

The discovery and development of Cas12A

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Abstract. CRISPR-Cas12a stands as a transformative tool in genome editing, offering unparalleled precision and efficiency. Its utilization of an RNA-guided Cas12a enzyme enables targeted double-strand DNA breaks, allowing for the manipulation of genes with remarkable accuracy. Notable features such as high specificity, multi-target editing capabilities, and a variety of Cas12a variants provide researchers with a versatile toolkit for diverse applications. From aiding in drug discovery through large-scale genetic screens to enabling precise corrections of disease-causing mutations in gene therapy, CRISPR-Cas12a holds immense potential across various fields. However, challenges such as off-target effects, lower editing efficiency compared to Cas9, and financial limitations pose significant considerations. Stringent biosecurity measures and ethical dilemmas surrounding germline editing and accessibility further underscore the need for cautious advancement. Nonetheless, CRISPR-Cas12a continues to pave the way for transformative advancements in biotechnology, medicine, and our understanding of biology.

Keywords: High Specificity; T-rich PAM sequence; Off-target effects; Multi-target Editing; crRNA array.

1. Introduction

CRISPR, an acronym for Clustered Regularly Interspaced Short Palindromic Repeats, represents a groundbreaking discovery in the field of molecular biology. Initially identified as a bacterial immune system mechanism to defend against viral infections, CRISPR has since been repurposed into a revolutionary genome editing tool [1]. By harnessing the precise targeting capabilities of CRISPR-associated (Cas) enzymes, particularly Cas9 and Cas12a, scientists can now manipulate DNA with unprecedented accuracy and efficiency. This technology holds immense potential for applications ranging from basic research to therapeutic interventions, offering hope for treating genetic disorders, combating infectious diseases, and advancing our understanding of biological systems [1]. The versatility and accessibility of CRISPR have sparked a paradigm shift in genetic engineering, paving the way for transformative advancements in biotechnology and medicine.

PAM stands for Protospacer Adjacent Motif. It's a short, specific sequence of nucleotides that is crucial for the function of certain CRISPR-associated (Cas) enzymes, particularly in the CRISPR-Cas9 and CRISPR-Cas12a systems [2]. In the context of CRISPR genome editing, PAM sequences are essential because they serve as recognition sites for the Cas enzymes to bind to the target DNA. Once the Cas enzyme locates the PAM sequence adjacent to the target DNA sequence, it initiates the process of DNA cleavage or modification. The PAM sequence requirements differ depending on the specific Cas enzyme being used. For example, the commonly used Cas9 enzyme from *Streptococcus pyogenes* recognizes a specific PAM sequence of "NGG" (where N represents any nucleotide), while Cas12a (Cpf1) recognizes a different PAM sequence, typically "TTTN." These PAM sequences provide specificity to the CRISPR-Cas system, ensuring that the Cas enzyme only binds and acts on the intended target DNA sequence, minimizing off-target effects and enhancing the precision of genome editing [2]. Understanding the PAM sequence requirements is crucial for designing effective CRISPR-based experiments and applications.

CRISPR-Cas12a, a novel RNA-guided endonuclease derived from prokaryotic adaptive immunity, has emerged as a promising alternative to the well-established CRISPR-Cas9 system for genome editing applications. Its relaxed PAM requirement, T-rich PAM recognition, and simplified single-guide RNA structure offer distinct advantages over CRISPR-Cas9, expanding the targetable genomic regions and simplifying the design and delivery of editing constructs. With its staggered cleavage

mechanism and smaller size, CRISPR-Cas12a presents opportunities for precise DNA manipulation and packaging into viral vectors for delivery [3]. While efforts continue to mitigate off-target effects, CRISPR-Cas12a has already demonstrated utility in various genome editing applications, including gene knockout, gene insertion, and base editing. As research progresses, CRISPR-Cas12a is poised to further revolutionize the field of molecular biology, offering new avenues for precise and efficient genome manipulation.

