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From Genetic Engineering to Genome Engineering: What Impact Has it Made on Science and Society?

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ABSTRACT

Genetic engineering, or genetically modified organism (GMO), has made great advances in technology and research, starting with the discovery of DNA cloning by Stanley Cohen and Herbert Boyer in 1973 that led to the first transgenic (genetically modified) mouse in 1980. However when we experiment with Mother Nature there are consequences, both good and bad. In animal research, scientists have been able to understand diseases better and develop new and better drugs. That's excellent news today, but the long-term effects of GMOs are unknown. Public trust and acceptance of GMO food has been unenthusiastic at best. It would be beneficial for all stakeholders to understand why GMO foods are not well received in the marketplace and apply that understanding to devising ways for genome engineering advocates to overcome consumer reluctance to GMO products.

1. Introduction

Curing diseases is the ultimate goal in developing advances in the life sciences. Scientists have made great strides in the field of genetic and genome engineering, but there is still a long way to go. Anticipating the potential impact of incorporating groundbreaking scientific discoveries into everyday life is an important element of fostering the public's acceptance of those discoveries. This article will cover: 1) The history of genetic engineering; 2) Its impact on the agriculture industry; 3) Discussion on whether genome engineering can improve life and health; and 4) The

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impact of genome engineering on the future of agriculture and medicine.

2. History of Genetic Engineering

Genetic engineering dates back to 1953, when James Watson (American), Francis Crick (British), and Maurice Wilkins (New Zealand/ British) first developed a 3D model of DNA. Their discovery opened the door to understanding what we know today about genes and gene traits in organisms such as plants, animals, humans and microorganisms. The three shared the 1962 Nobel Prize for physiology/medicine.

Gene modifications were accomplished by inserting a single desired gene from one species into another non-related species, in hopes that the host would acquire the desired trait that would result in the creation of transgenic organisms (plants, animals and microorganisms).

3. The Impact on the Agriculture Industry

In response to the growing worldwide population and the increased demand for food, scientists have tried to improve crop yields by modifying specific genes to induce specific desirable traits and produce better yields by improving resistance to insects, disease, herbicides and harsh weather conditions such as drought, flood, and freezing temperatures. Scientists are also working on adding enhanced nutritional value to food products.

Bacillus thuringiensis (Bt), a soil bacterium and a bacterial disease in insects, is used in plants to produce a toxin that kills certain insects when they eat the altered plant, in effect helping the plant to build resistance to insects (WHO, 2014). Another bacterium was used to build herbicide resistance and viruses were used for disease resistance.

3.1 Repercussions

Crop yields have prospered, but many consumers were not in favor of GMO foods when they discovered that the foods contained a bacterium called Bt that produces a toxin. One anti-GMO advocate describes Bt as producing "a protein that ruptures the stomach when ingested by pests," (GMO, 2014) implying that this may occur when humans ingest food that contains this bacteria and toxin.

Bt has been used as a microbial spray pesticide to control caterpillars and certain types of beetles, as well as mosquitoes and black flies, for years. Since the introduction of the first registered Bt plant-incorporated protectants for use in the United States in 1995, the use of synthetic pesticides for control of the European corn borer has dropped by about one-third, according to the Environmental Protection Agency (EPA) (EPA, 2002).

Organic food advocates were certainly not in favor of GMO foods and organic farmers feared that GMO grains could potentially cross-contaminate fields located nearby to a GMO farm.

Further negative publicity for GMO foods occurred when Monsanto's GMO corn did not kill the rootworm and the rootworm formed resistance in four mid-west states (Kaskey, 2011). Additionally, there was fear of the potential to create “super weeds” that might materialize due to cross-resistance that could occur over time.

Environmentalists were concerned that Bt corn was responsible for the death of the Monarch Butterfly. University scientists found that Bt corn pollen, like natural corn pollen, can blow onto milkweed leaves, which are the exclusive diet of Monarch caterpillars (Friedlander, B., 1999) (USDA 2002). Steps were recommended to avoid cross-contamination of crops.

StarLink, a Bt corn registered for use in animal feed only and not for human consumption due to unresolved questions concerning StarLink's potential allergenicity, was found in trace amounts in taco shells in 2000 (EPA, 2002). The USDA, FDA, and EPA worked to test and remove any potentially contaminated corn seeds from the market. Since then, the EPA will no longer issue split registrations (animal feed only and not for human consumption) for pesticide products.

