhance the specificity of genetic modifications (Hussen et al. 2023). Alongside CRISPR/Cas, emerging technologies such as base editing, prime editing, and multiplex genome editing are expanding the toolkit for genome editing, offering new possibilities for precise genetic modifications (Dong et al. 2021).

1.18. Future Prospects and Areas for Further Research

The future prospects of genome editing are exceptionally promising, with several exciting areas for further research and exploration: The development of personalized treatments based on individual genetic profiles is a rapidly advancing field. Genome editing will play a pivotal role in the future of precision medicine, offering tailored therapies for a wide range of diseases. Gene therapy, a field where genome editing plays a crucial role, is poised for remarkable growth. Innovations in gene therapy are expected to lead to effective treatments for a broader range of genetic disorders. As global challenges related to food security and climate change persist, CRISPR-edited crops are expected to play a significant role in agricultural sustainability (Wang and Doudna, 2023). Researchers will continue to develop crops with improved characteristics and resilience. Genome editing holds promise for addressing environmental challenges, such as invasive species and habitat restoration. It offers tools for protecting biodiversity and mitigating ecological issues. Biotechnology is on the cusp of transformation, with genome editing techniques offering more precise and versatile methods for a range of applications, from biofuel production to the development of novel biopharmaceuticals. Ongoing efforts will focus on refining regulatory and ethical frameworks to ensure the responsible use of genome editing, especially in clinical applications and agriculture (Kumar et al. 2023). Ensuring equitable access and addressing dual-use concerns will be central to these frameworks. Genome editing technologies will continue to drive scientific discoveries across various fields of biology (Fig. 4). They will be instrumental in uncovering the mechanisms of disease, the intricacies of genetics, and the potential for novel therapeutic targets (Liu et al. 2017; Wang et al. 2018; Wang et al. 2020; Broughton et al. 2020; Dong et al. 2021; Zhang 2021).

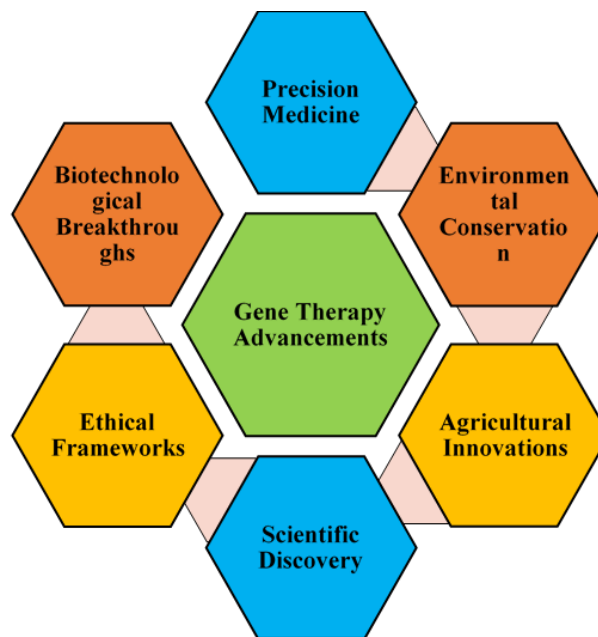


Fig. 4: Future prospects and areas for further research.

2. CONCLUSION

CRISPR/Cas technology has opened unprecedented possibilities in genome editing and genetic engineering. Its potential is far-reaching, from treating genetic diseases to enhancing agriculture and addressing environmental challenges. The current state of genome editing reflects substantial progress, but there are still challenges to address and room for improvement. As we look to the future, the prospects for genome editing are exceptionally bright. Precision medicine, gene therapy, agricultural innovations, and environmental conservation are just a few of the areas where CRISPR/Cas technology is set to make a significant impact. With ongoing research and innovation, we can expect to witness groundbreaking developments that will continue to redefine the boundaries of what is possible in the world of genetics and biology. The journey of genome editing is far from over, and its potential is poised for realization in the years to come.

