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Research Paper

Crispr - Cas9 in Gene Editing

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Abstract: *Clustered Regularly Interspaced Short Palindromic Repeats or simply CRISPR is a basic gene editing tool that is present among many bacteria as an immune system, allowing them to fight off against invading bacteriophages. However, with the advancement in biotechnology and gene editing tools, scientists and genetic engineers can modify it to edit parts of a genome to get desired traits. This research paper looks into the history and functioning of the CRISPR mechanism, its applications in addressing many global problems, and ethical considerations that must be taken into account. When we have a clear picture of such information, we realize the potential it has in shaping the world around us while delving into the fascinating world of molecular biology.*

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I. Introduction:

CRISPR is one of the most popularly and intensively studied topics from the last decade due to the many challenges and problems it has the potential to address, and as a result, is very significant in genetic engineering. The study aims to understand the history behind it that lead to its birth into being one of the most simple, effective, versatile and yet the most precise method in bio tech engineering. Not only this but while reading the report, we come to know about the various implications it has on the ecosystem, and its drawbacks. Since this research also discusses about its implications in living organisms including humans, the study also discusses various ethical considerations that must be thought of before proceeding further with genetic manipulation.

I. History of Genetic Engineering

Genetic engineering or genetics as a subject owe their origin to the discovery of the 'Double Helix Model' put forward by James Watson and Francis Crick (after observation using the X-ray diffraction method made by Rosalyn Frank), which described the structure of DNA as a twisted ladder. Since then, researchers tried different approaches to edit genomes (entire set of DNA instructions found in a cell) with the help of molecular widgets that could either cut or join together DNA bases.

Fig. shows James Watson and Francis Crick (from left)

It was not until 1972 when Herbert Boyer and Cohely created the world's first recombinant DNA, by linking a gene resistant to antibiotics with the plasmid or chromosome of a cell not as resistant, known as *Salmonella typhimirium.* This allowed scientists to, let's say, cut and delete the genes of a virus like HIV, and paste it with a gene from human cell, which made it act as an antibiotic against its virulent form. In this way, it could alleviate the patient of its symptoms. This process would form the basis of gene therapy, and by the early 2000s, it would be tested in hundreds of clinical trials across the US to treat genetic disorders like Parkinson's disease, sickle cell anemia, etc. However, this method had its drawbacks. Since it involved adding a gene at a spot where it wasn't familiar with, and also the unpredictable actions that could be taken by the regulatory gene, it was more or less dangerous. The risks of transforming a healthy cell into a cancerous one held scientists back in their place, and although later on artificially-prepared proteins could target specific mutations, their preparation was cumbersome, taking up years or even months. It was not nearly after a decade when in 2012, the CRISPR-Cas9 mechanism was discovered.

II. Discovery of CRISPR

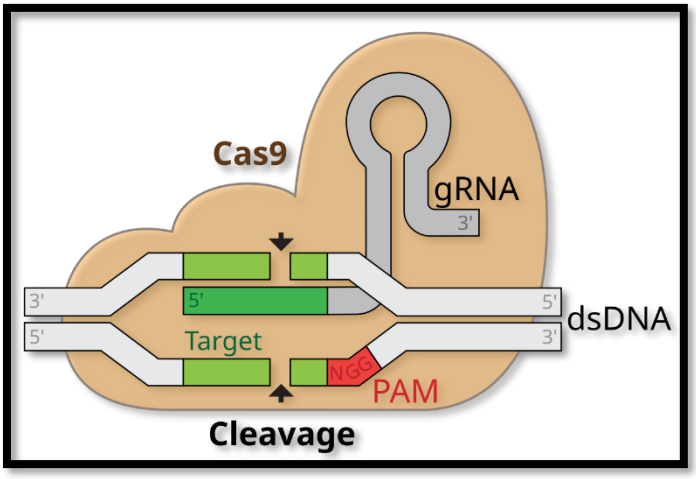
The **CRISPR Technology** was first identified as an unusual sequence in the 3' end of the iap gene of E. colin 1987 by the researcher Nakata and in 2002, Janson termed this sequence CRISPR- Clustered Regularly Interspaced Short Palindromic Repeats. However, the functional mechanism of **CRISPR-Cas9** was being revealed and in 2011, Siksnys transferred the CRISPR system from Streptococcus thermophilus to E. coli, demonstrating its potential as a defense mechanism against external infections while suggesting nonhost bacterium involvement is not required. But the major breakthrough occurred when in 2012, Jennifer Doudna and Emmanuelle Charpentier purified Cas9 from *S. thermophilus* and *S. pyogenes*, enabling cleavage of prokaryotic DNA in vitro and after further more testing, they concluded that the CRISPR system can silence genes in different situations, allowing them to target and edit genes by changing the nucleotide sequence.

