VETERINARY TODAY
INTERNATIONAL
ISSN 2583-8288

Rewriting the Code of Life with CRISPR-Cas9

Dr. Monika Khakhlary[1], Dr. Jianpui Pamei[1], Dr. Gunda Sai Rakesh[2], Dr. Lalhmangaihzuali[3], Dr. Priyanka Patir[4]

[1]PhD Scholar, Division of Biochemistry, Indian Council of Agricultural Research – Indian Veterinary Research Institute, Bareilly, U. P., India

[2]Assistant Professor, Department of Veterinary Microbiology, M.R. College of Veterinary Science & Research Centre, Jhajjar, Haryana, India

[3]PhD Scholar, Division of Medicine, Indian Council of Agricultural Research – Indian Veterinary Research Institute, Bareilly, U. P., India

[4]PhD Scholar, Division of Animal Nutrition, Indian Council of Agricultural Research – Indian Veterinary Research Institute, Bareilly, U. P., India

[1]*Corresponding author's email: monikakhakhlary@gmail.com*

doi.org/10.5281/VeterinaryToday.21498324

The Dawn of CRISPR

In 2012, a team of scientists led by Jennifer Doudna and Emmanuelle Charpentier explained how a basic immune response used by bacteria to fight off viruses could be reprogrammed. They showed that this mechanism could be turned into a highly accurate pair of molecular scissors, capable of cutting specific pieces of DNA in almost any organism. Eight years later, in October 2020, this discovery earned them the Nobel Prize in Chemistry. Today, CRISPR-Cas9 has moved out of basic research labs and into clinical trials, fundamentally shifting how we treat genetic diseases, run diagnostic tests, and engineer living tissue.

How Nature Built the Perfect Defense

Before scientists turned CRISPR into a genetic editor, it functioned purely as a cellular shield. When a virus attacks a bacterium, the microbe needs a way to remember the threat. It does this by moving through three clear phases:

1. Collecting the Evidence (Spacer Acquisition): When a bacterium encounters a virus incorporates a part of the foreign DNA (protospacer sequences) within the spacer region of the CRISPR array during the acquisition or adaptation phase. These protospacers are recognized and selected with the help of certain motifs in the foreign DNA strand that are present downstream, known as protospacer adjacent motif (PAM).

2. Building the Weapon (Biogenesis): When the same foreign genetic material slips back into the bacterial cell, it triggers the transcription of the archived CRISPR array, generating a long molecule known as pre-crRNA. This precursor strand contains repeating regions that match up perfectly with a separate, structural RNA molecule called tracrRNA. Once transcribed, the tracrRNA docks onto the Cas9 protein first. Next, the pre-crRNA pairs up with the tracrRNA through complementary base pairing, creating a double-stranded RNA structure that locks the entire complex onto the Cas9 enzyme. From there, a two-step trimming process begins: an auxiliary enzyme called RNase III handles the primary processing by cutting the pre-crRNA chain, while Cas9 steps in for the secondary processing to slice away any leftover repetitive or spacer code. Once these two steps are complete, the crRNA is fully mature and ready to zero in on its genetic target. Ultimately, this combination of the structural tracrRNA

backbone and the targeting crRNA sequence is what we call the single guide RNA (sgRNA).

3. Striking Back (Interference): The sgRNA-Cas9 complex then binds to the complementary sequences in the template DNA strand and brings about double stranded breaks (DSB).

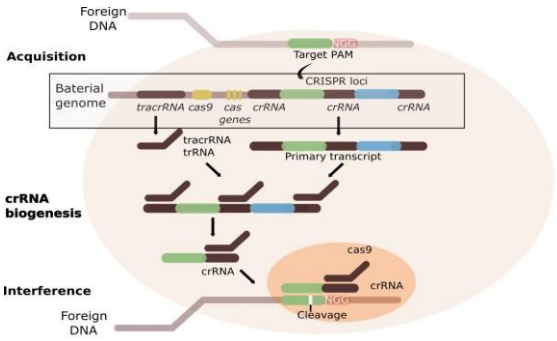


Figure 1: Mechanism of CRISPR Cas9 technology
Figure 2: DNA Repair mechanism after DSB by Cas9

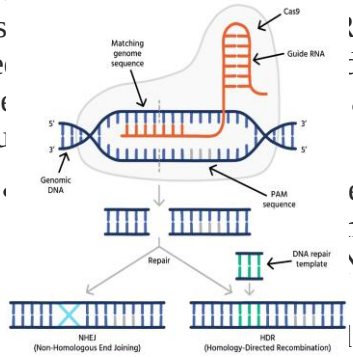
Fixing the Break: How Cells Edit Themselves

