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Review on
CRISPR AS TOOL OF GENE EDITING

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Abstract:

Gene editing, the ability to make highly specific changes in the DNA sequence of a living organism, essentially customizing its genetic makeup. Gene editing is performed using enzymes, particularly nucleases that have been engineered to target a specific DNA sequence, where they introduce cuts into the DNA strands, enabling the removal of existing DNA and the insertion of replacement DNA. Key among gene-editing technologies is a molecular tool known as CRISPR-Cas9, a powerful technology discovered in 2012 by American scientist Jennifer Doudna, French scientist Emmanuelle Carpenter, and colleagues and refined by American scientist Feng Zhang and colleagues. CRISPR-Cas9 functioned with precision, allowing researchers to remove and insert DNA in the desired locations.

Introduction:

Genome editing (also called gene editing) is a group of technologies that give scientists the ability to change an organism's DNA. These technologies allow genetic material to be added, removed, or altered at particular locations in the genome. Several approaches to genome editing have been developed. A recent one is known as CRISPR-Cas9, which is short for clustered regularly interspaced short palindromic repeats and CRISPR-associated protein 9. The CRISPR-Cas9 system has generated a lot of excitement in the scientific community because it is faster, cheaper, more accurate, and more efficient than other existing genome editing methods.

CRISPR-Cas9 was adapted from a naturally occurring genome editing system in bacteria. The bacteria capture snippets of DNA from invading viruses and use them to create DNA segments known as CRISPR arrays. The CRISPR arrays allow the bacteria to "remember" the viruses (or closely related ones). If the viruses attack again, the bacteria produce RNA segments from the CRISPR arrays to target the viruses' DNA. The bacteria then use Cas9 or a similar enzyme to cut the DNA apart, which disables the virus.

The CRISPR-Cas9 system works similarly in the lab. Researchers create a small piece of RNA with a short" guide" sequence that attaches (binds) to a specific target sequence of DNA in a genome. The RNA also binds to the Cas9 enzyme. As in bacteria, the modified RNA is used to recognize the DNA sequence, and the Cas9 enzyme cuts the DNA at the targeted location. Although Cas9 is the enzyme that is used most often, other enzymes (for example Cpf1) can also be used. Once the DNA is cut, researchers use the cell's own DNA repair machinery to add or delete pieces of genetic material, or to make changes to the DNA by replacing an existing segment with a customized DNA sequence.

Genome editing is of great interest in the prevention and treatment of human diseases. Currently, most research on genome editing is done to understand diseases using cells and animal models. Scientists are still working to determine whether this approach is safe and effective for use in people. It is being explored in research on a wide variety of diseases, including single-gene disorders such as cystic fibrosis, hemophilia, and sickle cell disease. It also holds promise for the treatment and prevention of more complex diseases, such as cancer, heart disease, mental illness, and human immunodeficiency virus (HIV) infection.

Ethical concerns arise when genome editing, using technologies such as CRISPR-Cas9, is used to alter human genomes. Most of the changes introduced with genome editing are limited to somatic cells, which are cells other than egg and sperm cells. These changes affect only certain tissues and are not passed from one generation to the next. However, changes made to genes in egg or sperm cells (germline cells) or in the genes of an embryo could be passed to future generations. Germline cell and embryo genome editing bring up a number of ethical challenges, including whether it would be permissible to use this technology to enhance normal human traits (such as height or intelligence). Based on concerns about ethics and safety, germline cell and embryo genome editing are currently illegal in many countries.

CRISPR (clustered regularly interspaced short palindromic repeats) is a family of DNA sequences found within the genomes of prokaryotic organisms such as bacteria and archaea. These sequences are derived from DNA fragments of viruses that have previously infected the prokaryote and are used to detect and destroy DNA from similar viruses during subsequent infections. Hence these sequences play a key role in the antiviral defense system of prokaryotes.