2. The DEVELOPMENT of CRISPR-Cas12a BASED TECHNOLOGY

2.1. What is the technology, how does it work?

Cas12a, also known as Cpf1, stands as a prominent member of the class 2 CRISPR-associated endonucleases, contributing significantly to the field of genome editing. Its functionality relies on the presence of a guide RNA (gRNA), a synthetic molecule designed to guide the Cas12a enzyme to a specific DNA sequence. Upon finding the target sequence, Cas12a initiates binding to the DNA, prompting the creation of a staggered double-strand break – a departure from the simultaneous cleavage characteristic of Cas9, another well-known CRISPR enzyme. Following this initial cut, Cas12a undergoes a distinctive conformational change, activating its nonspecific endonuclease activity. This property enables Cas12a not only to cleave the target DNA but also nearby non-targeted single-stranded DNA, demonstrating a capacity for collateral cleavage. The collateral cleavage activity has proven to be advantageous in diagnostic applications, where it has been exploited for the detection of specific DNA sequences. The versatility of Cas12a, with its unique cleavage properties, positions it as a potent tool in genetic engineering, offering novel opportunities for precision modifications within the CRISPR-Cas system. Researchers and scientists continue to explore and harness its capabilities for a broad range of applications, further expanding the landscape of genome editing technologies.

The journey begins with the design and synthesis of a guide RNA (gRNA), complementary to the target DNA sequence of interest. Guided by the gRNA, Cas12a searches for a protospacer adjacent motif (PAM) sequence in the target DNA, triggering a conformational change in Cas12a that allows it to bind to the DNA [3]. Once bound, Cas12a exhibits endonuclease activity, cleaving the target DNA in a staggered manner and generating fragments with sticky ends. The resulting double-strand breaks can be repaired by the cell's machinery through the error-prone non-homologous end joining (NHEJ) pathway or the precise homology-directed repair (HDR) pathway [3]. Depending on the repair mechanism, genetic modifications are introduced, leading to altered gene expression or function. These modifications can result in gene disruptions, knockouts, or the precise introduction of desired changes. Cas12a's unique features, including its recognition of diverse PAM sequences and generation of staggered cuts, make it a versatile and powerful tool for genome editing, with broad applications in biotechnology, medicine, and functional genomics.

Furthermore, CAS12A stands out in the field of genome editing due to its ability to facilitate site-directed integration, thanks to staggered overhangs. Research conducted on *F. novicida* and *L. bacterium* ND2006 nucleases, both variants of Cas12a, has revealed a significant occurrence of homology-directed repair (HDR) in rice. This suggests that Cas12a holds an advantage over Cas9 in the context of targeted gene integration. The mutation efficiencies vary based on the selected PAM sequence, ranging from 10% to 35% with "TTTV" and 5–10% with "TTV" in rice [4]. However, direct comparisons with Cas9 are challenging due to inherent variations in site-specific editing efficiencies.

2.2. Who discovered it? How was it discovered?

The discovery of the CRISPR-Cas12a system, also known as Cpf1, was made by Dr. Jennifer Doudna and Dr. Emmanuelle Charpentier, however, the adaptation of Cas12a as a gene-editing tool for targeted genome editing was developed by Dr. Feng Zhang's research group. Dr. Zhang's team

demonstrated the utility of CRISPR-Cas12a for gene editing in human cells, and their work was published in the journal *Cell* in 2015.

Dr. Jennifer Doudna is a biochemist and professor of Chemistry and Molecular and Cell Biology at the University of California, Berkeley. Born on February 19, 1964, in Washington, D.C., she is renowned for her groundbreaking work on the CRISPR-Cas9 gene editing system. Along with Emmanuelle Charpentier, she co-discovered the CRISPR-Cas9 technology, a revolutionary method for precise gene editing [5]. The discovery has had profound implications for genetic research, medicine, and biotechnology.

2.3. Pioneers in the discovery of CRISPR-Cas12a system

2.3.1. Who is Dr. Jennifer Doudna

Doudna's journey in academia has been marked by a pursuit of knowledge and a commitment to advancing our understanding of molecular processes. Her work has been important in revealing the specifics of RNA and DNA, with a particular focus on the development and applications of CRISPR-based technologies.

