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Genome Editing by CRISPR/Cas9

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Abstract

Genetic engineering is moving through quick advancement holding the hand of CRISPR gene editing technology as it throws lights upon biotechnology with the discovery of CRISPR and Cas9 endonuclease functions. Both in natural and artificial way of its function CRISPR enhances the immune system of bacteria against virus (natural) and in human beings (artificial) providing permanent relief from various diseases. In this review paper Gene editing process is discussed followed by introduction of CRISPR-Cas9 and its related terms defined briefly. Its natural technology that occurs in bacteria and its lab based technology and the genome editing technology is also discussed with diagram and proper explanations.

Keywords : CRISPR, cas9, bacteriophage, DNA, RNA, editing, Genome, PAM.

List of abbreviation for the Editing of Genome by CRISPR/CAS9

Abbreviations

Clustered Regularly Interspaced Short Palindromic repeats.

Protospacer Adjacent Motif.

Non-Homologous End Joining.

Homology Directed Repair.

Deoxy Ribonucleic Acid.

Ribonucleic Acid.

CRISPR Associated.

CRISPR From *Prevotella* and *Francisella 1*.

Introduction

Gene, a functional unit of heredity, made up of DNA. Genes were thought to be manipulated in selective breeding before DNA was invented. After discovery of DNA, there was many applications and experiments performed to modify DNA by scientists, writers and philosophers. Numerous studies and research were practiced on understanding DNA expression, regulation, characterization, processing etc. After some decades a new gene editing technology was described which is known as CRISPR-Cas9, for utilization because of its potentiality in

biotechnological purposes in industry and genetic engineering. This resulted in the ability beneficial to the economy, and also involving present and future products regulation concerning for national security, ethics, social matter in the utilization of technology.

Cas9 protein

The CRISPR associated protein 9 having 160 kilodalton mass works as defense system of certain bacteria against plasmids and DNA viruses. In genetic engineering and biotechnological purpose it is utilized laboriously. It functions in cutting the DNA strand and thus altering the genome. It is also called as bacterial RNA guided endonuclease.

PAM sequence – The PAM or the protospacer adjacent motif is a small sequence of DNA (2-6 bp) which follows the DNA position of template which is being targeted for cleavage formation by CRISPR is CRISPR-Cas9. The PAM is used for an enzyme Cas nuclease for cutting and normally 3-4 nucleotides towards downstream. The PAM is required for a Cas nuclease to cut and is generally found 3-4 nucleotides downstream from the cut site.

Guide RNA – The specific RNA sequence that helps in recognition of target DNA site and Cas nuclease is directed by it for editing. It consisting of two parts – crRNA and tracrRNA.

What is CRISPR/Cas9 technology?

CRISPR-Cas9 is a unique gene editing technology by adding, removing or altering DNA sequences which showed considerable improvement rather than other gene editing technologies which are beneficial in its efficacy, cost and functioning. A simplest, adaptable, brief procedure of gene manipulation. It is believed by many scientists and engineers and other communities that through CRISPR-Cas9 there would be many advancements in the disease diagnostic, treatment and prevention, improvement of crop yields, new variety production, in biotechnological uses for processing of biofuels, bioplastics, adhesives etc.

CRISPR Repeats

First CRISPR repeats discovered by an accident of a researcher of Osaka University Yoshizumi Ishino and his colleagues in 1987 in the genome of *E. coli* bacteria when gene analysis was performed in phosphate metabolism. Same kind of repetitive sequence was also found in other strain of *E. coli* and enterobacteria, *Salmonella dysenteriae* and *Salmonella enteric*. The study of CRISPR-Cas9 discovery showed special dimensions and interest for researchers because of its advancements towards gene editing, scientists starts thinking it as a strong therapeutic tool which might be highly helpful in treating diseases caused by genetic mutations.

Works of CRISPR

In the LAB

CRISPR is known as the defence system of bacteria which has been used as genome editing tool by modification for increasing its utilization. The efforts made by some scientists provided the view of CRISPR's role for genome editing in the laboratory. A guideRNA or gRNA must be produced from the cells to be engineered. A paper published by Doudna's group in the journal science where it has been reported that from the native CRISPR system the tracrRNA and crRNA sequences could be fused to form single RNA sequence called gRNA by which Cas9 has been directed to a specific site in the genome. This is the important till the gRNAs design be considered as simple that directs Cas9 or Cpf1 for targeting DNA sequences.

Cas9 which is used in the laboratory, the PAM sequence is NGG (where N denotes any of the four bases). PAM sequence is recognised by Cas9 and then it binds to the PAM sequence. The Cas9 checks whether there is base pairing complementary present in between the bound gRNA and the DNA stand. If there pairing complementarity is found then a blunt, double strand cut is made at position 3 base pairs upstream of 3' end of PAM sequence. After the double stranded DNA cut is completed, two ways are their for the cells to repair the damage known as non-homologous end joining (NHEJ) or homology directed repair (HDR). The faster among them is NHEJ but it has the possibility of error occurrence more than HDR. NHEJ repair is performed by the cells to repair the damage resulting into insertion of nucleotide at the break of DNA strand. This indicates that a mutant non-functional gene is expressed because of some cells which have damaged DNA permanently. This also meant to say that with the use of CRISPR/Cas9 to prepare double strand breaks in DNA for huge no. of cells, there will be misrepair of the damage and permanent loss of function mutations in the gene which is the targeted one. Thus the application of CRISPR fulfilled the aim of the researchers for rapid and efficient removal or deletion of genes.

