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Review Article

CRISPR-Cas Systems: Mechanisms, Variants, and Biomedical Applications: A Comprehensive Review

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Abstract

Objectives: This review aims to provide a comprehensive synthesis of the mechanisms, classification, emerging variants, and biomedical applications of CRISPR-Cas systems, while critically evaluating current limitations and future directions in genome editing.

Data Sources: Peer-reviewed literature was sourced from PubMed, Google Scholar, and institutional databases. Sources included primary research articles, systematic reviews, clinical trial reports, and authoritative commentary up till 2025.

Study Selection: Studies were selected based on relevance to CRISPR-Cas mechanism, classification, therapeutic application, and emerging technologies. Priority was given to high-impact journals in molecular biology, genetics, and clinical medicine.

Summary: CRISPR-Cas systems, originally characterized as adaptive immune mechanisms in prokaryotes, have been repurposed as highly precise genome engineering platforms. The two major system classes, defined by multi-protein versus single-effector complexes, encompass diverse types with distinct nuclease activities and target specificities. Key variants, including base editors, prime editors, and diagnostic platforms such as SHERLOCK and DETECTR, have substantially expanded functional capabilities. Biomedical applications span therapeutic gene correction in monogenic disorders, cancer immunotherapy, antiviral strategies, functional genomics, and disease modelling. Persistent challenges include off-target effects, delivery limitations, immune responses, and ethical concerns surrounding germline editing.

Conclusion: CRISPR-Cas technology represents a paradigm shift in molecular biology. Continued refinement of editing fidelity, delivery systems, and ethical frameworks will be essential for its safe and equitable clinical translation.

Keywords: CRISPR-Cas systems, genome editing, Cas9 nuclease, gene knockout, gene therapy, base editing, prime editing

1. Introduction

CRISPR-Cas (Clustered Regularly Interspaced Short Palindromic Repeats and CRISPR-associated proteins) systems constitute an adaptive immune mechanism conserved across bacteria and archaea, enabling prokaryotes to recognize and neutralize invading genetic elements such as bacteriophages and plasmids through sequence-specific targeting.[1,2] CRISPR loci comprise conserved repetitive sequences interspaced with unique spacer sequences, fragments derived from prior viral or plasmid encounters, which, together with Cas proteins, form a programmable surveillance and cleavage apparatus providing heritable immunity across generations.[3]

The translation of this natural defense machinery into a genome engineering tool represents one of the most consequential advances in modern biology. The discovery that the Type II CRISPR-Cas9 system could be repurposed for precise genome editing in vitro marked a paradigm shift in molecular biology.[4,5] By employing a single-guide RNA (sgRNA) to direct Cas9 nuclease activity toward a complementary DNA sequence adjacent to a protospacer adjacent motif (PAM), targeted double-strand breaks (DSBs) can be induced, enabling gene disruption, insertion, or correction through endogenous repair pathways.[6]

Since its adaptation for genome engineering, CRISPR-Cas has catalyzed advances across functional genomics, disease modelling, therapeutic gene editing, diagnostics,

and agricultural biotechnology.[7,8] Its simplicity, versatility, and efficiency have democratized gene editing, enabling precise manipulations previously constrained by more complex technologies such as zinc-finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs).[9] Nonetheless, challenges including off-target cleavage, delivery constraints, and ethical considerations, particularly regarding human germline editing, underscore the need for continued critical evaluation of the field.[10,11]

This review synthesizes the historical development, mechanistic basis, classification, emerging variants, and biomedical applications of CRISPR-Cas systems, while critically addressing current limitations and future directions.

2. Historical Development of CRISPR-Cas Systems

The discovery of CRISPR-Cas spans more than three decades, beginning with the identification of unusual repetitive DNA sequences in *Escherichia coli* by Ishino *et al.* in 1987, whose biological significance was initially unclear.[12] These sequences, subsequently named Clustered Regularly Interspaced Short Palindromic Repeats, were observed across diverse bacterial and archaeal species, suggesting a conserved biological function.[13]

A pivotal advance occurred when Barrangou *et al.* demonstrated that CRISPR sequences confer adaptive immunity in prokaryotes by integrating foreign genetic material as molecular spacers into the host genome.[1] These spacers serve as immunological memory, guiding Cas proteins to neutralize previously encountered bacteriophages and plasmids, establishing CRISPR as a naturally occurring, sequence-specific immune system.