Jennifer Doudna has received numerous awards and honors for her contributions to science, including the Breakthrough Prize in Life Sciences, the Japan Prize, and the Kavli Prize in Nanoscience.

Beyond her research, Doudna is deeply committed to education and advocacy. Actively involved in teaching, she plays a crucial role in mentoring the next generation of scientists and instilling in them a sense of responsibility in the application of technologies. She has also been a vocal advocate for ethical considerations in gene editing technologies, participating in discussions and initiatives to ensure responsible and transparent practices in the field.

2.3.2. Who is Dr. Emmanuelle Charpentier?

Dr. Emmanuelle Charpentier was born in Juvisy-sur-Orge, France, and she pursued her academic career with a focus on microbiology and biochemistry. She earned her Ph.D. in microbiology from the Institut Pasteur in Paris in 1995 [6]. Over the years, she has held research positions at several renowned institutions, including the Max Planck Institute for Infection Biology in Berlin.

One of the most significant achievements in Dr. Charpentier's career came in collaboration with Dr. Jennifer Doudna. In 2012, they co-published a groundbreaking paper in the journal *Science*, where they detailed the development and application of the CRISPR-Cas9 gene-editing system. This technology allows researchers to precisely edit genes, offering unprecedented potential for treating genetic diseases, modifying organisms for agricultural purposes, and advancing our understanding of biology.

The CRISPR-Cas9 breakthrough has not only led to numerous scientific advancements but has also gained widespread attention for its ethical implications. As a result, both Dr. Charpentier and Dr. Doudna have been actively engaged in discussions surrounding the responsible use of gene-editing technologies, emphasizing the importance of ethical considerations and regulatory frameworks.

In recognition of their work, Dr. Charpentier and Dr. Doudna were awarded the 2020 Nobel Prize in Chemistry [4]. This prestigious honor highlighted the transformative impact of their research on the field of molecular biology and its potential to reshape the future of medicine and biotechnology.

2.3.3. Who is Dr. Feng Zhang?

Dr. Feng Zhang is a Chinese-American bioengineer and professor known for his pioneering work in the field of genome editing using CRISPR-Cas9 technology. Born on October 22, 1981, in Shijiazhuang, China, Zhang has made significant contributions to the development and application of CRISPR-based gene-editing tools.

Cas12a, initially known as Cpf1, emerged through computational biology methods pioneered by Dr. Eugene Koonin, a colleague of Dr. Zhang. This approach involved meticulous analysis of bacterial and archaeal genomes to identify sequences bearing resemblance to known CRISPR systems. Dr. Zhang's lab, however, primarily focused on refining the Cas9 system for effective use in human

cells during the early 2010s, coinciding with the discovery of Cas12a by Dr. Koonin. While Dr. Zhang's team was not directly involved in the initial discovery of Cas12a, their groundbreaking work on Cas9 optimization laid the foundation for the broader advancement of CRISPR technology.

Zhang's significant contribution lies in pioneering the practical application of CRISPR-Cas9 for precise gene editing, which revolutionized the field of molecular biology. By developing innovative methods to deliver and utilize CRISPR-Cas9 in human cells, his lab made this powerful gene editing tool more accessible and impactful for researchers worldwide. While Cas12a was not the primary focus of Zhang's lab, their expertise and contributions likely played a pivotal role in elucidating the potential of Cas12a as an alternative gene editing tool. This collaborative effort underscores the interdisciplinary nature of scientific progress, where computational biology insights, combined with experimental research, drive innovation and advancement in gene editing technologies [7].

