The CRISPR Apple on the Tree of Knowledge

with Arvin M. Gouw, "The CRISPR Apple on the Tree of Knowledge Conference Highlights: CRISPR in Science, Ethics, and Religion"; Arvin M. Gouw, "Introducing the Brave New CRISPR World"; Roger R. Adams, "Moral Decisions about Human Germ-line Modification"; Constance M. Bertka, "Navigating the Future in a Sea of CRISPR Uncertainty"; and Linda Groff, "CRISPR, CRISPR on My Mind."

INTRODUCING THE BRAVE NEW CRISPR WORLD

by Arvin M. Gouw

Abstract. Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) has been the buzzword for genome editing in the past few years, especially with the birth of Lulu and Nana, twin girls who were genetically edited using the CRISPR/Cas system. To discuss this, a group of scientists, theologians, and ethicists gathered at the 2019 Institute on Religion in the Age of Science (IRAS) conference to discuss the implications of CRISPR gene editing. It became quickly apparent through our discussions that this CRISPR revolution will impact not only human medicine, but any application that involves DNA in every organism from bacteria to plants and animals. Moreover, there are multiple stakeholders in this technology—not only the scientific community, but also the business, legal, and religious communities, to name a few. As a scientist myself, I am providing a brief overview of the *scientific* hopes and concerns about this powerful technology.

Keywords: bioethics; CRISPR; genetics

What Is CRISPR?

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) is a new gene-editing tool that dramatically improves on previous technologies such as Zinc Finger Nucleases (ZFNs), and transcription activator-like effector nucleases (TALENs) (Gupta et al. 2019). The CRISPR/Cas system was originally studied as a mechanism by which bacteria respond to viral invasion (Haurwitz et al. 2010; Wiedenheft, Lander, et al. 2011). A segment of the foreign viral DNA is incorporated into the bacterial genome, where subsequent encounters will lead to the activation of the Cas enzyme,

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which cleaves that foreign DNA (Wiedenheft, van Duijn, et al. 2011). Scientists were quick to realize that the CRISPR/Cas system can be guided to cleave specific gene targets if they synthesize and introduce single guide RNA (sgRNA) that are unique to a gene(s) of interest (Wright et al. 2015; Kundert et al. 2019). Unlike ZFNs and TALENs, CRISPR is a much more robust system and easy to use. This has allowed for the proliferation of CRISPR applications in various species (Wright, Nunez, and Doudna 2016; Knott and Doudna 2018).

Moreover, CRISPR is able to target multiple genes. This was previously difficult if not impossible using TALENs and ZFNs. The ability to target multiple genes allows CRISPR to be used as a screening tool. The affordability of CRISPR also makes it possible to knock out every gene in the genome and study the outcome of knocking out those genes (Neff 2020; Shang et al. 2020). For example, we can use CRISPR to subsequentially knock out every gene in liver cancer, to determine which genes suppresses liver cancer cell survival (Sun et al. 2018; Wang et al. 2018). The span of CRISPR applications is limitless, and that is why scientists from all sectors of life sciences are employing CRISPR into their toolbox.

What Can We Use CRISPR For?

He Jiankui, a physicist by training, went to Stanford for a short postdoctoral fellowship. After less than two years at Stanford, he returned to China to attempt the first CRISPR application in humans. In December 2018, the twins Lulu and Nana were born with CRISPR modifications on the gene called CCR5. He's intention was to make Lulu and Nana resistant to HIV, because CCR5 is the receptor that HIV uses to infect human blood cells (Gouw 2019).

This news shocked scientists and ethicists all around the world, and led to immediate condemnations from various scientific communities. Human embryonic genetic modification of CRISPR is out of the question according to most scientists because it is still considered experimental and it usually involves embryo destruction. In December 2019, He was sentenced to three years in prison and three million yuan ($430,000) fine by the Shenzhen Nanshan District People's Court. With regard to He's work in particular, many scientists disagree on editing CCR5 because much about the gene's purpose remains unknown. A CCR5 knockout mouse model developed prior to He's work was shown to have less ability to fight herpes simplex virus (Sorensen and Paludan 2004; Carr et al. 2006). Other studies have shown that CCR5 reduction led to increased memory in mice (Zhou et al. 2016). This implies that Lulu and Nana might be intellectually enhanced. Given the various unknowns of deleting CCR5, scientists do not agree that CCR5 should be CRISPR-ed in humans.

While CRISPR has great potential to be used for prevention and treatment, this raises an obvious question regarding enhancement. Can CRISPR be used to create a more intelligent person, or one with blonde hair and blue eyes? Theoretically speaking, yes that is possible, but only if we fully understand the genetic information behind such traits and whether they are genetically determined. None of these complex traits can be attributed to a single gene. The determination of something as simple as eye color is highly complex genetically. Height is also another trait where multiple genes play a role, and we are still far from knowing everything that determines it. Moreover, any traits involving interactions with the environment (intelligence, friendliness, sense of humor, ethics, and so on) are far from the reach of genetics alone.

