

Recent Trends in Gene Editing Technology CRISPR Cas9

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Abstract

Gene editing has undergone a revolutionary transformation with the advent of CRISPR-Cas9 technology, fundamentally altering the landscape of molecular biology and genetics. CRISPR-cas9, a bacterial immune system repurposed for precise genetic modification, allows for targeted alterations in the DNA of various organisms with unprecedented accuracy and efficiency. This technology has rapidly become a cornerstone in genetic research, therapeutic development, and agricultural biotechnology. Recent advancements in CRISPR-Cas9 have expanded its capabilities beyond simple gene knock-out and knock-in applications. Researchers are now utilizing CRISPR to perform complex edits, such as gene regulation and epigenetic modifications, providing deeper insights into gene function and regulation. The development of CRISPR activation (CRISPRi) and CRISPR activation (CRISPRa) has enabled the fine-tuning of gene expression, which is crucial for understanding gene networks and cellular processes. Additionally, the integration of CRISPR with other emerging technologies, such as single-cell sequencing both basic and applied research in therapeutic applications, CRISPR-Cas9 holds promise for treating genetic disorders, with early clinical trials showing potential in correcting mutations associated with diseases like sickle cell anemia and muscular dystrophy. Efforts are underway to enhance the delivery mechanism of CRISPR components to target cells more efficiently and reduce off-target effects, which remain a significant challenge. In agriculture CRISPR technology is being harnessed to create modified crops with improved traits such as diseases resistance and enhanced nutritional profiles. The ethical and regulatory implication of CRISPR-Cas9, particularly concerning germline editing and bioadversity. Overall, CRISPR-Cas9 technology represents a paradigm shift in genetic engineering, offering transformative potential across various fields. ongoing research and development are expected to refine its applications, unlock opportunities for scientific and medical advancements.

Keywords: CRISPR/Cas9 • Application • Challenges in CRISPR technology

Introduction

The exponential development of genome editing technology has dramatically changed the landscape of biological and medical research, heralding a new era of precision medicine based on genome editing [1]. Target DNA can be cut by CRISPR-based nucleases, which then cause Double-Strand Breaks (DSBs) and introduce random mutations by Non-Homologous End-Joining (NHEJ) or Homology-Directed Repair (HDR) for precise editing [2]. The therapeutic potential of CRISPR-based tools has been investigated using the mouse models of various human diseases [3]. Nevertheless, accurate gene correction for *in vivo* therapeutic value is still difficult, in part because HDR-mediated DNA replacement is not very effective. Post-mitotic cells typically cannot use this tactic because HDR primarily happens during the S/G2 phase of cell division [4]. Precise genome editing tools have been developed and continuously optimized by fusing activity-impaired Cas nucleases with

deaminases, called base editors, or with reverse transcriptases, called Prime Editors (PEs) [5].

Ideas behind CRISPR-Cas9

CRISPR-Cas9 is a revolutionary genome editing tool that allows for targeted modifications to DNA. It utilizes the Cas9 endonuclease enzyme, which is guided to a specific location in the genome by a short RNA molecule. Recent advances in genome editing allow the manipulation of any gene at its own locus in a broad variety of species and tissues, including cultured cells and animal organs. Genome editing is a powerful tool for biomedical research and provides hope for correcting some inherited diseases [6]. Repeated DNA segments called clustered regularly interspaced short palindromic repeats, or CRISPRs, were first identified in bacterial species. In bacteria and archaea, the adaptive immune system uses CRISPR and CRISPR-associated (Cas) proteins to protect against invasive nucleic acids from phages and plasmids.

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Historical Background and Development of CRISPR Cas9

CRISPR-related gene-editing technology is currently one of the hottest biological tools. Since 2013, explosive growth has been recorded in the study of CRISPR technology, with tens of thousands of CRISPR-related articles published. In October 2020.