2.4. How have people used it for drug discovery?

Researchers utilize Cas12a for large-scale functional genomics screens to identify genes associated with specific diseases or drug responses, facilitating the discovery of potential drug targets or pathways. In drug target validation, Cas12a is employed to precisely manipulate target genes, confirming their relevance before investing in further drug development efforts. Additionally, Cas12a aids in drug resistance studies by introducing specific mutations associated with resistance, enabling the investigation of resistance mechanisms and the development of strategies to overcome it. The technology is also instrumental in disease modeling, allowing for the creation of cellular or animal models that mimic specific diseases, facilitating a deeper understanding of disease mechanisms and the testing of potential therapeutics. Cas12a's use in generating functional genomic libraries provides a systematic approach to studying the effects of gene perturbations on cellular processes, aiding in the identification of novel drug targets. Furthermore, its adaptation for epigenome editing allows researchers to modify epigenetic marks on genes, contributing to the study of the role of epigenetics in diseases and the identification of potential epigenetic drug targets.

2.5. Pros vs Cons

Cas12a's elevated specificity, primarily attributed to its strict adherence to a T-rich protospacer adjacent motif (PAM), underscores its remarkable precision in targeting DNA sequences [8]. This stringent requirement enables Cas12a to discriminate effectively between intended genomic loci and off-target sites, minimizing the risk of unintended genetic alterations [9]. By predominantly recognizing T-rich PAM sequences, Cas12a exhibits a reduced propensity for off-target cleavage events compared to other CRISPR systems, such as Cas9. This heightened specificity enhances the safety and efficacy of gene editing procedures, particularly in therapeutic contexts where precision is paramount. As a result, Cas12a emerges as a promising tool for precise genome editing, offering greater confidence in the accurate modification of target genes while mitigating the potential for unintended genetic changes.

The enhanced precision afforded by Cas12a's specificity not only promotes the accuracy of gene editing but also expands the scope of potential therapeutic applications. With its reduced off-target effects, Cas12a holds considerable promise for the development of safer and more effective gene therapies, enabling targeted corrections of disease-causing mutations while minimizing the risk of adverse effects. Moreover, Cas12a's superior specificity enhances its utility in precision medicine approaches, facilitating the precise modulation of gene expression levels to treat a diverse array of genetic disorders and diseases [9]. As research continues to elucidate the intricacies of Cas12a-mediated gene editing and optimize its delivery methods, the prospect of harnessing this CRISPR system for therapeutic interventions grows increasingly promising, ushering in a new era of precision genomic medicine.

Additionally, Cas12a's ability to process its own CRISPR RNA (crRNA) array represents a significant advancement in the field of genome editing, particularly in the realm of multi-target editing. Unlike other CRISPR systems that necessitate individual crRNAs for each target gene,

Cas12a streamlines the editing process by allowing multiple target sequences to be included within a single RNA transcript [10]. This simplification not only reduces the complexity of experimental design but also enhances the efficiency of gene editing workflows. By consolidating multiple targets into a single transcript, Cas12a facilitates more streamlined delivery methods, minimizing the logistical challenges associated with introducing multiple editing components into target cells. Consequently, researchers can expedite the experimental process and allocate resources more efficiently, accelerating the pace of genetic research and therapeutic development.

Cas12a's capability for multi-target editing offers researchers a powerful tool for making comprehensive genetic modifications, which is crucial in both biotechnology and biomedical research. By targeting multiple genes at once, Cas12a simplifies the process of unraveling complex biological processes or disease pathways [3]. This is particularly valuable for understanding conditions influenced by multiple genes, like polygenic diseases, where editing several genes simultaneously may be necessary to produce significant effects [3]. Additionally, in fields such as metabolic engineering and synthetic biology, Cas12a's ability to edit multiple genomic locations in one go streamlines the development of new strains and optimization of biological pathways. Overall, Cas12a's multi-target editing ability revolutionizes genetic manipulation, providing researchers with enhanced precision and flexibility to explore the intricacies of the genome and develop novel therapeutic approaches.

However, while Cas12a demonstrates improved specificity compared to Cas9, there is still uncertainty regarding its off-target effects profile [11]. Off-target effects occur when the gene-editing tool inadvertently modifies genomic sequences other than the intended target, which can lead to unintended consequences such as disrupting normal gene function or causing mutations. While Cas12a's stringent requirement for a specific PAM sequence enhances its ability to discriminate between intended and unintended targets, there is still uncertainty regarding its off-target effects profile.

