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CRISPR/CAS 9: A Novel Technique for Genome Editing

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ABSTRACT

Genome editing is an approach in which a specific target DNA sequence of the genome is altered by adding, removing, or replacing DNA bases. Recently, genome editing based on the bacterial defence mechanism.CRISPR/Cas9 has emerged as an easily applicable and reliable laboratory tool. Combining organoids and CRISPR/Cas9 creates exciting new opportunities to study organ development and human disease in vitro. The potential applications of CRISPR in organoids are only beginning to be explored. The development of antibiotic resistance in bacteria is a major public health threat. Infection rates of resistant pathogens continue to rise against nearly all antimicrobials, which have led to development of different strategies to combat the antimicrobial resistance. In this review, we discuss how the newly popular CRISPR-cas system has been applied to combat antibiotic resistance in both extracellular and intracellular pathogens. Recent investigations have revealed the implications of the CRISPR–Cas system as a promising tool for targeted genetic modifications in plants. This technology is likely to be more commonly adopted in plant functional genomics studies and crop improvement in the near future.

KEY WORDS: CRISPR, CAS9, GENOME EDITING, SGRNA.

INTRODUCTION

Due to recent advances in genome engineering technologies are evolving a new revolution in biological research. Researchers can now directly edit or modulate the function of DNA sequences in their endogenous context in virtually any organism of choice, permitting them to elucidate the functional organization of the genome at the systems level, as well as identify causal genetic variations. Genome editing is also referred to as genome engineering or gene editing. It is a technique that is used precisely and efficiently to modify DNA within a cell. So, genome engineering refers to the process of making targeted modifications to the genome, its context, or its outputs (e.g. transcripts). The development of effective ways to make accurate targeted modifications to the genome of organisms is an established objective in biological research. In recent times, a new method for genome editing based on a bacterial CRISPR-associated protein-9 nuclease (Cas9) from Streptococcus pyogenes was developed which created a buzz in the scientific world.

The main purpose of CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) and cas9 in some bacteria and archaea is to develop a defence mechanism towards an invasion of plasmids and viruses through adaptive immunity. It was proved that Streptococcus thermophilus acquired resistance towards a bacteriophage through incorporating a genomic part of a virus into its CRISPR locus. This type of technology enables the researchers to manipulate the genome by adding, removing, or varying the DNA sequence. It can be used to control gene expression in plants, animals, and even humans. It has the potential to delete detrimental traits and add desirable traits with more accuracy, effortlessly

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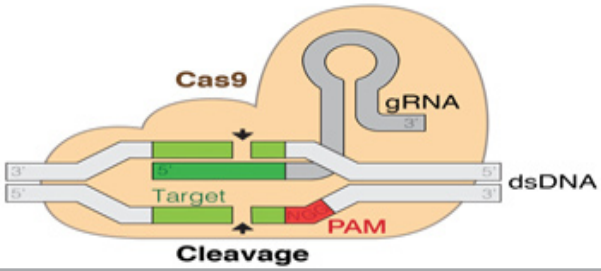
than before. Not only CRISPR can be used to "silence" genes by removing them but also to substitute desired genes by harnessing repair enzymes. So, for this, Cas9 enzyme can be modified to snip out disease-causing genes and insert a "good" gene to replace it.

What is the meaning of CRISPR?: CRISPR which stands for 'Clustered Regularly Interspaced Short Palindromic Repeats'. Early in 1987. It was originally discovered in E. coli and later in many other bacterial species. This molecule is made up of short palindromic DNA sequences that are repeated along the molecule and are regularly-spaced. Between these types of sequences, there are "spacers", foreign DNA sequences from organisms that have previously attacked the bacteria. The CRISPR molecule also includes CRISPR-associated genes or Cas genes. These can encode proteins that unwind DNA, and cut DNA, called helicases and nucleases, respectively.

Generally, the CRISPR-Cas systems are divided into two different classes based on their organization style and structural variation of the Cas genes: Class 1: Multiprotein effector complexes Class 2: Single effector protein The most frequently used subtype of CRISPR systems is the type II CRISPR/ Cas9 system, which mainly depends on a single Cas protein from Streptococcus pyogenes (SpCas9) targeting particular DNA sequences. CRISPR-Cas9 is the most common, cheap, and efficient system used for genome editing. This Cas9 stands for CRISPR-associated protein 9 and is the nuclease part that cuts the DNA / chops the DNA. CRISPR is the DNA-targeting part of the system which consists of an RNA molecule, or 'guide', designed to bind to specific DNA bases through complementary base-pairing.

