DRAWING THE LINE IN GENOMICS WITH CRISPR TECHNOLOGY

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Imagine a world where every mother selects the eye color of the child she is expecting; where every patient diagnosed with terminal cancer no longer receives a death sentence; or where every mushroom has been engineered not to brown. This world, though seemingly dystopian, is inching closer to reality with recent advancements in genomic editing. CRISPR-Cas9, a newly developed genome editing technology, gives scientists the ability to induce certain traits and cure genetic disease by directly editing DNA. Because of its unprecedented precision and simplicity, it is a revolutionary discovery that imapcts both scientists and everyday people alike.

The field of genome editing, though constantly advancing, is relatively new. Broadly speaking, genome editing involves the insertion, deletion, or replacement of DNA within the genome of a living organism. Most editing techniques utilize engineered nucleases which are nicknamed "molecular scissors." These nucleases create double-stranded breaks in the genome, cutting through both strands of DNA directly and rejoining them in order to edit the sequence. As researchers continue to improve their understanding of how DNA functions and how it can be manipulated, the editing techniques consequently become more refined and specific.

For quite a long time, the dominant editing technique was RNA interference (RNAi). RNAi is so named because the editing process is carried out by two types of small RNA: siRNA and microRNA. These RNA bind to proteins and form a complex that is targeted to a mRNA sequence, which is the sequence of nucleotides that will be translated into proteins. The proteins then degrade the mRNA to edit the sequence.[2] This process has been harnessed by scientists; by engineering sequences of siRNA, they can suppress or express a desired phenotype.

However, scientists quickly realized that RNAi has plenty of limitations in terms of useful applications. Namely, RNAi's off-target effects are numerous and challenging to eliminate entirely because the engineered siRNA can perform edits in non-target regions. Additionally, RNAi can only edit and silence genes - when considering gene therapy applications, RNAi cannot induce activation of genes, nor stably introduce gene segments. Other prominent editing techniques include zinc-finger nucleases (ZFN) and transcription activator-like effector nucleases (TALEN). Both, though able to directly edit DNA, lack in efficiency and ease of designing target sequences.[6]

THE TECHNOLOGICAL BREATHROUGH

Though editing technologies continue to improve, on the whole they have lacked potential for broad and useful applications - until now. Named CRISPR-Cas 9, this new technique allows researchers to edit DNA at precise locations, modify genes in living cells, and eventually correct mutations that cause genetic disease. CRISPR was initially discovered in archaea and bacteria as part of their immune system. Unlike RNAi, CRISPR directly edits DNA rather than working as a post-transcriptional modifier - it targets DNA as opposed to RNA or proteins.

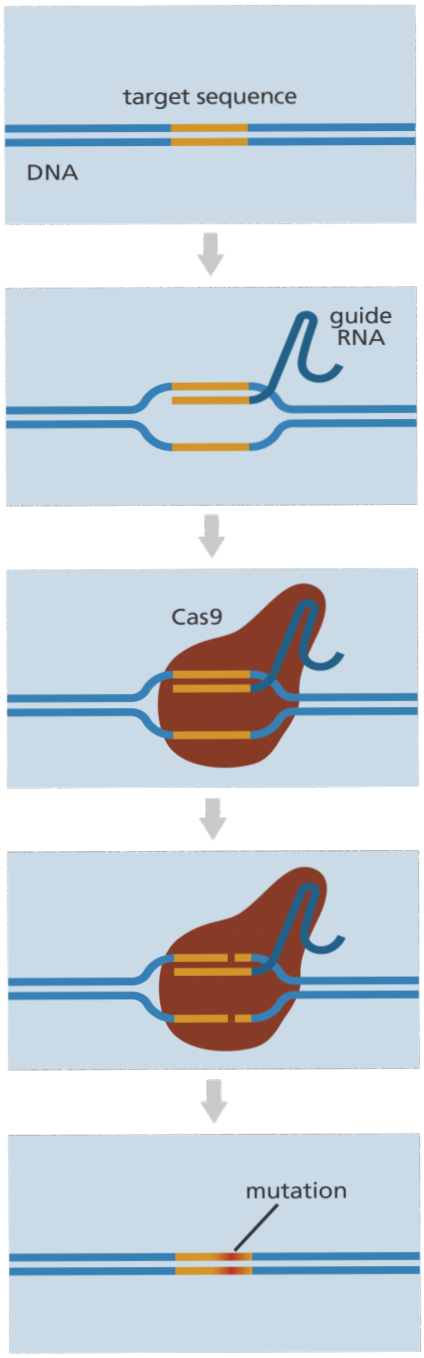

Figure 1: The pathway by which CRISPR edits the target sequence

The process by which CRISPR performs edits centers around guide RNA sequences (gRNA), which are short nucleotide sequences that are complementary to the target DNA sequence. The gRNA binds to the target sequence and the Cas9 protein binds to the DNA, forming a complex. Cas9 then cuts both strands, and a new sequence can be inserted. Enzymes are used to repair the cuts so that the sequence can reform.[1] Another unique feature of CRISPR is a protospacer adjacent motif (PAM) sequence, which is a two to six base pair sequence that CRISPR requires to recognize a target DNA. CRISPR can essentially be directed to any PAM-adjacent sequence, making editing versatile and flexible.[3] CRISPR is efficient and specified - it is also simple in the sense that gRNA is designed readily and modifications to the Cas system are easily introduced. Additionally, minimizing CRISPR's off target effects has been more successful because of the nature of the complex it forms.

