Vol: 4, Pg:29-34, Yr: 2023-AJABTR

CRISPR-Cas-9: A Recent Tool for Gene Editing

A Review on Discovery and Application

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Abstract: From the existence of life to modern days, prokaryotes have had an immense influence in our knowledge and understanding of molecular mechanisms. For a long time scientists thought that the bacteria are incapable of raising adaptive immunity. Advancements in vector science revealed a novel feature or molecular machinery, CRISPR or Clustered Regularly Interspaced Short Palindromic Repeat of bacteria and archaea by which they display antiviral capabilities. These primitive but superefficient organisms were able to activate adaptive immunity by inactivating attacking viral DNA thereby interrupting its ability to proliferate in host body. These breakthrough studies led to a new era of using much more sophisticated gene editing tools which have inordinate scope for correcting genetic disorders such as Alzheimer's disease, Huntington's, Parkinson's, hemophilia, β-thalassemia, cystic fibrosis, Duchene muscular dystrophy, Tay-Sachs, etc; autoimmune disorders such as multiple sclerosis, lupus and for treatments for different types of cancer as well as for treatments aimed at improving patient quality of life and prolonging lifespan.

This review discusses briefly the history of discovery, mechanism of gene therapy utilizing lesson learnt from the molecular mechanism used by the bacteria and the bioethical issues centered around the use of CRISPR-Cas-9 technique.

Keywords: CRISPR-Cas-9, Gene-editing, Adaptive Immunity

1. Introduction

Molecular biology techniques play a critical role in human life and society. Prokaryotes including bacteria and archaea provided us with the knowledge of not only the molecular mechanism, but also with the techniques to manipulate gene(s) most efficiently. Two

gene-editing techniques using restriction enzymes used before the discovery of Clustered Regularly Interspaced Short Palindromic Repeat or CRISPR Cas-9, were zinc finger nucleases (ZFN) and Transcription activator-like effector nucleases (TALENs) [1]. In both cases, the difficulty of protein engineering, expense, and time-consuming efforts were the major challenges for researchers and manufacturers as well. CRISPR-Cas-9 brought a new era in molecular biology and biotechnology with promising results for clinical applications as well as public health.

2. Background

Early works of two young scientists paved the path towards the discovery of CRISPR-Cas-9 system. Ishino, while doing his research with alkaline phosphatase in *E coli* sequenced a gene and noticed it had some extra length [2,3]. While looking for molecular mechanism for successful survival of a halophile *H. mediterranei*, a group of archaea who can survive in an extremely saline gradient, noticed those archaea had repetitive sequences in their DNA that were consistent from generation to generation [4,5].

These regularly spaced clusters in the DNA provide bacteria and archaea with antiviral properties. These clusters of repeated sequences were found to be conserved evolutionarily giving the prokaryotes a special advantage of protection and survival. For archaea these repeated sequences have been found in 90% of them and in about 50% of bacteria which gave these primitive organisms a superefficient power to activate adaptive immunity by inactivating attacking viral DNA thereby interrupting its ability to proliferate in host body [1,2].

The names of these structures changed a number of times, from Short Regularly Spaced Repeats (SRSRs), to Spacers Interspersed Direct Repeats (SPIDRs), Large Cluster of Tandem Repeats (LCTRs) and then finally in 2002 scientific community agreed to call it CRISPR or Clustered Regularly Interspaced Short Palindromic Repeat.

3. Mechanism of Action

There are three steps of the CRISPR defense mechanism to protect bacteria from repeated viral attacks: adaptation by spacer acquisition, expression by crRNA synthesis, and target interference. CRISPR loci are an array of short repeated sequences found in

chromosomal or plasmid DNA of prokaryotes. Cas gene is usually found adjacent to CRISPR that codes for nuclease protein (Cas protein) responsible to destroy or cleave viral nucleic acid [4,6].

Genetic analysis showed that spacer regions located on bacteria's genome acts as a memory system guided by RNA. When infected by virus, the genetic machinery acted like an efficient search to locate the identical sequence of viral DNA using

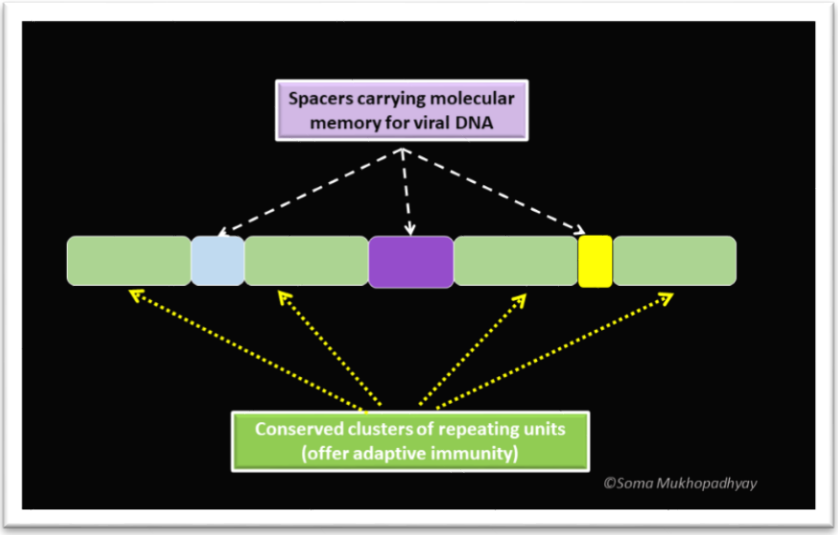

Fig 1: Animation of arrangement of CRISPR-Cas9 system in bacteria with interspersed variable spacers.

