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A Break in Science: Gene Editing with CRISPR-Cas9

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Abstract

Ground-breaking innovation has dominated the Clustered regularly interspaced short palindromic repeats for genome editing. Here we discussed the use of CRISPR-Cas9 in both animals and plants. This methodology is used to target genes that directly cause disease and to understand the biology of unknown species. On a national and international level, the issue of whether genetically engineered products, particularly CISPR/Cas, should be regulated is now being considered. This versatile platform that changes the game has given rise to many new plant biotechnologies that simplify gene regulation and protein production. Because of its effectiveness, adaptability, and lack of transgenes, CRISPR-Cas is intriguing. In order to reduce the off-target effects, a thorough evaluation of the CRISPR-Cas9 process is also necessary.

Keywords: CRISPR-Cas9, Gene Editing, Plant Genome Editing, Animal Genome Editing

Introduction

CRISPR-Cas are the prokaryotic adaptive immune system clustered regularly interspaced short palindromic repeats. It is found in the genomes of archaeal and other bacteria (Jansen et al., 2002). It is a streamlined RNA-guided Cas9 protein system for genome editing. This tool set now proves great scientific possibility for healing genetic malfunction, generating beneficial genetic characteristics, and developing novel methods for live-cell imaging, precise genome analysis, and point-of-care testing, among other things (Haroon et al., 2022a). Researchers have employed dCas9 coupled with fluorescent reporters to image the repetitive genomic regions in the live cells. CRISPR-Cas technology enables biological research and biotechnological applications in many sectors by providing an available and versatile means to edit, regulate and visualize genomes (Haroon et al., 2022b).

The research has been advanced by CRISPR-Cas 9, from understanding the biology of previously undiscovered species to identifying the genes that directly cause disease. Numerous Cas9-based medical studies are ongoing or will begin soon, with the outcomes likely directing onward action of Cas9-based biotechnology for somatic cell editing (Knott & Doudna, 2018; Zuo et al., 2017). In order to develop advanced cancer immunotherapies, the scientist designed T-cells and inactivated an endogenous retrovirus in pigs (Knott & Doudna, 2018).

Emerging Applications in Animals

The disruption of an EGFP (Enhanced Green Fluorescent Protein) transgene in mouse and transgenic zebrafish lines was demonstrated by Huang and colleagues. This initial/pioneer study was the first to show that CRISPR-Cas9 could be used in-vivo (Shen et al., 2013). The predominant editing technique in mice is CRISPR-Cas, which has higher activity and requires less RNA to accomplish multiple gene-targeting events(Wu et al., 2013).

CRISPR-Cas was effectively employed to engineer the haploid stem cells from mice and rats, resulting in triple-mutant clones in a single step (Horii et al., 2013) (Li et al., 2014). The CRISPR-Cas system was utilized to knock out the dead-end (*dnd*) gene and develop a sterile fish (salmon) (Güralp et al., 2020). This technology might solve the fish farming industry's numerous problems (Okoli et al., 2021). Through direct zygote injection, the CRISPR-Cas system effectively creates monoallelic and biallelic mutants for the vWF gene in pigs (Hai et al., 2014; Zhou et al., 2015). Research on

pig-to-human xenotransplantation has significantly advanced due to the adoption of the CRISPR-Cas system in genetic engineering (Ryczek et al., 2021). Recent research has shown that knock-in mice can be created with up to 50% efficacy using targeted Cas9 delivery with synthesized dual RNA (Sung et al., 2014). Human biological products have also been produced using livestock as bioreactors. Alternative means to generate human serum albumin have long been researched due to a lack of human blood supply and infection dangers (Peng et al., 2015). CRISPR-Cas9 holds the potential to edit the genes and generate site-specific mutations in the zygote (Navarro-Serna et al., 2020). Furthermore, the non-homologous end joining character is highly unpredictable, ideal for giant litter animals where valuable modification can be picked (West & Gill, 2016).

Method

CRISPR-Cas9 is a gene editing method that can precisely and successfully target any gene in the germ line and somatic cells from various organisms. CRISPR-Cas9 technology is used to treat neurodegenerative disease in non-human primate models.

