

A Review of the CRISPR-Cas System: Mechanism, Applications, and Future Perspectives

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ABSTRACT

The CRISPR-Cas system has revolutionized molecular biology as a powerful genome editing tool derived from a natural bacterial immune mechanism. This review explores the biological mechanism of CRISPR-Cas systems, particularly the widely used CRISPR-Cas9, along with other Cas variants. Emphasis is placed on its development as a genome-editing platform, its wide-ranging applications in medicine, agriculture, and biotechnology, and the associated ethical and technical challenges. Advances in CRISPR technologies promise unprecedented capabilities in treating genetic disorders, enhancing crop traits, and engineering synthetic organisms. However, concerns over off-target effects, gene drives, and germline editing warrant careful regulatory oversight. This review provides a comprehensive overview of the CRISPR-Cas system and highlights future directions in this rapidly evolving field.

INTRODUCTION

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated (Cas) proteins represent an adaptive immune mechanism found in prokaryotes, primarily bacteria and archaea. This system protects the organism from viral invasions by recognizing and cutting foreign genetic material. First discovered in the late 1980s and functionally characterized in the early 2000s, CRISPR-Cas systems have since been repurposed as precise genome-editing tools (Barrangou et al., 2007). The adaptation of CRISPR-Cas9 for genome editing, especially after the groundbreaking work of Jinek et al. (2012), opened a new era in biotechnology. CRISPR's simplicity, cost-effectiveness, and versatility set it apart from previous genome editing technologies like zinc-finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs). CRISPR's transformative impact is not limited to research laboratories but extends to real-world applications in disease treatment, crop development, and synthetic biology. With continued evolution of the CRISPR toolkit, its accessibility and effectiveness have improved drastically. Moreover, CRISPR's democratization through open-source protocols and low-cost reagents has allowed researchers from diverse backgrounds to engage in cutting-edge genetic editing.

Mechanism of the CRISPR-Cas System

The natural CRISPR-Cas system operates in three stages: adaptation, expression, and interference. During adaptation, bacteria incorporate segments of foreign DNA (spacers) into their own genome between repeat sequences. In the expression phase, these CRISPR loci are transcribed into precursor crRNA, processed into mature crRNAs. During interference, the crRNAs guide Cas proteins to complementary sequences in foreign DNA, where the Cas endonuclease introduces double-strand breaks. In biotechnology, the CRISPR-Cas9 system from *Streptococcus pyogenes* is the most widely used. This system uses a single-guide RNA (sgRNA) to direct Cas9 to a specific genomic target adjacent to a protospacer adjacent motif (PAM), typically "NGG." Cas9 induces double-stranded breaks at the target site. The cell repairs the break via non-homologous end joining (NHEJ), which is error-prone and can cause gene disruption, or homology-directed repair (HDR), which can be used to insert specific sequences. Variants like base editors and prime editors further enhance precision by modifying DNA without creating double-strand breaks, reducing off-target damage and broadening therapeutic scope (Anzalone et al., 2019).

Types and Variants of CRISPR-Cas Systems

CRISPR systems are diverse, and their classification helps understand the broad applicability and mechanisms of different

variants. They are broadly categorized into Class 1 and Class 2 systems. Class 1 systems (Types I, III, and IV) rely on multi-subunit protein complexes for interference. In contrast, Class 2 systems (Types II, V, and VI) employ a single, multi-domain protein to perform the interference function (Makarova et al., 2020). Cas9, the most well-known enzyme, belongs to Type II and has been the foundation for most genome-editing breakthroughs. Cas12 (Cpf1) enzymes, from Type V systems, offer advantages such as simpler guide RNA requirements and staggered DNA cuts. Cas13 proteins, under Type VI, target RNA rather than DNA, opening possibilities in transcriptome editing, RNA knockdown, and diagnostics.

In recent years, scientists have discovered novel Cas proteins through metagenomic mining of microbial genomes. Examples include Cas14, a smaller protein with DNA targeting capabilities, and CasX, a compact protein suitable for delivery through viral vectors. The discovery and engineering of new CRISPR-Cas tools continue to broaden the genome editing landscape, enabling precision medicine, portable diagnostics, and RNA editing applications.

Applications of the CRISPR-Cas System

Biomedical Applications

CRISPR-Cas technology is rapidly transforming medical research and treatment. Its most direct use in medicine involves the correction of genetic mutations responsible for diseases. Preclinical and clinical trials have demonstrated the successful use of CRISPR in treating blood disorders such as sickle cell disease and β -thalassemia. In these cases, autologous hematopoietic stem cells are harvested, edited *ex vivo*, and reintroduced into the patient to restore normal function (Frangoul et al., 2021).

CRISPR is also pivotal in developing gene therapies for inherited retinal diseases, immunodeficiencies, and cancer. CAR-T cells, genetically modified using CRISPR to enhance their tumor-targeting capacity, have demonstrated improved safety and efficacy in cancer immunotherapy.