Figure 1: CRISPER-Cas9

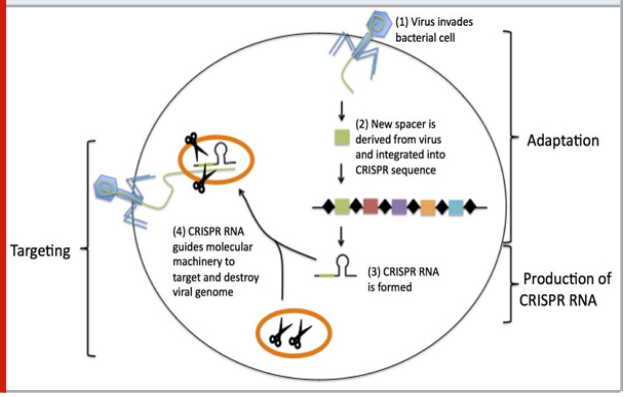


General Principle of CRISPER/Cas9: Genome editing can be achieved in vitro or in vivo by delivering the editing mechanism in situ, which powerfully adds, deletes, and "corrects" genes as well as performs other highly targeted genomic modifications. These can be used by cells to repair harmful breaks that occur on both strands of DNA, known as double-strand breaks. Targeted DNA alterations begin from the generation of nuclease-induced double-stranded breaks (DSBs), which leads to the stimulation of highly efficient recombination mechanisms of cellular DNA in mammalian cells. Nuclease-induced DNA DSBs can be repaired by one of the two major mechanisms that occur in almost all cell types and organisms: homology-directed repair (HDR) and non-homologous end-joining (NHEJ), resulting in targeted integration or gene disruptions, respectively.

Genome editing mainly relies on this concept of DNA double stranded break (DSB) repair mechanics. First report of CRISPR clusters by Ishino et al. in E. Coli bacteria in 1987. Among these, CRISPR/Cas9 is the most popular and efficient technique. It has two components such as Cas9 protein and a guide RNA (cr-RNA). RNA (cr-RNA) guides the Cas9 protein to the complementary sequence on target DNA which is subsequently cleaved by Cas9 proteins. The mechanisms of the CRISPR/Cas9 system can be easily understood by three different stages. The first stage is an adaptation, which leads to the insertion of new spacers in the CRISPR locus. In the second stage, expression, the system gets ready for action by expressing the cas genes (cas operon) and transcribing the CRISPR into a long precursor CRISPR RNA (pre-crRNA).

The pre-crRNA is subsequently processed into mature crRNA by Cas proteins (endonuclease activity) and accessory factors. In the third and last stage, interference, the combined action of cr-RNA and tracr-RNA(transactivating cr-RNA) fused into single sg-RNA(small guide RNA) which interact with their target DNA through complementary base pairing and Cas proteins associated with it, recognized the PAM (Protospacer Adjacent Motif) region in target DNA and ultimately degrade/destroy the target DNA and make them inactive. The application of CRISPR/Cas9 is being used at the molecular level like gene deletion/ insertion/ replacement, base editing, transcription modulation, and DNA labeling, etc and as a practical application level, it is being used in biological research, human medicine, biotechnology, and agriculture.

Figure 2: The steps of CRISPR-mediated immunity.



Types of CRISPR/ Cas Systems: Three different types of CRISPR/Cas systems are present: A) type I, B) type II and C) type III. Types I and III are found in both bacteria and archaea but, type II is unique in bacteria. The type II CRISPR/Cas system is the most studied and best characterized in which Cas9 protein is the critical component. The Cas9 endonuclease is a four-component system that includes two small RNA molecules named

CRISPR RNA (crRNA) that is specific to the targeted DNA, and trans-activating CRISPR RNA (tracrRNA) sequence that interacts with the Cas9 protein, that has DNA endonuclease activity. This complex will affect target-specific double-stranded DNA cleavage and will be repaired by the DNA repair mechanism by non-homologous end joining (NHEJ) or homology-directed repair (HDR). Cas9 endonuclease was re-engineered into a more controllable two-component system by joining the two RNA molecules into a "single-guide RNA"(sgRNA) that, when united with Cas9, could find and cut the DNA target specified by the guide RNA (Jinek, et al., 2012).