It is worth noting that Cas9 does not actually change or fix a gene—it only damages it. The actual edit happens when the host cell tries to repair the broken DNA strand. Cells use two primary methods to fix these cuts:

- **Non-Homologous End Joining (NHEJ):** An error-prone, template-independent repair pathway that directly ligates broken DNA ends. This process frequently introduces random insertions or deletions (indels), which disrupt the open reading frame and introduce premature stop codons to achieve a gene knockout. Because NHEJ is highly active throughout the cell cycle, it is the standard method for targeted gene disruption.
- **Homology-Directed Repair (HDR):** A high-fidelity repair pathway that utilizes an exogenous donor DNA template with flanking homology arms to introduce precise genetic changes. The cell copies this template directly into the double-stranded break, enabling seamless gene corrections or targeted knock-ins. However, because HDR is largely restricted to the late S and G2 phases of cell division, its efficiency is significantly limited in mature, non-dividing tissues.

Getting the Payload Inside the Cell

To execute target modifications, the Cas9 endonuclease and its tracking guide RNA must cros researchers utilize thre depending on the expe and precision requ



ector sequences nd the gRNA are NA molecules for and translation. cost-effective for baseline *in vitro* applications , the resulting prolonged expression window increases the frequency of off-target activity and potential insertional mutagenesis.

- **mRNA Delivery:** Cas9 is introduced as a transient messenger RNA transcript paired with synthetic gRNAs. This bypasses genomic transcription, allowing rapid cytoplasmic translation. Because the mRNA degrades rapidly, the brief expression window minimizes off-target events and avoids integration risks.
- **Ribonucleoprotein (RNP) Complexes:** Purified Cas9 protein is pre-assembled directly with guide RNA *in vitro* and delivered as a fully functional, active complex. Bypassing both cellular transcription and translation , RNPs offer the fastest editing kinetics and clear rapidly from the cell. This transient presence minimizes off-target cuts and immunogenicity, making RNPs the preferred choice for precise clinical therapeutics.

Therapeutic Translational Milestones

- **Immune Cell Engineering in Oncology:** In CAR-T and TCR-T

adoptive therapies, CRISPR-Cas9 prevents synthetic tumor-targeting receptors from mispairing with native T-cell receptor α and β chains (encoded by *TRAC* and *TRAB*). Knocking out these native loci increases functional receptor density on the cell surface, enhancing tumor clearance.

- **Modulating Tumor Thermotolerance:** Solid tumors escape heat ablation by overexpressing Heat Shock Protein 90α (HSP90α), which acts as a protective thermal shield. Delivering a CRISPR-Cas9 payload via hypoxia-responsive gold nanorods directly to the tumor core silences the *HSP90α* gene, removing this defense and enabling low-temperature infrared destruction while protecting healthy surrounding tissue.
- **Modeling Diseases in 3D Organoids:** CRISPR-Cas9 deployment within 3D organoids allows accurate replication of human pathology. Simultaneous knockout of *p53*, *PTEN*, *RB1*, and *NF1* maps oncogenesis in specific breast cancer subtypes, while deleting *HSP1* in lung organoids replicates the epithelial dysfunction and matrix deposition characteristic of pulmonary fibrosis.
- **Correcting Hemoglobinopathies:** Sickle cell disease and β-thalassemia are caused by adult β-globin gene mutations. Casgevy utilizes Cas9 to cleave the *BCL11A* erythroid enhancer in patient stem cells, disabling this genetic repressor to reactivate healthy fetal hemoglobin (γ-globin) production and permanently restore normal erythrocyte function.
- **In Vivo Therapeutics:** In May 2025, a custom single-nucleotide base editor successfully treated an infant with life-threatening carbamoyl phosphate synthetase 1 (CPS1) deficiency. Packaged into liver-targeted lipid nanoparticles, the intravenous payload corrected the metabolic point mutation directly *in vivo*, establishing a rapid framework for patient-specific monogenic disease interventions.

Technical Obstacles and Structural Refinements

Despite major progress, standard Cas9 deployment presents key safety limitations:

- **Off-Target Activity:** Guide RNAs can tolerate base pair mismatches, accidentally directing cleavage to non-target genomic loci and risking oncogenic mutations.
- **Double-Stranded Break Pathogenicity:** Cutting completely through the DNA duplex can trigger large unexpected deletions, chromosomal translocations, or p53-mediated cell death.
- **Genetic Mosaicism:** Variable cutting speeds during embryo editing or transgenic animal modeling generate mosaic tissues containing mixed edited and unedited genotypes.