Ishino first described the CRISPR structure in 1987 [7], and Jansen proposed the acronym CRISPR in 2002 following the discovery of many structures that were identical in other bacteria and archaea [8]. The discovery of hyper-variable spacers with sequence similarity to foreign plasmids and viruses in 2005 was a significant development. Afterwards, Mojica and associates conjectured that the CRISPR structure and associated protein may have immune defense properties and be important defense mechanisms against genetic elements that are transmissible. Subsequently, Charpentier and Doudna's groups reported the combination of crRNA and tracrRNA into a single sgRNA, which can more efficiently help Cas9 to play its editing role *in vitro*. In 2013, Zhang's lab first applied the CRISPR/Cas9 system to perform genome editing in eukaryotic cells. From then on, the new generation of CRISPR gene-editing technology, especially represented by the CRISPR/Cas9 system, has been well developed and widely applied in the field of life sciences, such as to produce gene-edited animal models, gene therapy to treat genetic disease, and animal and plant genetic trait improvement and biological breeding [9]. In 2020, for the epoch-making technological innovation and great contribution to life sciences, two scientists, Charpentier and Doudna, devoted most to the CRISPR/Cas9-based gene editing were awarded the Nobel prize in Chemistry. The delivery of Cas9 into cells is an important consideration in gene editing. Cas9 can be delivered in the forms of DNA, mRNA, or protein (Figure 1). Each format has pros and cons. The delivery of Cas9 in the form of plasmid DNA offers a cost-effective option. Only a standard laboratory set-up is required for plasmid preparation. Plasmid DNA-driven Cas9 expression also yields a longer expression time in cells, which may be advantageous if sustained expression is required for editing. However, because transcription and translation are required for the synthesis of the Cas9 protein the plasmid DNA format has the slowest onset of editing when compared with that of mRNA and protein. Sustained expression of Cas9 in cells also increases the chance of off-target effects. Furthermore, plasmid DNA poses a risk of insertional mutagenesis.

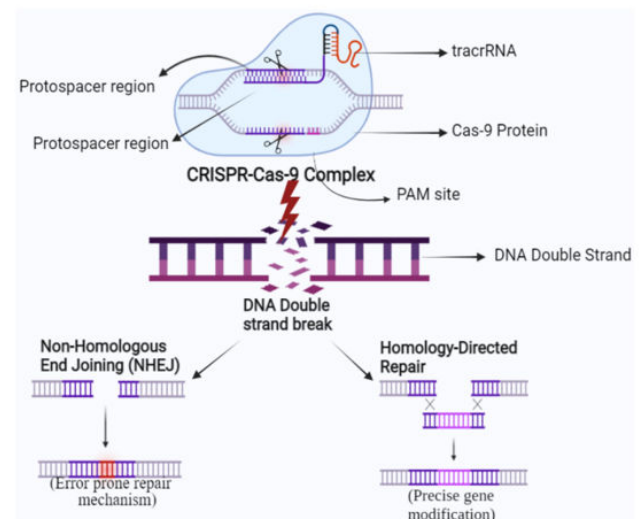


Figure 1. CRISPR-CAS 9 mediated genome engineering method.

The homing endonucleases have revolutionized Genome-Editing (GE) studies with the advent of techniques such as "Zinc-Finger Nuclease (ZFNs)", Transcription Activator-Like Effector Nucleases (TALENs), and CRISPR/Cas systems that can precisely induce mutations in DNA and have transformed the genetic modification techniques of plants in recent years. The CRISPR/Cas9 system was primarily found in bacteria as an RNA-mediated adaptive-immune system activated in response to different viral infections nowadays, CRISPR-based tools have become an alternative to TALENs and ZFNs, due to their high efficiency and simplicity in genome engineering technologies. From CRISPR-Cas9 and CRISPR-Cpf1 to CRISPR-assisted gene activation, epigenetic modulation, RNA-guided RNA degradation, and synthetic biology, researchers have explored a variety of approaches to enhance plant resistance from CRISPR-Cas9 and CRISPR-Cpf1 to CRISPR-assisted gene activation, epigenetic modulation, RNA-guided RNA degradation, and synthetic biology, researchers have explored a variety of approaches to enhance plant resistance. The CRISPR-based genome-editing technologies have been widely adapted to study plant-pathogen interactions that include host defense response against viruses, bacteria, fungi, and other pathogens. In the realm of epigenetics, modifying the histone code in plants through CRISPR-based tools has been explored as a means of enhancing plant resistance. By modulating the chromatin accessibility of the genomic DNA, researchers have successfully augmented the transcriptional output of defense-related genes, thereby fortifying the plant's innate immunity against various pathogenic agents. The CRISPR/Cas systems have been classified on the bases of structural, functional, and phylogenetic characteristics of Cas proteins into two main classes that are "Class 1 (types I, III and IV)" and "Class 2 (types II, V and VI)". These different CRISPR/Cas systems have different mechanisms for the target interference and biogenesis of guide RNA. The CRISPR/Cas systems have been classified on the bases of structural, functional, and phylogenetic characteristics of Cas proteins into