Thus, by manipulating the nucleotide sequence of the single-guide RNA, the synthetic Cas9 system could be programmed to target any DNA sequence for cleavage. A recent improvement is the use of the modified version of CRISPR/Cas9 system i.e., Cas9 to target protein domains for transcriptional regulation and epigenetic modifications. CRISPR/Cas9 expertise has altered the outlook of genome editing, allowing an earlier unachievable stage of genome targeting, high efficacy, easiness, and flexibility of the system. Many laboratories around the world are using the technology for variety of applications including use as a basic biology research tool, development of biotechnology products, and potentially to treat diseases in plants, animals and humans.

Following initial demonstration of genome editing by CRISPR-Cas9 by Feng Zhang's and George Church's groups simultaneously, in human cell cultures for the first time. It has since already been successfully adopted to target important genes in many cell lines and organisms, including yeast, Xenopus tropicalis, Candida albicans, zebrafish, fruit flies, ants, mosquitoes, nematodes, mice, rabbits, monkeys, pigs, human embryos and plants (Arabidopsis, rice, wheat, sorghum, tobacco). Though, initial success was achieved by the use of CRISPR/ Cas9, it takes time for routine use of this technology in humans, plants and animals. To a large extent, research is still focusing on its use in plant and animal models or isolated human cells to treat diseases. The CRISPR/ Cas9 system is widely used in biomedical research to edit the human genome and try to knock out genetic diseases like Huntington's disease or cystic fibrosis, breast and ovarian cancers (BRCA-1 and 2 mutations), hypertrophic cardiomyopathy, Down Syndrome, and HIV infections out of T cells.

Three Common Strategies have been developed for Genome Editing with the CRISPR/Cas9 Platform

1. Plasmid-based CRISPR/Cas9 strategy, where a plasmid is used to encode Cas9 protein and sgRNA, assembles Cas9 gene as well as sgRNA into the same plasmid in vitro. this strategy is longer lasting in the expression of Cas9 and sgRNA, and it prevents multiple transfections. The Key challenge in this system is the introduction.
2. Encoded plasmid needs to be introduced inside the nucleus of target cells, which is a key challenge in this system.
3. Direct intracellular delivery of Cas9 messenger RNA (mRNA) and sgRNA, the greatest drawback of which lies in the poor stability of mRNA, which results in transient expression of mRNA and a short duration of gene modification. 3. Direct delivery of Cas9 protein and sgRNA which has several advantages, including rapid action, great stability, and limited antigenicity.

How CRISPER/Cas9 system works: The CRISPR immune system is a mechanism which protects the bacteria from repeated virus attacks through three different steps:

1. Adaptation: When DNA from a different viruses invade the bacteria, the viral DNA is processed into short segments and is made into a new spacer between the repeats. These will serve as a genetic memory of previous infections.
2. Production of CRISPR RNA: The CRISPR sequence undergoes transcription, including spacers and Cas genes, creating a single-stranded RNA. The resulting single-stranded RNA is called CRISPR RNA, which contains copies of the invading viral DNA sequence in its spacers.
3. Targeting: This CRISPR RNAs will identify viral DNA and guide the CRISPR-associated proteins to them. The protein then cleaves and destroys the targeted viral material.

Uses of CRISPR system

1. Gene Knock-Out: Gene silencing using CRISPR starts with the use of a single guide RNA (sgRNA) to target genes and initiate a double-stranded break using the Cas9 endonuclease. These type of breaks are then repaired by an repair mechanism, the non-homologous end-joining (NHEJ). However, NHEJ is error-prone and results in genomic deletions or insertions, which then translates into permanent silencing of the target gene.
2. DNA-Free Gene Editing: CRISPR can be used for DNA-free gene editing without the use of DNA vectors, requiring only RNA or protein components. This system can be a good choice to avoid the possibility of undesirable type of genetic alterations due to the plasmid DNA integrating at the cut site or random vector integrations.
3. Gene Insertions or "Knock-ins": The CRISPR-induced double-strand break can also be used to create a gene "knock-ins" by exploiting the cells' homology-directed repair. The precise insertion of a donor template can alter the coding region of a gene. Previous studies which have demonstrated that single-stranded DNA can be used to create precise insertions using the CRISPR-Cas9 system.
4. Transient Gene Silencing: By modifying the Cas9 protein so it cannot cut DNA, transient gene silencing or transcriptional repression can also be done. The modified Cas9, led by a guide RNA, targets the promoter region of a gene and reduces transcriptional activity and gene expression. Transient activation or upregulation of specific genes can be effectively done.

