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An Analysis of CRISPR-Cas Gene Editing in Agriculture

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Abstract

The CRISPR-Cas system is a promising form of gene editing, especially for the agriculture industry. The ability to make single-nucleotide edits within a gene of interest, without the need to introduce foreign DNA, is a powerful tool for designing healthier and more efficient crops and food animals. This system provides opportunity for increased nutritional value, decreased food waste, and more economically and environmentally sustainable food production. Though this biotechnology is facing mechanistic limitations due to off-target effects and inefficient homology-directed repair, vast improvements have already been made to improve its efficacy. The CRISPR-Cas system is already the most advanced form of gene editing available. This paper also discusses the regulation of gene-edited agricultural products. While countries such as Australia recognize that gene editing cannot be distinguished from natural mutations and evolution, other entities such as the European Union treat these food products as genetically modified organisms, which subjects them to strict regulatory processes and testing. These conflicting policies will lead to novel effects on international trade. Though the CRISPR-Cas system is facing many mechanistic and regulatory challenges, and significant factors such as the public's opinion still need to be considered, it still has great potential to improve the global agriculture industry and provide a more sustainable future.

Introduction

Gene editing, which is a field of biotechnology that has only existed since the late 1900s, has grown dramatically in efficacy and range of applications. Gene editing is the ability to modify the DNA within a living organism through insertion, deletion, and substitution events. Since its introduction, this novel practice held the potential to safely alter DNA in a targeted manner. This differed from previous engineering practices such as genetic modification because it does not require the introduction of foreign DNA. Gene engineering takes advantage of naturally occurring cellular processes and uses them for directed changes to the genetic material.

The major components of gene editing are site-specific nucleases (SSNs), double-stranded DNA breaks, and the repair of these breaks (Lino et al., 2018). The two potential repair mechanisms are non-homologous end joining (NHEJ) and homology-directed repair (HDR) (Lino et al., 2018). The former is the most common type of repair because it can occur at any stage in the cell cycle, and simply re-ligates the strands directly, or with small insertions or deletions (Hefferin & Tomkinson, 2005). On the other hand, HDR involves a template for repair, and is a much more precise mechanism (Lino et al., 2018). However, it only occurs when there is an extra copy of the cell DNA present to be used as the template, which is during the S and G2 phases of cell growth (Lino et al., 2018). Homology-directed repair is especially important because it provides an avenue for more desirable gene products.

It is the SSN involved and mechanism by which the repair occurs that determines the gene products resulting from a double-stranded break (Lino et al., 2018). NHEJ can only lead to gene knockout, resulting from frameshifts through insertion and deletion events, or gene deletion, through the use of paired SSNs on either side of the gene of interest (Lino et al., 2018). In contrast, with its use of genetic templates, HDR can produce precise gene corrections or

additions (Lino et al., 2018). Multiple site-specific nucleases can be used to cut DNA and obtain any of these gene products. Zinc finger nucleases (ZFNs), for example, were introduced in 2002, but they had limitations in regards to high-affinity binding and sequence selection (Lino et al., 2018). Transcription activator-like effector nucleases (TALENs) made improvements to this system, and are less likely to produce off-target cutting (Lino et al., 2018). However, TALENs are much larger than ZFNs, and therefore more difficult to introduce into living cells (Lino et al., 2018). Both of these systems utilize proteins with a DNA-binding domain that has been specifically engineered to match the sequence of interest (Bortesi & Fischer, 2015). The most promising SSN yet is the CRISPR-Cas system.

