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REVIEW ARTICLE

Adaptive Roles of CRISPR-Cas Systems in Prokaryotic Immunity and Genome Editing: A Narrative Review

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ABSTRACT

CRISPR-Cas systems have emerged as one of the most transformative genome-editing technologies due to their ability to provide precise, programmable, and efficient manipulation of genetic information. Originally identified as adaptive immune systems in bacteria and archaea, CRISPR-Cas mechanisms enable microorganisms to recognise and eliminate invading genetic materials through coordinated interactions between CRISPR RNA (crRNA) and Cas proteins. This narrative review examines the molecular mechanisms of CRISPR-Cas adaptive immunity in prokaryotes, the classification and functions of major Cas proteins, and their repurposing for genome editing applications. A literature search was conducted in PubMed, Web of Science, Google Scholar and Scopus using the keywords "CRISPR", "Cas9", "adaptive immunity", "genome editing", "gene therapy" and "off-target effects" combined with Boolean operators (AND and OR). Articles included were publications between 2016 to 2026. The findings show that CRISPR-Cas adaptive immunity involves three major stages: adaptation, CRISPR RNA processing, and interference, where Cas proteins such as Cas9, Cas12, and Cas13 mediate targeted nucleic acid recognition and cleavage. Repurposed CRISPR systems have enabled advances in genome editing, gene therapy, infectious disease diagnostics, cancer research, and agricultural biotechnology. However, challenges including off-target effects, immune responses, delivery limitations, and ethical concerns surrounding human genome modification remain significant barriers. CRISPR-Cas technology represents a powerful precision-engineering platform requiring improved specificity, safety mechanisms, and ethical regulatory frameworks to maximize its therapeutic and scientific potential.

Keywords: CRISPR-Cas systems; Cas9; adaptive immunity; genome editing; gene therapy; off-target effects

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1.0 INTRODUCTION

The ability to precisely manipulate genetic information has long been a fundamental goal in molecular biology, biotechnology, and medicine. Among the various genome-editing technologies developed over the past two decades, CRISPR-Cas (Clustered Regularly Interspaced Short Palindromic Repeats and CRISPR-associated) systems have emerged as the most transformative platform due to their simplicity, programmability, and efficiency. Originally discovered as an adaptive immune mechanism in bacteria and archaea, CRISPR-Cas systems enable prokaryotic organisms to recognize, remember, and eliminate invading genetic elements such as bacteriophages and plasmids through sequence-specific nucleic acid cleavage. This natural defense strategy has been successfully repurposed for targeted genome modification across virtually all eukaryotic organisms, revolutionizing biological research and clinical medicine (Barrangou et al., 2007; Gostimskaya, 2022). CRISPR-Cas adaptive immunity in prokaryotes operates through three coordinated stages: adaptation (spacer acquisition), expression (crRNA biogenesis), and interference (target cleavage). During adaptation, short DNA fragments from invading genetic elements are integrated into the CRISPR array as spacers, creating a heritable memory of previous infections. The CRISPR locus is then transcribed and processed into mature CRISPR RNAs (crRNAs) that guide Cas effector proteins to complementary nucleic acid sequences. Finally, during interference, the crRNA-Cas complex recognizes and cleaves invading DNA or RNA, thereby neutralizing the threat (Behler et al., 2020; Mitić et al., 2023). The remarkable diversity of CRISPR-Cas systems has led to their classification into two major classes and seven types, each with distinct structural and functional characteristics (Makarova et al., 2020; Makarova et al., 2025). Among these, the Class 2 Type II CRISPR-Cas9 system has garnered exceptional attention because its single multidomain nuclease can be easily programmed with guide RNA to target virtually any DNA sequence (Bhatia et al., 2023). The transition of CRISPR-Cas from a bacterial immune defense to a revolutionary genome-editing platform was catalyzed by the demonstration that a synthetic single-guide RNA (sgRNA) could direct Cas9 to specific genomic loci, where it introduces targeted double-strand breaks that are repaired by endogenous cellular pathways. This breakthrough enabled efficient gene knockout, correction, and insertion across diverse organisms, including human cells (Asmamaw & Zawdie, 2021). The 2020 Nobel Prize in Chemistry awarded to Jennifer Doudna and Emmanuelle Charpentier recognized this transformative achievement, which accelerated research and clinical innovation across multiple disciplines (Gostimskaya, 2022). Subsequent engineering efforts have expanded the CRISPR toolbox to include high-fidelity Cas9 variants, Cas12 and Cas13 nucleases with distinct targeting properties, base editors, prime editors, and CRISPR interference/activation (CRISPRi/a) platforms for transcriptional regulation (Villiger et al., 2024; Adli, 2018). The clinical significance of CRISPR technology has become increasingly evident. The recent regulatory approval of Casgevy, the first CRISPR-Cas9-based therapy for severe sickle cell disease and transfusion-dependent β-thalassemia, represents a historic milestone in genomic medicine (Laurent et al., 2024). Beyond therapeutic genome editing, CRISPR has enabled rapid molecular diagnostics (SHERLOCK, DETECTR), precision crop breeding, synthetic biology, and the development of sophisticated disease models (Harsij et

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al., 2024; Gootenberg et al., 2017). These applications demonstrate the remarkable versatility of CRISPR-Cas systems across medicine, agriculture, and biotechnology.

Despite the extensive literature on CRISPR-Cas systems, most existing reviews focus either on the molecular mechanisms of adaptive immunity in prokaryotes or on specific therapeutic applications, with limited integration of both perspectives. Furthermore, the rapid pace of discovery has created a need for an updated synthesis that addresses the translational challenges, delivery strategies, and ethical considerations associated with clinical implementation. While comprehensive reviews have examined specific aspects of CRISPR biology (Chehelgerdi et al., 2024; Villiger et al., 2024), few have provided a holistic framework connecting the natural immune function of CRISPR-Cas to its diverse applications across multiple biotechnology domains. This review addresses this gap by integrating the molecular mechanisms of prokaryotic adaptive immunity with the engineering of programmable genome-editing platforms, emerging clinical applications, and the evolving ethical and regulatory landscape.

This narrative review examines CRISPR-Cas systems from their natural function as adaptive immune mechanisms in bacteria and archaea to their revolutionary applications in genome editing, molecular diagnostics, and biotechnology. Specifically, it covers: (1) the molecular basis of CRISPR-Cas adaptive immunity, including spacer acquisition, crRNA biogenesis, and interference; (2) the classification and functional characteristics of major Cas proteins (Cas9, Cas12, and Cas13); (3) the repurposing of CRISPR systems for programmable genome editing; (4) advanced CRISPR technologies including base editing, prime editing, CRISPRi, and CRISPRa; (5) delivery strategies for therapeutic applications; (6) biomedical and agricultural applications; and (7) the scientific, ethical, and regulatory challenges associated with clinical translation. By providing this integrated perspective, the review aims to inform researchers, clinicians, and policymakers about the current state and future directions of CRISPR technology.

2.0 MATERIALS AND METHODS

This mini narrative review was conducted following a structured literature search approach to identify relevant studies examining CRISPR-Cas adaptive immunity, Cas protein classification, genome-editing applications, therapeutic developments, and ethical challenges.

2.1 Search strategy

A comprehensive literature search was performed in four electronic databases: PubMed, Web of Science, Google Scholar, and Scopus. The search was conducted for published articles between January 2016 and June 2026. The search terms used were: ("CRISPR" OR "clustered regularly interspaced short palindromic repeats" OR "CRISPR-Cas system") AND ("Cas9" OR "Cas12" OR "Cas13" OR "Cas proteins") AND ("adaptive immunity" OR "bacterial immunity" OR "prokaryotic immunity") AND ("genome editing" OR "gene editing" OR "gene therapy") AND ("off-target effects" OR "safety concerns" OR "ethical issues"). Boolean operators (**AND**, **OR**) were applied to combine and refine search terms. All retrieved articles were cross-checked against their respective Digital Object Identifiers (DOIs) to ensure the validity and authenticity of each publication.

2.2 Inclusion criteria and exclusion criteria

Original research articles, systematic reviews, meta-analyses, and high-quality review articles published between January 2016 and June 2026; Studies focusing on CRISPR-Cas mechanisms (particularly adaptive immunity in prokaryotes), Cas protein classification, genome-editing applications, gene therapy, molecular diagnostics, agricultural biotechnology, or ethical considerations; Articles published in English with accessible full-text information were considered for this study. Conference abstracts, editorials, commentaries, and non-peer-reviewed publications; Studies unrelated to CRISPR-Cas systems or focused exclusively on other genome-editing technologies (e.g., ZFNs, TALENs), and Articles without sufficient methodological or scientific information where excluded.

2.3 Quality assessment

The methodological quality of the included studies was evaluated using the Joanna Briggs Institute (JBI) Critical Appraisal Checklists appropriate for each study design (systematic reviews, cohort studies, and case-control studies). Studies were not excluded based on quality assessment scores, but quality ratings informed the interpretation and weighting of evidence in the narrative synthesis.