The goal of producing GMO foods was to yield better crops, resulting in lower food prices, greater benefit (longer shelf life or nutritional value), or both. But according to the World Health Organization (WHO), when the first GMO foods were introduced onto the European market, the products were not perceived by the consumer as having any direct benefit - not significantly cheaper, no increased shelf life, no enhanced flavor (WHO, 2014).

3.2 What about GMO wheat?

Wheat is used in numerous foods for human consumption, including cereal, bread, pasta, and desserts (cookies, cakes, pies, etc.). Is there GMO wheat? Wheat has a very complex DNA structure of 42 chromosomes, compared to humans with 23 chromosomes (Colorado Wheat, 2013). Monsanto, an agriculture company, spent nearly a decade-and-a-half researching GMO wheat. The company began field-testing a variety in 1998, but suspended operations in 2005, after determining that a super-wheat strain wasn't ready to be commercialized (RT News, 2014).

While there is no commercially approved GM wheat on the market, there has been an incident where GMO wheat was found in a farmer's unmodified wheat in Oregon (Bachman, 2013). It was found that the GMO wheat came from Monsanto (Mufson, 2013). Out-crossing can happen, whether it's through nature (seeds blowing into another field) or through seeds clinging to someone's clothes and accidently transferring to another crop as that person travels. In addition, Monsanto has been working on an herbicide-tolerant wheat that the company anticipates will be ready to farm in a few years (RT News, 2014).

Due to the controversy about GMO products and the incident of GMO wheat being found in an unmodified wheat field where there is no commercially approved GMO, a ban on GMO products is in effect in 26 countries (Bello, 2013).

4. Has genome engineering improved things?

Genome engineering is a definite improvement over genetic engineering (insertion of a gene and hoping that the host will acquire the trait) when trying to achieve increased specificity on its target, flexibility and adaptability of gene editing and identifying potential off-target effects.

Today, genome engineering is based on the use of engineered or programmable nucleases composed of sequence-specific DNA-binding domains bound to a nonspecific DNA cleavage module (Gaj et al, 2013). These nucleases are guided to a specific sequence within the genome to induce a double-strand DNA break (DSB). When a DSB is generated, the cell's intrinsic DNA repair system is activated and the genome is modified during the repair of the DSB. DSBs are typically repaired by either nonhomologous end joining (NHEJ) or homology-directed repair (HDR) (Harrison et al, 2014). There are four main DNA-binding proteins that have been engineered. The first three have issues with either specificity, or flexibility and adaptability of gene editing (Hsu et al, 2014).

1) Meganucleases derived from microbial mobile genetic elements

2) Zinc-finger nucleases (ZFNs) based on eukaryotic transcription factors

3) Transcription activator-like effector nucleases (TALENs) from Xanthomonas

bacteria

4) Clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 endonucleases-RNA-guided DNA endonuclease, type II bacterial adaptive immune system

In the CRISPR/Cas9 system, the protospacer-adjacent motif (PAM) is the critical element located at the 3' end of the DNA target site and dictates the search mechanism for the DNA target with Cas9 (Hsu et al, 2014). Several studies have demonstrated that PAM is involved in the binding of the Cas9 to the target and the DSB (Hsu et al, 2014). Target sequences without PAM do not induce DSB (addgene, 2014). Cas9 (formerly known as Cas5, Csn1, or Csx12) is the only enzyme within the Cas gene cluster that facilitates target DNA cleavage (Hsu et al, 2014).

The CRISPR/Cas9, type II system is emerging as the sequence-specific nuclease of choice for genome engineering for three reasons (Harrison et al, 2014): 1) Cas9 is guided by a single guide RNA (gRNA) that is easily engineered. The gRNA targeting sequence consists of 20 nucleotides (nt), which is homologous to the DNA target site and can be ordered as a pair of oligonucleotides and rapidly cloned; 2) The modular features of the CRISPR–Cas9 system and the short 20 nt length of the targeting gRNA makes these components advantageous in being able to target and cleave multiple target sequences simultaneously (multiplexing); and 3) the CRISPR–Cas9 system enables efficiency and high specificity with minimal off-target effects of unwanted chromosomal translocations when well designed gRNAs are used.