CRISPR-Cas System

CRISPR stands for clustered regulatory interspaced short palindromic repeat; essentially, it is a repetitive DNA sequence found in nature. Though this type of sequence was initially discovered in 1987 in *Escherichia coli*, and was found in many other bacterial and even archaeal species over the years, it was not until the early 2000s that scientists understood the important immune system role it played (Ishino et al., 2018). Around this time, it was also discovered that some genes encoding DNA repair proteins were closely paired with CRISPR, and were thus named CRISPR-associated (Cas) genes (Ishino et al., 2018). These components work as an acquired immune response in bacterial cells, by recognizing and attacking invading pathogens (Ishino et al., 2018). The system recognizes pathogens due to the spacers in the CRISPR sequence that were captured from invading microorganisms and thus are homologous to the invaders (Ishino et al., 2018). These spacers are transcribed into short crRNA molecules, which can then interact with a Cas protein to form an endonuclease complex (Ishino et al., 2018). The

complex uses the crRNA as a guide to recognize invaders and attack them (Ishino et al., 2018). It is important to note that the matches between the CRISPR-Cas system and the pathogen sequences do not need to be exact. In fact, the system has evolved to respond with flexibility; that is, the cell can protect itself against the initial pathogen and its relatives. This means the cell does not need to store as many homologous spacers, and it can be protected against invaders it has not necessarily encountered yet.

Though Cas proteins constitute a whole family of endonucleases, only certain members are useful for gene editing purposes (Ishino et al., 2018). The Type II system is most commonly used due to its relative simplicity and ease of manipulation (Ishino et al., 2018). More specifically, the Cas9 protein has been found to be ideal in gene editing, and has been used in a variety of genetic manipulations, including bacterial, plant, and human applications. The system has also been optimized for gene editing through the use of a guide RNA (gRNA) (Lino et al., 2018). This gRNA is used in place of the crRNA of the natural system, and can be designed with relative ease to be homologous to the gene target sequence (Lino et al., 2018).

Human Health Applications

These advances in gene editing and the CRISPR-Cas system are significant due to the impacts in many fields, ranging from human health to food production. In the United States and some other major countries, CRISPR systems are entering their first human clinical trials to treat fatal diseases, by applying the editing technology in somatic tissues. The first applications of the CRISPR-Cas system in human disease treatment focused on blood diseases, because of the ability to isolate and target haematopoietic stem and progenitor cells (HSPCs) (Dever et al., 2016). Some early studies aimed to treat sickle cell disease, as it is caused by a mutation in the

globin gene (Dever et al., 2016). If scientists are able to correct the mutation, then millions of people could be successfully treated (Dever et al., 2016). These studies produced promising results. For one, cytotoxic side effects and off-target frequency decreased significantly when introducing the system as a ribonucleoprotein (RNP) complex, as opposed to an mRNA (Dever et al., 2016). Using the RNP complex, the desired mutation was detected in an average of 29% of the HSPCs, and the on to off-target activity ratio was about 25 to 1 (Dever et al., 2016). Though the results were not perfect, and the system was not ready for human-implementation at the time, this study provided important information regarding methods of delivery, frequency of non-desirable effects, and potential improvements to the technology moving forward (Dever et al., 2016).

More recently, researchers have discussed the potential use of CRISPR-Cas for treating cystic fibrosis, a lung disease caused by a mutant CFTR allele (Hodges & Conlon, 2019). This would be very advantageous for patients due to potential as a disease correction, as opposed to ongoing treatment, which would also help lower overall medical costs (Hodges & Conlon, 2019). Cystic fibrosis is a more difficult disease to treat with this biotechnology due to challenges in delivering the system to this less-accessible tissue (Hodges & Conlon, 2019). In order to achieve the desired, one-time treatment, basal stem cells in the lungs would need to be targeted (Hodges & Conlon, 2019). These are not only more difficult to reach via airways than apical cells, but also cystic fibrosis patients exhibit “more abundant, thicker mucus, infection and inflammation” that make it even harder to deliver the CRISPR-Cas system (Hodges & Conlon, 2019). Hodges and Conlon (2019), provide an analysis of potential solutions to this problem. They emphasize the need to use animal models, and build upon current delivery systems that have showed limited success, such as adenovirus co-administered with surfactant (Hodges & Conlon, 2019). Despite

the obstacles that this treatment will face before it can be implemented, researchers remain hopeful that a successful system can be developed within the next few years (Hodges & Conlon, 2019).

