**CRISPR Fundamentals**

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**The abbreviation CRISPR has become widely known for its use in the alteration of genomes over the last decade. Clustered regularly interspaced short palindromic repeats (CRISPR) is being used to alter the genes of eukaryotes to provide advancements in agriculture, medicine and other disciplines. Applications are being implemented in gene therapy to remove mutations, for making diagnoses, in imaging, and now in creating pharmaceutical drugs, when prior it was only used as research tool. There are a variety of mechanisms to implement CRISPR from Cas1 to Cas13. Enhanced techniques are evolving, increasing precision and effectiveness. This application is changing science and is going to make a large, positive change in how medication is made and used.**

**Introduction**

Scientists have been researching the ability to change genes for many years with the intent of providing health benefits to humans directly, through nutrition, or other medical therapeutic approaches. CRISPR (clustered regularly interspaced short palindromic repeats) has been the most successful technique thus far and continues to become more advanced and precise. Initial applications included the use of nucleic acid binding proteins such as zinc fingers and DNA with large recognition sites. This left room for unintended targets to be affected. Most recently, genetic alterations are more simply made by recognizing DNA through RNA, where specific fragments of genetic information can follow RNA to DNA. The CRISPR-Cas tool uses microbes that guide nucleases to either attach and/or cut base pair sequences.

Another important tactic used to implement CRISPR is accomplished through the use of viral vectors which is specifically referred to as CRISPR Cas9. The latest research involves the use of new bacteria as vectors as well as Achaea. Newer approaches even allow differentiation of targeting single stranded or double stranded DNA (Doudna, J. & Knott, G., 2018) Certain promoters can be specifically chosen to activate cellular transduction and transfection, which is the introduction of DNA or RNA into eukaryotes via a bacteriophage or vector and led across a cell or between cells. (Berkhout, B., et. al., 2018) This has the potential of rectifying genetic mutations, creating favorable traits, and allowing pharmaceuticals to treat diseases more efficiently.

**Recent Progress**

CRISPR-Cas is changing how medicines are being made. This technique regulates the uptake or down take of gene activity. By doing this, it can be observed if certain genes induce or reduce disease. Perfect targets can be made for medicine producers by focusing on what proteins correlate with diseases and negative or positive reaction to their manipulation. Another advantage provided by CRISPR is the capability to duplicate diseases for safer clinical trials. They can predict with more evidenced support what the outcome of trials will be. Scientists are also using CRISPR-Cas to study what genes are found in relation to drug resistance, which also benefits drug research with editing of genes. These genes are removed then exposed to pharmaceutical materials and the level of sensitivity or resistance is recorded. Medicine that works best within those conditions can then be created. Scientists must take into consideration what mutual effects exist as well. Some genes can be directly targeted because of blatant disease progression, but at times, a more indirect approach of protein manipulation is required. Alternatively, rather than knocking out genes, DNA fragments can also be added in order to repair a gene. This ties into drug therapy as well because drugs could be made to increase a positive effect more efficiently. A final benefit is being able to create a drug that merely regulates genes or proteins by increasing or inhibiting activity to gain control over a disease. (Scotts, A., 2018) In Cas9 to Cas12 use, targets are created so that drugs can target diseases that are passed on genetically. CRISPR has benefited nutrition by genetically enhancing crops to discourage infection, disease, and pest invasion, resulting in more product of higher quality. Mutations in humans and animals can be removed by causing base pairs to be skipped, deactivated or altogether deleted. (Doudna, J. & Knott, G., 2018)

**Discussion**

This approach to treatment seems to have many advantages. Treatment has the ability to become more targeted and specific, rather than a shotgun approach that has an effect on other random targets that did not need altering. The concern that coincides with this treatment, as any other, is what adaptations and immunities will arise? Inhibitors for CRISPR have been identified and are being referred to as anti-CRISPRs (Acrs). These effect the performance of Cas9 in a negative way by blocking or decreasing functionality. Acrs are being discovered within self targeting genomes which can induce cleaving of a genome leading to the genome being degraded and deemed nonfunctional. Much of the time, cleavage is the target to allow insertion of genetic material or repair of a genome, but in cases where a gene is mistargeted, the results could be detrimental. On the positive side of this issue, inhibiting some genes from being expressed could be beneficial in some instances. Another plus is that while Acrs have been discovered in CRISPR Cas-9, they have not been found in more recent techniques such as Cas12. (Bai, H., et. al., 2018)

A second concern is the idea of our bodies building immunity to CRISPR as a viral vector. Immunity can arise spontaneously in response to a foreign entity in the body such as a vector being used to implant genetic information. The DNA fragments could be attacked and broken down before reaching the intended target or reach an unintended target. One of the current approaches in overcoming this obstacle is to reduce the distance to the target site as much as possible. (Doudna, J., et. al., 2017) With these concerns in mind, pharmaceuticals may be fighting the same battle as they currently are, trying to stay one step ahead of a new drug resistant infection. In this scenario through, it would become a genome alteration resistance. Science and medicine must continue to make advancements, so perhaps this is just part of the next phase of treatment which will have its own sets of benefits and challenges.

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