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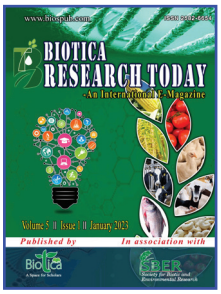

Biotica Research Today Vol 5:1 2023

62/64

How to cite this article?

Bhar, 2023. From Adaptive Immunity in Bacteria to Precise Genome Editing: The Story of CRISPR/Cas System. Biotica Research Today 5(1):62-64.

From Adaptive Immunity in Bacteria to Precise Genome Editing: The Story of CRISPR/Cas System

Anirban Bhar

Dept. of Botany, Ramakrishna Mission Vivekananda Centenary College (Autonomous), Rahara, Kolkata, West Bengal (700 118), India

Open Access

Corresponding Author

Anirban Bhar

e-mail: anirbanbhar@rkmvccrahara.ac.in, anirbanbhar06@gmail.com

Keywords

Agriculture, CRISPR-Cas, Genome editing, Plant biotechnology

Abstract

Plants are constantly facing enormous biotic and abiotic stress factors in nature. To secure the food for the ever-increasing population scientists are constantly put their effort to improve crop and generate stress tolerant plants. Genome editing is one of the cutting-edge technologies in plant biotechnology to improve plants. The Clustered Regulatory Interspaced Short Palindromic Repeats (CRISPR) technology is paramount due its high specificity and plasticity of application. CRISPR is existed in microbial system as the part of the immune response. The present article focusses towards development of this ground breaking technology and its application.

Introduction

Genome editing is a powerful tool in the modern era to modify organisms and get desired traits or even alter the genomic architecture of an organism to correct congenital disorders. There are many tools developed with time but the major constraint behind the successful widespread application of those tools is specificity. Tools like TALEN (Transcription Activator-Like Effector Nuclease), ZFN (Zinc Finger Nucleases), *etc*. are specific but CRISPR is the best available technology to date in terms of precision and robustness. This technique of genome editing is comparatively cost-effective and easy to apply. The wide possibility and enormous application of this groundbreaking invention brought the Nobel Prize to its inventors, Jennifer Doudna and Emmanuelle Charpentier in chemistry in 2020.

Bacterial Adaptive Immunity: The Birth of a Promising Technology

In 1987, Atsuo Nakata first observed the presence of repetitive palindromic sequences in the genome of *Escherichia coli*, which was termed Clustered Regulatory Interspaced Short Palindromic Repeats (CRISPR). It was then substantially observed in almost every bacterium both Gram positive and Gram negative exposed to viral infections. The virus is an infectious particle that required a living host to multiply them. The virus which infects bacteria (*e.g.*, bacteriophages) possesses short repetitive palindromic sequences within their genome. These sequences help the virus to attach itself to the bacterial genome for successful integration. In the integrated form the virus utilizes viral replication machinery to multiply its genome and is ultimately organized into mature viral particles. These matured newly formed viral particles then disrupt the bacterial cell and are released into the environment. During the release of viral DNA from the bacterial genome sometimes a short segment of viral DNA remained within the bacterial genome permanently as

Article History

Received on: 06th January 2023

Revised on: 21st January 2023

Accepted on: 22nd January 2023

E-mail: *bioticapublications@gmail.com*

a remnant. One bacterium may possess many such remnant viral DNA within its genome as a signature memory of the infecting virus. That means the bacterial genome exhibited as mosaic palindromic sequence interspaced with spacer sequences. These spacer sequences originated from mobile genetic elements (MGE) *e.g.*, transposons, viruses, *etc*. is called CRISPR array (Koonin and Makarova, 2019). After transcription of this mosaic genetic element produces pre-crRNA that contains both palindromic repeats flanked with spacer sequences. Then another short RNA, called tra-crRNA comes and binds with the corresponding sequence of palindromic regions. Simultaneously, a ribonuclease protein (RNase III) Cas9 (CRISPR-associated protein 9) comes in contact with the crRNA-tra-crRNA duplex to form a tripartite complex (Barazesh *et al.*, 2021). The nuclease then cleaved the crRNA within the palindromic region. As a result of that, multiple units of crRNA-tra-crRNA-Cas9 develop, which is termed an effector complex. When a new virus attacks the same bacteria, the effector molecule identifies the specific region of viral DNA complementary to the pre-existed viral DNA associated with the effector complex. This complementary sequence in a new viral particle is called PAM (Protospacer Adjacent Motif). The Cas9 nuclease cleaves the PAM and deactivates the virus (Heler *et al.*, 2015) (Figure 1).