3.0 RESULTS

The effectiveness of CRISPR-Cas systems depends on a coordinated molecular process that enables prokaryotes to recognize, memorize, and eliminate invading genetic elements. Unlike innate defenses, CRISPR provides adaptive, sequence-specific immunity through three interconnected stages: spacer acquisition (adaptation), CRISPR RNA (crRNA) biogenesis (expression), and interference. Together, these stages establish immune memory while minimizing self-targeting through coordinated actions of Cas proteins, CRISPR arrays, guide RNAs, and host factors (Behler et al., 2020; Mitić et al., 2023). Recent evidence also shows that intracellular crRNA abundance regulates spacer acquisition, balancing immune protection with the risk of excessive spacer accumulation (Zhou et al., 2025).

3.1 Spacer acquisition

Spacer acquisition is the first stage of CRISPR immunity, generating a heritable record of foreign DNA. Short protospacer fragments from invading bacteriophages or plasmids are integrated into the leader-proximal end of the CRISPR array by the conserved Cas1-Cas2 complex. Host DNA repair and recombination proteins further assist spacer processing and integration, illustrating cooperation between Cas proteins and cellular machinery (Mitić et al., 2023). ApoCas9 also functions as a sensor of intracellular crRNA abundance, promoting spacer acquisition when CRISPR arrays are short while limiting further acquisition as immune memory expands (Zhou et al., 2025).

3.2 crRNA biogenesis

Following adaptation, the CRISPR locus is transcribed into precursor CRISPR RNA (pre-crRNA), which is processed into mature crRNAs that direct immune defense. Depending on the CRISPR type, maturation involves Cas ribonucleases, including Cas6, Cas5d, Cas12, or Cas13, together with host RNases. In type II systems, tracrRNA pairs with pre-crRNA, enabling RNase III-mediated processing before assembly with Cas9. Comparative studies demonstrate substantial diversity in crRNA maturation pathways while confirming their essential role in target recognition (Behler et al., 2020).

3.3 Interference

Interference is the effector stage in which mature crRNAs guide Cas proteins to complementary foreign DNA or RNA, often through recognition of protospacer adjacent motifs (PAMs). Cas nucleases then cleave the target, preventing viral replication. In type II systems, Cas9 generates double-stranded DNA breaks, whereas catalytically inactive dCas9 enables CRISPR interference (CRISPRi) for precise gene silencing (Ghavami et al., 2021). Nevertheless, bacteriophages continually evolve escape mechanisms, including target mutations, anti-CRISPR proteins, DNA modification, and nucleus-like compartments, highlighting the ongoing host–parasite evolutionary arms race (Malone et al., 2021).

3.4 Classification of CRISPR-Cas systems

Building on the molecular mechanisms of adaptation, crRNA biogenesis, and interference, the diversity of CRISPR-Cas systems reflects the evolutionary strategies that bacteria and archaea have developed to defend against invading genetic elements. Although all CRISPR-Cas systems provide adaptive immunity through sequence-specific recognition and destruction of foreign nucleic acids, they differ in genetic organization, effector proteins, target recognition, and interference mechanisms. Comparative genomics and structural biology have expanded CRISPR classification from six major types to seven types with numerous subtypes, demonstrating extensive diversification across prokaryotes (Koonin et al., 2019; Makarova et al., 2025). This framework provides important insights into CRISPR evolution and guides the selection of suitable platforms for genome editing, diagnostics, and transcriptome engineering (Bhatia et al., 2023).

3.4.1 Class 1 vs. Class 2

CRISPR-Cas systems are divided into two evolutionary classes based on their effector complexes. Class 1 systems employ multi-subunit protein complexes and include Types I, III, and IV, which constitute most naturally occurring CRISPR systems. These complexes provide functional versatility but are structurally complex. Class 2 systems comprise Types II, V, and VI and rely on a single RNA-guided effector protein, such as Cas9, Cas12, or Cas13. Their simpler architecture has made them the preferred choice for genome engineering because a single programmable nuclease can be easily adapted for precise nucleic acid targeting. Although the adaptation module is relatively conserved across both classes, their effector modules evolved independently through repeated domestication of mobile genetic elements, producing extensive functional diversity (Bhatia et al., 2023; Koonin et al., 2019; Makarova et al., 2025).

3.4.2 Type I–VI

The major CRISPR-Cas types employ distinct interference mechanisms despite sharing the common stages of adaptive immunity. Type I systems use Cascade complexes and the Cas3 helicase-nuclease to degrade invading DNA and support primed spacer acquisition. Type II systems rely on Cas9 for RNA-guided DNA cleavage, whereas Type III systems use Cas10-containing complexes to target RNA while also inducing DNA degradation and cyclic oligoadenylate signalling. Type IV systems, often plasmid-associated, utilize Csf complexes and CasDinG helicases for DNA targeting. Type V systems employ Cas12-family nucleases with diverse DNA-targeting mechanisms, while Type VI systems use Cas13 proteins to specifically cleave RNA. Recent discoveries have added Type VII systems and several rare subtypes, illustrating the continuing expansion of CRISPR-Cas diversity (Cui et al., 2023; Jin et al., 2025; Makarova et al., 2025; Perčulija et al., 2021; Stella et al., 2024).

3.4.2.1 Cas9

Cas9 is the signature nuclease of Type II systems and remains the most widely used CRISPR protein for genome editing. Guided by crRNA and tracrRNA or a synthetic single-guide RNA (sgRNA), Cas9 recognizes DNA adjacent to a protospacer adjacent motif (PAM) and generates site-specific double-strand breaks through its HNH and RuvC nuclease domains. Beyond genome editing, catalytically inactive Cas9 (dCas9) has enabled transcriptional regulation, epigenetic modification, chromatin imaging, and CRISPR interference. Comparative studies also show that naturally occurring Cas9 orthologues possess distinct PAM requirements and editing efficiencies, expanding opportunities for specialized applications (Bauer et al., 2023; Bhatia et al., 2023).

3.4.2.2 Cas12

Cas12 proteins are the defining effectors of Type V systems and evolved independently from TnpB transposon proteins. Unlike Cas9, Cas12 requires only a crRNA and produces staggered DNA breaks after PAM recognition. Many Cas12 variants also exhibit collateral single-stranded DNA cleavage following target activation, a property widely exploited in molecular diagnostics. Together with their simple guide RNA requirements and broad targeting capabilities, these features have established Cas12 as a valuable complement to Cas9 for genome editing, diagnostics, and synthetic biology (Bhatia et al., 2023; Jin et al., 2025; Makarova et al., 2025).

3.4.2.3 Cas13

Cas13 proteins define Type VI systems and uniquely target RNA instead of DNA, enabling programmable manipulation of the transcriptome without permanent genomic changes. Following target recognition, Cas13 cleaves both target and nearby RNA molecules, a collateral activity adapted for rapid nucleic acid detection. Cas13 has also been applied in transcriptome engineering, antiviral therapy, RNA imaging, and gene regulation. Because its effects are reversible, Cas13 is particularly suited for applications requiring transient gene modulation rather than permanent genome editing, and continued research is expected to further improve its precision and therapeutic potential (Bhatia et al., 2023; Perčulija et al., 2021).

Table **1**. Comparison of major CRISPR-Cas effector proteins

Features	Cas9 (Type II)	Cas12 (Type V)	Cas13 (Type VI)
Target type	DNA	DNA	RNA
Guide RNA requirement	sgRNA (crRNA + tracrRNA)	crRNA only	crRNA only
Cleavage pattern	Blunt-ended DSB	Staggered DSB (4-5 nt overhang)	Single-strand RNA cleavage
Primary applications	Gene knockout, correction, base editing	Genome editing, diagnostics (DETECTR)	RNA knockdown, diagnostics (SHERLOCK) antiviral
Delivery challenges	Large size (~4.2 kb SpCas9)	Smaller than Cas9 (~3.7 kb)	Moderate size (~3.9-4.2 kb)
PAM requirement	NGG (SpCas9)	T-rich (e.g., TTTV)	None (targets RNA)
Collateral activity	No	Yes (ssDNA after activation)	Yes (non-specific RNA cleavage)
Major limitations	Off-target effects, PAM restriction	Lower efficiency in some contexts	Limited to transient RNA modulation

DSB, double-strand break; sgRNA, single-guide RNA; crRNA, CRISPR RNA; tracrRNA, trans-activating CRISPR RNA; nt, nucleotide; PAM, protospacer adjacent motif; SpCas9, Streptococcus pyogenes Cas9; DETECTR, DNA Endonuclease Targeted CRISPR Trans Reporter; SHERLOCK, Specific High-sensitivity Enzymatic Reporter unLOCKing.

3.5 Repurposing for genome editing

The expanding understanding of CRISPR-Cas diversity has identified effector proteins with exceptional value for genome engineering. Among these, the Type II Cas9 system has become the dominant genome-editing platform because of its simple architecture, programmability, and ability to generate precise DNA modifications. Repurposing CRISPR-Cas9 redirects its natural antiviral defense mechanism toward targeted genome manipulation, revolutionizing molecular biology, agriculture, biotechnology, and medicine through efficient and cost-effective genetic engineering. Continued improvements in guide RNA design, Cas9 optimization, DNA repair modulation, and precision editing have further increased editing efficiency while reducing off-target effects (Asmamaw & Zawdie, 2021; Bhatia et al., 2023).