What about Nonhuman Applications of CRISPR?

Arguably the nonhuman applications of CRISPR can be broader and larger in scale, because of the diminished ethical and regulatory issues that need to be addressed. Jennifer Doudna has been keeping a list of CRISPR-altered creatures, and up to a few years ago, she had about 40 entries (Ledford 2015b). Now, the list has expanded beyond what one list can track, ranging from prokaryotes to plants.

Just like any Genetically Modified Organism (GMO) technologies used on plants in the past, CRISPR can be used to modify plants to be pest resistant, to grow faster, to be more fruitful, and so forth. Gary Sherman, one of our speakers at the Institute on Religion in the Age of Science (IRAS) conference, argued that this is an acceptable option, given the dire food crises and climate challenges that we are currently facing. Regardless of the application, these plant modifications are not trivial scientifically. CRISPR modifications of rice and wheat have proven to be different than animal modification. CRISPR delivery cannot be done through viral infection or Homology-Directed Repair (HDR), for example (Shan et al. 2014). One would think that it would be simpler, but that is not always the case. However, scientists are making major strides in using CRISPR for agriculture purposes.

What about CRISPR-Terrorism?

Evolutionarily speaking, the best DNA delivery system in nature is a virus, because viruses naturally infect their host with viral DNA. Thus, for better or worse, viruses can be designed by CRISPR to carry whatever DNA we want it to deliver. It has been shown, albeit in mice, that it is possible to design a virus carrying a CRISPR system that can cause human lung cancer when inhaled (Maddalo et al. 2014). One can imagine that viruses can be designed to carry oncogenes (cancer-causing genes) where over time, the oncogene induces cancer. This would be very difficult to detect since the

point of contact would be impossible to determine. The television show *The Survivor* has an episode where CRISPR technology was used in racial biological warfare so that only people with darker skin color would be genetically CRISPR-ed. This presents a serious concern over the access of this technology.

CRISPR'S Power Over Life and Death of Whole Species

Mosquitoes can be more than just a simple nuisance when they start carrying deadly diseases such malaria, dengue fever, or more recently, the Zika virus. This has led to discussions and experiments that would allow us to exterminate specific disease-carrying mosquito species. Using CRISPR, it is possible to induce the CRISPR modifications done on one chromosome to copy itself to the other chromosomes, ensuring all the progenies of these mutant mosquitos will be CRISPR-ed (Lee and Fidock 2014; Hammond et al. 2016). This specific technology is called "gene drive," which is able to pass genomic modification to 100% of its progenies, greatly altering the population genetics within just a few generations (Baltimore et al. 2015). This technology could be used to cause the rapid extinction of specific species of mosquitoes. Due to the rise of concerns over gene drives, scientists have developed "reverse gene drives" using CRISPR to cut out the original genetic modification, rendering the genome back to the wild type (Akbari et al. 2015; DiCarlo et al. 2015).

On the other hand, George Church and Vincent Lynch have thought of using CRISPR to bring back extinct organisms, or de-extinction. Not unlike the movie *Jurassic Park,* they both retrieved the genetic information of woolly mammoths. Using CRISPR, Lynch's group was able to show that cells with mammoth genes can grow in low temperatures (Lynch, Bedoya-Reina, et al. 2015; Lynch, Nnamani, et al. 2015). Church's group intends to save endangered Indian elephants by modifying them with mammoth genes to make them cold resistant, so that they can be released with lots of space to roam in Siberia (Reardon 2016).

CRISPR Scientific Problems

Despite the great potential of CRISPR gene editing, it is necessary to understand the dangers of CRISPR scientifically. In general, there are three major concerns. First, scientists have to make sure that the sgRNA will target only one gene among the 20,000 to 30,000 genes that humans have. Not being able to target the intended gene of interest is sometimes referred to as an off-target effect. A lot of progress has been made in this field, and off-target effects have been reduced.

Second, though off-target effects may sound dangerous to laypeople based on experience with the side effects of pharmacological drugs, unintended on-target effects are scientifically more concerning. Unlike

off-target effects that can be analyzed and measured relatively quickly, on-target effects may take a long time to discover. A classic example of this is that targeting sickle-cell anemia causes loss of resistance to malaria. An unexpected on-target effect such as this may completely alter the cost-benefit analysis of modifying a particular gene of interest. Unfortunately, it is nearly impossible to know what all the consequences of altering a single gene among complex gene networks would be. The precautionary principle always asks scientists to wait for more information, but this should be balanced with the fact that waiting also means leaving many diseases untreated. It is always then a challenge to weigh the benefits and risks of a certain genetic modification. One possibility to remedy this would be to make the genetic modification reversible. This is indeed what scientists have been doing when creating transgenic mouse models for experimentation.

