

GENETIC REDESIGN: CRISPR-CAS AND MICROBIAL GENOME EDITING

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ABSTRACT :

Microbial biotechnology and genome editing have undergone a revolutionary transformation with the discovery and advancement of CRISPR-Cas systems. Initially recognized as an adaptive immune system in bacteria and archaea, these systems have been repurposed into highly effective tools for accurately modifying genetic material. In this comprehensive review, we delve into the remarkable influence of CRISPR-Cas systems on microbial biotechnology and genome editing. We explore their fundamental mechanisms, recent breakthroughs, existing challenges, and future prospects in this rapidly evolving field. By unraveling the intricate workings of CRISPR-Cas systems, we shed light on their ability to facilitate precise and efficient genetic manipulation. With a focus on microbial biotechnology and genome editing, we uncover how CRISPR-Cas systems have revolutionized these areas, presenting new possibilities for genetic engineering. Moreover, we discuss the challenges CRISPR-Cas systems face, such as off-target effects and delivery methods, while highlighting the ongoing research endeavors that aim to overcome these obstacles. By examining the transformative impact of CRISPR-Cas systems, this review reveals this groundbreaking technology's immense potential and promising future in microbial biotechnology and genome editing.

Keywords: *CRISPR-Cas systems, microbial biotechnology, genome editing, adaptive immune system, genetic material, precise manipulation, transformative impact, off-target effects, delivery methods, genetic engineering.*

INTRODUCTION :

Adaptive immune systems called CRISPR-Cas (Clustered Regularly Interspaced Short Palindromic Repeats-CRISPR-associated) systems are present in bacteria and archaea. They offer a mechanism of defence against genetic invaders like bacteriophages and plasmids. The science of genetic engineering has been transformed by CRISPR-Cas systems, which also have numerous applications in microbial biotechnology.

The discovery of CRISPR-Cas systems began in the late 1980s when researchers noticed a set of repetitive DNA sequences in the genomes of *Escherichia coli*. However, the function of these sequences remained unknown until 2005 when a group of scientists led by Francisco Mojica proposed that they may play a role in

bacterial immunity. Further studies revealed that these repetitive sequences were often accompanied by unique "spacer" sequences derived from genetic elements that had previously invaded the organism.

The mechanism of CRISPR-Cas systems involves three main steps: adaptation, expression, and interference. During adaptation, the system captures short fragments of DNA from invading genetic elements and incorporates them as new spacers into the CRISPR array. The expression phase involves the transcription and processing of the CRISPR array into short CRISPR RNAs (crRNAs). Finally, during interference, the crRNAs guide the Cas proteins to recognize and degrade the complementary nucleic acids of invading genetic elements.

IMPORTANCE OF CRISPR-CAS SYSTEMS IN MICROBIAL BIOTECHNOLOGY

CRISPR-Cas systems, particularly the Cas9 system derived from *Streptococcus pyogenes*, have become vital tools in microbial biotechnology. They offer simplicity, efficiency, and versatility in genome editing. The technology has revolutionized genetic engineering by enabling the precise manipulation of genes in various organisms. The applications of CRISPR-Cas systems in microbial biotechnology include:

1. **Gene editing:** Researchers can modify specific genes to study their function and develop improved microbial strains with desired traits.
2. **Disease modeling:** CRISPR-Cas systems aid in generating animal models for studying human diseases, allowing researchers to understand disease mechanisms and test potential therapies.
3. **Bioproduction:** Microbial strains can be engineered using CRISPR-Cas systems to produce valuable compounds like biofuels, pharmaceuticals, and industrial chemicals more efficiently.
4. **Disease treatment:** CRISPR-Cas systems show promise for treating genetic diseases by correcting or modifying disease-causing mutations in human cells.
5. **Diagnostic tools:** CRISPR-Cas systems can be repurposed as diagnostic tools for rapid and precise identification of pathogens or genetic variations, making them valuable in diagnostics.

