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CRISPR/Cas9: Method of Genetic Engineering

Shilpa[1] and Parveen Sharma[2]
[1]Senior Research Fellow, Department of Vegetable Science and Floriculture, CSK HPKV Palampur, Kangra, Himachal Pradesh
[2]Professor, Department of Vegetable Science and Floriculture, CSK HPKV Palampur, Kangra ,Himachal Pradesh

SUMMARY

CRISPR (clustered regularly interspaced short palindromic repeat) endonucleases are at the forefront of biotechnology, synthetic biology and gene editing. Methods for controlling enzyme properties promise to improve existing applications and enable new technologies. CRISPR enzymes rely on RNA cofactors to guide catalysis. CRISPR technology has been applied in the food and farming industries to engineer pro biotic cultures and to immunize industrial cultures versus infections. It is also being used in crops to enhance yield, drought tolerance and nutritional imbalance. CRISPR associated nucleases have shown to be useful as a tool for molecular testing due to their ability to specifically target nucleic acid sequences in a high background of non-target sequences. In 2016, the Cas9 nuclease was used to deplete unwanted nucleotide sequences. One other potential application is to create gene drives. These are genetic systems, which increase the chances of a particular trait passing on from parent to offspring. Eventually, over the course of generations, the trait spreads through entire populations, according to the Wyss Institute. Gene drives can aid in controlling the spread of diseases such as malaria by enhancing sterility among the disease vector female Anopheles gambiae mosquitoes according to the 2016 Nature Biotechnology article.

INTRODUCTION

The development of efficient and reliable ways to make precise, targeted changes to the genome of living cells is a long-standing goal for biomedical researchers. Recently, a new tool based on a bacterial CRISPR-associated protein-9 nuclease (Cas9) from Streptococcus pyogenes has generated considerable excitement. CRISPR/Cas9 is a technique that allows for the highly specific and rapid modification of DNA in a genome, the complete set of genetic instructions in an organism

CRISPR

(Clustered Regularly Interspaced Short Palindromic Repeats) is a family of DNA sequences found in the genomes of prokaryotic organisms such as bacteria and archaea. The CRISPR/Cas9 system allows for site-specific genomic targeting in virtually any organism. It is a specialized region of DNA with two distinct characteristics: the presence of nucleotide repeats and spacers. Repeated sequences of nucleotides — the building blocks of DNA — are distributed throughout a CRISPR region. Spacers are bits of DNA that are interspersed among these repeated sequences.

CRISPR RNA (crRNA)

Once a spacer is incorporated and the virus attacks again, a portion of the CRISPR is transcribed and processed into CRISPR RNA, or "crRNA." The nucleotide sequence of the CRISPR acts as a template to produce a complementary sequence of single stranded RNA.

Cas9:

The Cas9 protein is an enzyme that cuts foreign DNA. The protein typically binds to two RNA molecules: crRNA and another called tracrRNA (or "trans-activating crRNA"). The two then guide Cas9 to the target site where it will make its cut. This expanse of DNA is complementary to a 20-nucleotide stretch of the crRNA.

CRISPR

Using two separate regions, or "domains" on its structure, Cas9 cuts both strands of the DNA double helix, making what is known as a "double-stranded break," according to the 2014 Science article. Jennifer Doudna

and Emmanuelle Charpentier re-engineered the Cas9 endonuclease into a more manageable two-component system by fusing the two RNA molecules into a "single-guide RNA" that, when combined with Cas9, could find and cut the DNA target specified by the guide RNA. By manipulating the nucleotide sequence of the guide RNA, the artificial Cas9 system could be programmed to target any DNA sequence for cleavage.

Principle of CRISPR/Cas9

A single guide RNA (sgRNA), consisting of a crRNA sequence that is specific to the DNA target, and a tracr RNA sequence that interacts with the Cas9 protein

- Binds to a recombinant form of Cas9 protein that has DNA endonuclease activity
- The resulting complex will cause target-specific double-stranded DNA cleavage.
- The cleavage site will be repaired by the non homologous end joining (NHEJ) DNA repair pathway, an error-prone process that may result in insertions/deletions (INDELs) that may disrupt gene function.