The transformative leap from bacterial immunity to genome engineering emerged in 2012 when Jinek *et al.* harnessed the Type II CRISPR-Cas9 system for targeted DNA cleavage in vitro, demonstrating that a single-guide RNA could direct Cas9 to precise genomic loci.[4] Doudna and Charpentier's subsequent work formalized the framework for CRISPR-mediated gene editing, a contribution recognized with the 2020 Nobel Prize in Chemistry.[5] Further developments including optimization of PAM recognition, the engineering of Cas variants such as Cas12 and Cas13, and the introduction of base and prime editing, have progressively expanded the versatility and precision of CRISPR technologies.[8,14]

Today, CRISPR-Cas systems represent a paradigm shift in molecular biology and biotechnology, with a historical trajectory illustrating the dynamic interplay between fundamental research and translational innovation.[15,16]

3. Architecture and Classification of CRISPR-Cas Systems

3.1 Core Components

A functional CRISPR-Cas system consists of three principal components:

(a) CRISPR Array: A genomic locus containing direct repeats interspaced with unique spacers derived from previously encountered foreign DNA, serving as molecular memory of prior infections.[2,17]

(b) CRISPR RNA (crRNA): Transcribed from the CRISPR array, crRNA guides the Cas nuclease to complementary target sequences in invading genetic material, enabling precise cleavage.[4]

(c) CRISPR-Associated (Cas) Proteins: Nucleases that execute cleavage of target DNA or RNA. Among them, Cas9 is the most widely utilized in genome editing due to its simplicity and high efficiency, while Cas12 and Cas13 have been adapted for DNA and RNA targeting, respectively.[8,18]

3.2 Classification by Effector Complex

CRISPR-Cas systems are classified based on structural organization, effector complex composition, and interference mechanisms into two major classes, each comprising multiple types and subtypes.[19]

Class 1 Systems employ multi-protein effector complexes and include:

- **Type I:** Utilizes the Cascade (CRISPR-associated complex for antiviral defense) multi-protein complex; Cas3 is the signature nuclease responsible for target degradation. Widespread in bacteria and archaea.[20,21]
- **Type III:** Characterized by multi-subunit complexes with dual DNA and RNA targeting capabilities; Cas10 is the signature protein, enabling transcription-coupled RNA-mediated DNA targeting.[22,23,24]
- **Type IV:** Less well-characterized; often associated with plasmids and may mediate inter-plasmid competition rather than viral defence.[25,26]

Class 2 Systems are distinguished by a single, large effector protein and include:

- **Type II (Cas9):** The most widely employed system in genome engineering; Cas9, guided by sgRNA, introduces DSBs at target loci adjacent to an NGG PAM sequence.[4]
- **Type V (Cas12):** Introduces staggered cuts in target DNA and exhibits collateral single-stranded DNase activity, harnessed for diagnostic applications such as DETECTR.[27]
- **Type VI (Cas13):** Uniquely targets RNA, enabling programmable RNA editing and diagnostics, as exemplified by the SHERLOCK platform.[18]

4. Mechanism of CRISPR-Cas-Mediated Gene Editing

4.1 crRNA Biogenesis and Ribonucleoprotein Assembly

In Type II systems, transcription of the CRISPR array generates precursor crRNA (pre-crRNA), which is processed into mature crRNA containing spacer sequences complementary to prior foreign genetic elements. A trans-activating crRNA (tracrRNA)

associates with the crRNA to form a ribonucleoprotein complex that stabilizes the configuration required for target recognition and cleavage.[4] In practical genome editing, these two RNA components are fused into a single-guide RNA (sgRNA), simplifying the system.

4.2 Target Recognition and PAM-Dependent Specificity

Target recognition requires the presence of a protospacer adjacent motif (PAM), a short DNA sequence immediately flanking the target site. PAM sequences serve as critical determinants of specificity, enabling discrimination between self and non-self DNA to prevent autoimmunity. For Cas9, the canonical PAM is 5'-NGG-3', though requirements vary considerably among Cas orthologs.[28]

4.3 Endo-nucleolytic Cleavage

Upon PAM recognition and crRNA-guided alignment, Cas9 introduces a blunt-ended DSB at the target locus. Two nuclease domains mediate cleavage: the HNH domain cleaves the strand complementary to the guide RNA, while the RuvC domain cleaves the non-complementary strand.[4] This targeted cleavage constitutes the pivotal step in CRISPR-mediated genome editing, generating substrates for downstream cellular repair mechanisms.