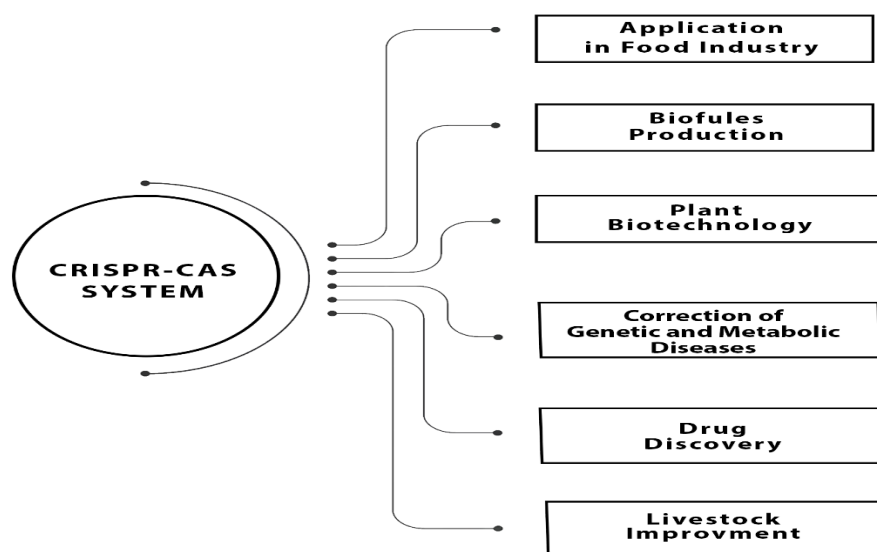


Fig 1. Applications of CRISPR-Cas system

STRUCTURE AND COMPONENTS OF CRISPER-CAS SYSTEMS

1. **CRISPR Array:** The CRISPR array is a bacterial or archaeal genome region consisting of repetitive DNA sequences interspersed with unique spacer sequences derived from previous encounters with foreign genetic elements.
2. **Cas Genes:** Cas genes encode for Cas proteins (CRISPR-associated proteins) that are involved in the various stages of the CRISPR-Cas immune response. There are several types of Cas proteins with different functions.
3. **CRISPR RNA (crRNA):** The crRNA is a short RNA molecule that is transcribed from the CRISPR array and processed to guide Cas proteins to the target sequence.
4. **Cas Nucleases:** Cas nucleases, such as Cas9 or Cas12a (also known as Cpf1), are enzymes that cleave the DNA at the target site. These nucleases can be guided to specific DNA sequences by the crRNA.

TYPES OF CRISPR-CAS SYSTEMS

CRISPR-Cas systems are classified into several types and subtypes based on the composition and organization of their Cas genes and the presence of specific signature proteins. The classification system includes the following types:

Type I: These systems employ a multi-subunit effector complex (Cascade) to target and cleave DNA.

Type II: This type includes the well-known CRISPR-Cas9 system, which uses a single RNA molecule (crRNA:tracrRNA or sgRNA) and a single effector nuclease (Cas9) for DNA cleavage.

Type III: These systems have a dual RNA molecule (crRNA:trans-activating CRISPR RNA or tracrRNA) and target RNA, rather than DNA.

Type IV: These systems are RNA-targeting systems with distinct Cas proteins and mechanisms.

Type V: Cas12 protein (previously Cpf1) can be programmed with a single crRNA to target and cleave specific DNA sequences, expanding genome editing options.

Type VI: Cas13a protein (previously C2c2) targets and cleaves RNA molecules, with applications in RNA editing and detection.