REFERENCES

- Abudayyeh O O, Gootenberg J S, Essletzbichler P, Han S, Joung J, Belanto J J, Verdine V, Cox D B, Kellner M J and Regev A, 2017. RNA targeting with CRISPR–Cas13. *Nature*, 550(7675), 280-284.
- Al-Attar S, Westra E R, Van Der Oost J and Brouns S J, 2011. Clustered regularly interspaced short palindromic repeats (CRISPRs): the hallmark of an ingenious antiviral defense mechanism in prokaryotes.
- Ali A, Zafar M M, Farooq Z, Ahmed S R, Ijaz A, Anwar Z and Maozhi R, 2023. Breakthrough in CRISPR/Cas system: Current and future directions and challenges. *Biotechnology Journal*, 2200642.
- Ali M, Shaikat F, Khan W, Syed A, Maqsood J, Kamal H and Zafar M M, 2023. Microsatellite-based diversity analysis and the development of core-set germplasm in Pakistani barley lines. *Cellular and Molecular Biology*, 69(10), 100-108.
- Bao M, Chen Q, Xu Z, Jensen E C, Liu C, Waitkus J T, Yuan X, He Q, Qin P and Du K, 2021. Challenges and opportunities for clustered regularly interspaced short palindromic repeats based molecular biosensing. *ACS sensors*, 6(7), 2497-2522.
- Bondy-Denomy J, Garcia B, Strum S, Du M, Rollins M F, Hidalgo-Reyes Y, Wiedenheft B, Maxwell K L and Davidson A R, 2015. Multiple mechanisms for CRISPR–Cas inhibition by anti-CRISPR proteins. *Nature*, 526(7571), 136-139.
- Brinegar K, Yetisen KA, Choi S, Vallillo E, Ruiz-Esparza G U, Prabhakar A M, Khademhosseini A and Yun S-H, 2017. The commercialization of genome-editing technologies. *Critical Reviews in Biotechnology*, 37(7), 924-932.
- Broughton J P, Deng X, Yu G, Fasching C L, Servellita V, Singh J, Miao X, Streithorst J A, Granados A and Sotomayor-Gonzalez A, 2020. CRISPR–Cas12-based detection of SARS-CoV-2. *Nature Biotechnology*, 38(7), 870-874.
- Chaudhry A, Hassan A U, Khan S H, Abbasi A, Hina A, Khan M T and Abdelsalam N R, 2023. The changing landscape of agriculture: role of precision breeding in developing smart crops. *Functional & Integrative Genomics*, 23(2), 167.
- Cobb R E, Wang Y and Zhao H, 2015. High-efficiency multiplex genome editing of *Streptomyces* species using an engineered CRISPR/Cas system. *ACS Synthetic Biology*, 4(6), 723-728.
- Concordet J-P and Haeussler M, 2018. CRISPOR: intuitive guide selection for CRISPR/Cas9 genome editing experiments and screens. *Nucleic Acids Research*, 46(W1), W242-W245.
- DiEuliis D and Giordano J, 2018. Gene editing using CRISPR/Cas9: implications for dual-use and biosecurity. *Protein & Cell*, 9(3), 239-240.
- Dong H, Cui Y and Zhang D, 2021. CRISPR/cas technologies and their applications in *Escherichia coli*. *Frontiers in Bioengineering and Biotechnology*, 9, 762676.
- Dwivedi S, Purohit P, Vasudeva A, Kumar M, Agrawal R, Sheikh N A, Misra R, Kishore S and Misra S, 2022. Gene therapy and gene editing in healthcare. In *Biotechnology in Healthcare* (pp. 147-175). Elsevier.
- Eskandar K, 2023. Revolutionizing biotechnology and bioengineering: unleashing the power of innovation. *J Appl Biotechnol Bioeng*, 10(3), 81-88.
