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BIOENGINEERING: GENOME EDITING AND CRISPR

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THE PROBLEM OF CONGENITAL DISEASE

Medicine today is constantly evolving. From Jonas Salk's revolutionary polio vaccine in 1955 to the University of Pittsburgh's Zika Virus cure announced just at the beginning of October this year, science has been rapidly responding to each successive viral outbreak. Treatments for other disease and injuries have also vastly improved over the years, evident in the increasing survival rates of cancer patients in the past decades [1]. Medicine has become a better and better match for the maladies of everyday life, but it is still very incomplete and always looking to progress forward. For instance, in the matter of congenital, or genetically inherited, disease, medicine is only able to diagnosis the problem and attempt to abate symptoms. Current medical technology can do nothing to resolve the actual problem, as the abnormality is not caused by an externality, but rather it is embedded in the afflicteds' very own DNA.

Solving such an issue certainly seems nigh-impossible, because medicine is reactionary. The problem occurs first, and then medicine is consulted and mostly so just to treat just the symptoms or help the body heal itself. Because of this, there is much difficulty in fixing persistent problems. Rather than merely finding temporary solutions or taking a secondary role, medicine must be able to tackle the root of the disease itself by finding permanent answers to the flaws in one's DNA. Though this may seem like a concept far into the future, scientists have already opened an entire branch of science created specifically to solve the problem of genetics.

GENETIC ENGINEERING AND GENE THERAPY

The discipline of genetic engineering began in the 1970's when 2 scientists, Herbert Boyer and Stanley Cohen, successfully modified the genes of a live bacteria. Ever since, the field has developed at an incredible pace, working its way through more complex organisms and developing more complex methods. In just 4 short decades, genetic engineering has already realized such feats as cloning, in vitro fertilization, and genome editing. From its inception, genetic engineering has been taken to be a practical field; it heavily relies on the theory and research of other fields like biology and chemistry to advance its work, in a manner similar to physics' reliance on math.

Currently, genetic engineering pervades modern culture – from GMO (genetically modified organism) rice to GMO salmon, virtually all items of consumption have been altered in some way. Genetic engineering is also beginning to have implications for humans as well. In 2003, the human genome was successfully sequenced, finally enabling the genetic modification of humans along with other animals and plants [2]. This new specialization aimed to apply genetic engineering to medicine in order to solve the problems like congenital disease that traditional medicine could not. Enter gene therapy.

Shrouded in bioethical concerns, this new, powerful approach to medicine offers solutions to an incredible number of diseases, hereditary or acquired, by seeking to directly edit a patient's DNA. Most importantly, gene therapy can create cures for diseases that are currently non-curable, namely congenital diseases, which are cause by mutations in genes. If you can read a patient's DNA and find the mistake, genetic engineering can then edit the mistake out. Genetic engineering will be deeply intertwined with Biological engineering, my field of choice. Exciting advances in genetic engineering will surely affect my line of work and currently, the most recent and most promising technology discovered to accomplish this is CRISPR-Cas9 [3].

THE MECHANICS OF CRISPR-CAS 9

CRISPR stands for "clustered regularly interspaced short palindromic repeats", and Cas9 stands for "CRISPR-associated nuclease 9." Together, CRISPR-Cas9 refers to a new technology in gene therapy whereby bodies are made to mimic bacteria. In order for bacteria to fight off viral infections, they employ what is now called the CRISPR system. This self-defense mechanism is similar to a human's antibody response, except it goes one step further. After the virus is neutralized, bits of the viral

DNA are spliced by Cas9 directly into the bacteria's genome, creating a genetic vaccination. Misreads of the added information are prevented by caps of the actual clustered repeats, which serve as markers and directions on how to execute the new gene. The significance lies mostly in the Cas9 protein. With it, extremely precise, double-stranded cuts can be made anywhere in the Human DNA sequence. Extreme accuracy and precision are provided by an accompanying 20 base string of RNA that lines Cas9 up with the desired cut site. Such cuts prompt the body's own natural response to mend the break by either reattaching the ends or filling the hole with another sequence of DNA. This presents two solutions to the matter of congenital disease. First, unwanted series of DNA may be cut out, and second, any desired sequence may be inserted.

CRISPR is delivered into the body by adeno associated vectors (AAV), a type of virus. Viruses in general infect by inserting their own DNA into the cells of its host, and AAV's were chosen specifically for their ability to infect non-mitotic cells and to elicit only a mild immunogenic response. By "infecting" AAV's themselves with CRISPR, the AAV's can be used to deliver CRISPR to the all cells of the patient. Together the two form a potent combination, able to deliver with both efficacy and potency.

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PRACTICAL POSSIBILITIES OF CRISPR

With over 19,000 protein producing genes, consisting of over 3 billion editable bases, the possibilities for CRISPR are near endless. To start, CRISPR-Cas9 provides a very conceptually simple way to "cure" congenital disease. So long as one can find the mutation in DNA causing the malady, one can use CRISPR-Cas9 to fix it.

