CRISPR Cas9 – a legitimate tool for future medicine.

By Rakshith H and Spandana M Hegde

ABSTRACT:

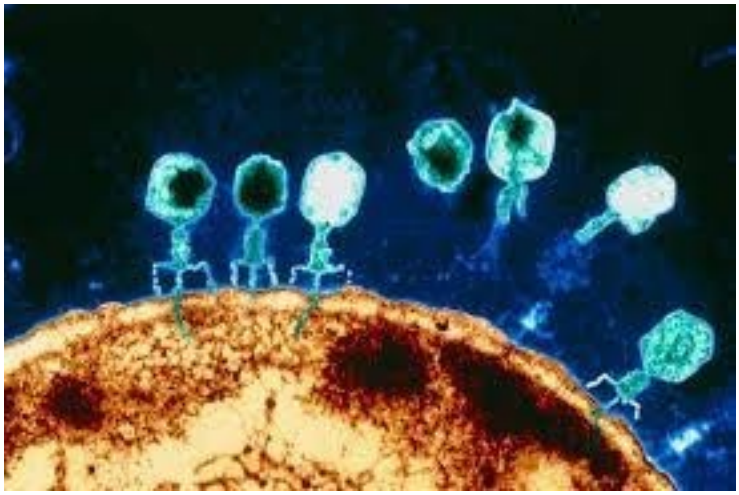
Clustered regularly interspaced short palindromic repeats, popularly known as CRISPR in scientific community has been one of the greatest observations that scientists have made. CRISPR-Cas9 is a unique technology that enables researchers to edit the parts of genome or by altering sections of DNA sequence. This technique is easy, cheap and specific. There are immense applications of this technique in medicinal as well as agricultural field.

HISTORY:

Basically, CRISPR is the acquired immunity widely found in the archea and bacteria .The first glimpse was found in1987 when an unusual repetitive DNA was found in the bacteria *Escherichia coli* during the analysis of genes involved in phosphate metabolism. Similar sequences were found in some halophilic bacteria which emphasized the evolutionary importance of these clusters of repeated sequences.

CRISPR can be simply understood in a broad manner as the immune system of bacteria which evolved along with time to defend themselves from the viral infections (Bacteriophages).

Bacteriophage infecting the bacterial cell is shown in below figure.



STRUCTURE OF CRISPR AND Cas-9GENES:

CRISPR consists of short DNA sequences which are palindromic in nature and in between these short palindromic sequences, there are some small DNA sequences which are unique and non-identical since they are present in spaces between the palindromic sequences ,they are referred as “spacer DNA” or (Sp.DNA).The importance of these spacer DNAs was not known at that time but after some series of experiments, in the early 20s, scientists found that the spacer DNAs were resembling and are of complete match with the viral DNA which are like the memory copies of the virus similar to the memory cell in human immune system which can trigger the secondary immune response rapidly upon infection and also along with this they found some genes associated with the CRISPR genes and called them as “Cas-Genes”, which stands for CRISPR associated genes and plays a vital role in CRISPR mechanism.

The Cas genes transcribe and translate a protein called as Cas protein. If the memory of the viral DNA is not there in the CRISPR system the Cas genes produces the different classes of Cas proteins which cuts the viral DNA and stores a copy of that as spacer DNA.

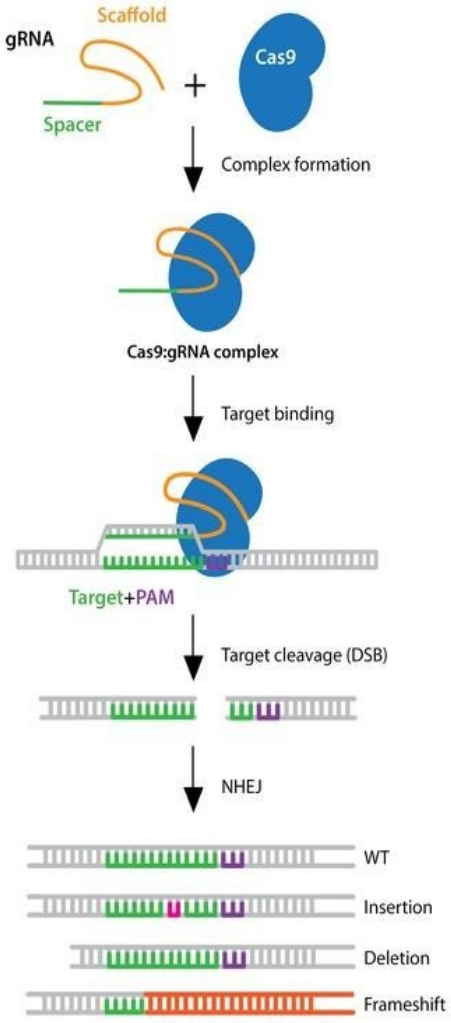
After the observation of this unique system from the living bacterial cell, scientists borrowed this system and laid emphasis on the study of CRISPR Cas 9 system.