two main classes that are “Class 1 (types I, III and IV)” and “Class 2 (types II, V and VI)”. These different CRISPR/Cas systems have different mechanisms for the target interference and biogenesis of guide RNA. The CRISPR-based class 2 endonucleases have been broadly used for manipulating nucleic acid sequences. Type-II CRISPR/SpCas9 has been widely used and best characterized for targeted genome editing in plants. This system has been originated from *Streptococcus pyogenes* and is composed of 3 major elements (a small mature “CRISPR RNA (crRNA)”, “SpCas9 nuclease”, and “trans-activating small RNA (tracrRNA). However, the modern and updated CRISPR system has only two components guide RNA (gRNA, which is made by artificial fusion of both crRNA and tracrRNA) and SpCas9 nuclease.

The SpCas9 nuclease reaches the targeted site of DNA with the help of gRNA that has 17–20 complementary nucleotides (nt) which recognize a “Protospacer Adjacent Motif (PAM) sequence”, 5'-NGG-3'. The selection of target sequences is mainly restricted due to the need for the specific PAM sequence. Two major approaches have been developed to overcome this restriction: (a) The variations of Cas9 protein to identify various PAM regions and (b) The use of different Cas proteins obtained from different organisms. The modified or engineered xCas9, SpCas9-NG, SpRY, and SpG that recognize the 5'-NG-3' PAM sequence can increase the efficiency of SpCas9 mediated.

About CRISPR-Cas9

The CRISPR/Cas systems play significant roles in protecting bacteria from invading foreign DNA originated from either bacteriophages or exogenous plasmids. In recent years, the CRISPR/Cas systems, especially CRISPR/Cas9 and CRISPR/Cas13 have been programmed to develop versatile gene-editing tools that could modify, delete or correct precise regions of the target DNA. Moreover, these two systems are also employed to develop highly sensitive and rapid isothermal diagnostic tools that are capable of detecting viruses, bacteria and cancer mutations. In recent years, the CRISPR/Cas systems, particularly CRISPR/Cas9 and CRISPR/Cas13, have been programmed to develop versatile gene-editing tools that could modify, delete, or correct precise regions of the target DNA. Additionally, these two systems are used to develop highly sensitive and rapid isothermal diagnostic tools that can detect viruses, bacteria, and cancer mutations. The CRISPR/Cas systems play significant roles in protecting bacteria from invading foreign DNA originated from either bacteriophages or exogenous plasmids.

Working principle of CRISPR-Cas9

In the last ten years, CRISPR-Cas9 technology has gained widespread recognition as a groundbreaking influence in the domain of genome editing. It has acted as a catalyst, sparking a revolution in the field of molecular biology. Among these CRISPR/Cas systems, type II CRISPR/Cas9 and type VI CRISPR/Cas13 systems have been widely exploited as genetic modification tool and diagnostic tool, respectively. Cas9 protein recognizes and binds to foreign nucleic acids primarily through Watson-Crick base pairing between its guide