One particular use for CRISPR is for curing achromatopsia (ACHM), a rare congenital version of colorblindness. While most types of colorblindness affect vision of only certain colors, ACHM renders the afflicted completely unable to see any color – that is, they see in true grey scale [4]. In addition, achromats also suffer from incidental side effects such as photophobia (hypersensitivity to light), hyperopia (far-sightedness), nystagmus (uncontrolled eye movement), and poor visual acuity (typically 20/200). Currently, there is no cure; the only treatment available is a prescription of special shades that filter out most lights and the advice to spend minimal time outdoors. However, CRISPR provides a solution. As with many other congenital diseases, the gene mutations associated with ACHM have all been identified: GNAT2, CNGA3, CNGB3, PDE6C, PDE6H, and ATF6 [5]. These genes are all integral to the process of phototransduction of the cones (responsible for color vison), whereby light is converted to an electrical signal, which in turn is interpreted by the brain as sight. Should any of the genes mutate, the process will be obstructed and no color will be registered. Because ACHM is usually caused by a simple single-gene mutation, CRISPR would allow geneticists to simply splice out the bad gene and replace it with a copy of its correct version. It really is as simple as that, and already scientists are working on animal models with a high degree of success.

Currently, animal trials are being conducted around the globe. In 2015, researchers (Ye et al.) conducted experiments on 120 mice with malformed CNGB3 genes [6]. The correct expression of the gene was delivered via AAV in 1, low, and 2, high, dose variants. 31% of the low dose group experienced improved cone function, compared to the 91% of the high dose group. During adjustment however, rod function temporarily worsened but returned to normal by the end of the 13 week trial. An early 2016 study (Liu et al.) performed a similar experiment placing emphasis on safety rather than efficacy. [7]. Varying the level of dosage, they concluded that though high dosage has higher potential to restore cone function, it also has higher potential to cause damage to rod (responsible for day and night vision) function. Sustained low dose treatments seem the safest way to proceed. Successful AAV trials have also been conducted with canine models (Komaromy et al.), which is significant because in past retinal disease trials, canine results translated well to human results [8].

So far, CRISPR-Cas9 in conjunction with AAV's has experienced almost universal success in the both theoretical and animal trials. Despite this, its use in medicine may be long delayed in this final transition from animal to human testing. While CRISPR is certainly capable of a world of good, some see the flip side, that CRISPR can cause many more problems than it can solve.

PROFESSIONAL AND PERSONAL EVALUATION

There is absolutely no question as to whether CRISPR-Cas9 works. Beyond a shadow of a doubt, it has proven on all fronts to be one of the most efficient and capable tools in cellular technology today in both lab and real world settings. So why is it not being employed already? There are two reasons: 1. It is still an infant technology – CRISPR was only discovered in the early 2000's, so there is still much to learn about it, and 2. Because CRISPR has so much raw potential, it has sparked a raging bioethical debate.

Brendan Fohlt writes on the conservative news site National Review that we should be extremely weary of CRISPR due to the moral concerns it raises [9]. Because CRISPR edited genes can be passed down from parent to

offspring, Fohlt cites scientific historian Daniel Kevles in seeing CRISPR as, "a potential new type of eugenics to harm minorities and the disabled," the idea being that the goal to create the optimal human will marginalize the minorities and handicapped. Both Fohlt and Kevles worry that unrestricted use of CRISPR will inevitably lead to abuse, resulting in a complete disregard for human life and rights. One such abuse is designer babies. With the ability to directly edit a genome, scientists could, in theory, modify the first cells of life to code for specific physical traits and enhancements. This topic will be explored in a follow-up paper on the ethics of CRISPR.

Another journalist and TED-speaker, Jennifer Khan, is a full proponent of CRISPR. In her TED-Talk speech, she outlines its potential use as a gene drive to eliminate malaria in a single year, bringing annual malaria-caused deaths from 1 million down to zero for future years [10]. Jennifer Doudna, one of the cofounders of CRISPR, also sees the pros outweighing the cons [11]. However, she calls for a "global pause" to discuss the ethical side to CRISPR, such as the dialogue held in the 1970's about cloning. Such a conversation would elucidate the mysteries of CRISPR and clarify any misconceptions held by the public. "This is no longer science fiction," Doudna says, emphasizing the need to bring together a global audience to discuss the global implications of her new technology.

Of the stances listed, I align with Doudna and Khan. CRISPR has astounding potential that can truly alter the course of human history. Acknowledging it as a panacea is in no way disregarding the ethical issue. Instead we should, as Doudna says, start a conversation to both inform and elucidate. That way, the world together can work through the problem together, and decide on different rules and regulations. Banning CRISPR may actually be more dangerous, as it will lead to unregulated and unmonitored use. Also, it is not as if CRISPR will vanish from existence should we choose not to use it. The tool exists right now in the present, and if we do not choose to use it for good, someone will choose to use it for otherwise. As with any new advance in technology, there are risks that you must carefully deliberate over, but ultimately, progress moves forward. Something even more impactful than CRISPR might arise in the future; we will need to face these issues sooner or later.

CRISPR IS THE ANSWER

Tens of millions of people suffer from congenital disease, and millions die from that same disease. Current medicine is only able to depress symptoms, but CRISPR is the new-found panacea for all of these ailments. While it is accompanied by a host of ethical concerns, together we can have a discussion on how to move forward through those dilemmas. CRISPR should be judged for its intended use and the good of which it is capable, not by how it might be abused. Practically, CRISPR has the ability to save millions of lives, both those alive now and those yet to be born. My family suffers from a history of diabetes, but this genetic disposition can be cured simply by snipping out several pieces of DNA. I hope to be able to utilize CRISPR to solve this problem for not only my family, but all others as well. Yes, adopting CRISPR is a risk, but how will we ever move forward without taking those risks? As Jennifer Khan aptly put it, "It can be frightening to act, but sometimes not acting is worse."

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