Dr. Jennifer Doudna and Dr. Emmanuelle charpentier Et.ol are the main streams scientists of this area and are awarded noble prize for chemistry this year for their work on *Streptococcus pyogenes* which has only one Cas protein that is Cas9. The Cas 9 protein is composed of 2 major parts. The first part is the main body of the molecule the Cas enzyme which has nucleases that can cut the DNA and the second part is the CRISPR RNA(Cr-RNA) which is associated with the

tracer RNA called as called as G-RNA which stands for guide RNA. This all together forms the Cas complex.

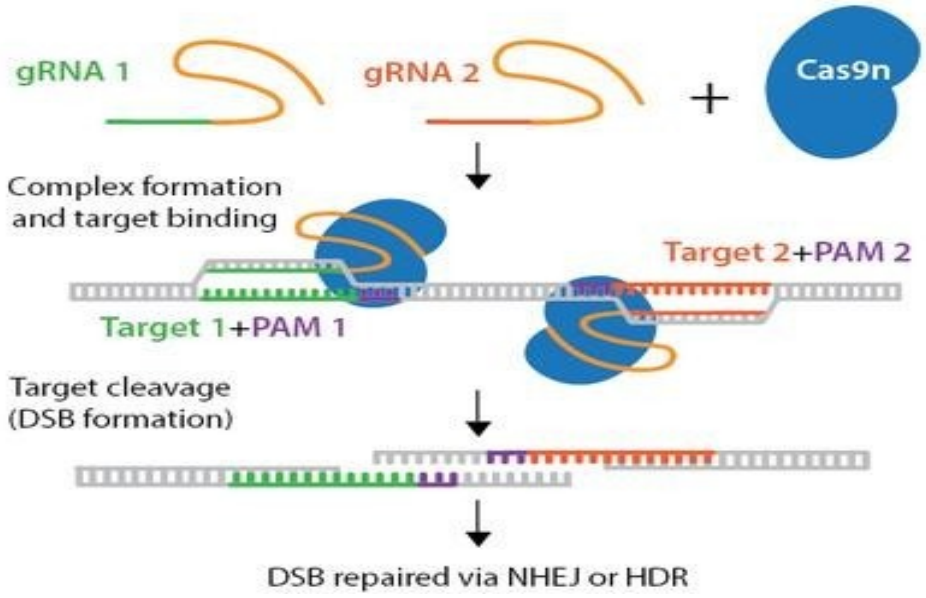
The Cr-RNA acts as Sp-RNA and unwinds the the part of DNA and the nucleases (molecular scissors) can cut the targeted part of DNA precisely while the tracer-RNA holds the Cr-RNA in the system. And after this operation the cells repairs the DNA and by the process of mutation the whole defective gene will be knocked off.

The detailed mechanism is shown in the below figure.



The most advantageous part of CRISPR is that we can alter the Cas complex by adding the desired spacer RNA we can make the complex to work in our favor and in this way we can treat as well cure a huge number of deadly genetic disorders.

Some researchers are engineering the complex by deactivating the cutting parts and introducing different enzymes to transport the Cas 9 complex to alter the specific DNA sequences. Like Cas 9 is infused with deaminase which can mutate the specific DNA base eventually replacing cytidiene with thymidine .This is a very precise gene editing which can convert defective gene to a healthy gene.

