

Chapter 10

CRISPR-Cas9 Genome Editing in Zebrafish for Modeling Human Diseases: A Narrative Review

Tejaswini Rah Raman^a, V.Jayashree^{b*}

¹*B.Pharm Student, Department of Pharmacology, School of Pharmaceutical Sciences, Vels Institutes of Science, Technology & Advanced Studies, Chennai*

²**Associate Professor, Department of Pharmacology, School of Pharmaceutical Sciences, Vels Institutes of Science, Technology & Advanced Studies, Chennai*

** Corresponding Author: jeya.sps@vistas.ac.in*

Abstract

Genome editing technologies have come a long way in terms of modern biological research by now allowing precise modification of genetic material. Various tools are under review, among which, CRISPR-Cas9 has emerged as one of the most efficient and widely used systems. This is due to its simplicity, The zebrafish, *Danio rerio*, has become a prominent vertebrate model for genetic studies because of its rapid embryonic development, optical transparency, and strong genetic similarity to humans. In recent years, CRISPR-Cas9 has been extensively applied in zebrafish to investigate gene function, generate targeted mutations, and establish models of human disease. This review summarizes the fundamental principles of CRISPR-Cas9-mediated genome editing and discusses its application in zebrafish research. Particular emphasis is placed on experimental workflows, including guide RNA design, embryo microinjection, and mutant screening strategies. In addition, the review highlights major research applications such as functional genomics, disease modeling, and

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drug discovery. Despite its numerous advantages, certain technical challenges, including off-target effects and mosaic mutations, remain important considerations. Continued improvements in genome editing strategies are expected to further enhance the utility of zebrafish as a model system for biomedical research. Overall, the integration of CRISPR-Cas9 technology with zebrafish biology provides a powerful platform for understanding gene function and advancing studies related to human health and disease.

Keywords: CRISPR; CRISPR-Cas9; genome; zebrafish; modification; genome editing.

1. Introduction

Genome editing technologies have transformed modern biological research by enabling precise manipulation of genetic material. One of the most influential breakthroughs in this field is the development of CRISPR-Cas9, a genome editing system derived from a bacterial adaptive immune mechanism that protects microorganisms against invading viral DNA [1,2]. The discovery and development of CRISPR-Cas9 have revolutionized molecular biology by providing researchers with a powerful tool capable of introducing targeted genetic modifications with high precision and efficiency [3,4].

Compared with earlier genome editing technologies such as zinc-finger nucleases and transcription activator-like effector nucleases, CRISPR-Cas9 offers several advantages, including easier design, reduced cost, and the ability to edit multiple genes simultaneously [5,6]. These characteristics have enabled the rapid adoption of CRISPR technology across a wide range of research disciplines, including genetics, biotechnology, and biomedical sciences [7,8]. In recent years, CRISPR-based genome editing has been applied

extensively to investigate gene function and to develop experimental models for human diseases [9].

One organism that has significantly benefited from the implementation of CRISPR technology is the zebrafish, *Danio rerio*. Zebrafish have become an important vertebrate model organism due to their rapid embryonic development, optical transparency during early life stages, and genetic similarity to humans [10]. Approximately seventy percent of human genes have at least one zebrafish orthologue, making this organism highly suitable for modeling human diseases and studying gene function in vivo [11].

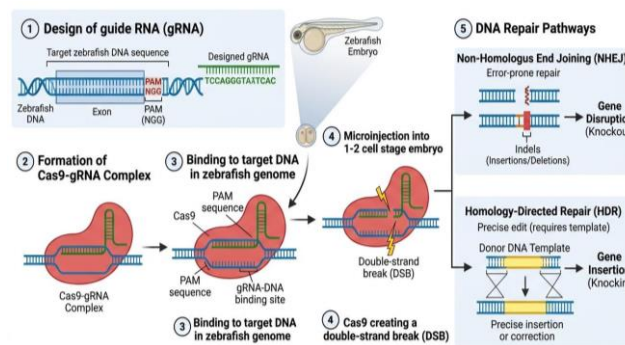


Figure 1: CRISPR-Cas9 Mechanism applied to Zebrafish Genome Editing

The integration of CRISPR-Cas9 genome editing with zebrafish biology has accelerated the generation of targeted genetic mutations and disease models. This technology allows researchers to efficiently knock out genes, introduce disease-associated mutations, and study the resulting phenotypic effects in living organisms [12,13]. Consequently, CRISPR-based zebrafish models have become valuable tools for investigating the genetic basis of diseases and for identifying potential therapeutic strategies.

This review discusses the mechanisms underlying CRISPR-Cas9 genome editing and highlights its applications in zebrafish models for

studying human diseases. The review also examines current challenges associated with CRISPR technology and explores potential future developments in genome editing research.