RNA and the foreign DNA carrying a short Protospacer Adjacent Motif (PAM). Once binding to the foreign DNA, the two nuclease domains including HNH and RuvC from Cas9 protein cleave the target DNA site at the complementary and non-complementary DNA strands, respectively. The foreign DNA is usually cut from both strands as a result of this kind of cleavage, resulting in site-specific Double Strand DNA Breaks (DSBs) that can be fixed by the Homology-Directed Repair (HDR) or Nonhomologous End-Joining (NHEJ) pathways. The previous repair route often leads to repair mistakes, which in turn cause frame-shifting insertions and deletions (also known as indels) that alter gene function. By adding a donor template, the later repair pathway can be used to produce the appropriate target gene alternations (Figure 2). Thus, by changing the guide RNA sequence, the CRISPR/Cas9 system may potentially modify any target DNA location that contains a PAM pattern. This novel system's ease of use makes it a desirable tool for high-throughput sequence-specific genome. Instead of targeting DNA, CRISPR/Cas13 system has been verified to recognize and bind to RNA. Intriguingly, the RNAase activity of this RNA-guided and RNA-targeting CRISPR effector Cas13a, also termed as C2c2, is activated once guide RNA binds to its target RNA, which then results in cleavage of nearby non-targeted RNAs. Thus, the CRISPR/Cas13 system has been employed as molecular diagnostic tools for visualizing, degrading, or binding RNA in a programmable, multiplexed fashion, as well as used in forward transcriptomic pooled screens. As mentioned previously, crRNA plays an essential role in recognizing the target sites. It has been reported that the crRNA usually contains a spacer and a repeat region. The spacer region bears a sequence complementary to the target DNA and the repeat sequence bases pair with a trans-activating (tracrRNA) to form a mature crRNA. Thus, both spacer and repeat sequence information are required when designing the crRNA. CRISPR/Cas systems can be delivered into cells through viral vectors, lipid vesicles, gold nanoparticles, carbon nanotubes, microinjection, electroporation, acoustoporation, magnetotransfection and laser optoporation. After transfection, single cells need to be isolated in order to generate clonal lines that can be verified as complete knockouts. Two methods including dilution cloning and Fluorescence-Activated Cell Sorting (FACS) can be employed to obtain single cell-derived clones. FACS is generally more effective in isolating double-positive cells compared with the dilution cloning. However, dilution cloning is cheaper and may result in less cellular stress than sorting.

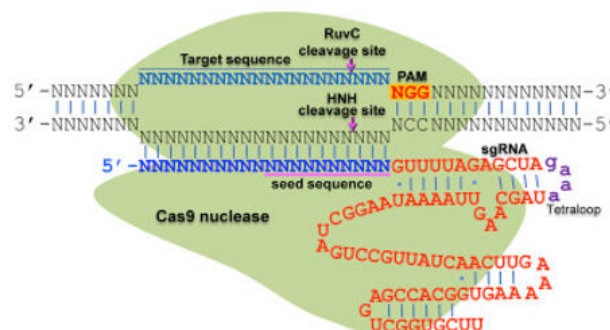


Figure 2. Illustration of the Cas9/sgRNA Ribonuclease.

Materials and Methods

Fundamental component of CRISPR-Cas9 gene editing tools

Based on the structure and functions of Cas-proteins, CRISPR/Cas system can be divided into Class I (type I, III, and IV) and Class II (type II, V, and VI). The class I systems consist of multi-subunit Cas-protein complexes, while the class II systems utilize a single Cas-protein. Since the structure of type II CRISPR/Cas-9 is relatively simple, it has been well studied and extensively used in genetic engineering. Cas-9 consists of two regions, called the recognition (REC) lobe and the Nuclease (NUC) lobe. The REC lobe consists of REC1 and REC2 domains responsible for binding guide RNA, whereas the NUC lobe is composed of RuvC, HNH, and Protospacer Adjacent Motif (PAM) interacting domains. The RuvC and HNH domains are used to cut each single-stranded DNA, while PAM interacting domain confers PAM specificity and is responsible for initiating binding to target DNA.

The CRISPR-Cas9 system stands as a groundbreaking genome editing tool, relying on two critical components: The Cas9 protein and the guide RNA (gRNA). A deeper understanding of this system can be attained by further exploring the structure and function of these components. The Cas9 protein, originating from bacterial immune systems, assumes the role of molecular scissors, executing precise DNA cleavage at specific target sites. Comprising two nuclease domains accountable for DNA strand cleavage and a recognition domain that interacts with the gRNA, Cas9 undergoes a structural alteration when it binds to both the gRNA and the target DNA sequence, culminating in the creation of a Double-Strand Break (DSB) at the designated location. The gRNA, serving as a guiding beacon, directs the Cas9 protein to the desired location within the genome for editing. This synthetic RNA molecule features a scaffold region and a customizable guide sequence. The Cas9 protein is guided to the appropriate region of the genome for editing by the gRNA, which acts as a guiding beacon. This artificial RNA molecule has a guide sequence that may be customized and a scaffold region. As seen in Figure 3, the guide sequence is carefully engineered to complement the target DNA sequence, making it easier for Cas9 to engage and cleave DNA. Understanding and utilizing this crucial element will be greatly aided by practical insights into gRNA selection and design that take into account target specificity, the Protospacer Adjacent Motif (PAM) sequence, and efficient gRNA design criteria. Through the utilization of the Cas9 protein's cutting ability and the specificity of gRNA, the CRISPR-Cas9 system facilitates accurate alterations to the genome (Figure 4).