Diagnostic tools based on CRISPR, such as SHERLOCK and DETECTR, have enabled rapid detection of viral pathogens including Zika virus, Dengue, and SARS-CoV-2. These tools combine CRISPR's sequence specificity with isothermal amplification to create affordable, accurate, and field-deployable diagnostic systems (Chen et al., 2018).

Agricultural Applications

CRISPR offers a powerful and precise method to enhance crop and livestock traits. It is used to develop disease-resistant crops, improve stress tolerance, and increase nutritional content. For example, CRISPR has enabled the development of tomato and banana varieties resistant to common pathogens, as well as rice strains tolerant to drought and salt stress (Zhang et al., 2014).

Moreover, CRISPR has accelerated domestication of wild plants, allowing rapid adaptation of species to agricultural conditions. Livestock genome editing is being explored to improve resistance to diseases such as porcine reproductive and respiratory syndrome (PRRS) in pigs. These advances promise more sustainable agriculture and improved food security, although consumer acceptance and regulatory approval remain critical factors.

Industrial and Synthetic Biology

Industrial biotechnology leverages CRISPR for engineering microbial strains used in the production of biofuels, enzymes, and pharmaceuticals. CRISPR enables rapid strain optimization by facilitating the deletion or insertion of multiple genes simultaneously. In synthetic biology, CRISPR has allowed the construction of genetic circuits and programmable cellular functions. Examples include engineered yeast producing high-value compounds like artemisinin, a malaria drug precursor. In bacteria, CRISPR has enabled the construction of biosensors and metabolite-responsive control systems, expanding the potential of engineered organisms in biomanufacturing.

Challenges and Ethical Considerations

Despite its potential, CRISPR faces several technical and ethical challenges. One major technical issue is the risk of off-target effects—unintended edits at genomic sites similar to the target. These can cause deleterious mutations. Improved Cas variants like SpCas9-HF and computational tools to predict off-target sites have reduced these risks (Kleinstiver et al., 2016).

Ethically, germline editing raises concerns because changes made to embryos can be inherited by future generations. The international scientific community largely agrees that germline editing should not proceed without broad societal consensus. The case of gene-edited babies in China highlighted the need for strict regulatory oversight and ethical boundaries (Cyranoski, 2018).

In environmental biotechnology, gene drives—selfish genetic elements that bias inheritance—pose ecological risks. Although promising for eradicating disease vectors like mosquitoes, their release into the wild must be approached with extreme caution due to the potential for irreversible ecosystem disruption (Esvelt et al., 2014).

Global Regulation and Policy Landscape

Regulation of CRISPR technologies varies globally. The United States takes a product-based regulatory approach, assessing the end product rather than the editing method. As a result, some CRISPR-edited crops are exempt from GMO regulations. The European Union, however, considers CRISPR modifications as genetic engineering and subjects them to stringent GMO regulations.

In the context of human therapies, regulatory agencies like the U.S. FDA and EMA (European Medicines Agency) have issued guidelines for clinical trials involving gene editing. However, discrepancies in regulatory frameworks across nations present challenges for international research and commercialization. A harmonized regulatory approach may be necessary to ensure safety while promoting innovation.

Future Perspectives

The future of CRISPR is expansive and multidimensional. Emerging tools such as prime editing offer unparalleled precision by inserting or deleting DNA sequences without double-strand breaks. Base editors are being refined to increase efficiency and reduce off-target activity, opening pathways for treatment of single-nucleotide diseases such as Huntington's and Tay-Sachs.

Nanoparticle delivery, engineered viral vectors, and cell-penetrating peptides are being explored to overcome the challenge of delivering CRISPR components into specific tissues. Combined with machine learning models for sgRNA design and outcome prediction, CRISPR's accuracy and applicability will continue to improve.

Synthetic biology is increasingly integrating CRISPR with logic circuits, enabling cells to make decisions based on environmental inputs. This could revolutionize therapeutic delivery, biosensing, and programmable biomanufacturing. Additionally, the application of CRISPR in personalized medicine—where therapies are tailored to an individual's genetic makeup—is becoming increasingly feasible.

Continued exploration of natural CRISPR systems may yield novel Cas enzymes with unique properties, further enriching the genetic engineering toolbox. As CRISPR technologies evolve, their responsible development will depend on interdisciplinary collaboration, public engagement, and ethical foresight.

CONCLUSION

The CRISPR-Cas system represents one of the most groundbreaking innovations in life sciences. From bacterial defense mechanisms to genome editing marvels, CRISPR has redefined what is possible in biology, medicine, and agriculture. The system's precision, adaptability, and efficiency make it an indispensable tool in the modern scientific arsenal. However, as with any powerful technology, CRISPR demands thoughtful governance. Addressing off-target effects, establishing robust ethical guidelines, and promoting equitable access will be critical to harness its benefits responsibly. The next decades will likely witness CRISPR's integration into routine clinical practice, global food production, and synthetic biology. It is imperative that scientific, regulatory, and public communities collaborate to ensure that this transformative technology uplifts global health and sustainability.

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