4.4 DNA Repair and Editing Outcomes

Following DSB formation, cellular repair machinery governs the genetic outcome through two principal pathways:

- **Non-Homologous End Joining (NHEJ):** An error-prone pathway that frequently introduces insertions or deletions (indels), effectively disrupting the target gene. Widely used for gene knockout applications.[29]
- **Homology-Directed Repair (HDR):** A high-fidelity mechanism utilizing an exogenously supplied DNA template to introduce precise nucleotide changes, insertions, or corrections. Suitable for targeted gene correction but limited in efficiency in post-mitotic cells.[8]

5. Emerging CRISPR Variants and Technologies

5.1 Base Editing

Base editors enable the direct chemical conversion of one nucleotide into another without generating DSBs or relying on HDR. This technology fuses a catalytically impaired Cas nuclease (nickase or catalytically dead Cas) to a cytidine or adenosine deaminase enzyme. The complex is directed by an sgRNA to the target site, where the deaminase catalyzes a precise chemical modification of the target base, achieving predictable single-nucleotide substitutions.[8] Base editing holds considerable promise for correcting point mutations underlying monogenic diseases while minimizing unintended genomic disruptions.

5.2 Prime Editing

Prime editing represents a further refinement, offering versatile genome modification including insertions, deletions, and all twelve possible base-to-base conversions with minimal off-target effects. This system combines a Cas9 nickase with a reverse transcriptase enzyme and a prime editing guide RNA (pegRNA) encoding the desired sequence modification. Upon target recognition, the reverse transcriptase synthesizes the edited sequence directly onto the target DNA, which is then incorporated through endogenous repair pathways.[30] Prime editing overcomes many limitations of conventional CRISPR-Cas9 editing, particularly in cell types or loci where HDR efficiency is low.

5.3 CRISPR-Based Diagnostics

Beyond genome editing, Cas12 and Cas13 variants have been exploited for molecular diagnostics. Cas12 exhibits collateral single-stranded DNase activity upon target DNA recognition, enabling rapid and sensitive nucleic acid detection, as exemplified by the DETECTR platform.[27] Similarly, Cas13 targets RNA with high specificity and has been employed in the SHERLOCK (Specific High-sensitivity Enzymatic Reporter unLOCKing) platform for programmable RNA detection.[31] These systems offer point-of-care diagnostic capabilities for infectious diseases, genetic disorders, and viral pathogens, illustrating the versatility of CRISPR well beyond traditional genome engineering.[32]

5.4 Expanded Cas Orthologs

A range of Cas orthologs, including Cas12a, Cas12b, and Cas13 variants, have been characterized, each with distinct PAM requirements, cleavage patterns, and substrate specificities.[18,27] This diversity enables precise tailoring of CRISPR tools to experimental or therapeutic objectives, optimizing efficiency while mitigating off-target activity.

6. Biomedical Applications of CRISPR-Cas Systems

6.1 Functional Genomics: Gene Knockout and Knock-In

CRISPR facilitates targeted gene disruption (knockout) or precise sequence insertion (knock-in) by directing nucleases to specific genomic loci. Knockouts are typically achieved via NHEJ, whereas knock-ins exploit HDR to insert exogenous sequences or correct mutations.[8] These approaches are indispensable in functional genomics, enabling investigators to dissect gene function, interrogate regulatory networks, and generate custom cellular and animal models for experimental research.[33]

6.2 Disease Modelling

By introducing defined mutations into cells or model organisms, CRISPR enables the generation of highly accurate disease models. Patient-specific mutations can be replicated in induced pluripotent stem cells (iPSCs) or animal models, providing platforms to study pathophysiology and evaluate therapeutic strategies. CRISPR-engineered models of Duchenne muscular dystrophy, cystic fibrosis, and diverse cancer genotypes have facilitated mechanistic insights and drug discovery in a controlled, reproducible manner.[33]

6.3 Therapeutic Gene Editing: Monogenic Disorders

CRISPR-Cas systems have demonstrated significant clinical promise in correcting mutations responsible for monogenic diseases. Clinical studies have reported successful ex vivo editing of hematopoietic stem cells in sickle cell disease and β-thalassemia, restoring functional hemoglobin production and substantially alleviating disease phenotypes.[34] Preclinical evidence further supports CRISPR-mediated correction in cystic fibrosis, Duchenne muscular dystrophy, and other hereditary disorders, highlighting its potential for genomic curative interventions.

6.4 Cancer Immunotherapy

CRISPR is increasingly applied in oncology to enhance both understanding and treatment of malignancies. It facilitates the generation of disease-specific cancer models, functional screens for oncogenes and tumor suppressors, and targeted modifications of immune cells for adoptive cell therapy. CRISPR-edited T cells engineered to express chimeric antigen receptors (CAR-T) have demonstrated improved tumor recognition and reduced immunogenicity, broadening the therapeutic repertoire of immuno-oncology.[35]

6.5 Antiviral Strategies

CRISPR-based interventions have been explored for combating viral infections by directly targeting viral DNA or RNA genomes. Cas9 and Cas12 variants have been employed to excise integrated viral sequences, including those from human immunodeficiency virus (HIV), while Cas13-mediated RNA targeting offers programmable strategies against RNA viruses, including influenza and SARS-CoV-2.[36] These approaches offer adaptable antiviral modalities complementing conventional therapeutic strategies.