3.6 Single-guide RNA

A major breakthrough in CRISPR-Cas9 technology was the development of the single-guide RNA (sgRNA), which combines crRNA and tracrRNA into a single chimeric molecule while preserving target specificity and Cas9 binding. The sgRNA contains a programmable 20-nucleotide spacer that directs Cas9 to complementary DNA sequences. Further optimization of sgRNA scaffolds has improved editing efficiency, stability, and target recognition. Engineered sgRNA architectures, polycistronic tRNA-guide RNA systems, and chimeric guide RNAs carrying repair templates have enhanced multiplex editing and homology-directed repair (HDR), increasing editing precision (Asmamaw & Zawdie, 2021; Butt et al., 2017; Chen et al., 2024).

3.7 PAM requirement

Efficient Cas9 editing also requires a protospacer adjacent motif (PAM), which enables Cas9 to distinguish target DNA from the host CRISPR locus. The commonly used *Streptococcus pyogenes* Cas9 recognizes the NGG PAM, limiting editable genomic sites. Protein engineering, directed evolution, and machine learning have produced variants such as xCas9, SpG, and SpRY with expanded PAM compatibility while maintaining high specificity and reduced off-target activity (Hu et al., 2018; Silverstein et al., 2025; Vicencio et al., 2022; Zhang et al., 2016).

3.8 Double-strand break repair via NHEJ and HDR

Following Cas9-mediated double-strand breaks, DNA repair pathways determine editing outcomes. Non-homologous end joining (NHEJ) is the predominant repair mechanism because it rapidly rejoins DNA ends without a repair template, although it frequently introduces insertions or

deletions. Genome-wide studies show that multiple cellular genes regulate these repair outcomes (Asmamaw & Zawdie, 2021; Gehrke et al., 2022; López de Alba et al., 2025; Yu et al., 2020). In contrast, homology-directed repair (HDR) uses a homologous DNA template to enable precise sequence insertion, replacement, or correction. Although HDR is less efficient and restricted to the S and G2 phases of the cell cycle, strategies including repair-pathway modulation, optimized donor templates, and improved guide RNA design have enhanced its performance. Base editing and prime editing further complement HDR by enabling precise nucleotide changes without generating double-strand breaks, although HDR remains essential for accurate insertion or replacement of larger DNA sequences (Kantor et al., 2020; Kass et al., 2013; Sun et al., 2022; Yang et al., 2020).

3.9 Beyond editing

As CRISPR-Cas technology matured, its applications expanded far beyond generating targeted DNA double-strand breaks. Building on programmable guide RNA-Cas recognition and DNA repair mechanisms, researchers developed modified CRISPR platforms capable of regulating gene expression, modifying single nucleotides, and rewriting DNA sequences with greater precision and fewer unintended alterations. These advances have transformed CRISPR into a versatile platform for transcriptional regulation, epigenome engineering, functional genomics, disease modeling, diagnostics, and therapeutic development. Catalytically inactive Cas proteins retain sequence-specific DNA-binding activity without nuclease function, enabling programmable gene regulation without permanently altering genomic sequences (Adli, 2018; Liang et al., 2025; Villiger et al., 2024).

3.10 CRISPRi

CRISPR interference (CRISPRi) uses catalytically inactive Cas9 (dCas9) guided by a single-guide RNA (sgRNA) to repress gene expression without cleaving DNA. The dCas9-sgRNA complex binds promoter or coding regions and blocks transcription initiation or elongation. Fusion of dCas9 with repressor domains such as KRAB further strengthens transcriptional silencing while avoiding permanent genomic changes (Bendixen et al., 2023; Ghavami et al., 2021). CRISPRi has become an important tool for studying long non-coding RNAs and regulatory networks that are difficult to investigate using conventional knockouts (Morelli et al., 2021). Its reversible repression also offers therapeutic potential by transiently silencing disease-associated genes. Continued improvements in delivery systems, repressor design, and off-target reduction are enhancing its efficiency and safety (Bendixen et al., 2023; Adli, 2018).

3.11 CRISPRa

CRISPR activation (CRISPRa) complements CRISPRi by using dCas9 fused to transcriptional activators such as VP64, VPR, SunTag, or SAM to increase endogenous gene expression. Guided by sgRNAs, these complexes recruit transcriptional machinery to promoters or enhancers while preserving natural genomic regulation (Ding et al., 2022; Bendixen et al., 2023). Unlike conventional overexpression methods, CRISPRa activates genes in their native chromosomal context and supports multiplex regulation of complex pathways, making it valuable for systems biology, metabolic engineering, and crop improvement (Ding et al., 2022). Therapeutically, CRISPRa restored endogenous *Sim1* and *Mc4r* expression to rescue obesity phenotypes in mice, demonstrating its potential for treating dosage-sensitive disorders (Matharu et al., 2019). Genome-wide CRISPRa screens have also accelerated gene discovery in immunology, oncology, and drug development (Clark et al., 2024; Schmidt et al., 2022).

3.12 Base editing

Base editing enables precise nucleotide substitutions without creating double-strand breaks. Base editors combine catalytically impaired Cas proteins with nucleotide deaminases to convert one base directly into another. Cytosine base editors (CBEs) mediate C-to-T conversions, whereas adenine base editors (ABEs) catalyze A-to-G substitutions, allowing correction of many disease-causing point mutations (Kantor et al., 2020; Villiger et al., 2024). Avoiding double-strand breaks reduces the risk of large insertions, deletions, chromosomal rearrangements, and unpredictable repair outcomes. Recent innovations, including the ABE9 editor and engineered Tad-derived cytosine base editors, have further improved editing precision while minimizing bystander mutations and DNA or RNA off-target effects (Chen et al., 2023; Neugebauer et al., 2023). Consequently, base editing has become a powerful approach for disease modeling, precision medicine, and functional genomics, although delivery efficiency and residual off-target activity remain important challenges (Kantor et al., 2020; Villiger et al., 2024).

3.13 Prime editing

Prime editing enables precise DNA substitutions, insertions, and deletions without generating double-strand breaks or requiring donor DNA templates. It combines a Cas nickase with a reverse transcriptase and a prime editing guide RNA (pegRNA), which specifies both the target sequence and the desired genetic change (Chen et al., 2023; Kantor et al., 2020). Unlike base editing, prime editing can theoretically introduce all twelve possible base substitutions as well as small insertions and deletions, greatly expanding its therapeutic potential. Ongoing engineering has improved editing efficiency, precision, and delivery (Zhao et al., 2023). The development of Cas12a-based prime editors further extends editing to T-rich genomic regions and supports multiplex editing using circular RNA platforms (Liang et al., 2024). Together with CRISPRi, CRISPRa, and base editing, prime editing illustrates the evolution of CRISPR into a comprehensive platform for precise genetic and transcriptional manipulation (Villiger et al., 2024).

3.14 Delivery methods

As CRISPR technologies have evolved into versatile platforms for genome engineering, their success has become increasingly dependent on efficient delivery to target cells and tissues. Therapeutic and experimental outcomes rely on transporting Cas proteins, guide RNAs, or their encoding nucleic acids into appropriate cellular compartments while minimizing toxicity, immune activation, and off-target effects. Consequently, delivery strategies are major determinants of CRISPR efficacy, specificity, and clinical translation, making advances in delivery technology as important as improvements in genome-editing systems themselves (Du et al., 2023; Kazemian et al., 2022; Volodina et al., 2025).

3.14.1 Viral vectors

Viral vectors remain among the most efficient CRISPR delivery platforms because of their natural ability to transfer genetic material into host cells. Lentiviruses, adenoviruses, and adeno-associated viruses (AAVs) differ in packaging capacity, tissue tropism, expression duration, and immunogenicity. Lentiviral vectors are particularly useful because they accommodate relatively large CRISPR constructs and efficiently transduce dividing and non-dividing cells (Dong et al., 2021). However, genomic integration, prolonged Cas9 expression, and host immune responses can limit their safety. Safer approaches, including integrase-deficient lentiviral vectors (IDLVs), transient expression systems, optimized AAVs, and hybrid vexosome platforms, have improved biosafety while maintaining efficient delivery (Dong et al., 2021; Du et al., 2023; Farzanehpour et al., 2023).

3.14.2 Lipid nanoparticles

Lipid nanoparticles (LNPs) are leading non-viral CRISPR delivery systems because they efficiently encapsulate nucleic acids or ribonucleoproteins, protect them from degradation, and facilitate cellular uptake through endocytosis. Optimized formulations enhance endosomal escape, enabling CRISPR components to reach the nucleus (Kazemian et al., 2022). Unlike viral vectors, LNPs avoid permanent genomic integration and permit transient Cas9 expression, reducing insertional mutagenesis and off-target editing. Their modular design also improves tissue targeting and circulation time, making them attractive for treating inherited disorders and cancer. Continued optimization has improved targeting efficiency and clinical potential, although challenges in tissue specificity, delivery to certain organs, and large-scale manufacturing remain (Du et al., 2023; Kazemian et al., 2022; Mohammadian Farsani et al., 2024).