Studies and analyses such as these were very important to the development of the CRISPR-Cas system for human use. As of April 2019, companies such as Vertex Pharmaceuticals Incorporated and CRISPR Therapeutics announced that they have begun FDA-approved clinical trials for two blood disorders: beta-thalassemia and sickle-cell disease ("CRISPR enters its first human clinical trials," 2019). In addition, the University of Pennsylvania, and some labs in China, are currently using T cells programmed with CRISPR to treat patients with reoccurring cancers ("CRISPR enters its first human clinical trials," 2019). It is important to note a number of caveats concerning the current use of CRISPR technology in human applications. For one, the people and diseases being treated are mostly using CRISPR-Cas as a final treatment option ("CRISPR enters its first human clinical trials," 2019). Meaning, these patients have exhausted other, more common methods that have been unsuccessful in treating their disease ("CRISPR enters its first human clinical trials," 2019). Also, the CRISPR-Cas system is currently being used only to treat somatic tissues ("CRISPR enters its first human clinical trials," 2019). In other words, this technology is not being implemented in embryonic stem cells. This helps reduce some of the risks associated with off-target effects, as the cells containing mutations, both desired and incidental, will not go on to produce an entire cell population with potentially deleterious effects ("CRISPR enters its first human clinical trials," 2019). Even while considering these conditions, many researchers are very hopeful about future human disease applications such as cystic fibrosis and Duchenne muscular dystrophy ("CRISPR enters its first human clinical trials," 2019).

Human disease studies faced a major set-back, however, in 2018 when Dr. He Jiankui announced he had developed the first ever gene-edited babies (Cyranoski, 2020). Dr. He's work is controversial for a number of reasons. For one, the goal of his experiment was to make the human babies less susceptible to inheriting HIV from their parents, which is unnecessary due to precautions that can already be taken with existing technologies (Cyranoski, 2020). The target gene was CCR5, and though it is known to be associated with HIV, it is not effective in treating all strains, and many researchers did not believe that Dr. He and his team took the necessary precautions to prevent off-target effects (Cyranoski, 2020). In addition, this lab disregarded the general worldwide scientific view that no experiments should be done on human embryos for reproductive purposes (Cyranoski, 2020). As a result of his actions, Dr. He was recently sentenced to three years in prison, a 3 million yuan (US$430,000) fine, and a ban from working with human reproductive technology for "illegal medical practice" (Cyranoski, 2020). Two of his colleagues, Dr. Zhang Renli and Dr. Qin Jinzhou, are the only other scientists to face consequences so far (Cyranoski, 2020). Not only did Dr. He shock the scientific community, he also sparked a reaction from the general public (Cyranoski, 2020). This experiment brought the CRISPR-Cas system to the forefront of news for the first time, and caused public backlash (Cyranoski, 2020). Many people became concerned with the concept of designer babies, which are embryos that "have been genetically enhanced, rather than merely correcting disease-causing mutations" (Ledford, 2017). Besides the bioethical issues of designer babies, they also represent a technology that would only be available to the exclusive few that could afford it, which brings in additional socioeconomic problems. As a result of the association between CRISPR human applications and these controversial fields, many researchers worry that the negative opinions

surrounding this work will have damaging effects on funding and regulation of this biotechnology moving forward (Cyranoski, 2020).

Agricultural Applications

Such issues should not affect the agricultural applications of the CRISPR-Cas system, because it has only been implemented in the somatic tissues of plants. While CRISPR has only just entered human clinical trials, large companies have been working to introduce CRISPR gene editing to the food industry for many years. This is an important step for agriculture because it provides an alternative to genetic modification. Genetic modification not only faced serious public backlash due to its use of foreign DNA to introduce novel characteristics into crops, it also suffered from strict regulation. Gene editing has the potential to both evade strict regulation and improve the effectiveness of food production (Gao, 2018). Gene editing in plants is viewed by most scientists as a breeding mechanism (Gao, 2018). That is to say, the insertion, deletion, and substitution events that occur as a result of the CRISPR system could occur in nature; scientists are merely speeding up evolution by more efficiently selecting advantageous traits (Gao, 2018). For this reason, researchers argue that it would be very difficult to distinguish CRISPR-edited products from more typical plant breeding techniques, and therefore would be a challenge to regulate these products (Gao, 2018). There are several other important implications of the use of CRISPR-Cas in agriculture. For one, this system can be used to prevent disease by targeting risk genes for DSBs followed by NHEJ and more complex applications using HDR (Gao, 2018).