The Cas9 requires precise homology between the gRNA and the targeted DNA sequence but it does allow a few mismatches of base pairs in the target sequence when a DSB is generated (addgene, 2014). Depending on the number, position and distribution of mismatches, this could affect specificity and the desired application (Hsu et al, 2014). These off-target effects and the long-term consequences of these effects are the current concerns with the CRISPR systems. Scientists are working on various methodologies such as Cas9 nickase to target single strand breaks on opposite sides of the targeted DNA (Hsu et al, 2014) (addgene, 2014), or choosing unique target sequences and optimizing gRNA and Cas9 to minimize this phenomenon (Cho et al, 2014).

CRISPR/Cas9 shows definite promise, but even with the current advances, scientists have just started to scratch the surface with gene editing that would ultimately enable correction of genetic disorders in humans, with minimal side effects.

5. What is the impact on the future of agriculture and medicine?

Using the CRISPR/Cas9 technology will certainly advance the field of genome engineering. But will it resolve public concerns regarding long-term safety and will it change our way of living?

5.1 GMO foods

Two safety concerns cloud the potential usefulness and acceptance by the public for transgenic, or GMO, foods. First, the insertion of a single foreign gene into a nonrelated species could set into motion a cascade of biological sequelae that we can neither predict nor manage. Second, the long-term effects that foreign genes might have on plants edible and inedible and the humans and animals that ingest those GMO foods, are completely unknown.

Genome sequencing with the CRISPR can help, due to its specificity in editing desired traits, which results in potentially fewer off-target effects. But the problem still remains that when an unexpected negative outcome happens, out-crossing could spread to non-altered crops, by nature or by man. Out-crossing could impact hundreds of millions, or even billions of people in our global economy.

Measures can be taken to minimize the occurrence of mishaps, but precipitating incidents would be nearly impossible to control on a large scale.

A recent survey conducted by North Carolina State University and University of Minnesota polled 1,117 U.S. consumers nationwide about their preference for choosing nanotech foods and GMO foods with qualifiers such as price, nutrition and taste. Survey responses showed that in general, consumers are willing to pay more to avoid these technologies in their food. However, they are more willing to buy these foods if there are health and safety benefits (News Desk 2014).

When we alter our foods, will we encounter the same issues with wheat (gluten), where we have difficulty digesting the grain completely? This partial digestion triggers the release of the protein Zonulin, that opens the tight junctions of our intestinal lining leading to a leaky gut, that could affect our immune system.

5.2 Research and developing new drugs

This is an area where genome engineering could advance basic research and our understanding of disease tremendously and promote the development of more effective drug therapies. Developing the right models for researching diseases and evaluating various drug therapies with speed and accuracy are the biggest hurdles in the pharmaceutical and biotech industry. Being able to knock-in or knock-out genes in mice with accuracy and speed and limit off-target effects with well-designed gRNA, are the ultimate goals. The mouse model is not a human model, but perhaps genome editing can get us a little closer.

5.3 Gene editing in curing single genetic disorders

It may be a while before we advance to gene editing in humans and we should proceed with extreme caution. If we can delete or modify a gene related to a single genetic disorder, we would cure that disease and do it with minimal side effects. Diseases involving more than one gene are so complex; implementation may require more advanced technology.

Life scientists are mindful that once gene editing is done, it's permanent and can't be undone. We can't predict with certainty the long-term effects. Should an unexpected negative outcome happen, we may not know how to correct it and this altered gene would be passed down for generations until scientists can figure out how to correct it.

When we cure diseases, patients live longer. The rising cost of healthcare would be reduced, but the financial burden would shift to the already cash-strapped social security system. Today, more Americans are living longer and retirees are collecting social security payments for a longer period of time than originally anticipated. If the retirement age is raised, will there be enough jobs to sustain all the people entering the work force each year? For several years now, it has been documented that recent college graduates are having difficulty in finding gainful employment. We must anticipate and plan for the impact that scientific discoveries might have on society.