Development of Disease Resistance in Plant Using a Novel Technique CRISPR / CAS9: The challenge for all disciplines of agriculture is to increases production and improves the quality of produce. As a global scenario, plant diseases are major challenge and biotic constraint that leads to significant crop yield losses in terms of both quantity and quality of the produce. Over the past few decades, the excessive & unnecessary use of chemical pesticides was the dominant form of disease control and subsequently created many problems such as the frequent emergence of fungicide resistance in pathogens and the harmful effects of fungicides to human health and negative impact on plants and environment. To overcome all these problems, adopting an integrated disease management strategy as an alternative tool for disease management. Integrated disease management (IDM) is a sustainable approach that combines all the suitable techniques such as biological, cultural, physical, and chemical control strategies holistically rather than using a single component strategy proved to be more effective and sustainable and minimizes economic, health and environmental risks.

But in the current scenario, due to the changing climatic conditions, the plant pathogenic organisms have developed different types of resistance mechanisms against pesticides and also the emergence of the new race of the pathogens in the environment through which diseases caused by the pathogens has become resistant which is a very difficult task to manage it effectively by adopting the traditional approaches including IDM. So, for this, scientists have evolved a novel, emerging, and a latest and most popular technique known as CRISPR/ CAS9 based genome editing technology through which plant disease can be managed by developing disease resistance in plants at the genetic level. CRISPR/CAS9 is an important tool for genome editing in an organism. Genome editing is a technology that can produce modifications such as insertion/deletion/substitution at specific sites in the genome of an organism. CRISPR stands for clustered regularly interspaced palindromic repeats. It is an array of short repeated sequences separated/ interspaced by spacers sequence derived from foreign DNA with unique sequences. First report of CRISPR clusters by Ishino et al. in E. Coli bacteria in 1987.

CRISPR is a defense system in bacteria which fight against the phage infection and provide sequence-specific adaptive immunity acts by integrating short virus sequences in the cell's CRISPR locus, allowing the cell to remember, recognize and clear infections. There are different tools for the genome editing process which includes Meganucleases, Zinc Finger Nucleases (ZFNs), Transcription Activator Like Effector Nucleases (TALENs), CRISPR/Cas9, and CRISPR/Cas12a (also called CRISPR/ Cpf1).In agricultural application, being used as improved yield, pest and disease resistance, herbicide tolerance, and improved nutritional quality. It is simple and cost-effective and has got broad spectrum applications in agriculture including plant disease management in the future. As a future perspective point of view, to improve the specificity of the CRISPR/Cas9 system to prevent off-target mutations and identifying smaller and more efficient Cas9 variants with distinct specificities. Scientists should be focused on more detailed studies on Homology Directed Repair mechanisms and also the development of more safe and efficient delivery mechanisms for Cas9/ sg RNA into organisms and exploring more potential applications of CRISPR/Cas System. There is a need to overcome the ethical and political barriers through proper awareness of society.

Advantages of the CRISPR/Cas9 System

1. Unlike ZFNs and TALENs, the CRISPR/Cas9 system can cleave methylated DNA in human cells, allowing genomic modifications that are beyond the reach of the other nucleases.
2. The main practical advantage of CRISPR/Cas9 compared to ZFNs and TALENs is the ease of multiplexing. The simultaneous introduction of DSBs at multiple sites can be used to edit several genes at the same time and can be particularly useful to knock out redundant genes or parallel pathways.
3. Applications of Cas9 as a Genome Engineering Platform

1. The Cas9 nuclease cleaves DNA via its RuvC and HNH nuclease domains, each of which nicks a DNA strand to generate blunt-end DSBs. Either catalytic domain can be inactivated to generate nickase mutants that cause single-strand DNA breaks.
2. Two Cas9 nickase complexes with spaced target sites can mimic targeted DSBs via cooperative nicks, doubling the length of target recognition.
3. Expression plasmids encoding the Cas9 gene and a short sgRNA cassette driven by the U6 RNA polymerase III promoters can be directly transfected into cell lines of interest.
4. Purified Cas9 protein and in vitro transcribed sgRNA can be microinjected into fertilized zygotes for rapid generation of transgenic animal models.
5. For somatic genetic modification, high-titer viral vectors encoding CRISPR reagents can be transduced into tissues or cells of interest.
6. Genome-scale functional screening can be facilitated by mass synthesis and delivery of guide RNA libraries.
7. Catalytically dead Cas9 (dCas9) can be converted into a general DNA-binding domain and it can be fused to functional effectors such as transcriptional activators. The modularity of targeting and flexible choice of functional domains enables rapid expansion of the Cas9 toolbox.
8. Cas9 coupled to fluorescent reporters facilitates live imaging of DNA loci for illuminating the dynamics of genome architecture.
9. Reconstituting split fragments of Cas9 via chemical or optical induction of heterodimer domains, such as the cib1/cry2 system from Arabidopsis, confers temporal control of dynamic cellular processes.