Most of all, CRISPR gene editing techniques hold the potential for sustainable agriculture (Gao, 2018). The world's population is growing at a rapid rate, and there are currently many

regions without a consistent supply of food. Whether due to climate and environmental factors, or economic limitations, these regions are unable to successfully produce the food necessary for the people there. Gene editing, especially the CRISPR-Cas system, can help solve this problem in a number of ways. Crops can be made more weather and pest resistant, which will allow them to grow in a wider range of environments and climate conditions (Gao, 2018). CRISPR is unique in that it can also provide the ability to target evolution and produce new traits that are very advantageous (Gao, 2018). For example, nitrogen fixation is currently only present in legumes, but CRISPR could help researchers drive the evolutionary process to produce this trait in other organisms (Gao, 2018). In addition, shelf-life can be improved by targeting the genes responsible for ripening, leading to reduced waste and more efficient use of the existing food supply. CRISPR technologies also have the potential to improve nutritional value of food crops by enhancing the genes responsible for the production of key vitamins and essential amino acids, and knocking out those responsible for decreased absorption (Rajendran et al., 2015). Other applications include the silencing of genes encoding common allergens such as gluten and β-lactoglobulin (Rajendran et al., 2015). According to Gao (2018), agriculture can also be made more sustainable because of "the relatively low cost and ease of use of CRISPR tools are spurring innovative research in academia and in companies of all sizes". The fact that CRISPR technologies can be used by both large corporations and relatively small farming operations is significant because it means that research can be done at all levels of production, which will promote the economic sustainability of the industry. This is a major difference between most human applications and the agricultural uses of CRISPR gene editing. The human treatment options, even those that are not embryonic, are most likely going to be expensive and relatively exclusive. On the other hand, agricultural applications can be applied at various scales of

production, from individual farmers, to conglomerates. Overall, the impacts of CRISPR-Cas technologies are significant in a broad range of ways, and the field has yet to see the full potential of such advances.

Compared to previous gene editing methods, the CRISPR-Cas system has several unique advantages. The CRISPR-Cas9 complex does not require any protein engineering like ZFNs and TALENs (Bortesi & Fischer, 2015). Instead, researchers can just manipulate the gRNA sequence, which is more versatile, and relatively easy to work with (Bortesi & Fischer, 2015). It was also discovered in human cells that this system has the ability to cleave methylated DNA (Bortesi & Fischer, 2015). If this ability is also present in regards to plant genomes, this would confer a significant advantage over ZFNs and TALENs, as plants have a large number of methylation sites (Bortesi & Fischer, 2015). Lastly, the CRISPR-Cas system is able to introduce double-stranded breaks at multiple sites at the same time (Bortesi & Fischer, 2015). This process, called multiplexing, is important because it allows for large engineered inversions, and makes it possible to edit multiple genes at once (Bortesi & Fischer, 2015). Though ZFNs and TALENs can also achieve this, it takes significantly more time and effort, as these systems require individually designed proteins at each target site (Bortesi & Fischer, 2015). Meanwhile, this ability seems more inherent to the CRISPR-Cas system due to the natural format containing repeating spacer regions (Cong et al., 2013).

Mechanistic Limitations

Despite the unique capabilities of this gene editing system, there are still a number of important limitations to consider. The mechanisms of this technology are not yet fully developed, and still require much improvement in regards to efficacy. As discussed earlier, non-

homologous end joining is the dominant form of DSB repair. This means that when researchers are attempting to take advantage of the HDR mechanism, NHEJ often still occurs because it is more rapid and prevalent in the cell (Gao, 2018). As a result, large amounts of template DNA are required to attempt to override this natural process (Gao, 2018). In order to effectively use the CRISPR-Cas system, improvements must be made to the efficiency of HDR and achieving the mutations of interest. One potential solution is to silence the mechanisms of NHEJ, by targeting the proteins involved in this process (Lino et al., 2018). Though some success was achieved, there still remain concerns about the cytotoxic side-effects of reducing NHEJ (Lino et al., 2018).