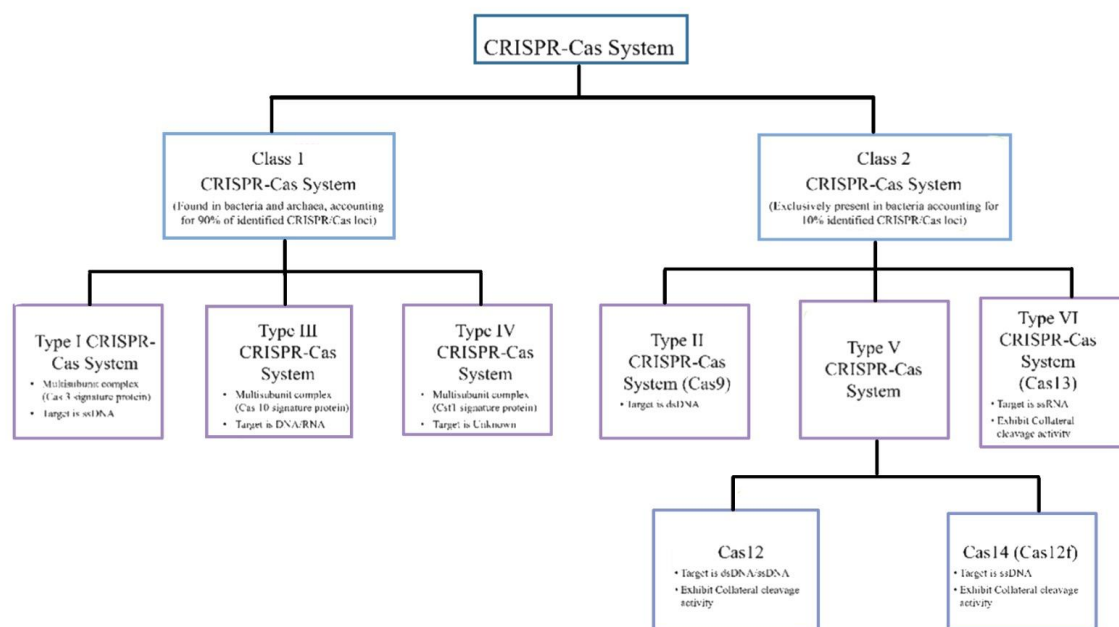


Fig.2 Types of CRISPER-Cas systems

CRISPER-CAS IMMUNE RESPONSE AND DEFENCE MECHANISM

The CRISPR-Cas immune response can be summarized in three main steps: adaptation, expression, and interference.

1. **Adaptation:** During this phase, the CRISPR-Cas system captures short DNA fragments (protospacers) from invading genetic elements, such as bacteriophages or plasmids. These protospacers are integrated into the CRISPR array as new spacers.

2. Expression: A lengthy precursor CRISPR RNA (pre-crRNA) is created from the CRISPR array and processed into individual crRNAs. Additional trans-activating CRISPR RNAs (tracrRNAs) or short RNAs may be needed in various CRISPR-Cas systems for processing and interference.
3. Interference: The crRNA associates with Cas proteins to form a ribonucleoprotein complex. This complex scans the cell's genetic material for sequences complementary to the crRNA spacer sequence. When a match is found, the Cas nuclease is activated and cleaves the target DNA or RNA, leading to degradation and the neutralization of the invading genetic element.

The CRISPR-Cas immune response and defence mechanism provide bacteria and archaea with adaptive immunity against foreign genetic elements, allowing them to recognize, target, and eliminate these elements. The ability to repurpose these systems for targeted genome editing has revolutionized genetic engineering and biotechnology.