To mitigate these risks, synthetic biologists are structurally engineering the editing machinery:

- **High-Fidelity Cas9 Engineering:** Engineered variants like SpCas9-HF1 and eSpCas9 feature amino acid substitutions that alter domain charges. This weakens non-specific bonds with the DNA backbone, requiring absolute guide-to-target complementarity before cleavage can occur.
- **Zero-Cut Base Editing :** Fusing base or prime editors to catalytically inactive "dead" Cas9 (dCas9) or nickases allows single-nucleotide conversions or new sequence insertions without introducing double-stranded breaks, preventing large-scale genomic rearrangements.

Conclusion

CRISPR-Cas9 has shifted from an intriguing microbial defense mechanism into the most transformative tool in modern biology. By allowing us to edit the genome with unprecedented accuracy, it has turned conditions that were once considered lifelong struggles—like sickle cell disease and rare metabolic disorders—into curable realities. While structural issues like off-target cuts and delivery barriers remain, the ongoing development of high-fidelity variants and zero-cut base editors shows that the platform is rapidly maturing. As delivery nanoparticles grow more sophisticated, CRISPR will continue to move beyond single-organ targeting, opening up safer, ultra-personalized cures that will shape human medicine for generations to come.

References

Böhm, S. et al. A gene therapy for inherited blindness using dCas9-VPR mediated transcriptional activation. Sci. Adv. 6, eaba5614 (2020).

Chen, L. et al. Recent progress in targeted delivery vectors based on biomimetic nanoparticles. Signal Transduct. Target Ther. 6, 225 (2021).

Cui, Q. et al. Modulating ROS to overcome multidrug resistance in cancer. Drug Resist. Updat. 41,1–25 (2018).

Fan, J., Cheng, Y. & Sun, M. Functionalized gold nanoparticles: synthesis, prop erties and biomedical applications. Chem. Rec. 20, 1474–1504 (2020).

Fan, Y., Marioli, M. & Zhang, K. Analytical characterization of liposomes and other lipid nanoparticles for drug delivery. J. Pharm. Biomed. Anal. 192, 113642 (2021).

Frangoul, H., Altshuler, D., Cappellini, M. D., Chen, Y. S., Domm, J., Eustace, B. K., Foell, J., de la Fuente, J., Grupp, S., Handgretinger, R., Ho, T. W., Kattamis, A., Kernytsky, A., Lekstrom-Himes, J., Li, A. M., Locatelli, F., Mapara, M. Y., de Montalembert, M., Rondelli, D., Sharma, A., Sheth S, Soni S, Steinberg MH, Wall D, Yen A, Corbacioglu, S. (2021). CRISPR-Cas9 Gene Editing for Sickle Cell Disease and β-Thalassemia. The New England journal of medicine, 384(3), 252–260. https://doi.org/10.1056/NEJMoa2031054

Hanlon, M.B., Shohet, J.M. & Wolfe, S.A. Selective targeting of genome amplifications and repeat elements by CRISPR-Cas9 nickases to promote cancer cell death. Nat Commun 16, 5126 (2025). https://doi.org/10.1038/s41467-025-60160-2

Himeda, C. L., Jones, T. I. & Jones, P. L. Targeted epigenetic repression by CRISPR/ dSaCas9 suppresses pathogenic DUX4-fl expression in FSHD. Mol. Ther. Methods Clin. Dev. 20, 298–311 (2021).

Hsu, M. N. et al. CRISPR interference-mediated noggin knockdown promotes BMP2-induced osteogenesis and calvarial bone healing. Biomaterials 252, 120094 (2020).

Hwang, HY., Lee, M., Yi, H. et al. Engineered Sdd7 cytosine base editors with enhanced specificity. Nat Commun 16, 5881 (2025). https://doi.org/10.1038/s41467-025-60789-z

Khurana, A., Sayed, N., Singh, V., Khurana, I., Allawadhi, P., Rawat, P. S., Navik, U., Pasumarthi, S. K., Bharani, K. K., & Weiskirchen, R. (2022). A comprehensive overview of CRISPR/Cas 9 technology and application thereof in drug discovery.

Journal of cellular biochemistry, 123(10), 1674–1698. https://doi.org/10.1002/jcb.30329

Larson, R. C. et al. CAR T cell killing requires the IFNγR pathway in solid but not liquid tumours. Nature 604, 563–570 (2022).

Li, T., Yang, Y., Qi, H. et al. CRISPR/Cas9 therapeutics: progress and prospects. Sig Transduct Target Ther 8, 36 (2023). https://doi.org/10.1038/s41392-023-01309-7

Li, X. et al. Hypoxia-responsive gene editing to reduce tumor thermal tolerance for mild-photothermal therapy. Angew. Chem. Int. Ed. Engl. 60,21200–21204 (2021).

Li, X. et al. Lactate metabolism in human health and disease. Signal Transduct. Target Ther. 7, 305 (2022).