As mentioned, the CRISPR system evolved to have flexibility in binding, so as to attack pathogens it had previously encountered, and any related sequences. This ability for mismatch in the homologous sequences is advantageous for cell immunity purpose in nature, but causes problems for gene editing applications (Lino et al., 2018). Decreased sequence specificity can cause difficulties in precise binding to the target gene, and can also lead off-target binding (Lino et al., 2018). Off-target effects are a major concern due to potential gene disruption and translocation events during the repair process. In humans, this could lead to activation of oncogenes or silencing of tumor suppressor genes, among other effects, and so CRISPR-Cas gene editing would therefore be too risky to implement (Cho et al., 2014). As a result, significant efforts are being made to improve gRNA design and Cas9 efficiency (Lino et al., 2018). For example, Cho et al. (2014) found that pairing nuclease and nickase (single-strand break proteins) activity lead to increased efficiency for target genes, and decrease negative off-target effects. This was a significant improvement to the efficacy of the system as a whole, but it also makes the process more difficult to execute, as two highly effective gRNA molecules are necessary for

success (Cho et al., 2014). Overall, the CRISPR-Cas system has advanced substantially, especially in the last 10 years, but there is still a lot of room for improvements.

Another solution that has shown promising results is the use of alternative Cas proteins. For example, one group tested the efficacy of using Cas12b as the endonuclease (Teng et al., 2018). This system, which is dual-RNA-guided, is used for a wide range of purposes in mammalian genome engineering, and has potential uses for both single and multiplex editing (Teng et al., 2018). The experiment here produced successful mutation at the gene of interest, and also showed zero off-target site effects, out of the 2657 potential sites that differed from the target sequence by five or less nucleotide mismatches (Teng et al., 2018). These results indicate not only that this particular system could be very useful, but also that the Cas proteins may be able to be modified to obtain these high levels of specificity (Teng et al., 2018).

Regulatory Limitations

Besides mechanistic drawbacks with this system, CRISPR could also face regulatory issues in agriculture. Though gene editing is more likely to avoid government regulation than genetic modification, there are still concerns, especially for applications that use gRNA (as some consider it to be foreign genetic material). Recently, many major countries have released decisions and updated regulatory frameworks regarding how they plan to regulate CRISPR-editing crops and food products. In March of 2018, the United States Department of Agriculture released the following statement:

> Under its biotechnology regulations, USDA does not regulate or have any plans to regulate plants that could otherwise have been developed through traditional breeding techniques … The newest of these methods, such as genome editing, expand traditional

> plant breeding tools because they can introduce new plant traits more quickly and precisely, potentially saving years or even decades in bringing needed new varieties to farmers. (*Secretary Perdue Issues USDA Statement on Plant Breeding Innovation*, n.d.)

Sonny Perdue, the Secretary of Agriculture, went on to reassure the public that the USDA would remain vigilant in its regulation of crops, but it would not interfere where there is no risk (*Secretary Perdue Issues USDA Statement on Plant Breeding Innovation*, n.d.). Besides the fact that gene editing is often indistinguishable from traditional breeding methods, the Secretary also cited other reasons to allow this innovative technology, such as improved disease and weather resistance, and increased availability of healthy and affordable food, all of which were major advantages discussed earlier (*Secretary Perdue Issues USDA Statement on Plant Breeding Innovation*, n.d.). Despite the USDA's strong stance of support, United States regulation of gene editing technologies is also dependent on the Food and Drug Administration and the Environmental Protection Agency. These three organizations work in tandem with the Coordinated Framework for the Regulation of Biotechnology to regulate gene editing in all its applications.