Medicine will change. Physicians will learn more efficient methods of diagnosing and treating diseases and many now common diseases might be eliminated. Prevention would be defined as editing a gene for a particular disease once it is diagnosed. Insurance providers will be more likely to pay for this new and highly specific preventive medicine because once a disease is cured, expenses will be eliminated, both short-term and long-term. The one area that science won't be able to change anytime in the extended future is the normal aging process.

5.4 Regenerative Medicine

With the introduction of 3D bioprinting using stems cells, the area of regenerative medicine has advanced significantly and scientists have been able to print tiny living kidneys, blood vessels, and livers in hopes of making organs available for transplant (Fraunhofer IGB, 2011) (Medical Design Tech, 2013)(Gizmodo, 2013). Bioprinting organs from a person's own stem cells will eliminate the risk of organ rejection and there would no longer be a waiting list for organ transplants.

But when we perfect this technology, at what point do we cross the line from practicing medicine to printing organs as if they are consumer goods? Currently, 3D models are used to strategize on the best approach for repairing a defected heart or knee. Will the mindset of practicing medicine change from repair to replacement? If so, would surgeons still need specialized training?

Researchers at the University of Toronto have discovered that the Sox2 gene, an on-off switch for a stem cell gene, is critical for determining the fate of the cell when it matures in mice (Univ. of Toronto, 2014). A major discovery for regenerative medicine. Could we get to the point where we can regenerate our own body parts if we lose a limb due to an accident, or regenerate damaged tissue? Scientists at the Gladstone Institute have found a way to transform non-beating cells into heart muscle cells after a heart attack with assistance from three genes, known together as GMT, in mice (Gladstone Institute, 2013).

If we all live longer, what are the consequences? Over-population or population control? Would we feel compelled to eliminate forests to build more single-family housing for a growing population, or build more multi-unit high-rises, referred to by some as concrete or glass-walled jungles? Another thought--What will happen to the wildlife and environment?

6. Conclusion

The take away message is, while advancing technology is important in curing diseases and improving crop yields, we must proceed with extreme caution. Scientists have the best intentions as they work diligently to advance medical research, but there are always consequences to everything we do, good and bad. In thinking about the ramifications for any type of advance technology, we need to think outside of our area of expertise as to who else may be affected both directly and indirectly.. Life is a cascade effect. Everything is related to and affected by a co-dependent element, much like systems biology.

Sometimes we can get so enamored by the technology that we may forget about the consequences, in science and to our society. Can we learn from the experience with GMO foods? While farmers, EPA and FDA are in favor of GMO foods due to the benefits to each organization, the consumer, an important stakeholder, feared GMO foods and did not see any benefits in terms of price, taste, or shelf life. Consumers are willing to pay more for non-GMO foods and lobbied to have GMO foods labeled. However, according to the survey, consumers are also willing to buy GMO foods if there are health and safety benefits. In order for any program to be successful, all stakeholders involved must be satisfied, and if they are not, resistance will results as in this case of GMO foods. Getting all stakeholders involved from the beginning can help to minimize repercussions down the road.

In medicine there is tremendous potential to curing diseases with gene editing. But again, we must proceed with extreme caution because any unintentional mishap is permament. And while we may not witness the consequences today, future generations will have to deal with trying to correct it.

References

addgene, CRISPR in the Lab: A Practical Guide, addgene website, (https://www.addgene.org/CRISPR/guide/)

Bachman, J. 2013. Genetically Modified Wheat Isn't Supposed to Exist. So What Is It Doing in Oregon? *Bloomberg Business Week*, May 30, 2013 (http://www.businessweek.com/articles/2013-05-30/genetically-modified-wheat-isnt-supposed-to-exist-dot-so-what-is-it-doing-in-oregon)

Bello, W. 2013. Twenty-Six Countries Ban GMOs—Why Won't the US? *The Nation Blog*, October 29, 2013 (http://www.thenation.com/blog/176863/twenty-six-countries-ban-gmos-why-wont-us#)

Cho, S.W., Kim, S., Kim, Y., 2014, Analysis of off-target effects of CRISPR/Cas-derived RNA-guided endonucleases and nickases, Genome Res. 24(1): 132–141.