Cas9 (or "CRISPR-associated protein 9") is an enzyme that uses CRISPR sequences as a guide to recognize and cleave specific strands of DNA that are complementary to the CRISPR sequence. Cas9 enzymes together with CRISPR sequences form the basis of a technology known as CRISPR-Cas9 that can be used to edit genes within organisms. This editing process has a wide variety of applications including basic biological research, development of biotechnology products, and treatment of diseases.

The CRISPR-Cas system is a prokaryotic immune system that confers resistance to foreign genetic elements such as those present within plasmids and phages that provides a form of acquired immunity. RNA harboring the spacer sequence helps Cas (CRISPR-associated) proteins recognize and cut foreign pathogenic DNA. Other RNA-guided Cas proteins cut foreign RNA. CRISPR are found in approximately 50% of sequenced bacterial genomes and nearly 90% of sequenced archaea.

Repeated sequences:

The discovery of clustered DNA repeats occurred independently in three parts of the world. The first description of what would later be called CRISPR is from Osaka University researcher Yoshizumi Ishino and his colleagues in 1987. They accidentally cloned part of a CRISPR sequence together with the "*iap" gene (isozyme conversion of alkaline phosphatase)* that was their target. The organization of the repeats was unusual repeated sequences are typically arranged consecutively, without interspersed different sequences. They did not know the function of the interrupted clustered repeats.

In 1993, researchers of *Mycobacterium tuberculosis* in the Netherlands published two articles about a cluster of interrupted direct repeats (DR) in that bacterium. They recognized the diversity of the sequences that intervened the direct repeats among different strains of *M. tuberculosis* and used this property to design a typing method that was named *spoligotyping*, which is still in use today.

At the same time, repeats were observed in the archaeal organisms of *Haloferax* and *Haloarcula* species, and their function was studied by Francisco Mojica at the University of Alicante in Spain. Although his hypothesis turned out to be wrong, Mojica's supervisor surmised at the time that the clustered repeats had a role in correctly segregating replicated DNA into daughter cells during cell division because plasmids and chromosomes with identical repeat arrays could not coexist in *Haloferax volcanii*. Transcription of the interrupted repeats was also noted for the first time.[16][17] By 2000, Mojica performed a survey of scientific literature and one of his students performed a search in published genomes with a program devised by himself. They identified interrupted repeats in 20 species of microbes as belonging to the same family.[18] In 2001, Mojica and Ruud Jansen, who were searching for additional interrupted repeats, proposed the acronym CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) to alleviate the confusion stemming from the numerous acronyms used to describe the sequences in the scientific literature.[17][19] In 2002, Tang, et al. showed evidence that CRISPR repeat regions from the genome of *Archaeoglobus fulgidus* were transcribed into long RNA molecules that were subsequently processed into unit-length small RNAs, plus some longer forms of 2, 3, or more spacer-repeat units.

CRISPR-associated systems:

A major addition to the understanding of CRISPR came with Jansen's observation that the prokaryote repeat cluster was accompanied by a set of homologous genes that make up CRISPR-associated systems or *cas* genes. Four *cas* genes (*cas* 1-4) were initially recognized. The Cas proteins showed helicase and nuclease motifs, suggesting a role in the dynamic structure of the CRISPR loci. In this publication the acronym CRISPR was used as the universal name of this pattern. However, the CRISPR function remained enigmatic.

In 2005, three independent research groups showed that some CRISPR spacers are derived from phage DNA and extrachromosomal DNA such as plasmids. In effect, the spacers are fragments of DNA gathered from viruses that previously tried to attack the cell. The source of the spacers was a sign that the CRISPR/*cas* system could have a role in adaptive immunity in bacteria. All three studies proposing this idea were initially rejected by high-profile journals, but eventually appeared in other journals.

The first publication proposing a role of CRISPR- Cas in microbial immunity, by the researchers at the University of Alicante, predicted a role for the RNA transcript of spacers on target recognition in a mechanism that could be analogous to the RNA interference system used by eukaryotic cells. Koonin and colleagues extended this RNA interference hypothesis by proposing mechanisms of action for the different CRISPR-Cas subtypes according to the predicted function of their proteins.

