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REWRITING OUR BIOLOGICAL DESTINY: THE INCREDIBLE POWER OF CRISPR/CAS9 SYSTEM

Shubham Khichi* and Yudhishthir Bhanwariya

Ph.D. Scholar, Department of Genetics and Plant Breeding, Agriculture University, Jodhpur, Rajasthan, 342304, India

INTRODUCTION TO CRISPR

The initialization of editing of gene was conducted in year 1996 when Kim et al. described ZFN (Zinc finger nucleases) for the first time which made from DNA binding Zinc finger for site recognition and FokI endonuclease for cleavage of that recognized/target site. The expensiveness and procedure to validate the technique make ZFN an undesired technique for many average institution and laboratories. Later in 2010, TALENs (Transcription-activator like effector endonuclease) has been introduced having DNA binding domain from bacterial genus *Xanthomonas*, and the cleavage domain from FokI endonuclease. TALENs introduction in public domain gained popularity which was drastically declined by introduction of CRISPR/Cas9 system.

CRISPR stands for Clustered Regularly Interspaced Short Palindromic Repeats. CRISPR systems allow prokaryotes to mount a memory-based adaptive immune response that targets previously encountered foreign nucleic acids for destruction. The CRISPR sequence was discovered earlier in 1986 by a Japanese molecular biologist "Yoshizumi Ishino" in *Escherichia coli*. Later on, two scientist named Dr. Jennifer Doudna and Dr. Emmanuelle Charpentier discovered the ability of CRISPR/Cas9 as tool for genome modification and won Nobel prize for their work in year 2020. This genome editing tool serves the researchers to manipulate the ongoing genetic system of microorganisms and in genetic improvement in crop plant. CRISPR/Cas9 system now been used for benefiting the mankind by developing climate resilient crop varieties, managing pest population, silencing the faulty gene and in vaccine and drug development. There are some challenges like off-target effects causing less precision in this system which is to be quired by making this technology more advance and user friendly.

Mechanism

When bacteriophage infect the bacteria with its genetic material, initially Cas1 and Cas2 protein is synthesizes. Cas1 and Cas2 are different protein but works together and recognizes the bacterial genome. These 2 proteins bind on a specific location of bacteriophage genome which is "PAM" (Protospacer adjacent motif). This sequence is NGG that is any nucleotide plus 2 Guanine. Now after recognizing PAM, Cas1 and Cas2 binds on PAM sequence and after binding it cut the double strand of DNA in that manner the upstream region of PAM which is protospacer region is cut.

Now, this isolated protospacer region is now incorporated into the bacterial DNA towards 5'prime end . This integration is also done by Cas protein; the process is called Spacer acquisition or Spacer integration.

After spacer acquisition, the CRSIPR array is transcribed by RNA poly-III and Cr-RNA (CRISPR RNA). On Cr-RNA the first ever enzyme come and react is RNAase-III, this enzyme cut the Cr-RNA into Fragments. After digestion with RNAase different fragment formed with different recognized protospacer. Now the fragment having CRISPR array + protospacer is now bind with tracer RNA (tr-RNA) and form a complex and after complex formation Cas9 is attached.

This complex travel towards bacteriophage genome, because the protospacer site in the complex is complementary to the protospacer in the bacteriophage genome. Cas9 recognize PAM site and bind on the bacteriophage genome, the protospacer helps the complex to travel to the recognition site that is why it is also known as Guide RNA (Fig 4). Tr-RNA helps in stabilizing the complex and Cas9 act as an endonuclease which will degrade the bacteriophage genome.

CRISPR/CAS9 as genome editing tool

Genome editing means to disturb or add ultimately modify. CRISPR/Cas9 complex Binds to the specific sequence (PAM site) of DNA segment (gene) which we want to digest or disrupt. Cas9 recognizes and cut two to three nucleotides above the PAM site which leads to the double strand break. After double strand break there are two strategies to overcome it first one is NHEJ which is non homologous end joining and second is homologous recombination.

When NHEJ followed breakdown DNA simply joined in which there is loss of 2 to 3 nucleotides forms each side so after joining originality of that gene earlier will not have in newly edited gene that means it got disrupted.

We want to add new gene for that there is a need of a homologous copy (doner DNA) of that gene. After double strand break homologous recombination takes place and it will do the gene modification by adding the desired gene.

Application of CRISPR/Cas-9 system

1. Genetic improvement of crops through CRISPR/Cas-9. Eg: Recently developed drought and salt tolerant rice variety "Pusa DST Rice 1".
2. In insect and pest management by altering doublesex (dsx) gene which leads to sterile insect. Eg: Mutation of doublesex in Hyphantria cunea results in sex-specific sterility (Li et al. 2020).
3. For functional genomics.
4. This genome editing tool can used to treat different genetic disorders by repairing or disrupting the faulty genes (gene silencing).
5. Novel drug development.

Challenges and future prospects

1. Unintended cutting of DNA segment that is off-target effect is a major challenge in this genome editing technology.
2. Ethical concerns regarding CRISPR/Cas9.
3. Combining the sterile insect technique (SIT) with the CRISPR/Cas9 system
4. The utilization of the CRISPR/Cas9 system for transcription regulation.
5. More advance genetic improvement of crops for nutrition trait enhancement (Biofortification) and climate resilient crops.
6. Overcoming the off target effects.

CONCLUSION

CRISPR is a powerful tool that has led to the development of diverse technologies for the control of pests, pest-associated diseases, climate resilient crop varieties, eliminating the faulty genes and novel drug development. Ultimately making this system of genome editing more sustainable for future thrust.

Corresponding E-mail:
shubhamaujodhpur@gmail.com