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Popular Article

Comparing Genome Editing Technologies: Mechanisms and Applications

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Abstract

Genome editing technologies have revolutionized genetic manipulation by offering precise tools to modify DNA sequences in various organisms. Genome editing holds significant promise for advancing research and applications in aquaculture species due to several practical advantages. This popular article compares and contrasts the mechanisms and applications of different genome editing technologies currently at the forefront of scientific and practical exploration. The discussion focuses on key methodologies such as CRISPR-Cas9, zinc-finger nucleases (ZFNs), and transcription activator-like effector nucleases (TALENs), elucidating their respective mechanisms of action. Furthermore, this popular article examines the diverse applications of these technologies across different fields, including agriculture, fisheries, medicine, and biotechnology. Genome editing technology like CRISPR/Cas9 have been used to knockout the genes in case of various cell lines including fish cell lines.

Introduction

Genome editing is a technique used to make precise changes to the DNA of living organisms, including plants, Fishes, animals, and even humans. It allows researchers to alter specific sequences of DNA in a targeted manner, which can have profound implications for various fields. Currently, there are various genome editing technologies such as zinc finger nucleases (ZFN), transcription activator-like effector nucleases (TALEN), and clustered regularly interspaced short palindromic repeats (CRISPR). Genome editing, which began development in the late 20th century and saw a significant advancement with the invention of CRISPR in 2009, for which scientist got noble prize in

2012. It is a precise technique for altering the DNA sequence of organisms. This process involves creating targeted cuts in DNA using custom-engineered nucleases molecular scissors and then modifying the DNA sequence according to specific needs (Singh et al., 2017). Unlike transgenes is, which involves transferring genes between organisms, genome editing allows for precise and often subtle modifications directly within the genome of the species under study. This technology enables researchers to add, remove, or alter DNA sequences to better understand biological processes in various organisms. Genome editing has been employed to enhance crop and livestock characteristics like yield, disease resistance, growth rate in fishes and drought tolerance in case of crops. Notably, technologies like CRISPR/Cas9 have been used to knockout the genes in case of various cell lines including fish cell lines.

How does genome edit technology like CRISPR-Cas9 works?

Genome editing uses a type of enzyme called an 'engineered nuclease/molecular scissor' which cuts the genome in a specific place (Luo et al., 2022). Engineered nucleases are made up of two parts: A nuclease part that cuts the DNA and a DNA-targeting part that is designed to guide the nuclease to a specific sequence of DNA. Genome editing allows precise alterations to an organism's DNA, whether human, animal, or plant, with CRISPR-Cas9 being the most widely employed method (Iqbal et al., 2023). The mechanism of action used in case of CRISPR-Cas9 Technology are given as below

1. **Target Identification**: First we have to make a guide RNA (gRNA) that matches a specific DNA sequence. This gRNA directs the Cas9 enzyme to the corrected location in the genome of an organism.
2. **DNA Cleavage**: Cas9 acts as molecular scissors to cut the double strands of DNA. It binds to the DNA at the designated site indicated by the gRNA and cuts both strands of the DNA.
3. **DNA Repair Mechanisms**: As we know that all organisms have a DNA repair mechanism so for that process two main pathways are generally used:
    - **Non-Homologous End Joining (NHEJ):** Often results in small insertions or deletions (indels) at the cutting site, potentially disrupting gene function via frame-shift mutations.
    - **Homology-Directed Repair (HDR):** This pathway enables precise DNA sequence insertion or substitution by providing a template DNA alongside CRISPR-Cas9 components.

4. Desired Genetic Modifications: By harnessing these repair pathways, Researchers can introduce

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specific genetic changes which include gene knockdown or gene knockout.