Experimental work by several groups revealed the basic mechanisms of CRISPR-Cas immunity. In 2007, the first experimental evidence that CRISPR was an adaptive immune system was published. A CRISPR region in *Streptococcus thermophilus* acquired spacers from the DNA of an infecting bacteriophage. The researchers manipulated the resistance of *S. thermophilus* to different types of phage by adding and deleting spacers whose sequence matched those found in the tested phages.[31][32] In 2008, Brouns and Van der Oost identified a complex of Cas proteins (called Cascade) that in *E. coli* cut the CRISPR RNA precursor within the repeats into mature spacer-containing RNA molecules (crRNA), which remained bound to the protein complex.[33] Moreover, it was found that Cascade, crRNA and a helicase/nuclease (Cas3) were required to provide a bacterial host with immunity against infection by a DNA virus. By designing an anti-virus CRISPR, they demonstrated that two orientations of the crRNA (sense/antisense) provided immunity, indicating that the crRNA guides were targeting dsDNA. That year Marraffini and Sontheimer confirmed that a CRISPR sequence of *S. epidermidis* targeted DNA and not RNA to prevent conjugation. This finding was at odds with the proposed RNA-interference-like mechanism of CRISPR-Cas immunity, although a CRISPR-Cas system that targets foreign RNA was later found in *Pyrococcus furiosus*. A 2010 study showed that CRISPR-Cas cuts both strands of phage and plasmid DNA in *S. thermophilus*.

Cas9:

Researchers studied a simpler CRISPR system from *Streptococcus pyogenes* that relies on the protein Cas9. The Cas9 endonuclease is a four-component system that includes two small RNA molecules named CRISPR RNA (crRNA) and trans-activating CRISPR RNA (tracrRNA). Jennifer Doudna and Emmanuelle Charpentier re-engineered the Cas9 endonuclease into a more manageable two-component system by fusing the two RNA molecules into a "single-guide RNA" that, when combined with Cas9, could find and cut the DNA target specified by the guide RNA. By manipulating the nucleotide

sequence of the guide RNA, the artificial Cas9 system could be programmed to target any DNA sequence for cleavage. Another group of collaborators comprising Virginijus Šikšnys together with Gasiûnas, Barrangou and Horvath showed that Cas9 from the *S. thermophilus* CRISPR system can also be reprogrammed to target a site of their choosing by changing the sequence of its crRNA. These advances fueled efforts to edit genomes with the modified CRISPR-Cas9 system.

Groups led by Feng Zhang and George Church simultaneously published descriptions of genome editing in human cell cultures using CRISPR-Cas9 for the first time. It has since been used in a wide range of organisms, including baker's yeast (*Saccharomyces cerevisiae*), the opportunistic pathogen *Candida albicans*, zebrafish (*Danio rerio*),[45] fruit flies (*Drosophila melanogaster*), ants (*Harpegnathos saltator* and *Ooceraea biroi*), mosquitoes (*Aedes aegypti*), nematodes (*Caenorhabditis elegans*), plants, mice, monkeys and human embryos.

CRISPR has been modified to make programmable transcription factors that allow scientists to target and activate or silence specific genes. The CRISPR-Cas9 system has shown to make effective gene edits in Human tripronuclear zygotes first described in a 2015 paper by Chinese scientists P. Liang and Y. Xu. The system made a successful cleavage of mutant Beta-Hemoglobin (HBB) in 28 out of 54 embryos. 4 out of the 28 embryos were successfully recombined using a donor template given by the scientists. The scientists showed that during DNA recombination of the cleaved strand, the homologous endogenous sequence HBD competes with the exogenous donor template. DNA repair in human embryos is much more complicated and particular than in derived stem cells.