Applications and Implications in Plant Breeding

1. Genome editing can accelerate plant breeding by allowing the introduction of predictable modifications directly in an elite background, and the CRISPR/Cas9 system is particularly beneficial because multiple traits can be modified .
2. NHEJ-mediated gene knockouts are the simplest form of targeted modification, and these could be used e.g., to eliminate genes that negatively affect food quality, to confer susceptibility to pathogens or to divert metabolic flux away from valuable end-products. Both TALEN and CRISPR/Cas9 technologies have been used to target the genes of the mildew resistance locus (MLO) in wheat and successfully knocked out all three MLO homoeoalleles, generating plants resistant to powdery mildew disease.
3. Site-specific nucleases also allow targeted molecular trait stacking, i.e., the addition of several genes in close vicinity to an existing transgenic locus. This makes it feasible to introduce multiple traits into crops with a low risk of segregation, which is difficult to achieve by classical breeding or even conventional genetic engineering. Once stacking has been achieved, the entire array of transgenes can be mobilized into another germplasm by crossing because it behaves as a single locus.

Drawbacks of CRISPR: CRISPR-mediated genome editing has drawbacks, though. The PAM requirement limits target sequences. Cas9 is large, so its gene is difficult to deliver to cells via vectors used in gene therapy. Scientists worry about off-target effects, although experts note that concerns about unintended mutations are often based on calculations from studies on improving editing. These studies may deliberately use low-specificity conditions to facilitate monitoring progress.

CONCLUSION

CRISPR/Cas9 based genome editing is a fundamental breakthrough technique. Application of genome editing tools in crop improvement to enhance yield, nutritional value, disease resistance and other traits will be a prominent area of work in the future. CRISPR has played a huge part in the increase in genome editing studies in recent years. This type of system has broad applications in plant and animal improvement, as well as in the medical field. Even though modifications to CRISPR have been made to minimize the possibility of off-target effects, it has yet to be proven. There is even a chance of genome vandalism viz., the system cuts on target without a precise edit. Once the precise and deeper mechanism underlying how the CRISPR/ Cas9 system works was understood, it could be harnessed for applications in molecular biology and genetics. Still there are many ethical issues raised by genome editing have to be addressed by researchers. Regulatory agencies will also need to regulate how best CRISPR/Cas9 technology can be exploited without hindering applicable research and development.

REFERENCES

Barrangou R, Doudna JA (September 2016). "Applications of CRISPR technologies in research and beyond". Nature Biotechnology. 34 (9): 933–941.

Doudna and Charpentier, (2014). https://www. ibiology. org/genetics-and-gene-regulation/crisprcas9/.

Ishino Y., Shinagawa H., Makino K., Amemura M. and Nakata A. 1987. Nucleotide sequence of the iap gene, responsible for alkaline phosphatase isozyme conversion in Escherichia coli, and identification of the gene product. Journal of Bacteriol. 169: 5429– 5433.

Jennifer, A., Charpentier, D.E. 2014. The new frontier of genome engineering with CRISPR-Cas9. 346 (6213).

Jinek, M. et al. (2012). A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunit. Science, 337 (6096): 816–821.

Li, M. et al. (2016). Reassessment of the four yield related genes Gn1a, DEP1, GS3, and IPA1 in rice using a CRISPR/Cas9 System. Frontiers in Plant Science, 7.

National Academy Press, Washington DC, USA. 2. Zhang, K., Raboanatahiry, N., Zhu B. and Li M. 2017. Progress in genome editing technology and its application in plants. Front. Plant science, 8:177.

Overton, J. (1996). Ecologically Based Pest Management-New Solutions for a New Century.

Sander, J.D. and Joung, J.K. (2014). CRISPR-Cas systems for editing, regulating and targeting genomes. Natural Biotechnology., 32: 347-355.

Wang, F. et al. (2016). Enhanced rice blast resistance by CRISPR/Cas9-targeted mutagenesis of the ERF transcription factor gene OsERF922. PLoS ONE 11, e0154027.

Zhou, H. et al. (2016). Development of commercial thermo-sensitive genic male sterile rice accelerates hybrid rice breeding using the CRISPR/Cas9- mediated TMS5 editing system. Scientific Reports, 6: 37395.