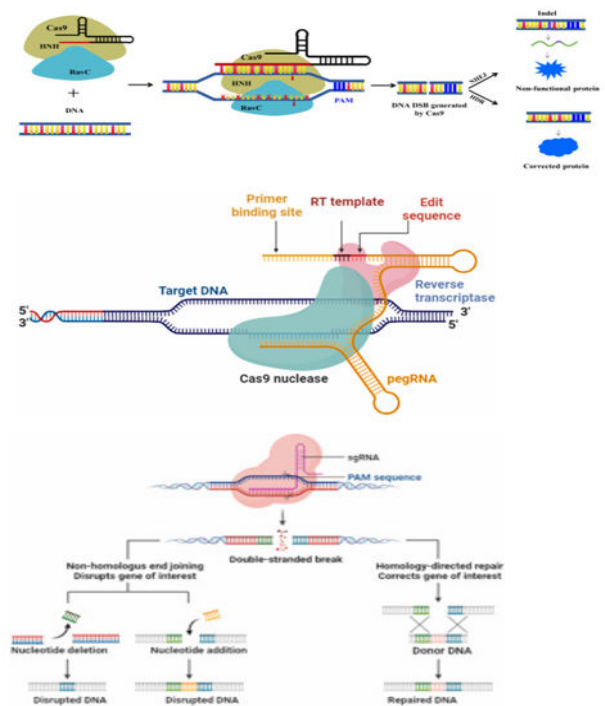


Figure 3. Working principle of CRISPR-Cas9 gene editing tool.

The fundamental component of CRISPR

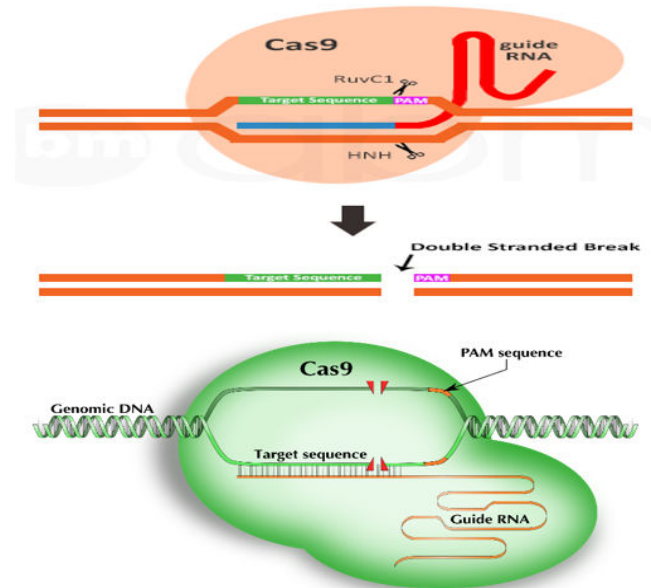


Figure 4. CRISPR CAS9/SgRNA.

Molecular mechanism of CRISPR-Cas9

CRISPR cleaves DNA at a specific location by using a general defense mechanism seen in bacteria and archaea. When a virus infects a bacterium, the bacteria insert a segment of the viral DNA into their own DNA and encase it in CRISPR (Clustered Regularly Interspaced

