Genetic Scissors: A Review of the Clustered Regularly Interspaced Palindromic Repeats

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Clustered regularly interspaced palindromic repeats (CRISPR), is a gene-editing tool that has revolutionized modern genetics. Often referred to as 'genetic scissors', CRISPR technology has enabled scientists to modify or modulate almost any gene in an organism's genome (Redman *et al.*, 2016). CRISPR technology consists of a guide ribonucleic acid (RNA) and a Cas9 (CRISPR-associated protein 9) endonuclease that cleaves the phosphodiester bond of deoxyribonucleic acid (DNA) nucleotides both precisely and efficiently (Berg *et al.*, 2002; Redman *et al.*, 2016). Using this novel technology, genomic editing ranging from gene deletion to gene addition is more readily achievable. Applications of genomic engineering include pharmaceutical drug development, crop improvement, gene therapy, and the potential for treating various diseases (Hsu *et al.*, 2014; Sano *et al.*, 2018). This paper will explore the history of CRISPR-Cas9 research, how this system functions at the molecular level, two practical applications, and conclude by addressing ethical implications of making this technology readily available and accessible for public use.

CRISPR and the *Cas* proteins have been an ongoing area of research for decades (Ishino *et al.*, 2018). CRISPR was first reported in 1987 following its discovery in the *Escherichia coli* bacterial genome (Ishino *et al.*, 1987). In 1993, CRISPR was similarly identified and reported in single-celled organisms classified under the Archaea domain of life (Mojica *et al.* 1993). These findings initiated a cascade of publications that reported CRISPR findings in other prokaryotic organisms. Despite the abundant research, there was still no general consensus of the function of these palindromic repeats. 2002 marked the first year that scientists reported CRISPR-associated (*Cas*) genes and their role as nucleases, as well as their involvement in DNA metabolism (Jansen *et al*., 2002). In 2007, Barrangou *et al*. (2007) conducted robust experiments which led his team to discover that the protein product of *Cas* genes enabled prokaryotic immunity against phages.

Following another few years of research, Jinek *et al.* (2012) made a revolutionary discovery in that the CRISPR associated Cas-9 protein induces double-stranded breaks in target DNA. 2013 marked the first year that genome editing was accomplished in eukaryotic cells (Cong *et al.*, 2013; Mali *et al.*, 2013). The CRISPR-Cas9 system continues to be an ongoing area of research with various public sectors now applying this system for society's betterment.

CRISPR-Cas9 is an exceptional tool at the fundamental level, but phenomenal on the molecular and chemical level. The way the enzymes and proteins work to orchestrate such a phenomena is nothing short of incredible, but how does it work? Say you wish to make an alphabet banner, but by mistake you switch around some of the letters. Thankfully, you can cut out the misprinted ones, print new ones using an alphabet guide and replace them in the correct alphabet ordering. In layman's terms, that's exactly how CRISPR-Cas9 works, except it requires a bit more detail and attention. CRISPR has two main components, a guide RNA for the targeted gene, and the endonuclease Cas9 which cleaves the DNA to enable modifications, and acts like a pair of scissors (Redman *et al.*, 2016). The guide RNA has two further components, tracrRNA, which provides a loop structure for the Cas9 protein, and crRNA, which pinpoints the targeted DNA strand and assists in binding it to the tracrRNA. The Cas9 endonuclease resembles a kidney bean shape and contains two active sites for the guide RNA and targeted gene to attach to (CRISPR Cas 9 Nuclease RNA-guided Genome Editing, n.d.) Based on the information from the Howard Hughes Medical Institute Biointeractive, the guide RNA will be introduced into the cell and the Cas9 structure will recognize and bind to a three-nucleotide sequence motif named PAM (protospacer adjacent motif), which is abundant throughout the genome. This PAM motif is located on the non-complementary strand and can consist of any nucleotide, followed by two guanines. The synthesized RNA sequence includes about 20 nucleotides that are complementary

to the targeted DNA strand, which can be any sequence as long as it is near a PAM motif. The guide RNA, once added to Cas9, will guide the Cas9 to the targeted sequence of DNA. Once it's bound, the Cas9 will unwind the DNA double helix, and will base pair in a complementary fashion with the RNA strand, if it is perfectly matched. If not, the Cas9 disengages and the DNA strand will zip back up. Since DNA is read 5' to 3', this is how the double helix strand is pulled apart, ahead of PAM closer to the 5' end. This is denoted in the below image, which shows both strands, the PAM site, the Cas9 endonuclease and the active sites , shown as scissors.