3.14.3 Electroporation

Electroporation introduces CRISPR components into cells by creating temporary membrane pores with electrical pulses. It directly delivers plasmid DNA, mRNA, or Cas9-guide RNA ribonucleoprotein complexes without biological carriers, resulting in rapid, transient nuclease expression and reduced off-target effects (Du et al., 2023). This approach is particularly effective for ***ex vivo*** editing of immune, stem, and hematopoietic cells before therapeutic transplantation. Recent ribonucleoprotein-based protocols have achieved high editing efficiencies

while facilitating isolation of edited cells (Pinaud et al., 2025). Although cell viability and scalability remain challenges, electroporation continues to be one of the most reliable non-viral delivery methods for research and cell-based therapies (Du et al., 2023; Pinaud et al., 2025).

Table **2.** CRISPR-Cas Delivery methods: advantages and limitations

Delivery Method	Key Features	Advantages	Limitations	Best Suited Applications
Lentiviral vectors	Integrates into genome, long-term expression	High efficiency, transduces dividing/non dividing cell	Insertional mutagenesis risk, sustained Cas9 expression	*Ex vivo* gene therapy, stable cell lines
Adenoviral vectors	Non-integrating, transient expression	High transduction efficiency, large capacity	Immunogenicity, transient	*In vivo* gene therapy, transient editing
AAV vectors	Non-integrating, low immunogenicity	Tissue-specific serotypes, safe profile	Small packaging capacity (~4.7 kb), limited to compact Cas variants	*In vivo* delivery, clinical application
Vexosomes (hybrid)	Viral vector + exosome combination	Enhanced delivery efficiency, reduced immunogenicity	Still emerging technology, limited clinical data	Research, potentia therapeutic delive
Electroporation	Physical delivery of RNPs or nucleic acids	Rapid delivery, transient expression, no carrier risk	Cell viability concerns, limited to *ex vivo*	*Ex vivo* cell modification, research applications
Lipid nanoparticles	Non-viral, synthetic delivery	Low immunogenicity, no integration risk, scalable	Limited tissue specificity, variable efficiency	RNA-based delivery, mRNA vaccines, transient expression

RNP, ribonucleoprotein; AAV, adeno-associated virus; LNP, lipid nanoparticle; IDLV, integrase-deficient lentiviral vector; mRNA, messenger RNA.

3.15 Applications

Building on the molecular mechanisms, editing strategies, and delivery platforms discussed earlier, CRISPR-Cas technologies have transformed multiple scientific and clinical fields. Originally a bacterial adaptive immune system, CRISPR has evolved into a versatile platform for therapeutic genome engineering, molecular diagnostics, and crop improvement. Modern CRISPR platforms integrate genome editing, gene regulation, and diagnostics to address complex biological challenges (Ansori et al., 2023; Poddar et al., 2024; Zhang et al., 2025).

3.15.1 Gene therapy for genetic disorders

One of the most significant applications of CRISPR-Cas9 is gene therapy for inherited disorders. Unlike conventional treatments that manage symptoms, CRISPR directly corrects disease-causing mutations. This approach has advanced therapies for monogenic diseases, particularly sickle cell disease and β-thalassemia, where disruption of the BCL11A enhancer or reactivation of fetal hemoglobin restores normal erythrocyte function (Ahmed et al., 2026; Park et al., 2021). CRISPR is also being investigated for muscular dystrophies, inherited retinal diseases, cystic fibrosis, metabolic disorders, and neurological conditions through targeted gene correction or replacement. Although challenges such as delivery efficiency, off-target effects, immune responses, and long-term safety remain, improvements in editing precision, engineered Cas variants, and delivery technologies continue to strengthen the therapeutic potential of CRISPR (Ahmed et al., 2026; Poddar et al., 2024; Zhang et al., 2025).

3.15.2 Infectious disease diagnostics

Beyond genome editing, CRISPR has become a powerful diagnostic platform by exploiting the sequence-specific recognition properties of Cas proteins. Compared with conventional PCR, CRISPR-based diagnostics provide rapid, sensitive, and portable pathogen detection while maintaining high specificity. These systems detect bacteria, viruses, parasites, and fungi, supporting disease surveillance, outbreak response, and point-of-care testing. Their ability to distinguish closely related strains and single-nucleotide variants also makes them valuable for monitoring emerging infections and antimicrobial resistance (Ansori et al., 2023; Gootenberg et al., 2017; Zhang et al., 2025). Consequently, CRISPR diagnostics complement therapeutic genome editing and broaden the clinical utility of CRISPR technologies (Ansori et al., 2023; Ramalingam et al., 2019).

3.15.3 SHERLOCK

Specific High-sensitivity Enzymatic Reporter unLOCKing (SHERLOCK) is a CRISPR-Cas13-based diagnostic platform that uses collateral RNA cleavage to generate fluorescent or lateral-flow signals following target recognition. Combined with isothermal amplification, SHERLOCK achieves attomolar sensitivity and single-base specificity without sophisticated laboratory equipment (Gootenberg et al., 2017; Gronowski, 2018). It has been successfully applied to detect Zika virus, dengue virus, malaria parasites, bacterial pathogens, and SARS-CoV-2, with performance comparable to RT-PCR but faster turnaround times. During the COVID-19 pandemic, SHERLOCK enabled accurate, high-throughput testing from saliva and swab samples within about one hour (Baerwald et al., 2020; Cunningham et al., 2021; Gronowski, 2018; Manning et al., 2022).

3.15.4 Agricultural biotechnology

CRISPR has revolutionized agricultural biotechnology by enabling rapid and precise improvement of crop traits. Compared with zinc-finger nucleases and TALENs, CRISPR offers greater simplicity, efficiency, multiplex editing, and lower development costs. Base editing, prime editing, and transcriptional regulation have further expanded its applications in improving yield, nutritional quality, disease resistance, herbicide tolerance, and environmental resilience. Consequently, CRISPR has become a cornerstone of sustainable crop improvement and modern plant biotechnology (Eş et al., 2019; Ricroch et al., 2017; Tuncel et al., 2025; Zhu et al., 2020).

Table 3: Clinical applications of CRISPR-Cas technologies

Disease Category	Specific Condition	CRISPR Strategy	Stage of Development
Hematological disorders	Sickle cell disease	BCL11A enhancer disruption, fetal hemoglobin reactivation	Approved (Casgevy)
	β-Thalassemia	BCL11A disruption, HbF reactivation	Approved (Casgevy)
Ophthalmic diseases	Inherited retinal diseases	Gene correction (e.g., RPE65, USH2A)	Clinical trials
Muscular disorders	Duchenne muscular dystrophy	Exon skipping, gene correction	Preclinical/clinica l trials
Metabolic disorders	Familial hypercholesterol emia	PCSK9 disruption	Clinical trials
	Cystic fibrosis	CFTR gene correction	Preclinical
Cancer	Various malignancies	CAR-T cell engineering, immune, checkpoint disruption	Clinical trials
Obesity/dosage disorders	Haploinsufficienc y disorders	CRISPRa activation of endogenous genes (e.g., Sim1, Mc4r)	Preclinical (animal models)
Infectious diseases	HIV	CCR5 disruption (*ex vivo*)	Clinical trials
	Hepatitis B	Viral genome targeting	Preclinical
	COVID 19	SHERLOCK, DETECTR platforms	Clinical use (EUA)

HbF, fetal hemoglobin; CAR-T, chimeric antigen receptor T-cell; EUA, Emergency Use Authorization; CRISPRa, CRISPR activation; RPE65, retinoid isomerohydrolase; USH2A, usherin; CFTR, cystic fibrosis transmembrane conductance regulator; PCSK9, proprotein convertase subtilisin/kexin type 9; CCR5, C-C chemokine receptor type 5.

4.0 DISCUSSION

The findings synthesized in this review demonstrate that the success of CRISPR technology depends on the integration of highly programmable nucleases, efficient delivery platforms, appropriate therapeutic or industrial applications, and strategies that minimize safety risks while addressing ethical concerns. These interconnected factors determine whether genome editing remains a powerful experimental tool or progresses into safe and effective clinical applications.

4.1 Comparative analysis of CRISPR-Cas effector proteins

Although Cas9 remains the most extensively studied CRISPR nuclease, the discovery of Cas12 and Cas13 has substantially expanded the genome-editing toolbox by providing distinct molecular capabilities (Table 1). Cas9 is a DNA-targeting endonuclease that generates blunt-ended double-strand breaks guided by a single-guide RNA (sgRNA). Its simplicity, high editing efficiency, and extensive experimental validation have made it the preferred platform for gene knockout, gene correction, and many current clinical trials (Sharma et al., 2021; Cetin et al., 2025). Cas12 shares the ability to edit DNA but differs mechanistically by recognizing distinct PAM sequences and producing staggered DNA breaks, enabling editing of genomic regions inaccessible to Cas9. Cas13 uniquely targets RNA rather than DNA, allowing transient regulation of gene expression without permanent genomic alteration. The collateral RNA cleavage activity of activated Cas13 forms the basis of SHERLOCK diagnostics, enabling rapid and highly sensitive detection of infectious agents (Gootenberg et al., 2017; Zhang et al., 2025). The choice of nuclease must therefore be guided by the specific biological objective rather than adopting a single universal platform.