Last but not least, the third concern that I will discuss here is the role of the epigenome in regulating gene expression. Despite popular belief, genes do not have the final say in whether we have a certain trait or not. Unlike the garden peas that the monk Gregor Mendell used to derive his principles of genetics, DNA in complex organisms does not dictate traits in a simple and direct manner. There are many other factors that influence the expression of a particular gene. Thus, having the gene alone does not necessarily lead to its expression and the associated trait with it. For example, it is possible for me to carry a gene that causes disease X. However, if the DNA segment that carries that gene is folded in a closed manner, that gene cannot be activated. Suppose then we insert a gene Y through CRISPR for a new trait. That Y gene can be inactivated by epigenetic factors, rendering the CRISPR modification useless. This is indeed what we have observed experimentally with genetic conditional models in cell lines and in transgenic mice.

The Real World Is More Complex Than Experimental Conditions

Any animal model of human disease is constructed using identical genetic backgrounds. For example, a genetically modified mouse carrying a cancer-causing gene shares identical genetic background, to ensure that when the mouse gets cancer, it is caused by the oncogene, and not by other genes in the background of the mouse. To ensure identical genetic background, mouse subspecies are interbred for 10 generations before being used for experimentation.

In the case of CRISPR applications, there is always the lurking question of whether a CRISPR modification will work the same in different genetic backgrounds, because unlike genetic animal models, every person has a different genetic background. Even people of the same race have as big, if not bigger, genetic variances as people of different races (Jorde and

Wooding 2004; Xing et al. 2009; Sudmant et al. 2015). Scientists could address this problem by experimenting with CRISPR modifications in animal models of various genetic backgrounds, but this is an expensive endeavor, since the experimental costs will be multiplied by the number of genetic background models that are being used. In the end, there is no guarantee that human genetic background variation will pose no problem, even if multiple genetic backgrounds in animal models have been tested.

In addition to the genetic background problem, CRISPR has resurrected concerns that were discussed in the stem cell debate, which is the use of embryonic stem cells for research or full modification and application. In the IRAS Conference "The CRISPR Apple on the Tree of Knowledge" (see the introductory article to this thematic section of *Zygon: Journal of Religion and Science*), Ted Peters and Lisa Fullam also discussed the similarities in the bioethical conundrums that CRISPR and stem cell research share. For example, since CRISPR transfection efficiency is a major problem, one way to bypass this problem is to do the modification early in the embryonic zygote, morula, or blastocyst stage, where there are still very few cells. However, this brings up the problem of not only designer babies, but also embryonic destruction, even if the stem cells are used for research purposes only.

Scientists are well aware of this and have proposed various mechanisms to address this issue. First, instead of modifying the zygote itself, scientists have proposed modifying the gametes (sperm and/or egg), which would produce the desired genetic modification when fertilized. Second, if human genetic modification is a concern, we could still use embryonic cells for research purposes for a short period of time, and then not implant them. Third, along similar lines, the source of embryos could be those left unused after in vitro fertilization (IVF) and assisted reproductive technology (ART). Fourth, though not as effective, it is possible to perform CRISPR modifications on induced pluripotent stem cells (iPS cells) as needed prior to transplantation. The use of iPS cells is promising, though it would not work for every scenario due to the lack of totipotency of iPS cells in general, and also the limited scope of the prevention. However, if such CRISPR-modified iPS cell is implanted, we then run into all the ethical problems of human cloning (Bosley et al. 2015).

CRISPR beyond Science

Usually with the advent of a new technology, price becomes a barrier to access. Only the rich are able to afford it. This is a major social justice critique of that CRISPR technology. Presumably, we could end up with the genetically enhanced *GenRich* and the unmodified *GenPoor* (Gouw 2018). However, I argue that CRISPR presents an interesting problem with regard to access. CRISPR is so easy to perform that high school students are able

to do CRISPR gene editing after a few days of training. This is partly due to the simplicity of the CRISPR protocol that biologists, or even nonbiologists like He Jiankui, follow. We might in fact have the very opposite problem with CRISPR, in which low barriers to access, coupled with a lack of deeper scientific understanding, lead to irresponsible experimentation and modifications (Doudna and Sternberg 2017).

As I mentioned in the background section, ZFN and TALEN were available prior to CRISPR. However, ZFN and TALEN were far more expensive and labor intensive. To do CRISPR, we only need to order the sgRNA, which costs as little as US$30. In fact one can buy a do-it-yourself (DIY) kit on Amazon for less than $200. This ease of accessibility has led to the democratization and revolution of the science (Ledford 2015b). There is a community of amateur biologists sharing resources to practice DIY CRISPR modification, earning them the nickname "biohackers." They might appear to be limited in terms of lab equipment, but CRISPR does not require much. One can either buy cheap used equipment on eBay, or do simpler CRISPR modifications, which require only a microwave and the DIY kit from Amazon. A small amount of money can go a long way in improving their lab working condition (Ledford 2015a), which will allow them to perform more complex CRISPR experiments.

Regardless of the scientific concerns that were brought up in this chapter, the CRISPR revolution goes beyond science. We need to start having discussions on genetic modification early as we learn what CRISPR is for and who will use it. Discussions across multiple disciplines between scientists, ethicists, policymakers, business leaders, attorneys, regulatory bodies, and patients are necessary. Only together can we and should we explore this brave new world.

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