Palindromic Repeats). The bacteria might be able to "remember" the virus if they save a portion of its genetic code. Upon re-infecting a bacterial cell, the virus cleaves its DNA and employs CRISPR-associated protein number 9 (CAS9) to eliminate the infection. The same CRISPR/Cas9 technology is utilized in the laboratory to identify and cut a particular bacterial RNA-guided Cas9 enzyme DNA sequence by generating an RNA sequence that matches the target DNA sequence. then searches the target genome for conserved three-nucleotide specific Protoadjacent Motifs (PAM). In the event that the Cas9-gRNA complex attaches, site-specific DSBs generate a blunt end, usually three base pairs prior to the PAM region, by recognizing DNA compatibility with the guide RNA. Following these DNA double-strand breaks, the breaks can be repaired by either Homology Directed Repair (HDR) or Non-Homologous End Joining (NHEJ). The error-prone method known as Non-Homologous End-Joining (NHEJ) involves just ligating the ends of the severed ends together. Tiny insertions and deletions (indels) that affect the function at the cleavage site can arise from this repair pathway. On the other hand, homologous DNA sequences are used as templates for flawless repair in Homology-Directed Repair (HDR). HDR executes the precise gene insertion or replacement by adding a donor DNA template with sequence homology at the predicted DSB site (Figures 5 and 6).

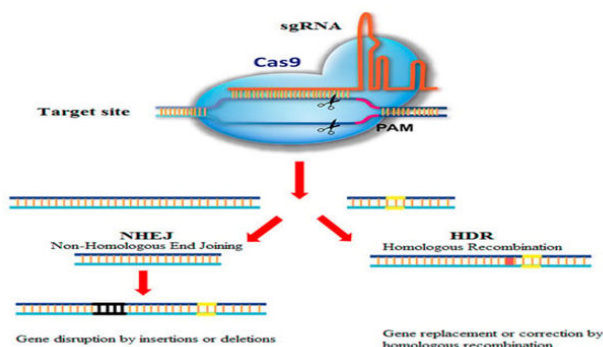


Figure 5. CRISPR-Cas9 gene editing mechanism showing DNA targeting and repair through NHEJ or HDR pathways.

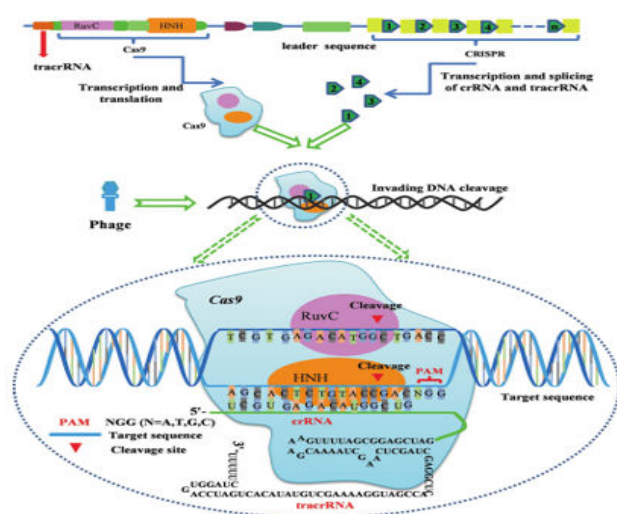


Figure 6. CRISPR/Cas9 genome editing technology.

Results

Application of CRISPR-Cas9 gene editing tool

The CRISPR/Cas-9 genome editing tool has been extensively studied for numerous uses in a short period of time since its discovery. It has had a significant impact on various fields such as biotechnology, agriculture, and medicine. Researchers anticipate that as technology develops in the future, it will be used to treat and cure illnesses, create more nutrient-dense crops, and eradicate infectious diseases. The following section discusses some of the most notable CRISPR/Cas-9 uses and ongoing clinical trials.

CRISPR/CAS9 in disease models: The CRISPR/Cas9 system has an extraordinary therapeutic potential for treating different diseases in which the genetic cause of dysfunction is known, or for the study of these diseases through the creation of cell or animal models. Therapy based on genome editing can lead to the restoration of gene function or compensation of the mutation. Single Nucleotide Polymorphism (SNP) editing has been approached with different strategies, such as: Knocking out the gene that causes the disease introducing a protective mutation or adding a therapeutic transgene ransgene. When the disease is caused by a virus, cleavage of viral DNA can be performed.