CRISPR not only used for DNA editing but also in modification of a two amino acids in the Cas9 and/or Cpf1 proteins, there will be conversion of the enzymes into mutants which doesn't have the ability to cut DNA. They just bind with the targeted DNA sequence in order to initiate or repress gene expression at that region of

the genome. This kind of proteins are known as nuclease-deficient endonuclease.

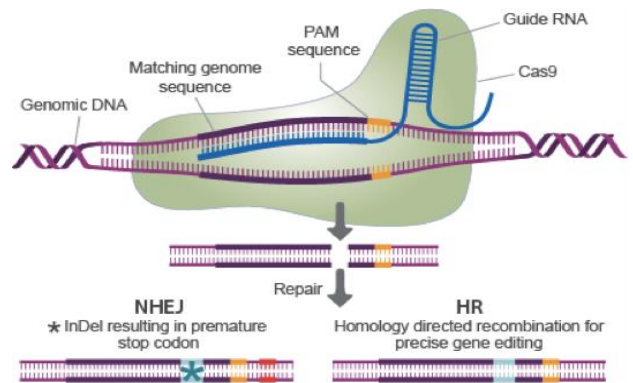


FIG 1.- CRISPR/Cas9 works in the laboratory.

How CRISPR works in nature?

CRISPR acts as defense system for bacteria. When a bacterium is attacked by a bacteriophage by injecting its DNA in the bacterial cell. Inside the bacterial cell the bacteriophage viral DNA invades the cell and starts replication to form multiple daughter DNA of bacteriophage from which new viral particles are produced followed by formation of head, tail fibre, sheath and then released from the cell of bacterium. In order to get resistance from attack of bacteriophage, bacteria utilizes some ingenious techniques to remove the bacteriophages. CRISPR is one of these kind of techniques. When a bacteriophage injects its DNA then the bacterial cell cleaves or cuts invading DNA of bacteriophage and reserves on its own genome. So that it can pull out the stored DNA of previously invading bacteria and utilized it to determine and destroys the new invader bacteriophage DNA. These are the steps followed to perform the techniques :-

- i. The DNA of the invader bacteriophage is introduced in the site of bacterial genome, called as CRISPR locus.
- ii. At the time of next attack of bacteriophage, DNA (A,T,G,C) in the site of CRISPR locus corresponding to the invader DNA of the bacteriophage which is transcribed into a small piece of RNA (A,U,G,C) known as CRISPR RNA (crRNA).
- iii. crRNA then binds to a large protein which is called as effector complex that guides in invader bacteriophage DNA, the sequence is complementary to the crRNA sequence. With the invader bacteriophage DNA the effector complex and its crRNA binds through base pairing.
- iv. The invader bacteriophage DNA is cut and is stored in the site or CRISPR locus for invasions later on.

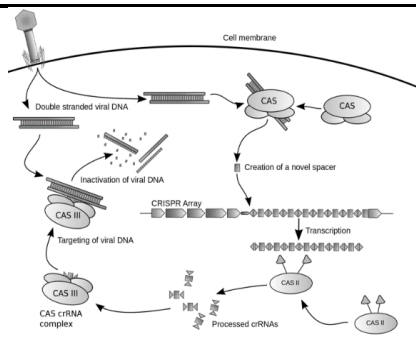


Fig 2. - CRISPR/Cas9 works naturally (in bacteria).

CRISPR/Cas9 Genome Editing Technology

An advanced technology in the stream of genetic engineering where genome editing can be performed in a brief and concise manner known as CRISPR-Cas9 genome editing and the tool associated with it known as CRISPR-Cas9 genetic scissor. This was observed by two researchers Noble Laureates Jennifer Doudna and Emmanuelle Charpentier in 2012 that the technology can be utilised in order to destroy and alter or replace genetic sequences by CRISPR Cas9 protein re programming.

Cas9 makes a double strand cut on the DNA helix at the previously recognised site. Cut after recognised in the DNA, it prepares to repair it by its own natural way but this shows possibilities of being error and despite of that gene function may be stopped. The next way out is to incorporate repair template of DNA molecule. The template comprises of a new code that can be incorporated into the DNA.

This CRISPR-Cas9 genome technology discovery shows lights in the in the field of medicine, pharmaceuticals and biotechnology, genetic engineering. Genetically linked diseases of human beings can be cured by this technology. But in order to avoid its negative effects genome editing usage must be regulated and manipulated strictly with proper measurements and controlled manner.

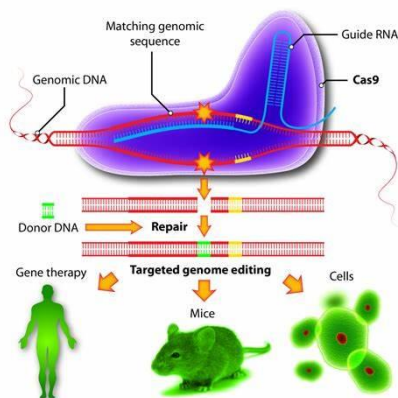


Fig 3. - Genome editing technology of CRISPR

Conclusions

This story reveals what a powerful mechanism of immunity defence carried out by a simple organism belonging from smallest group of organisms, the bacteria involves in the discovery of such an outstanding platform for genetic engineering and biotechnology industry. This helps the researchers to invent ways of disease resistance, prevention and complete treatment, and also curable way out for incurable diseases. Thus CRISPR/Cas9 technology of gene editing provides huge future aspects for not only in medicinal purposes but also in research advancements, unique inventions, genetic and biotechnological engineering purposes. Hopes are day by day increasing in between the researchers for further development and discovery with CRISPR's work.

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