2. Zebrafish as a Model Organism in Biomedical Research

The zebrafish has emerged as one of the most widely used vertebrate model organisms in biological and biomedical research. Several biological and experimental characteristics contribute to its popularity, particularly in genetic studies.

Zebrafish embryos develop externally and remain transparent during early developmental stages, allowing direct observation of cellular and developmental processes in real time [10]. Major organ systems such as the heart, brain, and digestive system develop rapidly, often within the first two to three days following fertilization. This rapid development enables efficient analysis of gene function and early disease phenotypes.

Another significant advantage of zebrafish is their high reproductive capacity. A single breeding pair can produce hundreds of embryos within a week, enabling large-scale genetic screening experiments and high-throughput analyses [11]. These characteristics make zebrafish particularly well suited for reverse genetics approaches using CRISPR-Cas9 genome editing.

Genetically, zebrafish share a considerable degree of conservation with humans. Many genes associated with human diseases have functional homologues in zebrafish, enabling researchers to investigate disease mechanisms using this model organism [13]. In addition, zebrafish are highly suitable for drug discovery studies because therapeutic compounds can easily be administered through the surrounding aquatic environment.

These advantages have established zebrafish as a powerful platform for studying developmental biology, disease pathogenesis, and therapeutic interventions.

3. Mechanism of CRISPR-Cas9 Genome Editing

The CRISPR-Cas9 genome editing system functions through an RNA-guided DNA cleavage mechanism. The system consists primarily of a Cas9 endonuclease enzyme and a single-guide RNA (sgRNA), which directs the Cas9 protein to a complementary DNA sequence within the genome [14,15].

The sgRNA contains a sequence that is complementary to the target DNA region, allowing the Cas9 enzyme to recognize and bind to specific genomic sites. Successful targeting requires the presence of a short DNA sequence known as the protospacer adjacent motif, or PAM, which is recognized by the Cas9 protein [4]. Once the sgRNA binds to its target sequence, Cas9 introduces a double-strand break in the DNA.

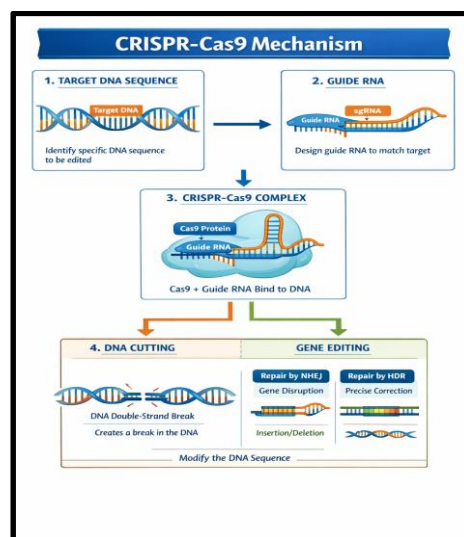


Figure 2: CRISPR-Cas9 Mechanism

Following DNA cleavage, cellular DNA repair mechanisms are activated to repair the damage. Two primary repair pathways are

involved in this process: non-homologous end joining and homology-directed repair. Non-homologous end joining is an error-prone repair pathway that frequently introduces insertions or deletions at the break site, often resulting in gene disruption [5]. This process is commonly used to generate gene knockout mutations.

In contrast, homology-directed repair utilizes a homologous DNA template to repair the break accurately. By providing an artificial donor DNA template, researchers can introduce precise genetic modifications such as point mutations or reporter gene insertions [17].

The combination of programmable RNA targeting and efficient DNA cleavage makes CRISPR-Cas9 one of the most versatile genomes editing technologies currently available.

4. CRISPR-Cas9 Editing Strategies in Zebrafish

Genome editing in zebrafish using CRISPR-Cas9 typically involves the microinjection of Cas9 mRNA or Cas9 protein along with sgRNA into fertilized embryos at the one-cell stage. Delivering genome editing components at this stage ensures that the modifications occur before extensive cell division, increasing the likelihood that the genetic changes will be present in most cells of the developing organism [10].

The experimental workflow begins with the identification of a target gene and the design of guide RNAs capable of recognizing specific genomic sequences. Bioinformatics tools are often used to minimize off-target effects and improve targeting efficiency [11].

Following microinjection, embryos are allowed to develop normally. Researchers then screen the embryos for mutations using molecular techniques such as polymerase chain reaction amplification and DNA sequencing [12].

Advancements in CRISPR technology have further improved genome editing efficiency in zebrafish. For instance, alternative Cas9 variants have expanded the range of genomic targets that can be edited [13]. These improvements have enhanced the ability of researchers to generate stable mutant lines for disease modeling studies.