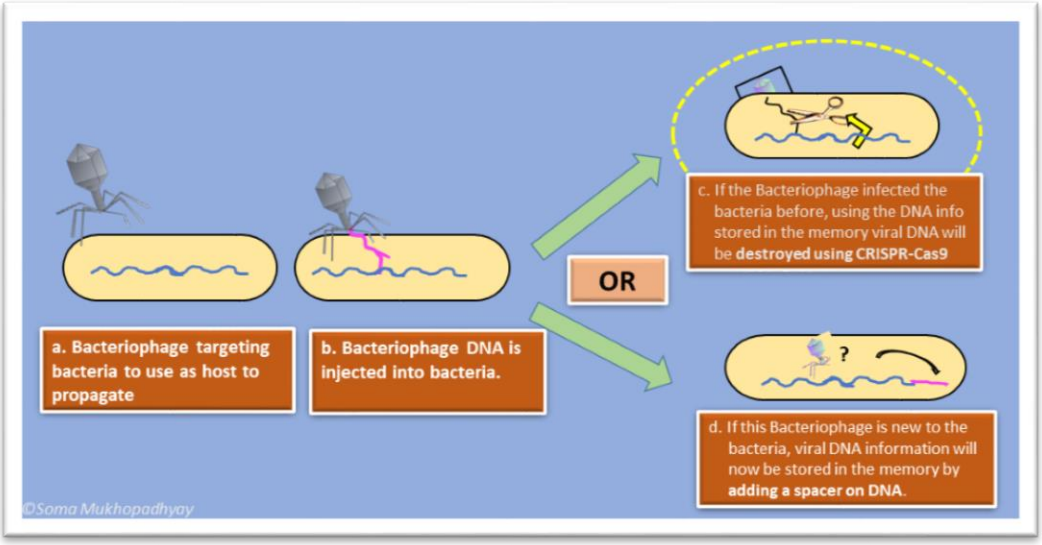

Fig 2: Mechanism of CRISPR-Cas-9 action in bacteria for protection

CRISPR guided by G-RNA (guiding RNA) and followed by Cas-9 (CRISPR associated protein) activity. Cas-9 proteins have helicase function to unwind the DNA to be manipulated and an endonuclease task. This endonuclease or the molecular scissor precisely cuts the DNA region to make the virus totally ineffective for infection.

Using this precise genetic tool as represented in figure 2, modification in zebra fish genome was performed in the lab. In case of human, there is a PAM (Protospacer Adjacent Motif) in every 50 base pair, which acts as the target sight identified by Cas-9 guided by g-RNA. When scientists know the gene in human genome they want to target for splicing, base-sequence of RNA is synthesized to match that DNA and then the molecular editing is done precisely by CRISPR-Cas-9 system.

At the initial stage of understanding, scientists contemplated that the molecular replacement mechanism could only affect a single gene at once. Later it was discovered that by one mechanism multiple genes could be manipulated, while on the other hand single base pair within a gene could be precisely removed or changed.

Scientists modified a gene in the mosquito which will prevent them from carrying the malarial parasite [3,9]; corrected a gene which showed improvement in curing the Duchene syndrome for muscular dystrophy [6,9], and corrected painful and deadly blood disorders like thalassemia and sickle cell, etc.

4. Ethical Issues and Approval:

For a long time there were controversies regarding inappropriate use of this genetic tool for germ line manipulation and other non-ethical purposes. After many years of ethical issue arguments, the Human Fertilisation and Embryology Authority (HFEA), a U.K. regulatory body, permitted the use of CRISPR on human embryos in the UK in 2016 [7]. In the same year, the National Institute of Health (NIH) approved clinical trials to start in the USA [7,8]. In 2017, the National Academy of Science approved CRISPR for use on the human germ line. This research gives us hope that we will not only correct genetic disorders like cystic fibrosis, but also discover the cause(s) and the solution(s) to curing infertility.

5. Conclusion

Bacteria acted as a Master Chef to show us how to combine ingredients efficiently to come up with a better recipe for molecular mechanism. The emergence of CRISPR has advanced our understanding of the genetic

basis of disease thereby opening new doors for basic molecular researches for more target specific gene therapies thereby using this tool for clinical applications. The existence of adaptive immunity in prokaryotes could be a new addition to our concept of defensive mechanisms in bacteria but with keen observation power, scientists envisioned the use of the CRISPR-Cas-9 technique would revolutionize biotechnology with its precision. This would allow for correcting and modifying errors in genomes in not only humans, but in plants, other animals and insects as well.

In 2020 the Nobel Prize in chemistry was awarded to Drs Jennifer Doudna and Emmanuelle Charpentier for development of a method for genome editing [4,8], but we have to remember a number of scientists' work paved the road towards the development of this sophisticated technique.

Like other biotechnological tools, there are still challenges to overcome in the practical applications and various improvements are needed to overcome obstacles as CRISPR might lead to varying levels of success.

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