ENVIRONMENTAL AND MEDICAL APPLICATIONS

CRISPR remains in the prelimary stages of research across the globe, which test its usage in a variety of applications. However, based on the efficiency of the technology, there is certainly much foresight into the potential CRISPR has to modify living organisms. For example, CRISPR could eliminate an invasive species from the planet. Scientists could develop a laboratory strain of the species with some problematic trait such as reduced fertility, and release the strain into the wild in order to slowly eliminate the population.[4] The hazards of such an operation include the possibility that off-target mutations could result in the adverse trait manifesting in nontarget organisms, which risks the unintentional global loss of a harmless species. At the same time, if the species of *Aedes* mosquito were wiped out in this manner, diseases like malaria and the Zika virus which are carried by this mosquito and

Figure 2: CRISPR Cas-9 protein 3D structure

continue to plague many underdeveloped communities would be greatly suppressed. But the conceivable applications of CRISPR don't stop there. CRISPR could also be very useful in the treatment of genetic disease; specifically, Down Syndrome has been heavily discussed in this regard. Children with DS have impaired language skills, learning difficulties, and both short and long term memory deficits. CRISPR could well be the specific tool required to alter the expression of DS, via either the silencing of an entire chromosome or deleting the specific gene associated with DS. Notably though, this treatment implicates gene editing from within the embryo, based on the fact that the main window to prevent cognitive impairment occurs before birth.[10] In addition to DS, CRISPR may also become an efficient form of cancer therapy. After further developments, CRISPR could be used to edit immune cells to make them better at fighting cancer, and these cells could be injected into cancer patients. On a similar note, more research on CRISPR could lead to the ability to edit a cell and completely delete the region that contributes to HIV - thereby curing a patient of a chronic disease.

THE ETHICAL CONTROVERSY

Regardless of its capacity to treat disease, the usage of CRISPR still raises a lot of ethical questions and brings together scientists, lawmakers, and the general public together to discuss how, if at all, CRISPR should be integrated into our lives. The primary ethical question centers around the juxtaposition of healing and enhancement: specifically, if scientists have the technol-

ogy to address muscle related illnesses for example, they could also improve the strength of a healthy person. Similarly, if researchers can edit cancer genes, they can also, say, edit genes for red hair.[7] This generalized ability to edit human phenotypes is overwhelming - as would be expected, the excitement for scientific discovery that accompanies the development of CRISPR is coupled with fear of the massive potential CRISPR holds. The National Institutes of Health released a statement in 2015 stating that their position is primarily restrictive of the use of CRISPR, especially in embryos. They describe the alternation of the human germline in embryos as "a line that should not be crossed." They also cite legislation against this process, such as the Dickey-Wicker amendment, which prohibits the use of creating or destroying human embryos for research purposes. The NIH points to what they see as a current lack of compelling applications to justify the use of CRISPR in embryos, bringing up issues such as "unquantifiable safety issues" and "affect[ing] the next generation without their consent."[9]
As to whether this is an astute judgement call is practically impossible to say. As of now, what we can say is that we simply don't know enough about the potential benefits of CRISPR nor the dangers of it to make a legitimate case either way. It would seem that as of now, it would be wise to pay close attention as this scientific breakthrough continues to grow within the constraints it has been given, and hope that ultimately there will be a way to introduce it into society to cure the sick and save lives, without irreversibly altering humanity in the process.

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IMAGE SOURCES

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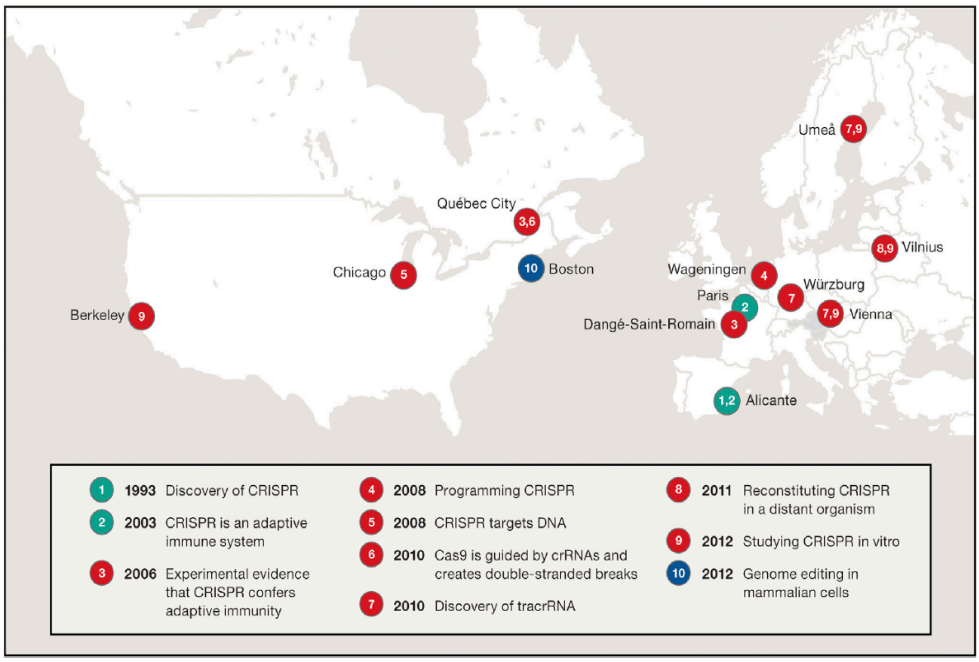

Figure 3: A map summarizing the international history and development of CRISPR