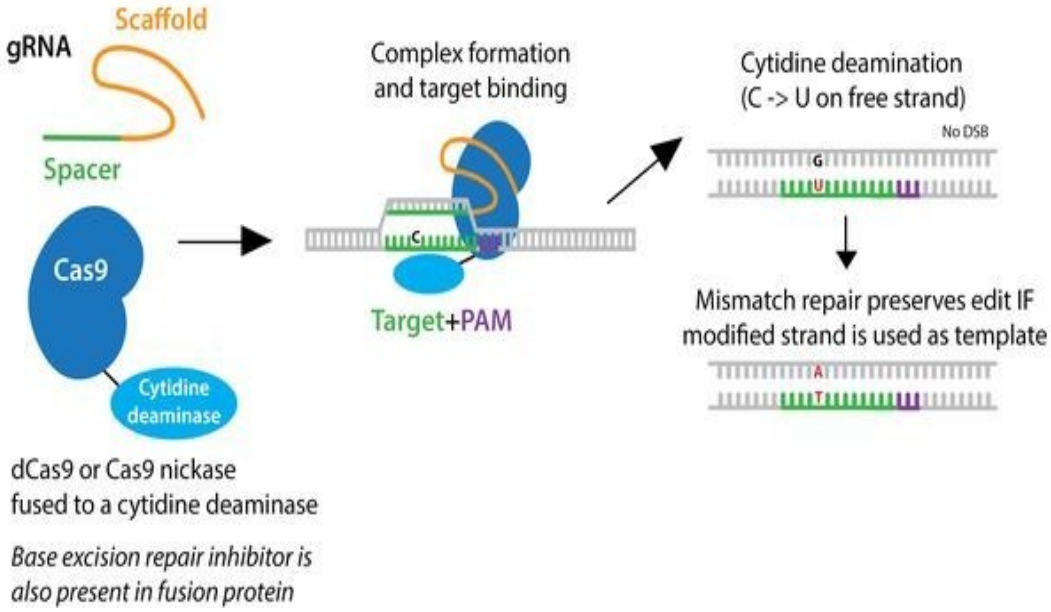


CRISPR is not only restricted to gene editing some scientist are using CRISPR to promote gene transcription and also in gene silencing which is a form of future medicine that is “Antisense therapy”.

APPLICATIONS:

In Medical field:

CRISPR is used to cure diseases due to point mutations like progeria (rapid aging diseases),sickle cell anemia and many more by



using CRISPR-Cas9 as base editors like molecular pencils which can erase and rewrite the nitrogen bases of DNA like letters on paper.

The study reports that in both tissue culture and in mouse, tumor growth stopped and there was a dramatic decrease in the volume of existing tumors when chemotherapy was combined with CRISPR-Cas9, which was used to disable a tumor gene known as NRF2. Previous studies have shown that the NRF2 gene controls cell functions in lung cancer tumors that helps them thwart the effect of chemotherapies that might otherwise reduce or eliminate them entirely. Scientists are trying to use CRISPR technique with chemotherapy to provide a safe, affordable way to give patients who are not responding to treatment, at least a fighting chance against this very challenging cancer.

Shaorang Deng at Hangzhou Cancer Hospital

Shaorong Deng of China gets an experimental treatment for cancer of the oesophagus that uses his own immune system cells. They have been genetically modified with the gene-editing CRISPR technique.

In Agriculture and Plant Biotechnology:

The prokaryote-derived CRISPR-Cas genome editing technology has altered plant molecular biology beyond all expectations, CRISPR-Cas allows precise genetic manipulation of crop species, which provide the opportunity to create germplasms with beneficial traits and to develop novel, more sustainable agricultural systems. Furthermore, the numerous emerging biotechnologies based CRISPR-Cas platforms have expanded the toolbox of fundamental research and plant synthetic biology.

CONCLUSION:

There are about 35,000 known diseases due to point mutations and thousands of known chromosomal aberrations and 100s of research papers have been published already from bacteria to mice from plants to primates. More than 6,000 base editor blueprints distributed to more than 1,000 researchers around the globe. All these unbelievable developments have taken place in less than 3 years! Which means in the historical time scale of science and evolution, it is like a blink of an eye! The potential of CRISPR is huge than what we thought. All these scientific sagas, seems to be like

tip of a monster huge ice berg which is submerged. And there are yet many things left behind waiting to be discovered.

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