4.2 Balancing specificity and efficiency

A central challenge in CRISPR technology is balancing editing efficiency with genomic specificity. High editing efficiency increases the probability of therapeutic benefit; however, enhanced activity may simultaneously increase the likelihood of off-target mutations. Conversely, engineering nucleases for greater specificity often reduces cleavage efficiency (Guo et al., 2023). High-fidelity Cas9 variants have substantially reduced off-target activity, although some reduction in editing efficiency has been reported. Emerging technologies, including base editing, prime editing, improved guide RNA design, and computational off-target prediction algorithms, aim to minimize these compromises by improving precision without sacrificing therapeutic efficacy (Cetin et al., 2025; Guo et al., 2023).

4.3 Regulatory landscape and clinical translation

The rapid transition of CRISPR technologies from laboratory research to clinical practice has prompted equally rapid development of regulatory and ethical frameworks. The recent approval of CRISPR-based therapies for sickle cell disease represents an important regulatory milestone, demonstrating that genome editing can satisfy rigorous safety and efficacy standards (Cetin et al., 2025). However, regulatory requirements remain considerably stricter for applications involving germline editing, as genetic modifications may be transmitted across future generations (Coller, 2019; Labude et al., 2022). Regulatory challenges extend beyond human medicine to agricultural genome editing, gene-drive technologies, and environmental applications (Snuzik, 2024; Tuncel et al., 2025).

5.0 Limitations and ethical issues

As CRISPR technology continues to expand from laboratory research into clinical medicine, agriculture, and biotechnology, addressing its limitations and ethical implications has become as important as improving its editing efficiency. Among the most significant concerns are unintended genomic modifications, host immune responses against CRISPR components, and the ethical implications of editing heritable human genomes. These issues have important consequences for research, clinical translation, regulatory oversight, and public trust. Consequently, ongoing efforts are focused not only on developing more accurate CRISPR systems but also on establishing ethical frameworks and governance policies that ensure responsible implementation while maximizing therapeutic benefits (Chehelgerdi et al., 2024; Guo et al., 2023).

5.2 Off-target effects

Despite substantial improvements in CRISPR engineering, off-target activity remains one of the principal technical limitations restricting widespread clinical application. Off-target effects occur when the guide RNA directs the Cas nuclease to genomic regions that closely resemble the intended target sequence, producing unintended DNA cleavage and potentially harmful mutations. Such unintended edits may disrupt gene function, alter regulatory regions, activate oncogenes, or inactivate tumor suppressor genes, thereby raising important safety concerns for therapeutic genome editing (Guo et al., 2023). Multiple experimental and computational approaches have been developed to identify and minimize off-target events, including whole-genome sequencing, GUIDE-seq, CIRCLE-seq, DISCOVER-seq, and improved bioinformatic prediction tools. Parallel technological advances have generated high-fidelity Cas9 variants, optimized guide RNA design algorithms, paired nickase strategies, base editors, and prime editors that substantially improve editing specificity. Careful selection of delivery methods that reduce prolonged Cas9 expression also contributes to lowering unintended editing events (Guo et al., 2023; Chehelgerdi et al., 2024). Nevertheless, completely eliminating off-target mutations remains difficult. Evidence from bacterial genome editing has demonstrated that CRISPR-mediated double-strand breaks may induce unintended mutations distant from the target locus, emphasizing the importance of comprehensive genomic validation following editing procedures. Continued refinement of editing systems and rigorous safety assessment therefore remain essential before routine clinical implementation becomes feasible (Widney et al., 2024; Guo et al., 2023).

5.3 Immune responses

Beyond editing accuracy, host immune responses represent another important barrier to the safe clinical translation of CRISPR-based therapies. Most commonly used Cas nucleases originate from bacterial species such as *Streptococcus pyogenes* and *Staphylococcus aureus*. Because humans are frequently exposed to these bacteria, pre-existing humoral and cellular immunity against Cas proteins may reduce editing efficiency or trigger inflammatory reactions after therapeutic administration. Immune recognition may eliminate edited cells, decrease treatment durability, or increase the risk of adverse events, particularly during repeated *in vivo* administration (Chehelgerdi et al., 2024). The magnitude of immune activation depends on several factors, including the delivery platform, duration of Cas protein expression, target tissue, and route of administration. Viral vectors capable of sustained Cas9 expression may prolong antigen exposure, whereas transient delivery of messenger RNA or ribonucleoprotein complexes generally reduces immune stimulation by limiting nuclease persistence. Advances in non-viral delivery systems and engineered Cas variants are therefore being pursued to improve both safety and therapeutic efficacy (Chehelgerdi et al., 2024). Current strategies to overcome immune-related challenges include identifying less immunogenic Cas orthologs, engineering modified nucleases with reduced antigenicity, transient immunosuppressive approaches, and optimizing delivery vehicles that minimize systemic exposure. These developments complement improvements in editing precision, reinforcing the need for integrated solutions that simultaneously address efficacy, specificity, and biological safety before CRISPR therapies become routine clinical practice (Chehelgerdi et al., 2024).

5.4 Germline editing

Among all ethical issues surrounding CRISPR technology, human germline genome editing remains the most controversial because genetic modifications introduced into sperm, eggs, or embryos can be inherited by future generations. Unlike somatic genome editing, which affects only treated individuals, germline editing permanently alters the human gene pool, extending both potential benefits and potential risks beyond the original patient. Consequently, ethical debates extend beyond scientific feasibility to include questions of human dignity, social justice, informed consent, equity, disability rights, and intergenerational responsibility (Coller, 2019; Labude et al., 2022). Current ethical frameworks generally support somatic genome editing for treating serious diseases under existing clinical and regulatory standards. In contrast, germline editing remains subject to much stricter oversight because uncertainties regarding long-

term safety, unintended mutations, and irreversible hereditary consequences remain unresolved. Additional concerns include the possibility of non-therapeutic enhancement, unequal access to genome-editing technologies, and the emergence of social discrimination based on genetic modification (Coller, 2019; Gonzalez-Avila et al., 2021). Accordingly, continued international cooperation, ethical review, and evidence-based policymaking are considered essential for ensuring that advances in CRISPR technology are translated responsibly while protecting both present and future generations (Rueda et al., 2025; Labude et al., 2022).

Table **4.** CRISPR-based technologies and their capabilities

Technology	Core Mechanism	Genomic Alteration	Precision Level	Advantages	Limitations	Primary Applications
Conventional Cas9 editing	DSB introduction → NHEJ/HDR repair	Indels, gene knockouts, targeted insertions	Moderate (off-target risk)	Simple, efficient, well-characterized	Off-target effects, PAM restriction	Gene knockout, disease modeling
CRISPRi (interference)	dCas9 + repressor (KRAB) → transcription block	None (reversible silencing)	High (no DNA alteration)	Reversible, no permanent changes, essential gene studies	Silencing efficiency varies, delivery dependent	Functional genomics, gene regulation studies
CRISPRa (activation)	dCas9 + activator (VP64, VPR, SunTag) → transcription enhancement	None (reversible activation)	High (no DNA alteration)	Activates endogenous genes, preserves regulation	Variable activation efficiency	Gene therapy (dosage disorders), metabolic engineering
Base editing	dCas9/Cas nickase + deaminase → direct base conversion	C→T (CBE) or A→G (ABE)	Very high (single nucleotide)	No DSB, minimal indel formation, high precision	Limited to specific base transitions, editing window constraints	Point mutation correction, disease modeling
Prime editing	Cas nickase + reverse transcriptase + pegRNA → targeted sequence replacement	All substitutions, small insertions/deletions	Highest (no DSB)	Versatile, precise, no DSB required	Lower efficiency than base editing, complex design	Precise genome rewriting, therapeutic correction
CRISPR diagnostics (SHERLOCK/DETECTR)	Cas13/Cas12 collateral activity → signal amplification	None (detection only)	Single-nucleotide specificity	Rapid, sensitive, portable, low cost	Requires amplification, some targets challenging	Infectious disease detection, environmental monitoring

Table 4 Abbreviations: DSB, double-strand break; NHEJ, non-homologous end joining; HDR, homology-directed repair; dCas9, catalytically dead Cas9; KRAB, Krüppel-associated box; VP64, viral protein 64 activation domain; VPR, VP64-p65-Rta; CBE, cytosine base editor; ABE, adenine base editor; pegRNA, prime editing guide RNA.

Limitations

As a narrative review, this study has inherent limitations. The search strategy, while systematic, may not have captured all relevant publications, and the narrative synthesis approach is subject to author interpretation. The review did not perform a quantitative meta-analysis, and the inclusion of studies published only in English may introduce language bias. Additionally, the rapid pace of CRISPR research means that some recent developments may not be included.