CRISPR/CAS9 in hematologic diseases: β -hemoglobinopathies, including Sickle-Cell Disease (SCD) and β -thalassemia, are caused by mutations in the β -globin (HBB) gene, and affect millions of people worldwide. Several studies have used CRISPR/Cas9 and donor sequences to achieve homologous recombination in the HBB gene in induced Pluripotent Stem Cells (iPSCs) and Hematopoietic Stem Cells (HSCs) (101-104). For instance, efficient correction of the Glu6Val mutation responsible of the SCD was achieved using progenitor cells that were derived from patients and differentiated to erythrocytes, resulting in the expression of the mRNA of adult β -globin (HbA) and confirming the intact transcriptional regulation of modified HBB alleles.

Role in gene therapy

Thus far, about 6000 genetic diseases have been identified. However, there are few viable therapy options for the bulk of the disorders. The method of modifying the mutant gene in its original position and substituting the damaged gene with foreign DNA is known as gene therapy. It represents the most recent advancement in the medical biotechnology revolution. 22 gene treatments, including the groundbreaking CRISPR/Cas-9, were authorized for the treatment of human diseases between 1998 and August 2019. Since its discovery in 2012, CRISPR/Cas-9 gene editing has held the promise of curing most of the known genetic diseases such as sickle cell disease, β -thalassemia, cystic fibrosis, and muscular dystrophy. CRISPR/Cas-9 for targeted Sickle Cell Disease (SCD) therapy and β -thalassemia have been also applied in clinical trials. SCD is an autosomal recessive genetic disease of red blood cells, which occurs due to point mutation in the β -globin chain of hemoglobin leading to sickle Hemoglobin (HbS) (Scientists have been also investigating CRISPR/Cas-9 for the treatment of cystic fibrosis. The genetic mutation of the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) gene decreases the structural stability and function of CFTR protein leading to cystic fibrosis.

Application of CRISPR-Cas9 in biotechnology

The innovative CRISPR-Cas9 technology has revolutionized the field of biotechnology, offering powerful tools for genome editing, gene regulation, and synthetic biology. This sub-section provides an overview of the applications and advancements of CRISPR-Cas9 in biotechnology. One of the main uses of CRISPR-Cas9 in biotechnology is genome editing. It makes it possible to precisely alter the DNA sequences of a variety of creatures, such as plants, animals, yeast, and bacteria. The creation of Genetically Modified Organisms (GMOs) with enhanced features or new functions has been sped up by this capacity. As an example, CRISPR-Cas9 was used to modify a yeast strain, showcasing the technology's potential for the synthesis of useful chemicals.

Application of CRISPR-Cas9 in animal breeding

Due to population-growth driven increasing demand, the production of animal-derived food products, especially milk and meat, has increased worldwide. Providing these products is vital for the health and fitness of people. Genome editing technology has made it possible to make precise changes in the animal genome to improve productivity and disease resistance. One of the genes that was first genetically modified in farm animals was myostatin. Changes in this gene can drastically improve economic efficiency of meat production. Farm animals that have been genetically engineered, thus far, include: pig, cattle, sheep, goat, and channel catfish. Nonetheless, because of its global economic value, multiparous nature, and comparatively short generation time, pig is the most genetically modified livestock to date. CRISPR-Cas9 plays a key role in improvement of livestock as the most prominent gene-editing technology today (Figure 7).

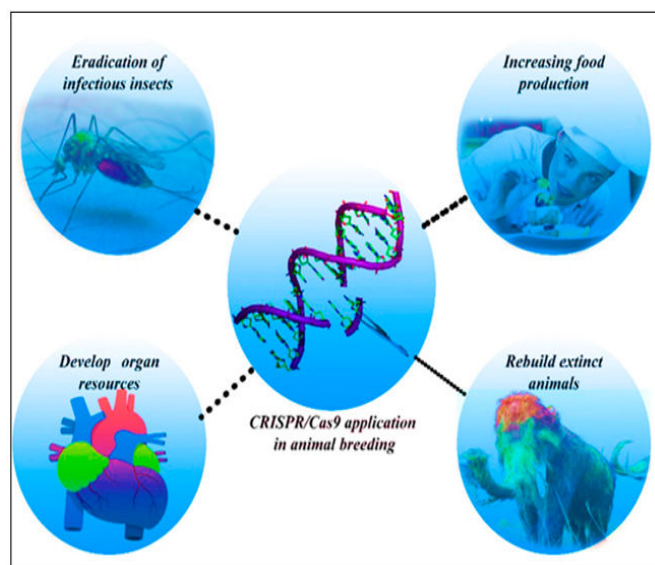


Figure 7. Exploitation of CRISPR-Cas9 in animal breeding and animal models.