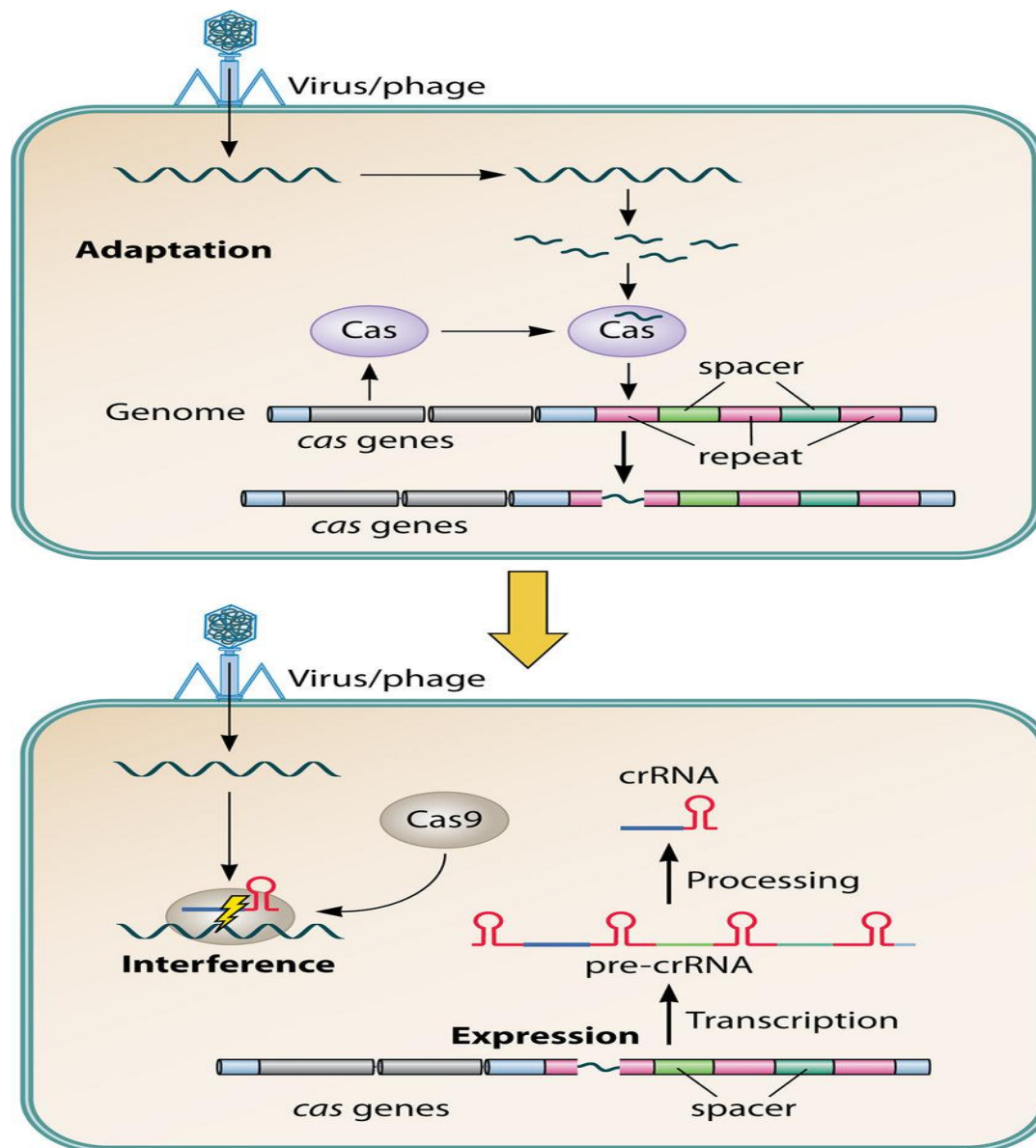


Fig 3.. The defence mechanism of the CRISPR-Cas system

Source: https://www.researchgate.net/figure/Process-of-CRISPR-Cas-acquired-immune-system-Top-Adaptation-The-invading-DNA-is_fig3_322642476

APPLICATIONS OF CRISPR-CAS SYSTEMS IN MICROBIAL BIOTECHNOLOGY

CRISPR-Cas systems have revolutionized microbial biotechnology, offering versatile applications in engineering microbial hosts, genome manipulation, and combating antibiotic resistance. These systems enable precise modifications of the microbial genome, facilitating the engineering of microbial hosts for the industrial production of various valuable compounds. By optimizing metabolic pathways and enzyme activity, CRISPR-