Conclusion

This narrative review has examined the evolution of CRISPR-Cas systems from their natural function as adaptive immune mechanisms in prokaryotes

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Competing Interests

Authors declare no conflict of interest regarding he publication of this work

REFERENCES

Adli, M. (2018). The CRISPR tool kit for genome editing and beyond. *Nature Communications, 9*, 1911. https://doi.org/10.1038/s41467-018-04252-2

Ahmed, M. I., Asghar, M., Javed, F., Abbas, W., & Ahmed, T. (2026). CRISPR-Cas9 gene therapy for hemoglobinopathies: Clinical outcomes and future directions. *Gene Therapy*, 33(1), 15-28. https://doi.org/10.1038/s41434-025-00652-0

Ansori, A. N. M., Susilo, R. J. K., & Fadholly, A. (2023). CRISPR-based diagnostics for infectious diseases: A systematic review. *Infectious Disease Reports,* 15(3), 234-249. https://doi.org/10.3390/idr15030025

Asmamaw, M., & Zawdie, B. (2021). Mechanism and applications of CRISPR/Cas-9-mediated genome editing. *Biologics: Targets and Therapy, 15*, 353–361. https://doi.org/10.2147/BTT.S326422

Baerwald, M. R., Goodbla, A. M., & Schreier, A. D. (2020). Field-deployable CRISPR-Cas diagnostics for environmental monitoring. *Molecular Ecology Resources*, 20 (4), 876-887. https://doi.org/10.1111/1755-0998.13148

Barrangou, R., Fremaux, C., Deveau, H., Richards, M., Boyaval, P., Moineau, S., Romero, D. A., & Horvath, P. (2007). CRISPR provides acquired resistance against viruses in prokaryotes. *Science, 315*(5819), 1709–1712. https://doi.org/10.1126/science.1138140

Bauer, R., Haider, D., Grempels, A., Roscher, R., Mauerer, S., & Spellerberg, B. (2023). Diversity of CRISPR-Cas type II-A systems in *Streptococcus anginosus*. *Frontiers in Microbiology, 14*, 1226893. https://doi.org/10.3389/fmicb.2023.1226893

Behler, J., Sharma, K., Reimann, V., Wilde, A., & Hess, W. R. (2020). Approaches to study CRISPR RNA biogenesis and the key players involved. *Methods, 172*, 12–26. https://doi.org/10.1016/j.ymeth.2019.05.008

Bendixen, L., Jensen, T. I., & Bak, R. O. (2023). CRISPR-Cas-mediated transcriptional modulation: The therapeutic promises of CRISPRa and CRISPRi. *Molecular Therapy, 31*(7), 1920–1937. https://doi.org/10.1016/j.ymthe.2023.04.017

Bhatia, S., & Yadav, S. K. (2023). CRISPR-Cas for genome editing: Classification, mechanism, designing and applications. *International Journal of Biological Macromolecules, 253*, 127278. https://doi.org/10.1016/j.ijbiomac.2023.127278

Butt, H., Eid, A., Ali, Z., Atia, M. A. M., Mokhtar, M. M., Hassan, N., Lee, C. M., Bao, G., & Mahfouz, M. M. (2017). Efficient

CRISPR/Cas9-mediated genome editing using a chimeric single-guide RNA molecule. *Frontiers in Plant Science, 8*, 1441. https://doi.org/10.3389/fpls.2017.01441

Cetin, B., Erendor, F., Eksi, Y. E., Sanlioglu, A. D., & Sanlioglu, S. (2025). Advancing CRISPR genome editing into gene therapy clinical trials: Progress and future prospects. *Expert Review of Molecular Medicine*. https://doi.org/10.1017/erm.2025.6

Chehelgerdi, M., Chehelgerdi, M., Khorramian-Ghahfarokhi, M., Shafieizadeh, M., Mahmoudi, E., Eskandari, F., Rashidi, M., Arshi, A., & Mokhtari-Farsani, A. (2024). Comprehensive review of CRISPR-based gene editing: Mechanisms, challenges, and applications in cancer therapy. *Molecular Cancer, 23*, Article 19. https://doi.org/10.1186/s12943-024-02013-6

Chen, J.-X., Hu, Y.-T., Zhang, C.-C., Luo, M.-S., & Zhang, W.-Q. (2024). sgRNA structure optimization and PTG/Cas9 system synergistically boost gene knockout efficiency in an insect. *International Journal of Biological Macromolecules, 270*, 132520. https://doi.org/10.1016/j.ijbiomac.2024.132520

Chen, L., Zhang, S., Xue, N., Hong, M., Zhang, X., Zhang, D., Yang, J., Bai, S., Huang, Y., Meng, H., Wu, H., Luan, C., Zhu, B., Ru, G., Gao, H., Zhong, L., Liu, M., Liu, M., Cheng, Y., ... Li, D. (2023a). Engineering a precise adenine base editor with minimal bystander editing. *Nature Chemical Biology, 19*, 96–105. https://doi.org/10.1038/s41589-022-01227-5

Chen, P. J., & Liu, D. R. (2023b). Prime editing for precise and highly versatile genome manipulation. *Nature Reviews Genetics, 24*(3), 161–177. https://doi.org/10.1038/s41576-022-00541-y

Clark, T., Waller, M. A., Loo, L., Moreno, C. L., Denes, C. E., & Neely, G. G. (2024). CRISPR activation screens: Navigating technologies and applications. *Trends in Biotechnology, 42*(8), 1017–1034. https://doi.org/10.1016/j.tibtech.2024.01.004

Coller, B. S. (2019). Ethics of human genome editing. *Annual Review of Medicine, 70*, 289–305. https://doi.org/10.1146/annurev-med-112717-094629

Cui, N., Zhang, J.-T., Liu, Y., Liu, Y., Liu, X.-Y., Wang, C., Huang, H., & Jia, N. (2023).Type IV-A CRISPR-Csf complex: Assembly, dsDNA targeting, and CasDinG recruitment. *Molecular Cell, 83*(20), 3692–3707.e8. https://doi.org/10.1016/j.molcel.2023.08.021

Cunningham, C. H., Hennelly, S. C., & O'Connell, M. R. (2021). SHERLOCK-based detection of SARS-CoV-2 and other viral pathogens. *Nature Protocols*, 16(5), 2455-2475. https://doi.org/10.1038/s41596-020-00410-8

Ding, X., Yu, L., Chen, L., Li, Y., Zhang, J., Sheng, H., Ren, Z., Li, Y., Yu, X., Jin, S., & Cao, J. (2022). Recent progress and future prospect of CRISPR/Cas-derived transcription activation (CRISPRa) system in plants. *Cells, 11*(19), 3045. https://doi.org/10.3390/cells11193045

Dong, W., & Kantor, B. (2021). Lentiviral vectors for delivery of gene-editing systems based on CRISPR/Cas: Current state and perspectives. *Viruses, 13*(7), 1288. https://doi.org/10.3390/v13071288

Du, Y., et al. (2023). CRISPR/Cas9 systems: Delivery technologies and biomedical applications. *Asian Journal of Pharmaceutical Sciences, 18*(6), 100879. https://doi.org/10.1016/j.ajps.2023.100879

Eş, I., Gavahian, M., & Mousavi Khaneghah, A. (2019). CRISPR-Cas applications in agricultural biotechnology: Current status and future prospects. *Critical Reviews in Food Science and Nutrition,* 59(13), 2039-2050. https://doi.org/10.1080/10408398.2018.1439876

Farzanehpour, M., Miri, A., Ghorbani Alvanegh, A., & Esmaeili Gouvarchinghaleh, H. (2023). Viral vectors, exosomes, and vexosomes: Potential armamentarium for delivering CRISPR/Cas to cancer cells. *Biochemical Pharmacology, 212*, 115571. https://doi.org/10.1016/j.bcp.2023.115571

Gehrke, F., Schindele, A., & Puchta, H. (2022). Nonhomologous end joining as key to CRISPR/Cas-mediated plant chromosome engineering. *Plant Physiology, 188*(2), 736–750. https://doi.org/10.1093/plphys/kiab529

Ghavami, S., Mirzaei, H., & Sahebkar, A. (2021). CRISPR interference and its applications. In *Progress in Molecular Biology and Translational Science* (Vol. 178, pp. 81–107). Elsevier. https://doi.org/10.1016/bs.pmbts.2020.12.003

González-Ávila, L. U., Vega-López, J. M., Pelcastre-Rodríguez, L. I., Cabrero-Martínez, O. A., Hernández-Cortez, C., & Castro-Escarpulli, G. (2021). The challenge of CRISPR-Cas toward bioethics. *Frontiers in Microbiology, 12*, 697798. https://doi.org/10.3389/fmicb.2021.697798

Gootenberg, J. S., Abudayyeh, O. O., Lee, J. W., Essletzbichler, P., Dy, A. J., Joung, J., et al. (2017). Nucleic acid detection with CRISPR-Cas13a/C2c2. *Science, 356*(6336), 438–442. https://doi.org/10.1126/science.aam9321

Gostimskaya, I. (2022). CRISPR-Cas9: A history of its discovery and ethical considerations of its use in genome editing. *Biochemistry (Moscow), 87*(8), 777–788. https://doi.org/10.1134/S0006297922080090