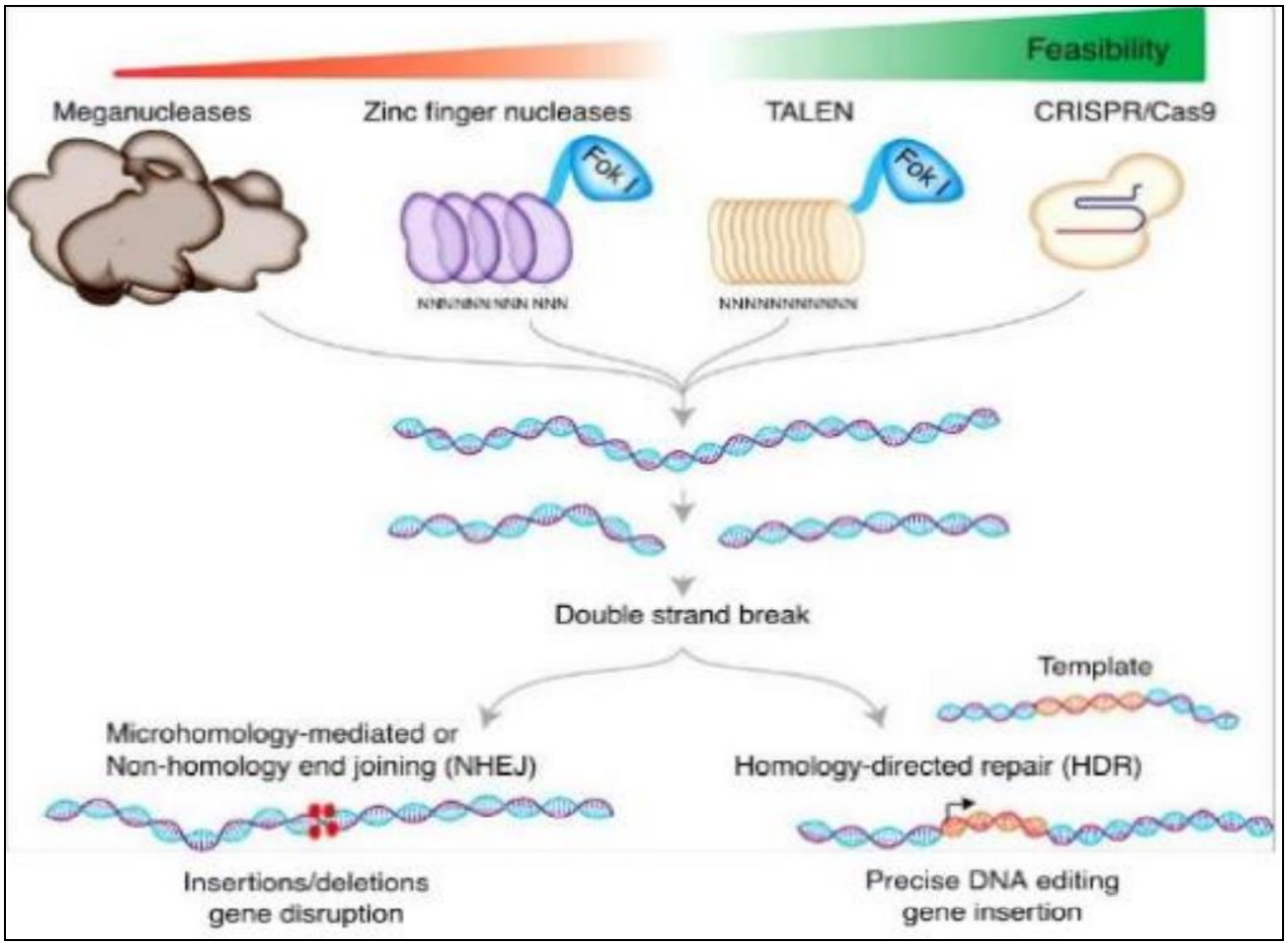

Fig.1. Overview of the mechanism of various genome editing technology (Gaj et al., 2016)

Different Genome editing platform

1. Mega nucleases

Mega nucleases, part of the LAGLIDADG family, are homing endonucleases used extensively in genome editing. They are notable for their ability to target and cleave substantial DNA segments (14-40 base pairs). These enzymes are considered "selfish genetic elements" due to their propensity to insert themselves into DNA. Compared to methods like ZFN, mega nucleases are advantageous because they typically cause less cellular toxicity. However, a significant drawback is the labour-intensive and expensive process of engineering specific enzymes for all potential target sequences (Daboussi, et al., 2015).

2. Zinc Finger Nucleases (ZFN)

Zinc finger nucleases (ZFNs) are a class of engineered DNA-binding proteins that facilitate targeted editing of the genome by creating double-strand breaks in DNA at user-specified locations.

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ZFNs are Highly-specific Genomic Scissors. Each Zinc Finger Nuclease (ZFN) consists of two functional domains: Zinc-finger nucleases (ZFNs) are engineered proteins that act as precise "genomic scissors." They consist of a DNA-binding domain derived from zinc fingers and a DNA-cleaving domain from the nuclease domain of Fok I. Researchers in the 1990s began utilizing ZFNs to enhance the specificity of genome editing and minimize unintended alterations.