Cas systems enhance the production yields of biofuels, pharmaceuticals, fine chemicals, and enzymes. They allow large-scale modifications across the genome, enabling the study of gene functions, the construction of designer microbial systems, and the creation of synthetic gene circuits and biosensors. In metabolic engineering, CRISPR-Cas systems optimize metabolic fluxes and enzyme activities, improving the production of specific compounds by modifying the microbial genome. Additionally, CRISPR-Cas systems offer potential solutions to combat antibiotic resistance by targeting and eliminating antibiotic-resistance genes in bacteria. These technologies show promise in maintaining the potency of current antibiotics by cleaving particular DNA sequences linked to resistance. Overall, CRISPR-Cas systems have revolutionized microbial biotechnology by offering strong tools for precise genome editing and enhancing study, bioproduction, and anti-antibiotic resistance methods.

CRISPR-CAS SYSTEM APPLICATIONS IN GENOME EDITING

The CRISPR-Cas system has revolutionized genome editing across different species. Its promise for human therapies is highlighted by discussions of its uses in gene function investigations, genetic disease research, precision medicine, and the treatment of genetic disorders. CRISPR-Cas permits precise changes in gene function investigations to examine gene functions and reveal genetic pathways. In genetic disease research, it facilitates the study of disease mechanisms and the development of targeted therapies by introducing disease-causing mutations. CRISPR-Cas also contributes to precision medicine by allowing personalized treatment approaches based on an individual's genetic makeup. Additionally, it holds promise for directly treating genetic disorders by correcting disease-causing mutations. Overall, the CRISPR-Cas system's precise genome editing capabilities have opened up new possibilities in understanding genetic functions, developing personalized treatments, and potentially curing genetic diseases.