In contrast to the USDA's statement, the FDA released a draft for procedures entitled "Regulation of Intentionally Altered Genomic DNA in Animals". Under the guidelines outlined in this draft, any animals whose genomes were intentionally altered using any modern biotechnology are subject to regulation as a "new animal drug" (*CVM GFI #187 Regulation of Intentionally Altered Genomic DNA in Animals*, 2017). This includes all gene editing techniques, even if the resulting alteration could have occurred in nature, such as insertion or deletion events resulting from CRISPR-Cas activity (*CVM GFI #187 Regulation of Intentionally Altered Genomic DNA in Animals*, 2017). This decision has prompted backlash in the scientific

community, because many researchers believe that these technologies do not pose unreasonable risk, and therefore should not be subjected to such strict regulations (Eenennaam et al., 2019). Eenennaam et al. (2019) also argue that this new policy directly contradicts previous regulatory procedures which stipulated that products should be assessed because of the risk they pose, not because of a particular technique used to create them.

These regulations have already caused extensive problems for the development of gene edited animals in the United States (Eenennaam et al., 2019). Not only have no transgenic food animals reached consumers, but those that are ready for market are incurring extensive regulatory costs, which is harming the CRISPR-Cas system's ability to be an affordable method for food production (Eenennaam et al., 2019). Additionally, even the AquAdvantage salmon, which was developed in 1989 and did not receive FDA approval until 2015, has not been made available to United States consumers (Eenennaam et al., 2019). This food animal product, developed in Canada, has been blocked from import and sale in the U.S. until a program is developed for informing consumers they are buying bioengineered products (Eenennaam et al., 2019).

So far, no official progress has been made in regards to labeling such food animal products. In 2018, though, the FDA releasing a statement that manufacturers could voluntarily label their products as containing or not containing gene-edited products using phrases such as "not genetically engineered," "not bioengineered," or "not genetically modified through the use of modern biotechnology" (Center for Food Safety and Applied Nutrition, 2018). Additionally, despite the draft policies previously released, this statement said the FDA "is not aware of any information showing that foods derived from genetically engineered plants, as a class, differ from other foods in any meaningful or uniform way. These foods also don't present different or

greater safety concerns than their non-genetically engineered counterparts." (Center for Food Safety and Applied Nutrition, 2018) These seemingly contradicting ideas make it difficult to predict the FDA's official policies regarding gene editing in agriculture. The only concrete labelling policy currently in effect is the requirement to label any gene-edited products that have "compositional differences resulted in material changes" (Center for Food Safety and Applied Nutrition, 2018). This refers to foods where the nutrition value may have changed, such as high oleic soybean oil and laurate canola oil (Center for Food Safety and Applied Nutrition, 2018).

Eenennaam et al. (2019) also claims that titling these gene editing food animals as "drugs" may also cause public outcry similar to that in response to genetically modified animals. This would lead to even more delays to gene edited food animal integration into the market, and be very detrimental to gene editing research and applications. Despite these harsh restrictions, it is still important to consider that the FDA's regulations were released in 2017 as a draft, and there is yet to be any official policies put into practice.

International Regulations

Other leading political entities, such as the European Union, Australia, and China, have also made key decisions regarding the regulation of genome-edited agricultural products. The EU, in particular, is known for its strict oversight of such biotechnology practices. This history of harsh regulations is a result of European consumers' lack of trust in the government to enforce food safety, stemming from inadequate response to issues such as mad cow disease (Accountability Office, 2001). Though the inadequate response had nothing to do with gene editing or biotechnology food, European consumers became wary of new foods (Accountability Office, 2001). In July of 2018, the EU Court of Justice released its decision to regulate

"organisms obtained by mutagenesis" as genetically modified organisms (*Organisms obtained by mutagenesis are GMOs and are, in principle, subject to the obligations laid down by the GMO Directive*, 2018). This decision, which is similar to the draft procedures of the FDA in the United States, asserts that all gene-edited organisms will be regulated to the same, strict degree as GMOs (*Organisms obtained by mutagenesis are GMOs and are, in principle, subject to the obligations laid down by the GMO Directive*, 2018). Conversely, the Australian Office of the Gene Technology Regulator took a more lenient approach to regulating CRISPR and other gene editing technologies. In fact, most CRISPR methods will require no government approval for use in plants and animals (*Overview of amendments to the Gene Technology Regulations 2001*, 2019). With the updates to their regulatory system, the Australian government will only restrict technologies that use nucleases from transgenes integrated into the genome, not the nucleases expressed from a transient transgene or those inserted directly (*Overview of amendments to the Gene Technology Regulations 2001*, 2019). Though the major political powers have varying regulatory practices, this area of CRISPR technologies could prove to be challenging in the evolution of the field.