This uncertainty arises because Cas12a may still cleave DNA at sequences with imperfect matches or non-canonical PAM sites, albeit at a lower frequency compared to Cas9. Imperfect matches refer to sequences that are similar but not identical to the intended target, while non-canonical PAM sites are sequences that deviate from the standard PAM motif recognized by Cas12a [12]. These off-target cleavage events can occur due to factors such as sequence homology or structural similarities between the target and off-target sites. As a result, further research is necessary to thoroughly characterize the off-target effects of Cas12a and develop strategies to minimize them.

Furthermore, the lower efficiency of Cas12a in making desired genetic edits poses a significant drawback, particularly in applications like gene therapy where the successful modification of a specific percentage of cells is paramount for therapeutic efficacy [13]. Gene therapy aims to correct or replace defective genes within target cells to treat genetic disorders or diseases. In this context, the efficacy of gene editing directly correlates with the proportion of cells that undergo the intended genetic modification. However, due to its lower efficiency compared to Cas9, Cas12a may result in a larger proportion of targeted cells failing to undergo the desired genetic edit.

This reduced efficacy can diminish the overall effectiveness of gene therapy treatments employing Cas12a, as a substantial portion of cells may remain unaffected by the intended genetic modification. Consequently, the therapeutic outcome may be compromised, leading to inadequate disease correction or symptom alleviation. In scenarios where high levels of gene correction are required to achieve therapeutic benefits, such as in severe genetic disorders or conditions with limited cellular turnover rates, the lower efficiency of Cas12a may present significant obstacles to achieving desired treatment outcomes [13]. Moreover, the reduced efficacy of Cas12a may necessitate higher doses of editing components or repeated treatment administrations to achieve sufficient levels of gene correction, potentially increasing the risk of adverse effects and treatment-related complications. Thus, addressing the lower efficiency of Cas12a is crucial for optimizing its utility in gene therapy applications and maximizing the therapeutic benefits for patients.

2.6. Limitations of the Technology

The implementation of CRISPR-based technologies undoubtedly presents a considerable financial burden, particularly for research laboratories and smaller institutions operating within constrained budgets. The expenses associated with CRISPR encompass a wide range of factors, each contributing to the overall cost barrier.

Firstly, the procurement of essential reagents and materials constitutes a significant portion of the expenses. This includes not only the Cas proteins and guide RNAs necessary for genome editing but also the various components required for experimental setup and validation. Additionally, the cost of specialized consumables, such as cell culture media, nucleic acid purification kits, and sequencing reagents, further adds to the financial strain.

Moreover, the acquisition and maintenance of sophisticated equipment essential for CRISPR experiments represent a substantial investment. This encompasses instrumentation for molecular biology techniques, such as PCR machines, electrophoresis systems, and DNA sequencers, as well as advanced imaging platforms for cell analysis and microscopy. The initial purchase cost, coupled with ongoing maintenance, calibration, and upgrades, contribute significantly to the overall expenditure. Personnel training also constitutes a noteworthy expense. Effective utilization of CRISPR technologies necessitates skilled personnel proficient in molecular biology techniques, bioinformatics, and genome editing protocols. Training researchers and technicians to master these techniques requires time, resources, and often external workshops or courses, all of which incur additional costs.

The high cost associated with CRISPR technologies can have profound implications for access and equity within the scientific community [14]. Smaller research institutions, under-resourced laboratories, and scientists in developing countries may face significant barriers to adopting CRISPR due to financial constraints. Consequently, limited access to CRISPR technologies may impede scientific progress and innovation, hindering the exploration of novel research avenues and the development of potential therapeutic interventions.

CRISPR experiments, particularly those involving CRISPR-Cas12a (Cpf1), entail the manipulation of genetic material within laboratory settings. These experiments may utilize viral vectors or bacterial cultures to deliver CRISPR components into target cells or organisms for genome editing purposes. While these techniques offer powerful tools for scientific research and potential therapeutic applications, they also present inherent risks related to biosecurity and environmental safety, especially concerning the possibility of accidental leakage or release of infectious materials.