Cpf1:

In 2015, the nuclease Cpf1 (also known as Cas12a) was characterized in the CRISPR/Cpf1 system of the bacterium *Francisella novicida*. The name, from a TIGRFAMs protein family definition built in 2012, reflects the prevalence of its CRISPR-Cas subtype in the Prevotella and Francisella lineages. Cpf1 showed several key differences from Cas9 including: causing a 'staggered' cut in double stranded DNA as opposed to the 'blunt' cut produced by Cas9, relying on a 'T rich' PAM (providing alternative targeting sites to Cas9) and requiring only a CRISPR RNA (crRNA) for successful targeting. By contrast Cas9 requires both crRNA and a transactivating crRNA (tracrRNA).

These differences may give Cpf1 some advantages over Cas9.

For example, Cpf1's small crRNAs are ideal for multiplexed genome editing, as more of them can be packaged in one vector than can Cas9's sgRNAs. As well, the sticky 5' overhangs left by Cpf1 can be used for DNA assembly that is much more target-specific than traditional Restriction Enzyme cloning. Finally, Cpf1 cleaves DNA 18-23 base pairs downstream from the PAM site. This means there is no disruption to the recognition sequence after repair, and so Cpf1 enables multiple rounds of DNA cleavage. By contrast, since Cas9 cuts only 3 base pairs upstream of the PAM site, the NHEJ pathway results in indel mutations which destroy the recognition sequence, thereby preventing further rounds of cutting. In theory, repeated rounds of DNA cleavage should cause an increased opportunity for the desired genomic editing to occur.

Genome editing means CRISPR to most people. Yet methods using zinc-finger nucleases, transcription activator-like effector nucleases (TALENs), and meganucleases have their own unique strengths. All of these techniques rely on cellular DNA-repair mechanisms. Options that don't-base editing, epigenetic editing, and site-specific recombinases-offer further advantages.

Conclusion:

Gene editing with CRISPR is most advanced method used in genetics. Genome editing has extended our ability to elucidate the contribution of genetics to disease by promoting the creation of more accurate cellular and animal models of pathological processes and has begun to show extraordinary potential in a variety of fields, ranging from basic research to applied biotechnology and biomedical research. Recent progress in developing programmable nucleases, such as zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) and clustered regularly interspaced short palindromic repeat (CRISPR)-Cas-associated nucleases, has greatly expedited the progress of gene editing from concept to clinical practice.

References:

1. Ormond KE(1), Mortlock DP(2), Scholes DT(3), Bombard Y(4), Brody LC(5), Faucett WA(6), Garrison NA(7), Hercher L(8), Isasi R(9), Middleton A(10), Musunuru K(11), Shriner D(12), Virani A(13), Young CE(3). Human Germline Genome Editing. Am J Hum Genet. 2017 Aug 3;101(2):167-176. PubMed: 28777929. Free full-text available from PubMed Central: PMC5544380.
2. Gupta RM, Musunuru K. Expanding the genetic editing tool kit: ZFNs, TALENs, and CRISPR-Cas9. J Clin Invest. 2014 Oct;124(10): 4154-61. doi: 10.1172/JCI72992. Epub 2014 Oct 1. Review. PubMed: 25271723. Free full-text available from PubMed Central: PMC4191047.
3. Hsu PD, Lander ES, Zhang F. Development and applications of CRISPR-Cas9 for genome engineering. Cell. 2014 Jun 5;157(6):1262-78. doi:10.1016/j.cell.2014.05.010. Review. PubMed: 24906146. Free full-text available from PubMed Central: PMC4343198.
4. Komor AC, Badran AH, Liu DR. CRISPR-Based Technologies for the Manipulation of Eukaryotic Genomes. Cell. 2017 Apr 20;169(3):559. doi:10.1016/j.cell.2017.04.005. Pub Med: 28431253.
   Lander ES. The Heroes of CRISPR. Cell. 2016 Jan 14;164(1-2):18-28. doi:10.1016/j.cell.2015.12.041. Review. PubMed: 26771483.
5. Mulepati S, Héroux A, Bailey S (2014). "Crystal structure of a CRISPR RNA–guided surveillance complex bound to a ssDNA target". Science. 345 (6203): 1479 1484. Bibcode:2014Sci...345.1479M. doi:10.1126/science. 1256996. PMC 4427192. PMID 25123481.
6. Barrangou R (2015). "The roles of CRISPR-Cas systems in adaptive immunity and beyond". Current Opinion in Immunology. **32**: 36–41. doi:10.1016/j.coi. 2014.12.008. PMID 25574773.
7. Horvath P, Barrangou R (January 2010). "CRISPR/Cas, the immune system of bacteria and archaea". Science. **327** (5962): 167–170. Bibcode:2010Sci...327..167H. doi:10.1126/science.1179555. PMID 20056882. S2CID 17960960.
8. Redman M, King A, Watson C, King D (August 2016). "What is CRISPR/Cas9?". Archives of Disease in Childhood. Education and Practice Edition. **101** (4):213–215. doi:10.1136/archdischild-2016-310459. PMC 4975809. PMID 27059283.
9. Jump up to:[a] [b] Barrangou R, Fremaux C, Deveau H, Richards M, Boyaval P, Moineau S, et al. (March 2007). "CRISPR provides acquired resistance against viruses in prokaryotes". Science. **315**(5819): 1709–1712. Bibcode:2007Sci...315.1709B. doi:10.1126/science.1138140. hdl:20.500.11794/38902. PMID 17379808. S2CID 3888761. (registration required)