Fig. shows Jennifer Doudna and Emmanuelle Charpentier (from left), co-founders of the CRISPR-Cas9 Technology

III. Functioning of the CRISPR-Cas9 Mechanism

When a viral DNA is transferred from a virus to a bacterium, the bacterium produces 2 short types of RNA after detection, one of which is the guide RNA (here, g. RNA). The g. RNA contains the sequence matching with the viral DNA and together with the other RNA, it forms a complex with a protein known as Cas9 (nuclease or enzyme that cuts DNA). The g. RNA then locks onto the sequence within the viral genome which enables Cas9 to cut the DNA using two tiny molecular scissors, disabling the virus. This specific instance can be further elucidated as the

Cas9 locks onto the short sequence known as PAM of DNA, unzipping the DNA after which the g. RNA matches to its target. When the DNA gets disabled, it tries to self-repair (non-homologous end joining) but is error prone that can lead to variations, which are random, that ultimately disable the gene.



Realizing this mechanism, Jennifer Doudna and her team were able to inject CRISPR with a specific g. RNA into some cells and when this g. RNA cuts its matching sequence, instead of the formation of mutations, they were able to replace and modify the cut part by inserting a new sequence. In simple words, the CRISPR-Cas9 mechanism is a 'cut and paste' tool, allowing scientists to modify the gene and therefore, quickly became a major revelation in the field of biotechnology.

IV. Advancements

A chronological list of advancements made since the discovery of the CRISPR-Cas9 mechanism have been listed:

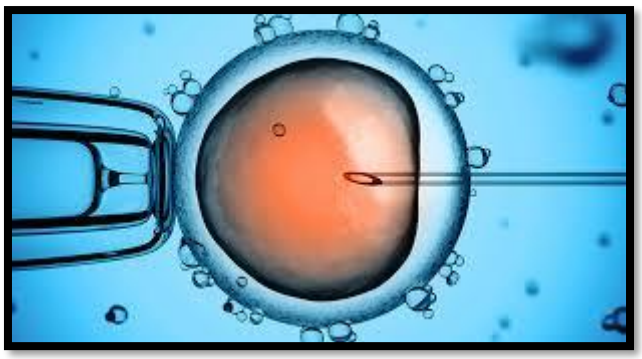
2012:- Elucidation of biochemical nature of CRISPR Technology by Jennifer Doudna, Emmanuelle Charpentier, and their teams

2013:- CRISPR Utility is displayed in Eukaryotic Cells by Feng Zhang

2014:- Potential of Developing Gene Drives with CRISPR Technology Is Being Discussed; Would help in eradication of vector borne diseases like malaria

2015:- First Genetically Modified Organism (GMO) made available for human consumption is sold in the form of salmon by the company AquaBounty

2015:- First Human Embryo Edited Using CRISPR Technology



2017:- CAR T Therapy for Cancer Is Approved; Potential to Replace Chemotherapy for Treating Cancer Due To Its Less Toxic Nature

2018:- First Human Trials for CRISPR are Approved Officially; Experimental Treatment to Potentially End Cases of β-thalassemia (read as beta thalassemia) and Sickle Cell Anemia