Specifically, CRISPR-Cas12a experiments often involve the use of viral vectors, such as adeno-associated viruses (AAVs) or lentiviruses, as delivery vehicles for introducing Cas12a proteins and guide RNAs into target cells. These viral vectors may contain genetic material derived from pathogenic viruses or genetically modified organisms, raising concerns about their potential to cause harm if accidentally released into the environment or exposed to individuals handling them [15].

Accidental leakage or release of infectious materials could occur due to various factors, including equipment malfunction, human error during experimental procedures, or inadequate containment measures. For example, spills or leaks from laboratory equipment or improper disposal of contaminated waste could result in the unintended release of viral vectors or genetically modified organisms into the surrounding environment.

The consequences of such incidents could be significant, ranging from potential exposure of laboratory personnel to infectious agents or genetically modified organisms to environmental contamination and ecological disruption [15]. Moreover, if the released materials contain genetic elements capable of replication or propagation, there is a risk of unintended spread and transmission to unintended hosts, including humans, animals, or plants.

To mitigate the risks associated with infectious materials leakage in CRISPR-Cas12a experiments, stringent containment protocols and biosafety measures must be implemented and adhered to rigorously. This includes conducting experiments within designated biosafety level (BSL) facilities appropriate for handling the specific organisms or materials involved. Additionally, researchers must

receive appropriate training in laboratory safety procedures and practices to minimize the likelihood of accidents or incidents.

Furthermore, the development and implementation of robust biocontainment strategies, such as engineered safety mechanisms or containment vectors, can help prevent unintended release or escape of genetically modified organisms from laboratory settings. Regular risk assessments and inspections of laboratory facilities are also essential to identify potential hazards and ensure compliance with safety regulations and guidelines.

2.7. Exploring the Diversity of Cas12a Enzymes for Genome Editing Applications

Cas12a (CRISPR-associated protein 12a), also known as Cpf1, has emerged as a powerful tool in the field of genome editing, offering researchers the ability to precisely modify DNA sequences with unprecedented efficiency and specificity. One of the remarkable features of Cas12a is its diversity, with various enzyme variants identified from different bacterial species. Among these variants, LbCas12a, originating from the *Lachnospiraceae* bacterium, has been one of the earliest and most extensively studied. Known for its robust genome editing activity, LbCas12a has demonstrated remarkable efficacy in a wide range of genetic manipulation tasks, including gene knockout, insertion, and base editing [16].

AsCas12a, sourced from *Acidaminococcus* sp. BV3L6, represents another prominent Cas12a variant characterized by its efficiency and versatility in genome editing endeavors. With its precise targeting capabilities and minimal off-target effects, AsCas12a has become a staple tool in the toolkit of genetic engineers, facilitating various applications from basic research to therapeutic interventions [17]. Furthermore, FnCas12a, derived from *Francisella novicida*, has garnered attention for its unique properties, including high specificity and broad targeting range [18]. This Cas12a variant exhibits efficient genome editing capabilities, making it a valuable asset for researchers seeking precise genetic manipulation with minimal off-target effects.

MbCas12a, is another Cas12a variant that has demonstrated efficient genome editing capabilities. With its robust cleavage activity and versatility, MbCas12a holds promise for applications in both basic research and biotechnological endeavors [19]. Additionally, CjCas12a, originating from *Campylobacter jejuni*, has been studied for its potential in genome editing and related applications. While further research is needed to fully explore its capabilities, CjCas12a offers researchers a valuable tool for targeted genetic manipulation.

Collectively, these diverse Cas12a variants offer a rich repertoire of tools for researchers to explore and harness in their quest to unlock the full potential of CRISPR technologies for genome editing and beyond.