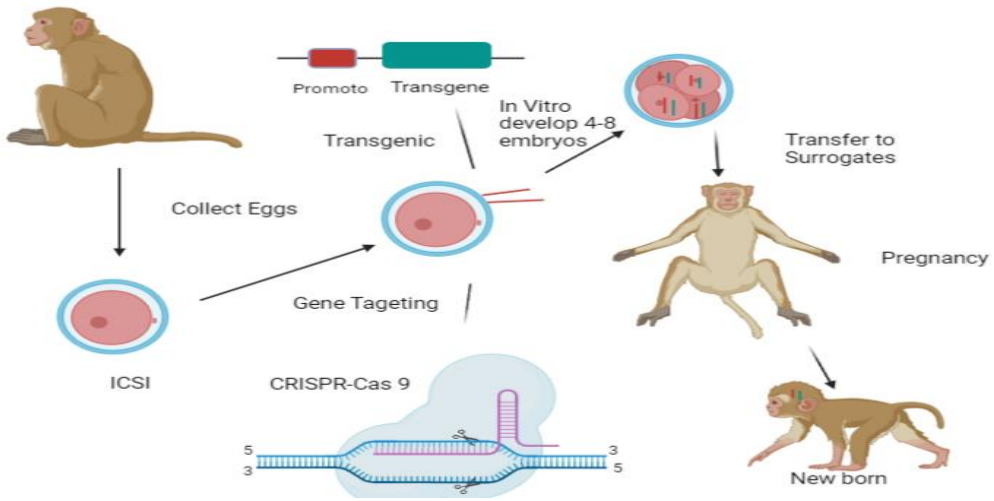

Fig. 1. Super ovulating females collect their eggs, fertilized in vitro via intracytoplasmic sperm injection. Lentiviral vectors are introduced into the fertilized eggs. The cytoplasm receives an injection of gRNA/Cas9. The implanted eggs were in vitro cultured. The newborn monkeys are examined to determine whether the mutations were produced. (Tu et al., 2015).

Emerging applications in Plants

The solution to tenable agriculture is to create genetic variations in plant crops (Griggs et al., 2013). For a very long period, the induction of genomic alterations was restricted by the characteristics of old gene editing tools. CRISPR-Cas, on the other hand, has changed plant breeding. (Jinek et al., 2012). The global population will rise to 9.6 billion people, with a 60% increase in the consumption of staple crops in 2050 (Tilman et al., 2011). The green revolution's pace of rising yields has been constantly reduced. Global warming is predicted to reduce crop yield. So, there is a need to develop climate-resilient crops. On the other hand, traditional breeding techniques are time-taking, labor-intensive, and complex. Consequently, there is a dire need to develop efficient and time-saving breeding strategies (Chen et al., 2019). Primitive sequence-specific nucleases, such as Transcription Activator-like Effector Nucleases (TALENs) and Zinc-Finger Nucleases (ZFNs), have been demonstrated to be useful for genome modification. However, their development necessitates complicated protein synthesis, thus limiting their use (Christian et al., 2010).

CRISPR/Cas is proficient in crop enhancement by modifying specific loci. This adaptable platform has produced new ways to facilitate plant gene regulation and protein production. Examples of CRISPR-Cas-related plant biotechnologies include in-situ-directed evolution, effective gene editing, and gene expression modification. In rice, for example, it is possible to simultaneously edit the eight agronomically significant genes (Shen et al., 2017).

By removing genetic regions that cause undesirable phenotypes or introducing beneficial mutation through precision genome editing, CRISPR-Cas technology can develop new varieties with desirable traits (Zhu et al., 2020). The efficacy of the CRISPR-Cas system also depends on the system's delivery in the living organism. In wheat, Agrobacterium provides a good alternative compared to the biolistic delivery method of the CRISPR-Cas system. This Agrobacterium complex delivery requires several transformation events compared to others. Wheat phenotypes with high yields were created by knocking down the cytokinin oxidase/dehydrogenase gene (Z. Zhang et al., 2019).

