The Future of Gene Editing

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The CRISPR-Cas9 System
The new advancements in genetics have led to a cutting-edge discovery of a system that has made genomic engineering easier than ever. The system is the combination of clustered regularly interspaced short palindromic repeats, known as CRISPR, and the enzyme Cas9. The CRISPR-Cas9 system has made the process of altering and moving genes easier, cheaper and faster (2). CRISPR-Cas9 can be used across the wide range of biosciences because the system can be used to alter plant genomes to improve our crops and livestock, or the system can edit the genes in a human embryo to prevent the expression of genetic diseases before they occur (2). This technology in gene editing can lead to the advancement of gene therapy too. Gene therapy is the process of inserting a "corrected" gene into a DNA sequence to change the gene entirely. CRISPR-Cas9 acts as the way to cut out the unwanted gene and advancements in gene therapy will be able to replace the unwanted gene with a specific gene to better the life expectancy of a recipient.

The History of CRISPR-Cas9
CRISPRs were first discovered in 1987 as a repetitive DNA sequence in the *Escherichia coli* genome while a scientist was researching the genes involved in phosphate metabolism (3). The repeated sequence patterns were discovered in other bacteria, so scientists believed the sequence played a significant role in the evolution of bacteria (3). Further developments did not arise again until the early 2000s. In 2005, scientists published a discovery about a “sequence similarity between the spacer regions of CRISPRs and sequences of bacteriophages, archaeology viruses, and plasmids finally shed light on the function of CRISPR as an immune system” (3). These scientists also found that several other genes, involved in coding DNA repair proteins, work closely with CRISPR (3). These genes were the Cas proteins. Cas9 is the most common and most studied multidomain effector protein (3). Cas9 is crRNA-dependent endonuclease that is made up of two unrelated nuclease domains, RuvC and HNH (3). These nuclease domains are responsible for cleavage of the nontarget and target DNA strands in the crRNA-target DNA complex (3). Further genomic analyses suggested that CRISPR and the Cas proteins act as an immune system in prokaryotic cells to protect the cells from invading viruses and plasmids (3). This system is a natural defense system for prokaryotes to defend against bacteriophages.

How does CRISPR-Cas9 work?
Guide RNA seeks out a targeted gene sequence along the DNA double helix where the gRNA then binds to the target sequence through a complementary sequence called a PAM sequence. The complementary sequence ensures that the guide RNA does not bind with other non-targeted genes. The Cas9 enzyme then binds to the guide RNA and cuts the two strands of DNA at the target sequence where the DNA is to be added or removed (10). The cell repairs the cut and “effectively silences the targeted gene by joining the cleaved DNA back together” through a process known as non-homologous end joining (2). See Figure 1.

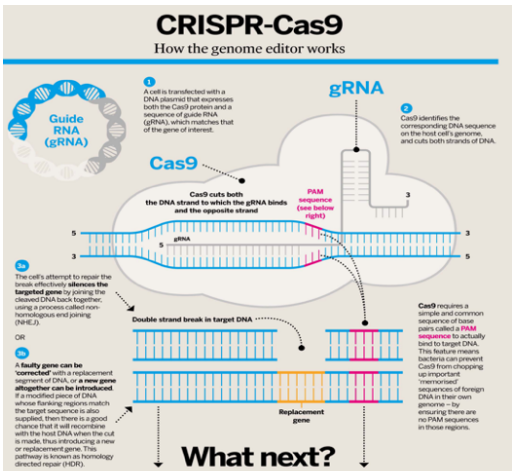

Figure 1: The process of CRISPR-Cas9 (adapted from “Focus on: Gene Editing”)

How will CRISPR-Cas9 help us in the future?
As research with CRISPR-Cas9 continues, the gene editing world as we know it will be changed forever in a positive way. CRISPR-Cas9 can lead scientists towards curing genetic diseases through going into human cells to cut out the infected DNA sequence to silence it or insert a new genetic sequence to keep the recipient from experiencing the genetic disease. In November of 2017, scientists tried to edit a gene inside Brian Madeux’s body in a hope to cure his Hunter syndrome (5). This attempt is the first time

scientists have tried to edit a gene inside a human to permanently change their DNA (5). Through further tests and extensive research, scientists will be altering human genomes to save lives and improve life expectancy in the not so distant future.

The Agricultural Impacts

Gene editing will impact the agricultural industry quicker than curing human diseases according to Jennifer Doudna, a leading researcher in the "CRISPR Revolution" (6). Doudna believes that gene editing will a have a large impact on agriculture because there have already been advances in the growing of some vegetables. Gene editing in crops can lead to "Accelerated crop growth, resistance to pests and inclement weather, and higher nutritional benefit" (7). Successful studies have been done with cabbage in Sweden that looks like regular cabbage, seed development and improvements in harvesting tomatoes (6). The Lippman group utilizes CRISPR in the modification of the number and branching patterns in tomatoes (1) as seen in figure 2. In 2020, a new genetically modified corn will hit the market, and this new corn variety will change how humans design agricultural crops (1). The genetically altered corn was modified using CRISPR-Cas9, and the corn will serve as a basis of future "CRISPR-aided drugs" (1). Scientists think that CRISPR can be used to alter all types of fruits, vegetables, and other crops.

Figure 2: CRISPR-Cas9 can be used to alter the branching style and the number of tomatoes from a tomato plant (from Zachary Lippman in Bomgardner article).

Scientists say, "Using CRISPR to add—or remove—a plant trait is faster, more precise, easier, and in most cases cheaper than either traditional breeding techniques or older genetic engineering methods" (1). The agriculture aspect of gene editing looks quite promising. As agricultural research improves, it will give scientists more of a basis to use in human genomes.

What are the risks of gene editing?

There are still many unknown factors of gene editing, however. Many studies have found a variety of factors that affect the CRISPR-Cas9 system, "such as Cas9 activity, target site selection and sgRNA design, delivery methods, off-target effects, and the incidence of HDR, [(which effects fidelity)]" (11). Once scientists figure out ways to overcome these pitfalls, they will be able to use the system more efficiently. The insertion of new genes may inadvertently affect other genes. This inadvertent effect is known as pleiotropy. Pleiotropy is the production by a single gene of two or more separate effects. In other words, a single gene can affect multiple characteristics. Scientists may not know every outcome of every gene; for example, the scientists may understand how a gene expresses a specific disease, but the scientists may not realize that the unwanted gene expresses other characteristics that a person may not want to get rid of permanently. Another risk is that farmers may go out of business if crops are genetically engineered, and this outcome may negatively affect the economy. Gene editing has a lot of ethical considerations around the process as well. Is it ethical to be able to change a child's life before they are even born? All these factors need to be considered when talking about gene editing.

How can gene editing be regulated?

Since many unknown factors surround the gene editing world, scientists and politicians have to be 100% sure that an experiment involving CRISPR-Cas9 does not have any flaws. Scientists have to establish a set process that has minimal adverse outcomes. Politicians may never allow scientists to do tests on humans that will help the patient gain years on the patient's life because some people may view it is as the scientists playing God, so scientists will have to be self-policing when conducting experiments with the system. Scientists will have to jump through many hoops before they can publish any works involving gene editing or gene altering because it is such a controversial topic. If experiments with CRISPR-Cas9 get out of hand, then politicians may have to pass laws to regulate the system's use. As research in the field continues, in future elections we may see future politicians having to address the issue of modifying genes in humans. Scientists and politicians will have to establish a basis of what is acceptable and what will not be appropriate when using CRISPR-Cas9.

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