Another important nation to consider is China, as it leads to world in number of CRISPR-related agricultural papers it publishes, twice as many as the United States, which is in the runner-up (Cohen, 2019). One of the reasons China is a leader in this field is Dr. Gao Caixia, who in 2013 was the first to use CRISPR to modify a plant genome (Cohen, 2019). She and her team are currently working on many crop projects, ranging from hardy tomatoes and aromatic rice, to fungus-resistant wheat and herbicide-resistant corn (Cohen, 2019). Despite having a top agricultural research community, China has yet to release any formal regulation decisions on how it will treat gene edited crops (Cohen, 2019). Most researchers believe that even though

China strictly regulates the import of genetically modified crops, it will soon side with the USDA in allowing gene edited crops as long as they contain no foreign DNA (Cohen, 2019). One indication of this regulatory decision is the country's recent purchase of Syngenta, one of the world's largest agribusinesses, which has an impressive CRISPR team (Cohen, 2019). Additionally, China may be pressured to support CRISPR agriculture products because, according to Dr. Li Jiayang, China must "feed 1.4 billion people with very limited natural resources" (Cohen, 2019). In other words, the country needs to maximize its resources by using pest and weather resistant crops to increase overall agricultural yield. Some experts have predicted that China is strategically waiting to release regulatory approval until state-owned companies such as Syngenta have products ready for market (Cohen, 2019). Even though no official regulations have been enacted, Gao and other researchers claim that once it happens, their CRISPR crops will be ready to release within months (Cohen, 2019).

In addition to investing in crop applications of CRISPR, China is also developing gene edited food animals (Cohen, 2019). For example, researchers are working to improve the pork industry (Cohen, 2019). In 2017, Dr. Zhao Jianguo and his team were successful in restoring a gene for a protein that helps form brown fat (Cohen, 2019). This is significant because this gene allowed newborn pigs to retain heat, thereby avoiding hypothermia and loss of product, and producing leaner meat (Cohen, 2019). Other teams have shown promising results in speeding the growth rate of pigs and even making them resistant to several devastating diseases that would otherwise significantly slow production (Cohen, 2019). Overall, though China has made no official decisions regarding the regulation of CRISPR in agriculture, geneticists there and around the world are confident that the use of gene edited crops and food animals will soon be approved for consumption (Cohen, 2019).

By comparison, as a result of Dr. He Jiankui's shocking disregard for the ban on human embryonic CRISPR experiments, China has put forth strict regulations on human applications of this technology (Normile, 2019). These regulations now label experiments including but not limited to " gene editing, the transfer of genes or attempts to regulate gene expression, the use of stem cells" as "high-risk" (Normile, 2019). These types of experiments now require individual approval by the State Council and informed consent of all participants (Normile, 2019). Additionally, proposals are subject to rejection if there are any issues with funding or conflict of interest (Normile, 2019). Even though these regulations may slow research in human applications of CRISPR, most researchers have had a positive response (Normile, 2019). Many are glad to have more clarity on where the Chinese government stands, and want to avoid a repeat of the chaotic backlash caused by Dr. He Jiankui's research (Normile, 2019). The overall trend in China, it seems, is that they are focusing their research efforts on the agricultural applications of CRISPR. This is wise for a number of reasons, including lower-risk research, their limited natural resources, and easier ability to implement and profit from agricultural uses of this biotechnology.