Application of CRISPR-Cas9 in single-base gene-editing technology

While some diseases caused by point mutations have been effectively repaired by CRISPR/Cas9 by cleaving the double strand for repair, this approach's inefficiency and unpredictability have limited its applicability. In 2016, Komor et al. made the case that fixing the mutant base, as opposed to removing it to permit random recombination, is a better way to treat genetic disorders. The deamination of cytosine into uracil, which then transforms back into thymine during replicative division, is catalyzed by cytidine deaminase. They thus combined rat-derived cytidine deaminase (APOBEC1) with CRISPR/dCas9, resulting in the effective conversion of C to U bases. To increase base substitution's accuracy and efficiency, this base-editing method was also refined. In addition to offering a reliable technique of base substitution, the development of single-base gene editing technology also creates a base substitution.

Discussion

Challenges in CRISPR/Cas9 technology

Off-target effects: One of the most significant concerns surrounding CRISPR/Cas9 is the risk of off-target effects, where the Cas9 enzyme inadvertently edits unintended sites in the genome. This can lead to unintended mutations, which may have harmful consequences, including the potential for tumorigenesis. Researchers are actively working to enhance the specificity of CRISPR systems by developing high-fidelity Cas9 variants and optimizing guide RNA (gRNA) designs to minimize these risks.

Delivery mechanisms: Efficient and safe delivery of CRISPR components to target cells remains a critical hurdle. Traditional viral vectors, such as Adeno-Associated Viruses (AAV), face limitations including low loading capacity and lack of specific targeting capabilities. Alternative delivery methods, including lipid nanoparticles and polymeric carriers, are being explored to improve the delivery of CRISPR systems, particularly for targeting internal organs and crossing the blood-brain barrier.

Ethical considerations: The ethical implications of gene editing, particularly in human embryos and germline cells, pose significant challenges. The potential for "designer babies" and unintended ecological impacts raises concerns among scientists, ethicists, and the public. Ongoing discussions and regulatory frameworks are necessary to address these ethical dilemmas.

Recent advances and considerations: Recent studies have highlighted innovative approaches to overcome these challenges. For example, the development of base editing and prime editing technologies aims to provide more precise editing capabilities without introducing Double-Strand Breaks (DSBs), thereby reducing off-target effects and enhancing safety.

Mode of delivery of CRISPR CAS 9

The ease of use and precision with which CRISPR technology allows for genome editing promotes its application in medicine. The CRISPR-Cas system has been proposed in numerous research as a viable treatment option for genetic illnesses. However, it is essential to make sure that the CRISPR chemicals are correctly delivered to the target cells in order to produce beneficial therapeutic activities. A better way to understand delivery is to divide it into two parts: The CRISPR cargo and delivery vehicle.

Conclusion

The CRISPR-Cas9 technology is a new and purposeful method in genome editing that has recently surpassed other methods due to its outstanding advantages. So that, it has significantly contributed to all fields of life science, including medicine, plant biotechnology and animal breeding, also expanding researchers' understanding of the basis of gene diversity and gene editing. Given its current central role in this landscape, ongoing research will likely focus on improving this technology further to enhance its specificity and efficiency. While CRISPR/Cas9 technology holds immense potential for revolutionizing gene therapy, addressing the challenges of off-target effects, delivery mechanisms, immune responses, ethical considerations, and long-term efficacy is crucial. Ongoing research and innovation are essential to unlock the full therapeutic potential of this groundbreaking technology and ensure its safe application in clinical practice. CRISPR/Cas9 has been considered one of the most powerful tools for Genomic Editing of various important crops, because of its high efficiency, relatively low cost, and ease of use compared with other GE techniques, such as ZFNs and TALENs. CRISPR/Cas9 has begun to revolutionize biological research, as the method of choice for targeting specific genome sequences in simple or complex organisms. Although significant progress has been made to increase its efficiency and target specificity, more work remains to be done to improve it.

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