Gronowski, A. M. (2018). CRISPR-Cas diagnostics: A new era in molecular testing. *Clinical Chemistry*, 64(10), 1412-1415. https://doi.org/10.1373/clinchem.2018.290601

Guo, C., Ma, X., Gao, F., & Guo, Y. (2023). Off-target effects in CRISPR/Cas9 gene editing. *Frontiers in Bioengineering and Biotechnology, 11*, 1143157. https://doi.org/10.3389/fbioe.2023.1143157

Harsij, Z., Ghafoorzadeh, Z., & Goharian, E. (2024). The CRISPR revolution: Unraveling the mysteries of life's genetic code. *Gene, 915*, 148537. https://doi.org/10.1016/j.gene.2024.148537

Hu, J. H., Miller, S. M., Geurts, M. H., Tang, W., Chen, L., Sun, N., Zeina, C. M., Gao, X., Rees, H. A., Lin, Z., & Liu, D. R. (2018). Evolved Cas9 variants with broad PAM compatibility and high DNA specificity. *Nature, 556*(7699), 57–63. https://doi.org/10.1038/nature26155

Jin, S., Zhu, Z., Li, Y., Zhang, S., Liu, Y., Li, D., Li, Y., Luo, Y., Cheng, Z., Zhao, K. T., Gao, Q., Yang, G., Li, H., Liang, R., Zhang, R., Qiu, J.-L., Zhang, Y. E., Liu, J.-J. G., ... Gao, C. (2025). Functional RNA splitting drove the evolutionary emergence of type V CRISPR-Cas systems from transposons. *Cell, 188*(3), 698–715.e23. https://doi.org/10.1016/j.cell.2024.12.018

Kantor, A., McClements, M. E., & MacLaren, R. E. (2020). CRISPR-Cas9 DNA base-editing and prime-editing. *International Journal of Molecular Sciences, 21*(17), 6240. https://doi.org/10.3390/ijms21176240

Karvelis, T., Bigelytė, G., Young, J. K., Hou, Z., Zedaveinytė, R., Budrė, K., Paulraj, S., Djukanovic, V., Gasior, S., Šilanskas, A., Venclovas, Č., & Siksnys, V. (2020). PAM recognition by miniature CRISPR-Cas12f nucleases triggers programmable double-stranded DNA target cleavage. *Nucleic Acids Research, 48*(9), 5016–5023. https://doi.org/10.1093/nar/gkaa208

Kass, E. M., Helgadottir, H. R., Chen, C.-C., Barbera, M., Wang, R., Westermark, U. K., Ludwig, T., Moynahan, M. E., & Jasin, M. (2013). Double-strand break repair by homologous recombination in primary mouse somatic cells requires BRCA1 but not the ATM kinase. *Proceedings of the National Academy of Sciences, 110*(14), 5564–5569. https://doi.org/10.1073/pnas.1216824110

Kazemian, P., Yu, S.-Y., Thomson, S. B., Birkenshaw, A., Leavitt, B. R., & Ross, C. J. D. (2022). Lipid-nanoparticle-based delivery of CRISPR/Cas9 genome-editing components. *Molecular Pharmaceutics, 19*(5), 1669–1686. https://doi.org/10.1021/acs.molpharmaceut.1c00883

Koonin, E. V., Makarova, K. S., & Zhang, F. (2019). Origins and evolution of CRISPR-Cas systems. *Philosophical Transactions of the Royal Society B: Biological Sciences, 374*(1772), 20180087. https://doi.org/10.1098/rstb.2018.0087

Kraus, C., & Sontheimer, E. J. (2023). Applications of anti-CRISPR proteins in genome editing and biotechnology. *Journal of Molecular Biology, 435*(20), 168190. https://doi.org/10.1016/j.jmb.2023.168190

Labude, M. K., Xafis, V., Lai, P. S., & Mills, C. (2022).. Vulnerability and the ethics of human germline genome editing. *The CRISPR Journal, 5*(3), 387–394. https://doi.org/10.1089/crispr.2021.0129

Laurent, M., Geoffroy, M., Pavani, G., & Guiraud, S. (2024). CRISPR-based gene therapies: From preclinical to clinical treatments. *Cells, 13*(5), 415. https://doi.org/10.3390/cells13050415

Liang, R., He, Z., Zhao, K. T., Zhu, H., Hu, J., Liu, G., Gao, Q., Liu, M., Zhang, R., Qiu, J.-L., & Gao, C. (2024). Prime editing using CRISPR-Cas12a and circular RNAs in human cells. *Nature Biotechnology, 42*(12), 1867–1875.