7. Limitations, Challenges, and Ethical Considerations

7.1 Off-Target Effects

One of the principal challenges in CRISPR-mediated genome editing is off-target cleavage, whereby Cas nucleases bind and cut genomic loci with partial sequence similarity to the intended target. Such unintended modifications can disrupt non-target genes, potentially leading to genomic instability, deleterious mutations, or oncogenic transformation. Mitigation strategies include the development of high-fidelity Cas variants, rigorous optimization of guide RNA design, and comprehensive computational prediction coupled with experimental validation.[37]

7.2 Delivery Constraints

Efficient and tissue-specific delivery of CRISPR components remains a significant technical challenge, particularly for in vivo applications. Viral vectors, notably adeno-associated viruses (AAVs), offer high transduction efficiency but are constrained by packaging limits and potential immunogenicity. Non-viral delivery systems, including lipid nanoparticles, electroporation, and polymer-based carriers, present alternatives with lower immunogenicity but often exhibit reduced efficiency or limited tissue targeting.[38] Selection of an appropriate delivery strategy is critical to achieving effective editing while minimizing adverse effects.

7.3 Immune Responses and Mosaicism

Pre-existing immunity to Cas proteins may elicit immune reactions that reduce editing efficiency or provoke adverse clinical effects. Additionally, incomplete or uneven editing during embryonic or zygotic applications can produce mosaic organisms with variable genotypes, complicating phenotypic interpretation and therapeutic predictability.[11]

7.4 Ethical and Regulatory Considerations

Germline editing and heritable genomic modifications raise profound ethical, legal, and societal concerns. The application of CRISPR to human embryos has prompted international calls for robust regulatory oversight and inclusive public deliberation.[39,40] Issues of equitable access, informed consent, and the boundary between therapeutic correction and enhancement remain central to ongoing ethical discourse.[41] Responsible deployment of CRISPR technologies necessitates frameworks that balance scientific innovation with principles of safety, justice, and public trust.[42]

8. Future Directions

The future trajectory of CRISPR-Cas technologies is defined by increasing precision, versatility, and clinical translatability. CRISPR-based transcriptional regulation and epigenome editing, using catalytically dead Cas9 (dCas9) fused to transcriptional activators, repressors, or epigenetic modifiers enable sophisticated modulation of gene expression without altering the underlying DNA sequence.[43] This capacity for gene regulation holds promise for diseases driven by aberrant transcriptional programs.

The development of integrase-coupled CRISPR systems, capable of inserting large DNA payloads at precise genomic loci, addresses longstanding limitations of HDR-based approaches in cells with low recombination activity. Concurrently, the convergence of CRISPR with single-cell sequencing, organoid technologies, and artificial intelligence-driven guide RNA design is accelerating both functional genomics discovery and therapeutic development.

The continued expansion of clinical trials targeting hematologic disorders, ocular diseases, metabolic conditions, and cancer immunotherapies reflects growing confidence in the safety and efficacy of CRISPR-based interventions.[34] Ongoing engagement with ethical, legal, and societal considerations will be essential to ensure that the benefits of these advances are equitably distributed.[44]

9. Conclusion

CRISPR-Cas systems have emerged as transformative tools in genome engineering, offering unprecedented precision, versatility, and efficiency across a spectrum of biomedical applications, from gene knockout and knock-in strategies to disease modelling, therapeutic gene

correction, and molecular diagnostics. Clinical translation is advancing rapidly, with proof-of-concept demonstrated for conditions including sickle cell disease and β-thalassemia.

Nonetheless, challenges persist: off-target cleavage, delivery limitations, immune responses, and ethical concerns surrounding germline modification highlight the need for continued, responsible innovation. Emerging technologies including base and prime editing, novel Cas orthologs, CRISPR-based diagnostics, and integrative synthetic biology approaches are progressively refining editing accuracy, expanding applications, and improving safety profiles.

CRISPR-Cas technology represents a cornerstone of next-generation biotechnology and precision medicine. Its responsible development, guided by rigorous scientific scrutiny and inclusive ethical deliberation, holds the potential to transform the management of genetic disease and fundamentally advance our understanding of the human genome.

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