Consequences for International Trade

Looking forward, it is important to consider the how conflicting regulations on gene editing may affect international trade. To evaluate this emerging issue, we can use the trade of genetically modified organisms as a framework example. In 2001, the U.S. Government Accountability Office released a report entitled "Concerns Over Biotechnology Challenge U.S. Agricultural Exports". This report summarized the conflicting regulations of GMOs between the United States and the European Union, emphasized which crop products would be most affected,

and described the potential challenges the U.S. would face in exporting GMO products (Accountability Office, 2001). Similar to the current regulations facing CRISPR and other gene-edited products, the United States was a leading entity in genetically modified crops, while the European Union was strictly against their production and import (Accountability Office, 2001). One major problem about exporting food products in that growing biotechnology age, was "traceability" (Accountability Office, 2001). Traceability was a policy supported by the EU that required documentation following GMOs throughout their production process (Accountability Office, 2001). Essentially, this would mean that every product that is or contains a biotechnology ingredient is subject to the strict regulations (Accountability Office, 2001). If this same policy is applied to gene-edited products, it will have a major impact on trade. Not only would high oleic soybean oil be restricted, but also any products, such as margarine, containing this oil would also be restricted. This could lead to reduced export profits and less overall trade with the European Union (Accountability Office, 2001). Also, in the case of GMOs, the only way the European Union would allow trade was if the "natural" and GMO products were kept entirely separate throughout production (Accountability Office, 2001). Given the structure of the production system in the United States, this was not a reasonable request (Accountability Office, 2001). Corporations were strongly against this request because, according to them, "any regulatory measure that would ultimately lead to segregation or traceability would raise handling costs and potentially undermine the efficiency and competitiveness of this system" (Accountability Office, 2001).

However, with gene-edited crops and products, regulation gets even more complex. As mentioned previously, the genetic changes made using CRISPR are simply insertions or deletions that could occur naturally with evolution, though CRISPR is targeted for selective

advantages, and occurs at a much faster rate (Gao, 2018). Because of this, it is currently virtually impossible to detect whether a mutation in DNA is a result of natural evolution, or CRISPR manipulation (Gao, 2018). This brings up the issue of how the European Union will go about enforcing its strict biotechnology regulations. There is no efficient way to test these products, so how can the EU keep all CRISPR-edited foods out of their market? The current answer is that European Union has no true way to enforce their regulations, and many gene-edited agricultural products will likely become available in member countries despite restriction efforts. While the United States may be worried about loss of trade products, European researchers are worried about the effect these regulations will have on plant biotechnology in their countries (Stokstad, 2018). Instead of being a financially affordable production method for all scales of farms, the expensive regulatory costs in Europe will cause gene-edited crops to only be possible for large companies (Stokstad, 2018). This process is expected to cost around $35 million, which is much too high for universities, nonprofits, and other small operations (Stokstad, 2018). Overall, the conflicting regulations on biotechnology will have consequences for international trade that are yet to be determined, but based on the framework of genetically modified organisms, these regulations will probably lead to decreased profits and difficulty enforcing policies.

Conclusions

In general, though this revolutionary gene editing system faces mechanistic and regulatory limitations, it has great potential to change the agriculture industry. This technology provides opportunity for increased nutritional value, decreased food waste, and more efficient and economic food production. For countries such as China, CRISPR food production offers the potential benefit of overcoming limited natural resources and feeding their continuously growing

population. Most of the mechanistic challenges are related to the specificity and efficiency of the CRISPR-Cas system. It is important to remember that this system is already the most advanced gene editing technology yet, and even so, scientists are constantly working to make improvements. Though some political entities are resistant to CRIPSR agricultural products, much of the world powers seem to embrace its potential. Other technologies such as RNA editing are emerging in this field, and are hoped to have advantages such as reversible and transient effects (Katrekar et al., 2019). Despite the possible advantages they hold, where they may be reduced risk in human health applications, these technologies will likely face all the same issues as CRISPR and gene editing, from off-target effects to regulatory set-backs. For this reason, CRISPR is still the foremost biotechnology, as it is the most advanced, developed, and ready for market. There is still a lot that remains to be seen about this technology, including the public's opinion, official regulation policies, and international trade effects. Overall, the CRISPR-Cas system is very promising for significant improvements in food production and sustainability.

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