- Fraczek M G, Naseeb S and Delneri D, 2018. History of genome editing in yeast. *Yeast*, 35(5), 361-368.
- Giordano A, 2023. From the lab to the field: CRISPR/Cas addressing challenges in agriculture. *Journal of Experimental Botany*, 74(12), 3399-3401.
- Gleditzsch D, Pausch P, Müller-Esparza H, Özcan A, Guo X, Bange G and Randau L, 2019. PAM identification by CRISPR-Cas effector complexes: diversified mechanisms and structures. *RNA Biology*, 16(4), 504-517.
- Haroon M, Tariq H, Afzal R, Anas M, Nasar S, Kainat N and Zafar M M, 2023. Progress in genome-wide identification of RBPs and their role in mitigating stresses, and growth in plants. *South African Journal of Botany*, 160, 132-146.
- Hartenian E and Doench J G, 2015. Genetic screens and functional genomics using CRISPR/Cas9 technology. *The FEBS Journal*, 282(8), 1383-1393.
- Hille F and Charpentier E, 2016. CRISPR-Cas: biology, mechanisms and relevance. *Philosophical transactions of the royal society B: Biological Sciences*, 371(1707), 20150496.
- Hussen BM, Rasul M F, Abdullah S R, Hidayat H J, Faraj G S H, Ali F A and Taheri M, 2023. Targeting miRNA by CRISPR/Cas in cancer: advantages and challenges. *Military Medical Research*, 10(1), 32.
- Kamal, H., Minhas, F. U. A. A., Farooq, M., Tripathi, D., Hamza, M., Mustafa, R., ... & Amin, I. (2019). In silico prediction and validations of domains involved in *Gossypium hirsutum* SnRK1 protein interaction with cotton leaf curl Multan betasatellite encoded β CI. *Frontiers in Plant Science*, 10, 656.
- Kamal, H., Minhas, F. U. A. A., Tripathi, D., Abbasi, W. A., Hamza, M., Mustafa, R., ... & Amin, I. (2019). β CI, pathogenicity determinant encoded by Cotton leaf curl Multan betasatellite, interacts with calmodulin-like protein 11 (Gh-CML11) in *Gossypium hirsutum*. *PLoS One*, 14(12), e0225876.
- Kantor A, McClements M E and MacLaren R E, 2020. CRISPR-Cas9 DNA base-editing and prime-editing. *International Journal of Molecular Sciences*, 21(17), 6240.
- Khanna K, Ohri P and Bhardwaj R, 2023. Nanotechnology and CRISPR/Cas9 system for sustainable agriculture. *Environmental Science and Pollution Research*, 1-16.
- Khurana A, Sayed N, Singh V, Khurana I, Allawadhi P, Rawat P S, Navik U, Pasumarthi S K, Bharani K K and Weiskirchen R, 2022. A comprehensive overview of CRISPR/Cas 9 technology and application thereof in drug discovery. *Journal of Cellular Biochemistry*, 123(10), 1674-1698.
- Knott G J and Doudna J A, 2018. CRISPR-Cas guides the future of genetic engineering. *Science*, 361(6405), 866-869.
- Koonin E V and Makarova K S, 2022. Evolutionary plasticity and functional versatility of CRISPR systems. *PLoS Biology*, 20(1), e3001481.
- Kumar M, Prusty M R, Pandey M K, Singh P K, Bohra A, Guo B and Varshney R K, 2023. Application of CRISPR/Cas9-mediated gene editing for abiotic stress management in crop plants. *Frontiers in Plant Science*, 14, 1157678.