These proteins are designed to bind to specific DNA sequences within the genome, where they induce targeted DNA cleavage. This allows scientists to either remove the targeted DNA sequence or introduce a new sequence through homologous recombination. Despite their advancement in genome editing success rates to approximately 10%, the process of designing, constructing, and producing functional zinc finger proteins is challenging and time-consuming. Each new target DNA sequence requires the engineering of a new ZFN, which adds complexity to their application in genetic research and therapeutic development (Zhang et al., 2019).

3. Transcription activator-like effector nucleases (TALENs)

In 2009, Transcription Activator-Like Effector Nucleases (TALENs) emerged as a novel class of proteins for genome editing. Derived from natural proteins, similar to ZFNs, TALENs can specifically bind to targeted DNA sequences. While both TALENs and ZFNs are similarly efficient in editing the genome, TALENs offer the advantage of being simpler to engineer. Compared to ZFNs technology, the process of TALENs is considerably easier (Zhang et al., 2019).

4, Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-Cas9

Researchers have had the knowledge and ability to edit genomes for many years, but CRISPR technology has brought major improvements to the speed, cost, accuracy, and efficiency of genome editing. CRISPR associated DNA sequences were first observed in bacteria in the early 1990s, but it was not until the 2000s that the scientific community understood its ability to recognize specific genome sequences and cut them via the Cas9 protein, a protein that works with CRISPR and that has DNA-cutting abilities.

In 2012, Jennifer Doudna and E. Charpentier from the University of California, USA, developed CRISPR-Cas9 gene editing technology (Doudna et al., 2014). Compared to earlier methods of genome editing such as ZFNs or TALENs, CRISPR has been shown to be six times more effective in creating targeted mutations in the genome of an organism. This breakthrough has significantly reduced the time and cost required for large-scale genomics projects, which previously necessitated many years and substantial financial investment.

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Applications of various Genome editing technology

Genome editing holds significant promise for advancing research and applications in aquaculture species due to several practical advantages. These include the accessibility of large numbers of externally fertilized embryos and their substantial size, which facilitates manual microinjection. CRISPR/Cas9 technology has already proven successful in vivo and in cell lines across various important aquaculture species such as Atlantic salmon, rainbow trout, carps (Rohu, grass, common carp), channel and southern catfish, Pacific oyster, Nile tilapia, and gilthead sea bream (Iqbal et al., 2023). Current genome-editing studies in aquaculture have targeted traits such as pigmentation enhancement in Atlantic salmon, sterility in Atlantic salmon and catfish, accelerated growth in channel catfish and common carp, muscle development in common carp, germ cell development in southern catfish, and improved immunity and disease resistance in rohu and grass carp (Yang et al., 2022). Looking forward, genome-editing technologies are poised to integrate into commercial aquaculture breeding programs, complementing selective-breeding efforts to carefully manage genetic diversity and mitigate potential inbreeding effects.

Gene editing is anticipated to revolutionize aquaculture by accelerating reproduction, breeding, and spawning processes to meet the demand for high-quality fish seed, controlling diseases, developing new therapeutic approaches, enhancing desirable traits, bolstering immunity, and promoting faster growth in captive environments. This technology represents a game-changing tool for enhancing the quantity, quality, and sustainability of aquaculture and seafood production. globally. However, public and regulatory acceptance are key to its potential being realized.

Conclusion

Genome editing holds significant promise for advancing research and applications in aquaculture species due to several practical advantages. Aquaculture is rapidly becoming more significant than traditional fishing as the fastest-growing sector in food production, crucial for ensuring food security, especially in developing nations. The application of genetics and breeding technologies is expanding swiftly within aquaculture, including advanced breeding programs for key species worldwide. Many farmed aquatic species closely resemble their wild ancestors, presenting a valuable opportunity to enhance sustainable seafood production through genetic improvements. Additionally, the high fecundity and external fertilization of aquaculture species provide promising avenues for detailed genetic research aimed at enhancing complex traits. Genome-editing technologies like CRISPR/Cas9 hold substantial promise for accelerating genetic enhancements related to production traits. Managing infectious diseases remains a primary challenge in aquaculture, making

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it a key focus for selective breeding and genome-editing initiatives.

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