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CRISPR-Cas9 Genome Editing System

Poonam Sharma, Parshant Kumar Sharma, Priya Sharma, O. P. Verma and Ashim Debnath*

Dept. of Genetics & Plant Breeding, Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya, Uttar Pradesh (224 229), India

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Corresponding Author
Ashim Debnath
e-mail: ashimdebnathdmr@gmail.com

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Abstract

CRISPR/Cas9 is a versatile genome-editing tool that has been used to investigate the function of genetic components, create genetically modified creatures, and conduct preclinical research on genetic illnesses. It is a new approach that may precisely and accurately change any section of any species without affecting other gene. In this article, we look at the molecular mechanism, applications, and problems of CRISPR/Cas9-mediated genome editing, as well as the future clinical pharmacological properties of CRISPR/Cas9.

Introduction

Researchers are currently grappling with the problem of determining the molecular process by which genes influence individual phenotypes. CRISPR-Cas9 (clustered regularly interspaced short palindromic repeats) was first identified in *E. coli*. In CRISPR/Cas system, invading foreign DNA is processed by Cas nuclease into small DNA fragments, which are then incorporated into CRISPR locus of host genomes as the spacers. The spacers are employed as transcriptional templates for generating crRNA, which leads Cas to cleave target DNA sequences of invading viruses and phages in response to viral and phage infections.

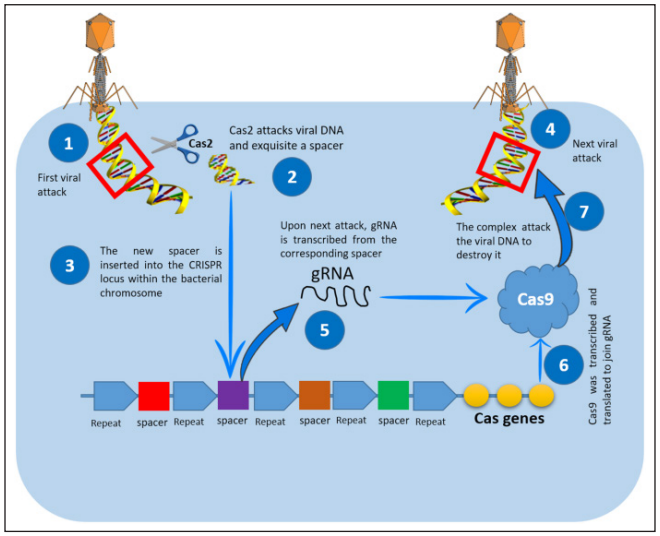

Figure 1: A description of the CRISPR/Cas bacterial immune system explained by Sabit *et al*. (2021)

Applications

Agriculture

CRISPR gene editing tools can edit crops without harming other gene. Recently, CRISPR-Cas9 mediated mutation in GRAIN WIDTH and WEIGHT 2 (GW2) locus improves aleurone layer and grain nutritional quality in rice (Achary *et al.,* 2021).

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E-mail: *bioticapublications@gmail.com*

Human Health

CRISPR-Cas9 system will revolutionize gene therapy and make it possible to treat large number of disease that would be impossible to treat without this technique. Recently, CRISPR-Cas9 system is important to the profound understanding and clinical transition of RBPs (RNA binding proteins) as cancer therapeutic target (Zhang *et al*., 2014).

Research Application

CRISPR-Cas9 system will allow the creation of new animal and cellular model which help us to learn more about disease and test new drugs and vaccines on these models. Novel combination of CRISPR-Cas9 based gene drives eliminates resistance and localizes spread which is considered to be invasive in the UK as a case for gene drive population control (Faber *et al.*, 2021).

Challenges

Off-Target Mutation

Multiple DNA sequences that are identical or very similar to target DNA sequences are frequently found in large genomes. CRISPR/Cas9 cleaves these identical or highly similar DNA sequences as well as target DNA sequences, resulting in alterations in unwanted site known as off-target mutations. Cell death can occur as a result of off-target mutations.

Pam Dependence

A 2-5 nt PAM sequence located directly downstream of the target region, in addition to gRNA/target sequence complementarily, is required for CRISPR/Cas9 specificity.

gRNA Production

Another key aspect of CRISPR/Cas9-mediated genome editing is gRNA creation. It is currently challenging to use RNA polymerase II for gRNA production due to substantial post-transcriptional processing and modification of mRNA produced by RNA polymerase II.

Conclusion

An ideal genome editing tool should feature a simple, efficient, and low-cost assembly of nucleases that can target any place in the genome without causing off-target changes. After these obstacles are resolved, CRISPR/Cas9 has the potential to become a reliable and simple genome editing technology. It opens the door to exposing gene function in biology and fixing gene abnormalities in illnesses, thanks to the simplicity and versatility of CRISPR/Cas9.

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