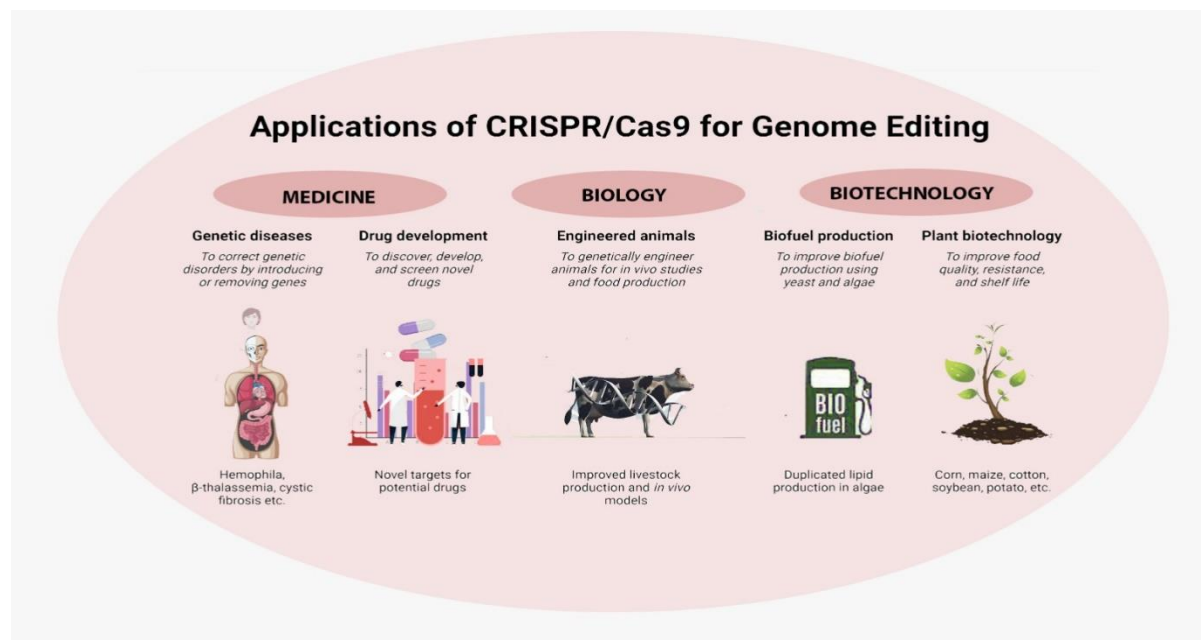


Fig 4. Applications of CRISPR-Cas for genome editing

CHALLENGES AND FUTURE PERSPECTIVES

Off-target effects and specificity concerns:

One of the primary challenges in harnessing CRISPR-Cas genome editing lies in mitigating off-target effects, wherein the Cas nucleases may inadvertently cleave unintended DNA sequences. Efforts are underway to enhance the specificity of CRISPR-Cas systems by employing refined guide RNA designs and Cas variants engineered to minimize off-target activity. Employing techniques such as high-throughput sequencing and computational algorithms allows for the identification and mitigation of off-target effects.

Delivery methods for CRISPR-Cas components:

The efficient delivery of CRISPR-Cas components into target cells is pivotal for successful genome editing. Various strategies encompassing viral vectors, lipid-based carriers, nanoparticles, electroporation, and other sophisticated approaches are being explored to facilitate the transfer of Cas proteins and guide RNAs into cells. Ongoing research endeavours aim to optimize delivery methods, enhance efficacy, and mitigate potential immunogenic responses.

Ethical considerations and regulatory challenges:

The utilization of CRISPR-Cas systems raises profound ethical considerations and regulatory challenges. Contemplation of heritable genome editing in human embryos, germline modifications, and potential unintended consequences on future generations necessitates meticulous evaluation and ethical scrutiny. The

development of robust regulatory frameworks is underway to ensure the responsible deployment of CRISPR-Cas technologies, encompassing rigorous oversight while judiciously balancing potential benefits against societal and ethical concerns.

FUTURE DIRECTIONS AND EMERGING APPLICATIONS

The field of CRISPR-Cas systems continues to evolve at a rapid pace, with ongoing research and development endeavors focusing on enhancing efficiency, specificity, and delivery methodologies. Future trajectories include exploring alternative Cas proteins, such as Cas13 for RNA targeting, and expanding the repertoire of attainable genetic modifications. Furthermore, CRISPR-based diagnostics and therapies, including gene therapies for genetic disorders, represent emerging frontiers of application. Exploiting CRISPR-Cas systems for epigenome editing, modulation of gene expression, and precise regulation of cellular processes emerge as captivating prospects for future investigations.

In summary, addressing off-target effects and refining specificity, optimizing delivery methodologies, navigating ethical and regulatory landscapes, and exploring emerging applications comprise pivotal facets in the progressive advancement and utilization of CRISPR-Cas systems. Sustained advancements in these domains will unlock the full potential of CRISPR-Cas systems for precise and ethically sound genome editing, heralding transformative possibilities across diverse domains of biotechnology and medicine.

CONCLUSION

CRISPR-Cas systems have brought about a transformative impact on microbial biotechnology, revolutionizing genome editing and offering versatile applications. These systems enable precise and efficient manipulation of genetic material in microbial hosts, leading to enhanced production of valuable compounds, genome-scale engineering, metabolic optimization, and combatting antibiotic resistance. The development of efficient microbial strains for industrial production and the acceleration of genome-scale engineering and synthetic biology have been facilitated by CRISPR-Cas systems. They have also shown potential in targeting and eliminating antibiotic resistance genes. This review paper provides a comprehensive overview of CRISPR-Cas systems' mechanisms, applications, challenges, and future prospects in microbial biotechnology and genome editing. By presenting a balanced assessment of the current state of knowledge, the review aims to inspire further advancements in the utilization of CRISPR-Cas systems for the betterment of society and scientific progress.

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