Colorado Wheat, 2013. Why is the Wheat Genome So Complicated? Colorado Wheat website, November 15, 2013 (http://coloradowheat.org/2013/11/why-is-the-wheat-genome-so-complicated/)

Environmental Protection Agency, 2002. Pesticides: EPA's Regulation of Bacillus thuringiensis (Bt) Crops, EPA website, May 2002 (http://www.epa.gov/pesticides/biopesticides/pips/regofbtcrops.htm)

Fraunhofer IGB Press Release, 2011. Blood Vessels from Your Printer, September 13, 2011 (http://www.igb.fraunhofer.de/en/press-media/press-releases/2011/blood-vessels-from-your-printer.html)

Friedlander, B, 1999, Toxic pollen from widely planted, genetically modified corn can kill monarch butterflies, Cornell study shows, *Cornell Chronical* April 19, 1999 (http://news.cornell.edu/stories/1999/04/toxic-pollen-bt-corn-can-kill-monarch-butterflies)

Gaj, T., Gersbach, C.A., Barbas, C.F., 2013. ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. Trends in biotechnology 31: 397-405.

Gizmodo, 2013, Scientists Have 3D-Printed Mini Human Livers for the First Time Ever, April 23, 2013 (http://gizmodo.com/5995271/scientists-have-3d-printed-mini-human-livers-for-the-first-time-ever)

Gladstone Institute Press Room, 2013. Gladstone Scientists Transform Non-Beating Human Cells into Heart-Muscle Cells, Press Release, August 22, 2013 (http://gladstoneinstitutes.org/pressrelease/2013-08-22/gladstone-scientists-transform-non-beating-human-cells-into-heart-muscle-cel)

GMO Awareness 2014, GMO Risks, GMO- Awareness Website, 2011 -2014 (http://gmo-awareness.com/all-about-gmos/gmo-risks/)

Harrison, M., Jenkins, B., O'Connor-Giles, K.M. 2014. REVIEW: A CRISPR view of development, *Genes and Development* 28:1859–187.

Hsu, P., Landers, E., Zhang, F. 2014 Leading Edge Review: Development and Applications of CRISPR-Cas9 for Genome Engineering, *Cell* 157, 1262–1278.

Kaskey, J, 2011. Monsanto Corn May Be Failing to Kill Bugs in 4 States, EPA Says, *Bloomberg Business Week*, December 6, 2011 (http://www.businessweek.com/news/2011-12-06/monsanto-corn-may-be-failing-to-kill-bugs-in-4-states-epa-says.html)

Medical Design Technology, 2013. Living Kidneys 3D Printed in China, China View Video, September 9, 2013 (http://www.mdtmag.com/videos/2013/09/living-kidneys-3d-printed-china)

Mufson, S. 2013. Unapproved genetically modified wheat from Monsanto found in Oregon field, *The Washington Post*, May 30, 2013 (http://www.washingtonpost.com/business/economy/unapproved-genetically-modified-wheat-from-monsanto-found-in-oregon-field/2013/05/30/93fe7abe-c95e-11e2-8da7-d274bc611a47_story.html)

News Desk, 2014. Most Would Accept Nanotechnology, Genetic Modification in Food for Nutrition, Safety, *News Safety News,* December 3, 2014 (http://www.foodsafetynews.com/2014/12/consumers-accept-nanotech-genetic-modification-in-food-for-nutrition-safety/)

RT News, 2014. Monsanto readies first-ever GMO wheat, RT website, January 15, 2014 (http://rt.com/usa/monsanto-gmo-wheat-crop-648/)

United States Department of Agriculture (USDA) Agriculture Research Services 2002, Bt Corn Not a Threat to

Monarchs, USDA website, February 2002 Vol 50, No.2, last modified 3/11/14 (http://www.ars.usda.gov/is/AR/archive/feb02/corn0202.htm?pf=1)

University of Toronto media room, 2014. University of Toronto cell biologists discover on-off switch for key stem cell gene, Media Room, December 16, 2014 (http://media.utoronto.ca/media-releases/university-of-toronto-cell-biologists-discover-on-off-switch-for-key-stem-cell-gene/)

World Health Organization, 2014. Food Safety: Frequently asked questions on genetically modified foods, WHO website 2014 (http://www.who.int/foodsafety/areas_work/food-technology/faq-genetically-modified-food/en/)