2019:- Possibility of Making Single Stranded Cuts using Prime Editing; Published By Dr. David Liu

2020:- The Year of CRISPR

-CRISPR Clinical Trials began with Victoria Gray the first patient to undergo sickle cell treatment

-All ten patients who had received CTX001 therapy earlier showed positive results; 7 treated for β-thalassemia and 3 for sickle cell anemia

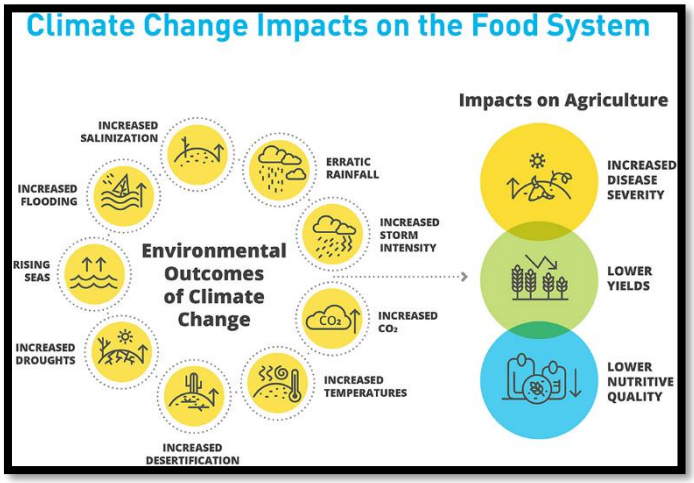
-In October 2020, co-founders Emmanuelle Charpentier and Jennifer Doudna made headlines after winning the Nobel Prize in Chemistry for the development of CRISPR.

V. Applications

CRISPR has various uses and applications across a wide range of fields, from medicine to environmental sciences. This report will look into its profound impact in 2 main sectors, namely Agriculture & Livestock, and Medicine.

1. Agriculture & Livestock:

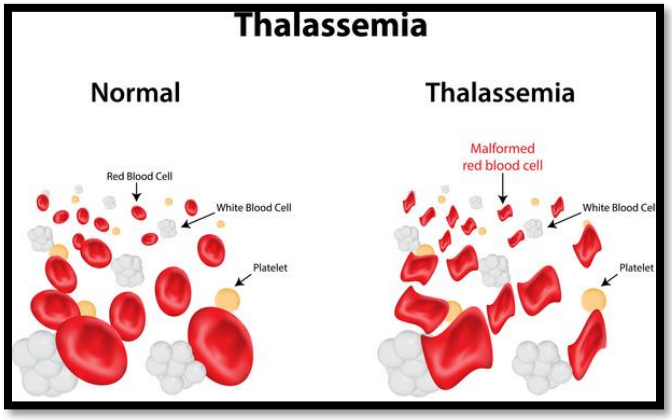
As said by Jennifer Doudna in her interview with the Guardian, creating genetically modified plants is one of the main challenges CRISPR aims to achieve in order to combat global warming. Plants that are resistant to drastic climatic conditions like drought or extreme cold is of utmost demand and with the development of CRISPR technology, we are more likely to see the rise in use of genetically modified plants like corn, rice, soyabean, potato and maize which are not only climate resistant, but are also modified to yield better nutritional value.



Similarly, genetic modifications can also be made in livestock (transgenic animals) such as cow and sheep to improve traits such as meat quality, disease and climate resistance, and animal welfare.

2. Medicine:

Already in use in the medical sector, it has helped in correcting genetic disorders by introducing or removing genes like β-thalassemia, cystic fibrosis, sickle cell anemia, etc. Not only this but CRISPR has also allowed the discovery, development and screening of novel drugs (for eg. inducing deoxyguanosine kinase (DGUOK) knockout to the stem cells which developed as hepatocytes. It produced hepatocyte mitochondrial dysfunction as a screening board for compounds.)