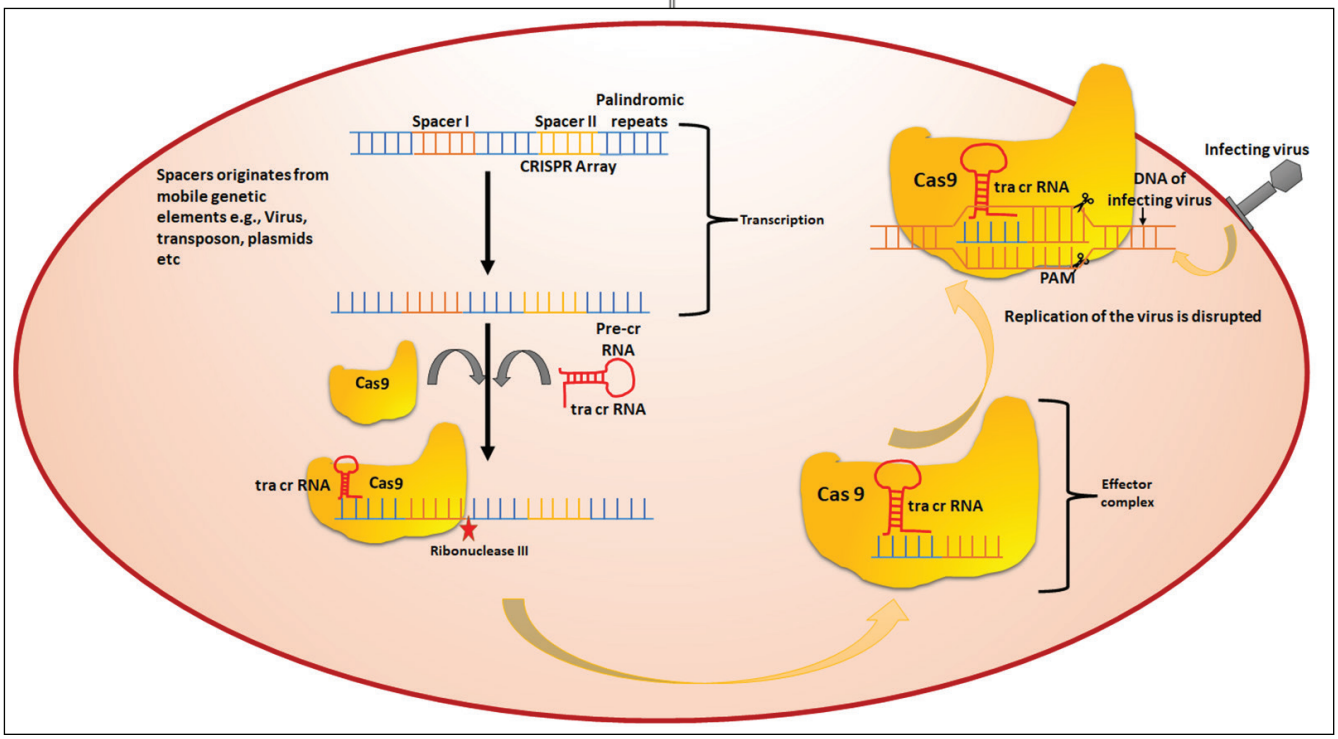

Figure 1: The schematic diagram showing step by step mechanism of CRISPR-Cas system in inducing immunity in bacterial cells

Jennifer Doudna and Emmanuelle Charpentier modify this natural immune response mechanism in bacteria into a revolutionized editing technology. In a natural system, crRNA and tra-crRNA are two separate units. Scientists had fused these two units into a single guide RNA (sgRNA) and by altering the short sequence (around 20 bp) of this sgRNA virtually any segment of the genome of any organism can be targeted precisely. By modifying the DNA repair mechanism of the targeted organism, those precise segments can be deleted, modified or new segments can be incorporated.

Application in Medical Science

The clinical application of the CRISPR/Cas9 system is enormous and many are under extensive trial. This technology has successfully been employed in cancer therapy and viral infections *e.g.*, human papillomavirus (HPV), Epstein Barr virus (EBV), Hepatitis B virus (HBV), *etc*. Recently, this technology has also been successfully employed in different congenital diseases *e.g.*, haemophilia, Duchenne muscular dystrophy, hemopoietic diseases, *etc*. (De Buhr and Lebbink, 2018). In most congenital issues, corrections are needed within germline cells. The application of such technology in germline cells has several ethical issues and norms. Each country has different ethical guidelines for human application of CRISPR technology which need to be further clarified. Uniform laws and regulation across the globe are urgently needed for their sustainable application in human welfare.

Application in Agricultural Science

The application of CRISPR technology in modifying plant health is comparatively easier than that of human health. CRISPR/Cas system has popularly been used

for the past few years in altering and manipulating desired characters in agriculturally important plants. Biotic and abiotic stress tolerant plant development is the primary notion of plant biotechnology. The CRISPR toolbox is successfully used to develop many biotic and abiotic stress tolerant plants by targeted genome editing. Besides, CRISPR technology is also used to alter the size, shape, and colour of the product according to consumer preferences. The quantitative trait loci (QTLs) can also be modified by this technology to improve yield (Tyagi *et al.*, 2020). In plant biotechnology, the laws regulating genetically modified organisms (GMOs) are strict in many countries. Whereas, CRISPR technology in many cases able to bypass these stringent laws and makes it easy to apply this technology in the next agricultural revolution.

Conclusion

The high specificity and flexibility enable CRISPR to modify almost any part of the genome of any organism. Several modifications and advancements have been done continuously for improving the applicability of this technology. Their application in medical as well as agricultural sector is quite extensive. Technological advancement supported by the amendments of existing rules and development of novel legislations are urgently needed to explore this technique in its full potential.

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