CRISPR/Cas9 Gene Editing:

DISRUPT

If a single cut is made, a process called non-homologous end joining can result in the addition or deletion of base pairs, disrupting the original DNA sequence and causing gene inactivation.

DELETE

A larger fragment of DNA can be deleted by using two guide RNAs that target separate sites. After cleavage at each site, non-homologous end joining unites the separate ends, deleting the intervening sequence

CORRECT OR INSERT

Adding a DNA template alongside the CRISPR/Cas9 machinery allows the cell to correct a gene, or even insert a new gene, using a process called homology directed repair. Scientists make use of the CRISPR-Cas9 systems' recognition of specific DNA sequences and apply it in the process of development of improved crops. Instead of viral DNA as spacers, scientists design their own sequences, based on their specific gene of interest. If a gene's sequence known, it can be easily used in CRISPR. It will then act just like a spacer for the system and guide the Cas9 protein to a DNA matching sequence.

What other techniques are there for altering genes?

- Over the years scientists have learned about genetics and gene function by studying the effects of changes in DNA.
- If you can create a change in a gene, either in a cell line or a whole organism, it is possible to then study the effect of that change to understand what the function of that gene is.
- For a long time geneticists used chemicals or radiation to cause mutations. However, they had no way of controlling where in the genome the mutation would occur.
- For several years scientists have been using 'gene targeting' to introduce changes in specific places in the genome, by removing or adding either whole genes or single bases.
- Traditional gene targeting has been very valuable for studying genes and genetics, however it takes a long time to create a mutation and is fairly expensive.
- Several 'gene editing' technologies have recently been developed to improve gene targeting methods, including CRISPR-Cas systems, transcription activator-like effector nucleases (TALENs) and zinc-finger nucleases (ZFNs).
- The CRISPR-Cas9 system currently stands out as the fastest, cheapest and most reliable system for 'editing' genes.

Using CRISPR/Cas9 to Study Plant Cell-Wall Associated Enzymes XylaM is a main component in the secondary cell wall that contributes to mechanical strength and cell wall recalcitrance. The structure of xylan consists of a β-1,4-linked xylopyranosyl (Xyl) backbone and often decorated by α-L-arabinopyranose (Araf) as a

single unit and sometimes substituted with 4-O-methyl-α-D glucuronic acid (GlcA) (Darvill et al., 1980). Previous studies identified two rice mutants, ss1 and ss2, that exhibited dwarf, thinner stems, and leaf tip necrosis phenotypes (Tu et al., 2015). A follow-up study found that these two mutants contain point mutations in the gene named OsXYN1, which encodes an endo-1,4β-xylanase (Tu et al., 2020). To confirm the role of OsXYN1, two OsXYN1 CRISPR mutant lines were produced that contained 1 and 2 bp deletions, respectively (Tu et al., 2020). As expected, both OsXYN1 mutants demonstrated similar phenotypes to ss1 and ss2. Furthermore, the ss mutants contained less lignin and downregulated genes related to xylan and lignin biosynthesis (Tu et al., 2020). Moreover, the ss mutants were likely to wilt under sunlight and demonstrated inefficient water uptake, which was caused by having a thinner middle lamella compared to WT. As a trade-off, genes in the aquaporin water channel pathway were found to be upregulated in the ss mutants (Tu et al., 2020).

Manipulating enzymes in the pectin degradation pathway can potentially enhance the postharvest life of fruits such as tomatoes (Kitagawa et al., 2005). A recent study successfully edited pectate lyase (PL), polygalacturonase 2a (PG2a), and β-galactanase (TBG4) to reveal their functions in pectin degradation and fruit ripening in tomato (Wang D. et al., 2019). The PL CRISPR line showed a firmer inner and outer pericarp. Both the PL and PG2a CRISPR lines showed higher juice and paste viscosity. The TBG4 CRISPR lines exhibited more separation of the intracellular spaces and larger fruit size, whereas TBG4 and PG2a CRISPR lines also showed a delay in fruit color changes during ripening (Wang et al., 2019).