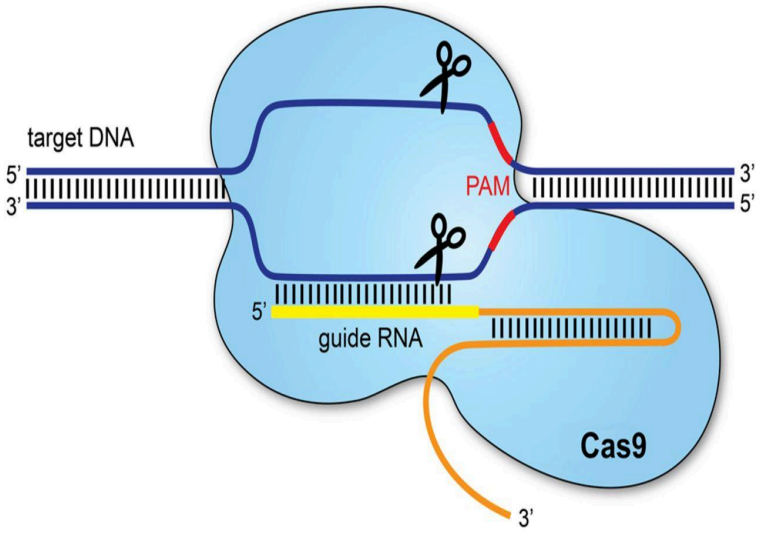

Figure 1. The CRISPR/Cas9 System

Once the guide RNA is aligned with target DNA, it will form a DNA-RNA helix. The formation of this helix activates the Cas9 nuclease - the scissor portion. It will make specific snips in the DNA three nucleotides upstream from the PAM site. The Cas9 contains two active sites, which are engaged once molecules bind to undergo chemical reactions, generate the snips and cleave both strands of the DNA, which results in a double-stranded DNA break. The final step of the CRISPR mechanism is repairing the broken DNA, which can either be repaired by nonhomologous end joining, or homology-directed repair. Nonhomologous end joining is frequently used, as it is faster and the cell does not require a template to join the ends; however, this method can cause rare errors, which can introduce further mutations the cell just worked to repair. Cas9 would recognize this, and repeat the

process in order to repair it. Homology-directed repair uses a homologous DNA template, like that from a sister chromatid, to repair the break. This method is less error-prone. Scientists can also manipulate this repair system, by using an excess of DNA and a Cas9-guide RNA (HHMI Biointeractive, 2018). Using this robust system, scientists are now applying CRISPR-Cas9 technology to better society. Two practical applications are discussed below.

One of the most popular CRISPR applications is therapeutic application. This technology has revolutionized modern health care by enabling scientists to cut, remove, and add genes. The CRISPR-Cas9 system has the potential to eliminate incurable diseases induced by mutated DNA and reduce suffering. For example, clonal hematopoiesis is a condition where a hematopoietic stem cell proliferates into cells that contain identical genetic mutations. This condition is known to increase the potential of cardiovascular disease and may even cause death. A group of scientists used CRISPR technology to assess whether a mutated *Dnmt3a* (DNA [cytosine-5]-methyltransferase 3a) gene in hematopoietic stem cells will result in cardiovascular disease (Sano *et al.*, 2018). The authors used lentivirus vectors to insert Cas9 and RNA into bone marrow cells of mice to inactivate the *Dnmt3a* gene. When these mice were faced with increased blood pressure (via an upregulation of angiotensin II), the researchers found that mice with an inactivated Dnmt3a gene experienced increased cardiac hypertrophy and reduced cardiac function (Sano *et al.*, 2018). Their research concluded that CRISPR-Cas9 can be used to delete genes and that this technology may be implemented to fight against various genetically linked diseases.

After the discovery of CRISPR-Cas9 in 1987, researchers broadened their vision to assess new ways to use this technology. In addition to therapeutic purposes, such as removing genetically-linked illnesses including cancer, it was discovered that CRISPR-Cas9 could be used

on crops to improve food quality and harvestation (Shakeel et al. 2021). This was accomplished by enhancing the immune system of the plants and resistance against diseases and insects (Shakeel et al. 2021). The researchers used various food plants during their experimentation but they primarily focused on daily food crops such as rice. Shakeel *et al.* (2021) enhanced the resistance of rice by targeting the susceptibility (*Su*) genes that encoded sugar transporters. Bacterial blight infection releases transcription activator-like effectors (TALEs) that increase the transcription of genes encoding sugar transporters (i.e., *OsSWEET11*, *OsSWEET13*, *OsSWEET14*, etc.) (Shakeel *et al.,* 2021). SWEET genes are susceptible to bacterial blight disease that allow bacterias to spread widely in plant tissues (Shakeel *et al.,* 2021). The researchers were able to enhance the resistance of the rice plants by applying the CRISPR-Cas9 system to disrupt the linkage between TALEs and SWEET genes. They successfully developed rice lines with broad-spectrum resistance to bacterial blight (Shakeel *et al*., 2021). It was further determined that all of these edits were cost effective and efficient.

To conclude, the CRISPR-Cas9 system has many practical applications but various ethical concerns related to the regulation and accessibility of this technology still need to be addressed. Since different countries have different policies, codes of ethics, regulations and restrictions, the ‘Global Medical Association’ has differentiated the therapeutic and enhancement purpose of CRISPR-Cas9 based on the global common ethical sense (Potter, 2020). However, due to different countries having diverse policies, beliefs and goals, it appears that this technology will not be released globally any time soon.. For example, Canada and America have more open minded markets for CRISPR while the European Union has very strict regulations. Canada and America laws focus on the final product result whereas the European Union laws focus on the “process”. In other words, Canada and America will allow the utilization of

CRISPR-Cas9 based on whether the final end product can be used or not. On the contrary, the European Union is establishing restrictions on the commercial usage of CRISPR-Cas9 which has resulted in some countries wanting to abandon the European Union system (Potter, A. 2020). Will CRISPR bring the world to an upper level quality life? Or will it cause issues? So far evidence has shown that CRISPR is promising and can bring more positive outcomes compared to negative ones. It will be interesting to see how CRISPR will revolutionize the world moving forward.

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