VI. Ethical Considerations

Because genome editing enables the modification, insertion and replacement of genes, it has led to new bioethical, social and legal issues, for which various factors must be taken into consideration before one proceeds with genome editing.

1) Ecological Imbalance

Gene drifts caused in a population of organisms will persist for a very long time and as a result, there is a very high chance that it leads to multiple mutations as generations progress. It can also lead to the transference of negative traits to the progeny of the organisms with every generation, thus making it hard to control the traits within a population of organism.

2) Regulations for Consumers

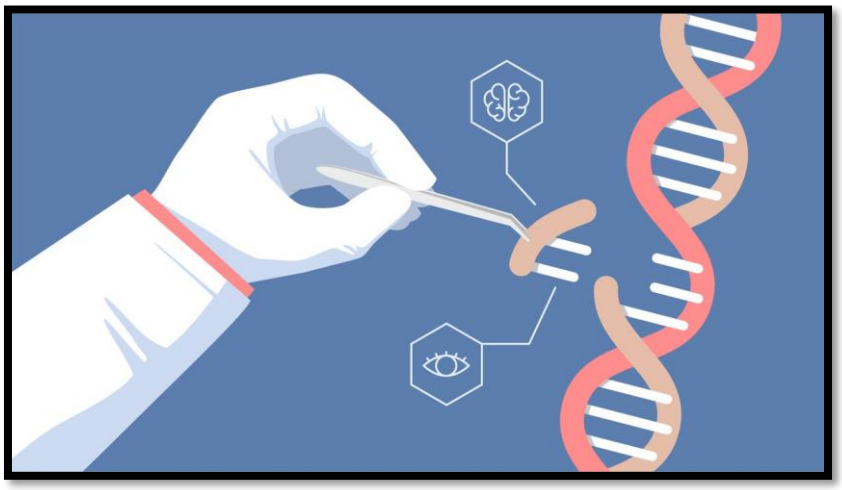
Obtaining the desired genetic modifications with the use of CRISPR-Cas9 make it very difficult to identify and maintain genetically modified organisms in the market after they leave the lab. Therefore, it is essential for regulatory agencies like the European Medicines Agency and US Food and Drug Administration to consider whether such organisms are suitable for human consumption or not.

3) Usage of Chimeric Animals for Organ Transplantation

Organ transplantation is the replacement of an organ that cannot function properly in an individual's body with a healthy organ from a living donor. Since patients have to wait for a very long time to receive from an appropriate donor, development of chimeric animals (animals that are derived from many zygotes from different sources) reduces time and saves effort. However, the main problem faced here is if the animals involved would be treated as humans or animals, and also that it affects human dignity because they transform such animals into becoming more human-like some argue.

4) Animal Welfare and Dignity

This is one of the most widely discussed bioethical concerns as firstly, editing genomes in animals for desired phenotypes can have side effects on animals and can even lead to mutations that will be passed on from one generation to the next. The second reason is that such transgenic animals would no longer be treated as animals but rather as objects whose only purpose is to serve human beings which is not ethical or morally acceptable many people argue. In this way, it affects 'animal dignity'.



II. Conclusion

Through this report, we understand the manner and sequence of events that led to the birth of genetic engineering or more specifically, CRISPR-Cas9 Mechanism. With experiments and tests to prove the efficiency of their discovery, Jennifer Doudna and her colleague Emmanuelle Charpentier revealed the CRISPR Technology to the whole world, which quickly garnered attention and became intensively studied. Its applications in addressing global problems like creating drought-resistant crops or treatment of genetic disorders like cystic fibrosis gives us the thought that CRISPR technology is to remain with humans for a very long time and from such advancements made, it is safe to say that it has high scope and potential to change the world around us, in way that is more suited to us humans. However, CRISPR technology has its drawbacks, from destabilizing the ecosystem to affecting animal dignity in a negatively. But if handled properly without misuse, CRISPR can shape the future for generations to come.

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