The CRISPR/Cas9 mediated genome editing approach has emerged as a powerful tool to modify cell wall biosynthesis pathways in several plant species. Recently, the base-editing property of CRISPR/Cas9 was exploited to generate double herbicide resistant rice plants (Hu Z. et al., 2019). C17 is a newly identified growth inhibitor that can disrupt cellulose biosynthesis by directly acting on CESA1 or CESA3. However, a C17 resistant mutant line has been identified in Arabidopsis that contained a C to T point mutation in CESA3. By transforming a BE3-CESA3S983F CRISPR/Cas9 base editor system with cytidine deaminase into another isoxaben resistant mutant (irx2-1) background, 9 out of 2,000 transgenic lines conferred C17 and isoxaben resistance, including seven chimeric mutants and two homozygous and Cas9-free mutant lines (Hu Z. et al., 2019).

CRISPR - Cas9 Applications

Researchers have found that the CRISPR - Cas9 system can be applied to nearly every organism. Early studies using CRISPR - Cas9 for gene editing have focused on crops important for agriculture. It was realized early on that the system could be used in crops to improve traits, such as yield, plant architecture, plant aesthetics, and disease tolerance.

CRISPR has been used to edit the genome of rice. The team of Ying Wang from Syngenta Biotechnology China designed several CRISPR sgRNAs and successfully deleted fragments of the dense and erect panicle1 (DEP1) gene in the Indica rice line IR58025B. Improvements in yield-related traits, such as dense and erect panicles and reduced plant height,were observed in the mutant plants produced.

- Researchers from Beijing Key Laboratory of Vegetable Germplasm Improvement, led by Shouwei Tian used CRISPR-Cas9 to target ClPDS, the phytoene desaturase in watermelon, to achieve the albino phenotype.
- Researchers from the Chinese Academy of Agricultural Sciences and National Center for Citrus Variety Improvement and Southwest University have also developed citrus plants resistant to citrus canker caused by Xanthomonas citri subsp. citri (Xcc), a serious disease of citrus, through CRISPR-Cas9.

CONCLUSION

Genome editing in general, and CRISPR-Cas9 in particular, is a revolutionary tool that can impact science, food production, and society. CRISPR-Cas9 has great potential for transforming agriculture by making plants tolerant to biotic and abiotic stresses and improving their nutritional value and yield. These attributes are necessary to meet the demand of an increasing world population. The shorter timeframe from genotype to phenotype makes CRISPR/Cas9 mutagenesis particularly valuable and appealing to generate higher-order mutants to discover gene functions for many cell wall-related gene families. Finally, while we have largely focused on cell wall biosynthesis here, this CRISPR/Cas9 approach is equally applicable to other genes whose products function in modifying and/or degrading the plant cell wall.

REFERENCES

Anders, C. Niewoehner O., DuerstA. and Martin Jinek. Structural basis of PAM- dependent target DNA recognition by the Cas9 endonuclease Nature 513, 569–573 (2014).

Bikard D, Jiang W, Samai P, Hochschild A, Zhang F, Marraffini L.A.. Programmable repression and activation of bacterial gene expression using an engineered CRISPR-Cas system. Nucleic Acids Res 41,7429-7437 (2013).

Biertumpfel C., Yang, W., and Suck, D. Crystal structure of T4 endonuclease VII resolving a Holliday junction. Nature 449, 616–620 (2007).

Bolotin A, Quinquis B, Sorokin A, & Ehrlich S.D. Clustered regularly interspaced short palindrome repeats (CRISPRs) have spacers of extrachromosomal origin. Microbiology 151, 2551–2561 (2005).

Cong L., Ran F.A., Cox D, et al. Multiplex genome engineering using CRISPR/Cas systems. Science 339(6121), 819–823 (2013).