- Liu C, Zhang L, Liu H and Cheng K, 2017. Delivery strategies of the CRISPR-Cas9 gene-editing system for therapeutic applications. *Journal of Controlled Release*, 266, 17-26.
- Ma D and Liu F, 2015. Genome editing and its applications in model organisms. *Genomics, Proteomics & Bioinformatics*, 13(6), 336-344.
- Mahler M, Costa A R, van Beljouw S P, Fineran P C and Brouns S J, 2023. Approaches for bacteriophage genome engineering. *Trends in Biotechnology*, 41(5), 669-685.
- McGinn J and Marraffini L A, 2019. Molecular mechanisms of CRISPR-Cas spacer acquisition. *Nature Reviews Microbiology*, 17(1), 7-12.
- Metzger J M, Wang Y, Neuman S S, Snow K J, Murray S A, Lutz C M and Gong S, 2023. Efficient in vivo neuronal genome editing in the mouse brain using nanocapsules containing CRISPR-Cas9 ribonucleoproteins. *Biomaterials*, 293, 121959.
- Naeem M, Majeed S, Hoque M Z and Ahmad I, 2020. Latest developed strategies to minimize the off-target effects in CRISPR-Cas-mediated genome editing. *Cells*, 9(7), 1608.
- Nussenzweig P M and Marraffini L A, 2020. Molecular mechanisms of CRISPR-Cas immunity in bacteria. *Annual Review of Genetics*, 54, 93-120.
- Piergentili R, Del Rio A, Signore F, Umani Ronchi F, Marinelli E and Zaami S, 2021. CRISPR-Cas and its wide-ranging applications: From human genome editing to environmental implications, technical limitations, hazards and bioethical issues. *Cells*, 10(5), 969.
- Rath D, Amlinger L, Rath A and Lundgren M, 2015. The CRISPR-Cas immune system: biology, mechanisms and applications. *Biochimie*, 117, 119-128.
- Razzaq A, Ali A, Zahid S, Malik A, Pengtao L, Gong W and Zafar M M, 2023. Engineering of cry genes "CryII and CryIh" in cotton (*Gossypium hirsutum* L.) for protection against insect pest attack. *Archives of Phytopathology and Plant Protection*, 56(5), 384-396.
- Ren M, Zafar M M, Mo H, Yang Z and Li F, 2019. Fighting against fall armyworm by using multiple genes pyramiding and silencing (MGPS) technology. *Sci China Life Science*, 62(12), 1703-6.
- Seruggia D and Montoliu L, 2014. The new CRISPR-Cas system: RNA-guided genome engineering to efficiently produce any desired genetic alteration in animals. *Transgenic Research*, 23, 707-716.
- Valdés E and Lecaros J A, 2023. Ethical Issues in Genetically Modified Foods: From Transgenesis to CRISPR-Cas9 Genome Editing Technology. In *Handbook of Bioethical Decisions. Volume I: Decisions at the Bench* (pp. 723-736). Cham: Springer International Publishing.
- Waddington S N, Privolizzi R, Karda R and O'Neill H C, 2016. A broad overview and review of CRISPR-Cas technology and stem cells. *Current stem cell reports*, 2, 9-20.
- Wang J Y and Doudna J A, 2023. CRISPR technology: A decade of genome editing is only the beginning. *Science*, 379(6629), eadd8643.
- Wang L, Deng A, Zhang Y, Liu S, Liang Y, Bai H, Cui D, Qiu Q, Shang X and Yang Z, 2018. Efficient CRISPR-Cas9 mediated multiplex genome editing in yeasts. *Biotechnology for Biofuels*, 11, 1-16.
- Wang M, Zhang R and Li J, 2020. CRISPR/cas systems redefine nucleic acid detection: principles and methods. *Biosensors and Bioelectronics*, 165, 112430.
- Xu Y and Li Z, 2020. CRISPR-Cas systems: Overview, innovations and applications in human disease research and gene therapy. *Computational and Structural Biotechnology Journal*, 18, 2401-2415.
- Yang Z and Blenner M, 2020. Genome editing systems across yeast species. *Current opinion in Biotechnology*, 66, 255-266.
- Zafar M M, Razzaq A, Farooq M A, Rehman A, Firdous H, Shakeel A and Ren M, 2020. Insect resistance management in *Bacillus thuringiensis* cotton by MGPS (multiple genes pyramiding and silencing). *Journal of Cotton Research*, 3(1), 1-13.
- Zafar M M, Mustafa G, Shoukat F, Idrees A, Ali A, Sharif F and Li F, 2022a. Heterologous expression of cry3Bb1 and cry3 genes for enhanced resistance against insect pests in cotton. *Scientific Reports*, 12(1), 10878.
- Zafar M M, Rehman A, Razzaq A, Parvaiz A, Mustafa G, Sharif F and Ren M, 2022b. Genome-wide characterization and expression analysis of Erf gene family in cotton. *BMC Plant Biology*, 22(1), 134.
- Zhang B, 2021. CRISPR/Cas gene therapy. *Journal of Cellular Physiology*, 236(4), 2459-2481.
- Zhang Y, Li W, Hu Y, Ding T, Zafar M M, Jia X and Wang W, 2022. Cotton flower metabolites inhibit SARS-CoV-2 main protease. *FEBS Open Bio*, 12(10), 1886-1895.