2.8. Investigating the Thermostability and Genome Editing Efficiency of LbCas12a for Enhanced CRISPR Applications

In this study, researchers delved into the properties of LbCas12a, a CRISPR enzyme, to assess its potential for genome editing applications. Firstly, they conducted experiments to evaluate the thermostability of LbCas12a under varying temperature conditions using a protein melt fluorescent assay. By analyzing the fluorescence data obtained from the CFX96 real-time System, they gained insights into how the protein behaves under different temperature regimes, shedding light on its robustness and suitability for practical applications [20].

Furthermore, they investigated the genome editing efficiency of LbCas12a in human embryonic kidney (HEK293T) cells and mouse neural progenitor cells (NPCs). Through meticulous nucleofection procedures and subsequent analysis of gene editing outcomes using techniques like DNA sequencing, they elucidated the enzyme's effectiveness in inducing targeted changes in the

Cellular genome [20]. These comprehensive investigations offer valuable insights into the functional characteristics of LbCas12a, providing a foundation for its optimization and utilization in CRISPR-based research and therapeutic interventions.

2.9. Ethical implications

The advent of CRISPR-Cas systems, with specific enzymes like Cas12a at the forefront, represents a seismic shift in genetic engineering capabilities. These tools allow for unprecedented precision in DNA modification, opening vast opportunities across various fields, including medicine, agriculture, and basic scientific research [3]. However, the power to edit genes, especially through processes like germline editing, introduces complex ethical and societal dilemmas. Germline editing involves modifications to sperm, eggs, or embryos, meaning these alterations are inheritable, affecting future generations [21]. This prospect, while holding the promise of eradicating hereditary diseases, raises profound ethical concerns, such as the morality of altering human evolution, the potential for unforeseen genetic consequences, and the societal impact of creating genetic disparities. The complexities of gene interactions mean that even well-intentioned edits could lead to unexpected outcomes, highlighting the need for caution and comprehensive understanding before proceeding with such interventions.

Furthermore, the accessibility and equitable distribution of CRISPR technologies pose significant challenges. The complexity and cost associated with these cutting-edge tools risk exacerbating existing health disparities, creating a divide where only the wealthy or those in developed nations can benefit from genetic therapies [22]. This potential for inequality necessitates a global conversation on how to ensure these revolutionary treatments can be made available to all, irrespective of socioeconomic status or geographical location. Ethical considerations around who gains access to these treatments and how they are prioritized are paramount to ensuring fairness and justice in the application of CRISPR technologies.

Eugenics is a set of beliefs and practices aimed at improving the genetic quality of the human population, historically by excluding people and groups judged to be inferior, and promoting those judged to be superior [23].

With the advent of new genetic technologies, such as CRISPR-Cas12a, which allows for precise editing of the genome, there is a renewed concern that such tools could be used for neo-eugenics purposes. CRISPR technology holds the promise for treating and preventing a wide range of diseases by directly editing genes. However, it also raises ethical concerns, especially regarding the potential for its misuse in attempting to engineer 'desirable' traits in humans, not just to cure diseases but to enhance physical or cognitive abilities, select for particular physical traits, or even to attempt to influence personality traits or intelligence [23].

It's important to note that the use of genetic technologies for eugenic purposes is widely condemned by the international community, with numerous ethical guidelines and regulations in place to prevent such applications. These regulations aim to ensure that genetic technologies are used responsibly, focusing on therapeutic and preventive health care, respecting individual rights and diversity, and avoiding the imposition of arbitrary standards of "genetic perfection" or the promotion of discriminatory practices.

3. Summary

CRISPR-Cas12a, a revolutionary RNA-guided endonuclease, stands as a powerful contender in the realm of genome editing. Its advantages over Cas9, including a relaxed PAM requirement and single-guide RNA structure, translate into expanded targetable regions and streamlined design processes. Furthermore, Cas12a's staggered DNA cleavage and smaller size offer unique opportunities for precise manipulations and efficient packaging into viral vectors for delivery. While ongoing research focuses on minimizing off-target effects, Cas12a has already proven its utility in various applications, such as gene knockout, insertion, and base editing. As research progresses, CRISPR-Cas12a is poised to revolutionize the field of molecular biology, offering unparalleled avenues for precise and efficient manipulation of the genome.

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