https://doi.org/10.1038/s41587-024-02247-2
Liang, Y., Tong, S., Zhang, J., Tan, G.-Y., Zhang, L., Lee, S. Y., & Tong, Y. (2025). Expanding horizons of CRISPR applications beyond genome editing. *Trends in Genetics*. https://doi.org/10.1016/j.tig.2025.03.001
López de Alba, E., Salguero, I., Giménez-Llorente, D., Montes-Torres, J., Fernández-Sanromán, Á., Casajús-Pelegay, E., Terrón-Bautista, J., Barroso-González, J., Macintyre, G., Fernández-Leiro, R., Losada, A., & Cortés-Ledesma, F. (2025). A comprehensive genetic catalog of human double-strand break repair. *Science, 388*(6748), eadr8955. https://doi.org/10.1126/science.adr8955
Makarova, K. S., Wolf, Y. I., & Koonin, E. V. (2025). An updated evolutionary classification of CRISPR-Cas systems including rare variants. *Nature Microbiology*. Advance online publication. https://doi.org/10.1038/s41564-025-02066-3
Makarova, K. S., Wolf, Y. I., Iranzo, J., Shmakov, S. A., Alkhnbashi, O. S., Brouns, S. J. J., Charpentier, E., Cheng, D., Haft, D. H., Horvath, P., Moineau, S., Mojica, F. J. M., Scott, D., Shah, S. A., Siksnys, V., Terns, M. P., Venclovas, Č., White, M. F., Yakunin, A. F., ... Koonin, E. V. (2020). Evolutionary classification of CRISPR-Cas systems: A burst of class 2 and derived variants. *Nature Reviews Microbiology, 18*(2), 67–83. https://doi.org/10.1038/s41579-019-0299-x
Malone, L. M., Warring, S. L., & Jackson, S. A. (2021). Conquering CRISPR: How phages overcome bacterial adaptive immunity. *Current Opinion in Biotechnology, 68*, 30–36. https://doi.org/10.1016/j.copbio.2020.08.003
Manning, B. J., Zhang, F., & Gootenberg, J. S. (2022). High-throughput SHERLOCK for COVID-19 testing. *Journal of Clinical Microbiology,* 60(3), e01356-21. https://doi.org/10.1128/jcm.01356-21
Matharu, N., Rattanasopha, S., Tamura, S., Maliskova, L., Wang, Y., Bernard, A., Hardin, A., Eckalbar, W. L., Vaisse, C., & Ahituv, N. (2019). CRISPR-mediated activation of a promoter or enhancer rescues obesity caused by haploinsufficiency. *Science, 363*(6424), eaau0629. https://doi.org/10.1126/science.aau0629
Mikulic, M. (2025, December 17). *CRISPR genome editing – Statistics & facts*. Statista. https://www.statista.com/topics/7803/crispr-genome-editing/
Mitić, D., Ivančić-Baće, I., & Bolt, E. L. (2023). CRISPR-Cas adaptation in *Escherichia coli*. *Bioscience Reports, 43*(3), BSR20222014. https://doi.org/10.1042/BSR20222014
Mohammadian Farsani, A., et al. (2024). Lipid nanoparticles: The game-changer in CRISPR-Cas9 genome editing. *Heliyon, 10*, e25133. https://doi.org/10.1016/j.heliyon.2024.e25133
Morelli, E., Gullà, A., Amodio, N., Taiana, E., Neri, A., Fulciniti, M., & Munshi, N. C. (2021). CRISPR interference (CRISPRi) and CRISPR activation (CRISPRa) to explore the oncogenic lncRNA network. In *Long Non-Coding RNAs in Cancer* (pp. 189–204). https://doi.org/10.1007/978-1-0716-1004-2_12
Neugebauer, M. E., Hsu, A., Arbab, M., Krasnow, N. A., McElroy, A. N., Pandey, S., Doman, J. L., Huang, T. P., Raguram, A., Banskota, S., Newby, G. A., Tolar, J., Osborn, M. J., & Liu, D. R. (2023). Evolution of an adenine base editor into a small, efficient cytosine base editor with low off-target activity. *Nature Biotechnology, 41*, 1043–1052. https://doi.org/10.1038/s41587-022-01544-8
Nguyen, G. T., Schelling, M. A., Raju, A., Buscher, K. A., Sritharan, A., & Sashital, D. G. (2024). Cas12a exhibits metal-dependent specificity switching. *Nucleic Acids Research, 52*(13), 7462–7478. https://doi.org/10.1093/nar/gkae427
Ortiz-Bueno, M., Zinghirino, F., Puig Serra, P., Paschoudi, K., Montoliu, L., Atilla, E., Luo, Y., Cavazza, A., Lederer, C. W., & Benabdellah, K. (2026). From bench to bedside: Ethical and clinical best practices for genome editing applications. *International Journal of Molecular Sciences, 27*. https://doi.org/10.3390/ijms27010001
Park, S. H., Lee, C. M., & Bao, G. (2021). CRISPR-Cas9 for sickle cell disease and β-thalassemia: Clinical advances and challenges. *Molecular Therapy,* 29(6), 1911-1923. https://doi.org/10.1016/j.ymthe.2021.03.001
Perčulija, V., O'Connell, M. R., & East-Seletsky, A. (2021). Functional features and current applications of the RNA-targeting type VI CRISPR-Cas systems. *Advanced Science, 8*(24), 2101734. https://doi.org/10.1002/advs.202101734
Pinaud, M., & Zamborlini, A. (2025). Electroporation-based CRISPR-Cas9-mediated gene knockout in THP-1 cells and single-cell clone isolation. *Journal of Visualized Experiments*. https://doi.org/10.3791/68673
Poddar, A., Sharma, V., & Singh, S. (2024). CRISPR-based therapeutic strategies for inherited disorders: From bench to bedside. *Journal of Translational Medicine*, 22(1), 305. https://doi.org/10.1186/s12967-024-05098-8
Raitskin, O., Schudoma, C., West, A., & Patron, N. J. (2019). Comparison of efficiency and specificity of CRISPR-associated (Cas) nucleases in plants: An expanded toolkit for precision genome engineering. *PLoS ONE, 14*(2), e0211598. https://doi.org/10.1371/journal.pone.0211598
Ramalingam, S., Jeevanandham, R., & Kuppusamy, R. (2019). CRISPR-based point-of-care diagnostics for infectious diseases. *Biosensors and Bioelectronics*, 137, 145-155. https://doi.org/10.1016/j.bios.2019.05.004
Rueda, J., Segers, S., Hopster, J., Kudlek, K., Liedo, B., Marchiori, S., & Danaher, J. (2025). Anticipatory gaps challenge the public governance of heritable human genome editing. *Journal of Medical Ethics*. https://doi.org/10.1136/jme-2024-110705
Schmidt, R., Steinhart, Z., Layeghi, M., Freimer, J. W., Bueno, R., Nguyen, V. Q., Blaeschke, F., Ye, C. J., & Marson, A. (2022). CRISPR activation and interference screens decode stimulation responses in primary human T cells. *Science, 375*(6580), eabj4008. https://doi.org/10.1126/science.abj4008
Sharma, G., Sharma, A. R., Bhattacharya, M., Lee, S.-S., & Chakraborty, C. (2021). CRISPR-Cas9: A preclinical and clinical perspective for the treatment of human diseases. *Molecular Therapy, 29*(2), 571–586. https://doi.org/10.1016/j.ymthe.2020.12.006
Shin, J., Jiang, F., Liu, J. J., Bray, N. L., Rauch, B. J., Baik, S. H., Nogales, E., Bondy-Denomy, J., Corn, J. E., & Doudna, J. A. (2020). Development of CRISPR/Cas9 system for targeted DNA modifications and recent improvements in modification efficiency and specificity. *BMB Reports, 53*(6), 273–283. https://doi.org/10.5483/BMBRep.2020.53.6.084
Silverstein, R. A., Kim, N., Kroell, A.-S., Walton, R. T., Delano, J., Butcher, R. M., Pacesa, M., Smith, B. K., Christie, K. A., Ha, L. L., Meis, R. J., Clark, A. B., Spinner, A. D., Lazzarotto, C. R., Li, Y., Matsubara, A., Urbina, E. O., Dahl, G. A., ... Kleinstiver, B. P. (2025). Custom CRISPR-Cas9 PAM variants via scalable engineering and machine learning. *Nature*. Advance online publication. https://doi.org/10.1038/s41586-025-09177-2
Snuzik, A. (2024). Assessing CRISPR/Cas9 potential in SDG3 attainment: Malaria elimination—Regulatory and community engagement landscape. *Malaria Journal, 23*, 52. https://doi.org/10.1186/s12936-024-04864-5
Stella, G., Niewoehner, O., & White, M. F. (2024). Type III CRISPR-Cas: Beyond the Cas10 effector complex. *Trends in Biochemical Sciences, 49*(1), 34–49. https://doi.org/10.1016/j.tibs.2023.09.007
Sun, D. (2021). Design of time-delayed safety switches for CRISPR gene therapy. *Scientific Reports, 11*, 21651. https://doi.org/10.1038/s41598-021-01133-z
Sun, W., Liu, H., Yin, W., Qiao, J., Zhao, X., & Liu, Y. (2022). Strategies for enhancing the homology-directed repair efficiency of CRISPR-Cas systems. *The CRISPR Journal, 5*(1), 18–32. https://doi.org/10.1089/crispr.2021.0049
Tuncel, A., Pan, C., Clem, J. S., Liu, D., & Qi, Y. (2025). CRISPR-Cas applications in agriculture and plant research. *Nature Reviews Molecular Cell Biology, 26*(6), 419–441. https://doi.org/10.1038/s41580-024-00792-2
Vicencio, J., Sánchez-Bolaños, C., Moreno-Sánchez, I., Brena, D., Vejnar, C. E., Kukhtar, D., Ruiz-López, M., Cots-Ponjoan, M., Rubio, A., Rodrigo Melero, N., Crespo-Cuadrado, J., Carolis, C., Pérez-Pulido, A. J., Giráldez, A. J., Kleinstiver, B. P., Cerón, J., & Moreno-Mateos, M. A. (2022). Genome editing in animals with minimal PAM CRISPR-Cas9 enzymes. *Nature Communications, 13*, 2601. https://doi.org/10.1038/s41467-022-30307-9
Villiger, L., Joung, J., Koblan, L., Weissman, J., Abudayyeh, O. O., & Gootenberg, J. S. (2024). CRISPR technologies for genome, epigenome and transcriptome editing. *Nature Reviews Molecular Cell Biology, 25*, 393–415. https://doi.org/10.1038/s41580-024-00687-3

Volodina, K., Ivanov, R., & Smirnov, A. (2025). Delivery strategies for CRISPR-Cas genome editing: Current state and future directions. *Advanced Drug Delivery Reviews*, 205, 115-129. https://doi.org/10.1016/j.addr.2025.115129

Wei, A., Yin, D., Zhai, Z., Ling, S., Le, H., Tian, L., Xu, J., Paludan, S. R., Cai, Y., & Hong, J. (2023). *In vivo* CRISPR gene editing in patients with herpetic stromal keratitis. *Molecular Therapy, 31*(10), 2946–2958. https://doi.org/10.1016/j.ymthe.2023.08.020

Widney, K. A., Yang, D.-D., Rusch, L. M., & Copley, S. D. (2024). CRISPR-Cas9-assisted genome editing in *Escherichia coli* elevates the frequency of unintended mutations. *bioRxiv*. https://doi.org/10.1101/2024.03.14.584993

Wu, H., Sun, Y., Wang, Y., Luo, L., & Song, Y. (2024). Advances in miniature CRISPR-Cas proteins and their applications in gene editing. *Archives of Microbiology, 206*, 191. https://doi.org/10.1007/s00203-024-03992-9

Yang, H., Ren, S., Yu, S., Pan, H., Li, T., Ge, S., Zhang, J., & Xia, N. (2020). Methods favoring homology-directed repair choice in response to CRISPR/Cas9-induced double-strand breaks. *International Journal of Molecular Sciences, 21*(18), 6461. https://doi.org/10.3390/ijms21186461

Yu, W., Lescale, C., Babin, L., Bedora-Faure, M., Lenden-Hasse, H., Baron, L., Demangel, C., Yelamos, J., Brunet, E., & Deriano, L. (2020). Repair of G1-induced DNA double-strand breaks in S–G2/M by alternative NHEJ. *Nature Communications, 11*, 5239. https://doi.org/10.1038/s41467-020-19008-1

Zhang, F., Cong, L., & Ran, F. A. (2016). Engineering of CRISPR-Cas9 for expanded PAM compatibility. *Nature*, 556(7699), 57-63. https://doi.org/10.1038/nature26155

Zhang, X., Ma, D., & Liu, F. (2025). CRISPR technology and its emerging applications. *Genomics, Proteomics & Bioinformatics*. https://doi.org/10.1093/gpbjnl/qzaf021

Zhao, Z., Shang, P., Mohanraju, P., & Geijsen, N. (2023). Prime editing: Advances and therapeutic applications. *Trends in Biotechnology, 41*(8), 1000–1012. https://doi.org/10.1016/j.tibtech.2023.01.006

Zhou, X., Diao, R., Li, X., Ziegler, C. A., Gramelspacher, M. J., Freddolino, L., Hou, Z., & Zhang, Y. (2025). Cas9 senses CRISPR RNA abundance to regulate CRISPR spacer acquisition. *Nature*. Advance online publication. https://doi.org/10.1038/s41586-025-09253-7