CRISPR-Cas has been successfully used in plant genomes to induce massive deletions (Durr et al., 2018). Inversions are the most prevalent large-scale chromosomal reconfiguration in all organisms (Schmidt et al., 2019). In *Arabidopsis*, homozygous and biallelic mutations caused by CRISPR-Cas have been described in first-generation transgenic plants (Feng et al., 2013). CRISPR-Cas has been used to improve the immune system of plants against the geminivirus. This technology may damage plant viruses' genomes and offer resistance against the virus (Ji et al., 2018)(Yao et al., 2018). CRISPR-Cas is used to produce herbicide-resistant germplasm of rice. It supports high agricultural output while avoiding soil degradation.

CRISPR-Cas is appealing because of its efficiency, versatility, and lack of transgenes. According to research on point mutation of the *ALS* gene, herbicide tolerance can be induced by specific amino acid alteration in acetolactate synthase (Powles & Yu, 2010). CRISPR-Cas can help restore self-incompatibility by causing mutations in genes like *farnesyl pyrophosphate synthase 2*. Activating parthenocarpy produces seedless fruit in citrus crops (Qin et al., 2018). In the absence of a reliable delivery method in the plant, numerous new tools for producing altered plants were created, allowing genome change without exogenous DNA. Using plant viruses to produce genetically modified plants instead of tissue culture is a promising method (Ali et al., 2015).

The CRISPR-Cas systems have been modified. Cas 9 expression can be controlled in specialized cell types using tissue-specific promoters, limiting gene editing to specific tissues or organs (Decaestecker et al., 2019). Using CRISPR-Cas9 technology, Dan Voytas and Lazaro combined agronomically beneficial qualities with advantageous characteristics of wild crops. In order to allow de novo domestication, they selected six growth and quality-related genes of domesticated tomatoes and edited them in cultivated tomato specie. The modified lines demonstrated an increase in fruit size and also a rise in fruit number in a single generation (Zsögön et al., 2018).

The off-target mutations are easy to handle in plant breeding as they can be eliminated by back-crossing in most species. The combination of the single-guided RNA (sgRNA) and Cas9 protein that target DNA determines the precision of CRISPR-Cas9 technology. For proficient DNA cleavage by Cas9, seed sequence (accurate pair between 8-12 bases of guided sequence) and the analogous area of the DNA target are critical (Cong et al., 2013) (Jiang et al., 2013). The sequencing, position, and background of the target may affect the efficacy of CRISPR-Cas9. Some DNA-binding proteins are known to be restricted by histone modification and DNA methylation. On the other hand, Cas9 has been shown to efficiently cleave methylated DNA in vitro and in vivo experiments (Hsu et al., 2013).