5. Applications of CRISPR-Cas9 in Zebrafish Disease Models

5.1 Cancer Research

Cancer research has benefited significantly from CRISPR-based zebrafish models. Targeted mutations in tumor suppressor genes and oncogenes can induce tumor formation in zebrafish, enabling researchers to study tumor initiation, progression, and metastasis in a living organism [16].

5.2 Cardiovascular diseases

Zebrafish are widely used to study cardiovascular diseases because their heart structure and development share similarities with humans. CRISPR-induced mutations in genes associated with cardiac development can lead to abnormalities in heart morphology and function, providing valuable insights into congenital heart diseases [17].

5.3 Neurological Disorders

Zebrafish models have also been developed for studying neurological disorders. CRISPR-mediated mutations affecting neuronal signaling pathways can produce behavioral and physiological phenotypes that resemble human neurological conditions [9]. These models are particularly useful for high-throughput screening of neuroactive compounds.

5.4 Metabolic Disorders

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CRISPR technology has also enabled the development of zebrafish models for metabolic and genetic disorders. Mutations affecting metabolic pathways can lead to phenotypes similar to human metabolic diseases, allowing researchers to study disease progression and evaluate therapeutic strategies [18].

6. Limitations and Challenges

Despite its advantages, CRISPR-Cas9 genome editing also presents several challenges. One significant concern is the occurrence of off-target mutations, which may introduce unintended genetic changes in the genome. Another limitation is mosaicism, which occurs when genome editing events happen after the first cell division, resulting in organisms containing both edited and unedited cells. This phenomenon can complicate early phenotypic analysis.[25]

In addition, precise genome editing through homology-directed repair remains relatively inefficient compared with non-homologous end joining, making accurate gene insertion experiments more difficult.

Ethical considerations related to genome editing technologies must also be considered as CRISPR approaches move closer to clinical applications [26].

7. Future Perspectives

Continuous improvements in genome editing technologies are expected to expand the applications of CRISPR-Cas9 in biomedical research. Emerging approaches such as base editing and prime editing allow precise nucleotide modifications without introducing double-strand breaks, potentially reducing off-target effects [17].

The integration of CRISPR genome editing with advanced genomic technologies and imaging techniques will likely enhance our

understanding of gene function and disease mechanisms.

Ultimately, insights gained from zebrafish disease models may contribute to the development of novel gene therapies and personalized medicine strategies in human healthcare [21].

8. Discussion

One of the most significant contributions of CRISPR-based zebrafish models is the ability to replicate human disease-associated mutations and observe their effects during development and adulthood. Unlike traditional genetic approaches that required extensive time and resources, CRISPR technology enables the rapid generation of targeted mutations, allowing researchers to study gene function more efficiently [4,10]. This has facilitated the development of disease models for cancer, cardiovascular disorders, neurological diseases, and metabolic conditions.

Despite these advantages, several challenges remain in the application of CRISPR technology. Off-target mutations represent a major concern because unintended DNA modifications may lead to inaccurate experimental results or unexpected phenotypes [25]. Although improvements in guide RNA design and high-fidelity Cas9 variants have reduced these risks, careful validation of edited organisms is still required.

Nevertheless, ongoing technological improvements continue to enhance the reliability and efficiency of CRISPR-based genome editing. Advances such as base editing, prime editing, and improved Cas9 variants are expected to address many of the current limitations and expand the potential applications of genome editing technologies in zebrafish models and other experimental systems [17].

9. Conclusion

In summary, the CRISPR-Cas9 genome editing system has become an indispensable tool for modern biological and biomedical research. Its ability to introduce targeted genetic modifications with high precision has significantly accelerated the study of gene function and disease mechanisms. The zebrafish model organism provides an ideal platform for applying CRISPR technology due to its genetic similarity to humans, rapid development, and suitability for large-scale genetic screening.

The integration of CRISPR-Cas9 with zebrafish genetics has enabled the generation of numerous disease models that replicate human genetic disorders. These models have provided valuable insights into the molecular basis of diseases and have facilitated the identification of potential therapeutic targets. In addition, zebrafish-based CRISPR studies have contributed to advances in drug discovery and translational research.

Although challenges such as off-target effects, mosaicism, and limited efficiency of precise genome editing remain, continued technological developments are expected to improve the accuracy and applicability of CRISPR-based approaches. Future innovations in genome editing tools and experimental methodologies will likely expand the role of zebrafish models in biomedical research.

Overall, the combination of CRISPR-Cas9 genome editing and zebrafish biology represents a powerful approach for advancing our understanding of human diseases and developing novel therapeutic strategies for improving human health.

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