10. Jump up to:[a] [b] Marraffini LA, Sontheimer EJ (December 2008). "CRISPR interference limits horizontal gene transfer in staphylococci by targeting DNA". Science. **322** (5909): 1843–1845. Bibcode:2008Sci...322.1843M. doi:10.1126/science.1165771. PMC 2695655. PMID 19095942.
11. Mohanraju P, Makarova KS, Zetsche B, Zhang F, Koonin EV, van der Oost J (2016). "Diverse evolutionary roots and mechanistic variations of the CRISPR-Cas systems"(PDF). Science. **353** (6299): aad5147. doi:10.1126/science.aad5147. hdl:1721.1/113195. PMID 27493190. S2CID 11086282.
12. Hille F., Richter H, Wong SP, Bratoviè M, Ressel S, Charpentier E (March 2018). "The Biology of CRISPR-Cas: Backward and Forward". Cell. **172** (6): 1239–1259. doi:10.1016/j.cell.2017.11.032. hdl:21.11116/0000-0003-FC0D-4. PMID 29522745. S2CID 3777503.
13. Zhang F, Wen Y, Guo X (2014). "CRISPR/Cas9 for genome editing: progress, implications and challenges". Human Molecular Genetics. **23** (R1): R40–6. doi:10.1093/hmg/ddu125. PMID 24651067.
14. CRISPR-CAS9, TALENS and ZFNS - the battle in gene editing https://www.ptglab.com/news/blog/crispr-cas9-talens-and-zfns-the-battle-in-gene-editing/
15. Hsu PD, Lander ES, Zhang F (June 2014). "Development and applications of CRISPR-Cas9 for genome engineering". Cell. **157** (6): 1262–1278. doi:10.1016/j.cell.2014.05.010. PMC 4343198. PMID 24906146.
16. Ishino Y, Shinagawa H, Makino K, Amemura M, Nakata A (December 1987). "Nucleotide sequence of the iap gene, responsible for alkaline phosphatase isozyme conversion in Escherichia coli, and identification of the gene product". Journal of Bacteriology. **169** (12): 5429–5433. doi:10.1128/jb.169.12.5429-5433.1987. PMC 213968. PMID 3316184.
17. Van Soolingen D, de Haas PE, Hermans PW, Groenen PM, van Embden JD (August 1993). "Comparison of various repetitive DNA elements as genetic markers for strain differentiation and epidemiology of Mycobacterium tuberculosis". Journal of Clinical Microbiology. **31** (8): 1987–1995. doi:10.1128/JCM.31.8.1987-1995.1993. PMC 265684. PMID 7690367.