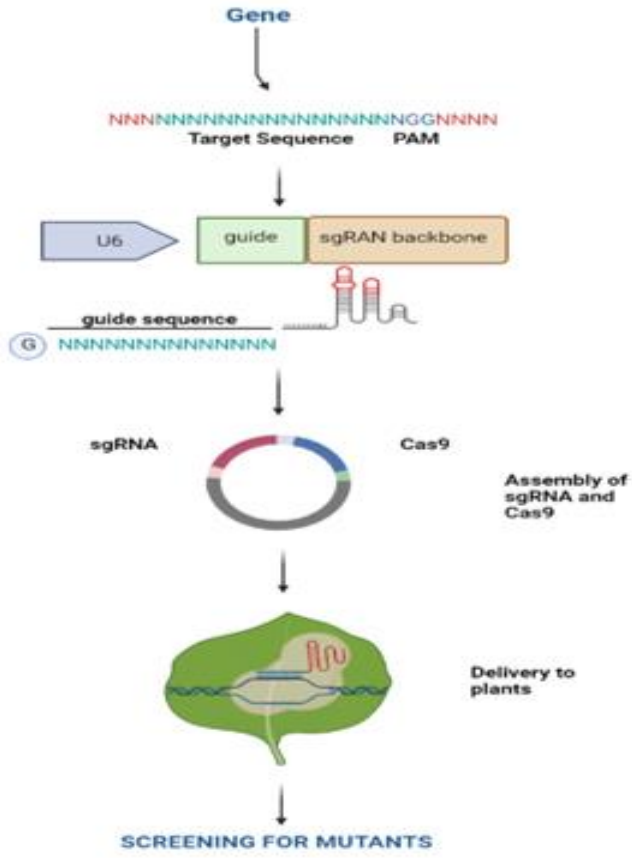

Fig 2: Process of producing of CRISPR-Cas 9 mutagenic plant
sgRNA from by the reaction of targeted DNA with U6 promoter and sgRNA with Cas9 assemble in plasmid and delivered in plant (Belhaj et al., 2015)

Future work

Although CRISPR/Cas has enormous potential, a few limitations, like health and ethical issues of using genetically edited tissue, organs, and animals, are still under consideration. One of the technical challenges is the transfer of genes into living cells using the CRISPR-Cas system. The researchers frequently use the virus vectors to transfer genes of interest in-vivo or in-vitro. Adeno-associated virus vectors are particularly appealing therapeutic delivery vehicles for the in-vivo system. Furthermore, the large size of Cas proteins does not enable packaging proteins into AVV vector. As a result, future progress in reducing the size of Cas proteins or discovering smaller Cas9 proteins is necessary.

Mosaic mutations, frequent in zygote injected-based genome editing, could be a problem in extensive animal genome editing (Vandana et al., 2021). The CRISPR/Cas-generated off-target mutations may be lethal for animals' health and raise questions about animal welfare (Singh & Ali, 2021). The CRISPR-Cas9 method is more likely to produce off-target mutations in human cells. Mutations outside the target location can cause gene malfunction and, in some instances, cell death (Ryczek et al., 2021). Also, due to health and ethical issues, more evidence of xenotransplantation's efficiency and safety is needed to achieve acceptability (Vandana et al., 2021).

Furthermore, a careful evaluation of the CRISPR-Cas9 technology needs to be done, as the technology has yielded crops with little or a fixed amount of virus resistance, allowing viruses to emerge quickly (Mehta et al., 2019). While the CRISPR-Cas domestication method has much promise, the approach still needs to have a few bottlenecks. In addition, wild cultivars are usually resistant to regeneration. Different transformation processes are needed to permit their domestication. Finally, creating an optimal crop requires changes to different loci, so other proficient multiplexed genome modification technologies are necessary.

CRISPR-Cas modifications are still in their early stages. To generate genotypes with mutations, recurrent mutagenesis and selection platforms are necessary. With more advancement, these methods will aid in identifying gene functions (Zhu et al., 2020).

For plant genome editing, harnessing homologous recombination for gene insertion remains a significant problem. The extent to which plant off-target mutations occur has yet to be systematically studied (Belhaj et al., 2015). To fine-tune gene function precisely, more development in gene regulation and precision genome editing will be required. Because the distribution of CRISPR-Cas reagents remains a crucial barrier to plant genome editing, more novel delivery strategies are needed. Nanomaterials offer potential transporters for CRISPR-Cas reagent delivery in diverse forms (H. Zhang et al., 2019). The first CRISPR-Cas-based directed evolution platform for plants was recently established. One can be optimistic that future technological advancements will produce far above our expectations (Butt et al., 2019).

Conclusion

CRISPR-Cas9 has become the most widely used method for gene editing compared to earlier technologies like TALENS and ZFNs, as it has simplicity, accuracy, and affordability. The advantage of CRISPR-Cas9 technology makes it highly adaptable in any molecular biology lab. The rapid use of CRISPR-Cas9 has driven new ethical, legal, and social issues in the environment, medicine, animal, and agriculture (Singh & Ali, 2021). Moreover, the CRISPR-Cas technology in vivo distribution tactics has yet to be tested entirely for safety, precision, and effectiveness. Furthermore, immunological rejections, low HDR, and off-target effects are becoming extremely pervasive. According to several groups, CRISPR-Cas9 creates unexpected significant deletion and complicated lesions in addition to expected indels and conversation. Therefore, further research is necessary to reduce these obstacles (Lee et al., 2020).

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