



Title	Caution required for handling genome editing technology
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Citation	Trends in Biotechnology, 32(5), 234-237 https://doi.org/10.1016/j.tibtech.2014.03.005
Issue Date	2014-05
Doc URL	http://hdl.handle.net/2115/57039
Type	article (author version)
File Information	Author_TiBEC(Ishii).pdf



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<http://dx.doi.org/10.1016/j.tibtech.2014.03.005>

Genome editing technology, although a robust tool for genetic engineering, is creating indistinct regulatory boundaries between naturally occurring and modified organisms. However, researchers must act with caution in research and development to avoid misleading society. Furthermore, appropriate regulations should be proactively discussed and established for handling genome editing technology.

Current conditions

Genome editing with engineered nucleases such as Zinc Finger Nuclease (ZFN), Transcription Activator-Like Effector Nuclease (TALEN), and Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR)/Cas demonstrate a precision genome engineering in higher organisms [1]. Although genome editing has received significant attention due to its potential applications in plant and/or animal breeding, it has also raised regulatory issues. The artificial nucleases may generate novel organisms that are extremely similar or identical to naturally occurring organisms. Currently, some countries have attempted to establish regulations for handling ZFN and TALEN, but not yet CRISPR/Cas. In contrast, some researchers advocate that organisms modified using genome editing do not fall under the genetically modified organism (GMO) regulations. Yet, caution is needed because inappropriate use of genome editing may cause societal problems and loss of opportunities for agricultural and environmental applications. Here we briefly review regulatory responses, scrutinize societal implications, and propose a future direction for the biotechnology of genome editing.

Technical aspects

The genetic material in an organism can be modified using various mutagenesis techniques. Older techniques, such as chemical mutagenesis, produce entirely random mutations, whereas newer techniques, such as those of genetic engineering, can produce site-specific mutations. A GMO is an organism modified using such genetic engineering techniques. The most common type of genetic engineering begins with extracellular DNA manipulation to construct a vector harboring a specific DNA sequence or gene that is intended to transfer. The vector is transduced into cells or directly into an organism using physical, chemical, or biological methods. The modified cells, such as protoplasts, callus cells, or embryonic stem cells, are used to generate a GMO that harbors the exogenous DNA sequence. When the sequence is derived from an unrelated organism, the process is referred to transgenesis. When DNA sequences are transferred between closely related organisms, the process is called cisgenesis, particularly in the genetic engineering of plants. Both transgenesis and cisgenesis can be labor intensive and require time-consuming screens to identify GMOs, especially when dealing with higher organisms. Building on the concept of transgenesis and cisgenesis, genome editing is an advanced genetic engineering technology that can directly modify a gene within a genome. This modification is achieved by enzymes that cause double-stranded breaks (DSBs) in a target sequences and induce DNA repair through Non-Homologous End Joining (NHEJ) or Homology-Directed Repair (HDR) (Box1). The repair systems can subsequently facilitate the efficient creation of the desired mutation even in the genomes of higher organisms. Genome editing efficiently causes genetic modifications in which one or a few bases are removed, an amino acid substitution of a protein occurs, or a mutation is completely repaired in the resultant organism genome without leaving marked genetic vestiges following the modifications.

Despite the advantages of genome editing, there are still some technical issues. Obtaining a GMO that has an intentional mutation from among arising variants, albeit less laborious than conventional transgenesis or cisgenesis, continues to require screening. The technology may also cause off-target mutagenesis after attaining the desired modification in a target sequence [1]. The nucleases may fail to induce a biallelic

modification in diploid organisms, thereby resulting in an organism with a monoallelic modification [2]. Furthermore, the microinjection of the nuclease mRNAs into zygotes may induce not only germline modifications, but also mosaic modifications in which wild type cells, including germline, and genetically modified cells coexist in the resultant organisms [3]. Therefore, the research processes using genome editing and the resultant organisms require careful screening and characterization.

Responses by regulatory agencies

In the Cartagena Protocol on Biosafety, the technical legal term that is close to the GMO, “living modified organism” is stipulated as “any living organism that possesses a novel combination of genetic material obtained through the use of modern biotechnology” [4]. The use of the nucleases such as ZFN may be outside the scope of current GMO regulations including the Cartagena Protocol because these regulations largely depend on the existence of an exogenous DNA sequence in the resultant organisms. At present, some countries have attempted to establish regulations for the agricultural use of three types of ZFN (Box1) and TALEN. The major issue is whether plants modified using genome editing fall under existing GMO regulations. However, there are two types of GMO regulations: product-based and process-based approaches [5]. For instance, the USA has adopted product-based regulations under which health and environmental risks associated with a GMO are assessed according to the final product. In contrast, in the EU, GMOs are subject to the process-based regulations involving a detailed procedure based on a scientific assessment of the risks to human health and the environment. The differences in these GMO regulatory approaches may be reflected in the regulations of genome editing technology.

Argentina

In 2011, a preliminary view of the regulatory criteria for new plant technologies including genome editing was expressed in a regulatory workshop [6]. Although plants developed using ZFN-3 would fall under their product and process-based regulations, ZFN-1 may not be regulated in the Argentinian regulatory framework (Box1). Moreover, it was also stated that ZFN-2 would be regulated on a case-by-case basis if its use entails the

introduction of coding sequences.

Australia and New Zealand

In 2012, the Food Standards Australia New Zealand GMO workshop concluded that plants generated using ZFN-3 should be regulated as GMOs [7]. In contrast, they concluded that ZFN-1 and 2 should not be regulated owing to their similarity to traditional mutagenic techniques. Against this backdrop, the Australian Office of the Gene Technology Regulator stated in a 2011 review of the current act that the product-based regulatory oversight of new organisms generated using tools such as ZFNs requires improvement [8], (Box1). In 2013, the New Zealand Environmental Protection Authority (EPA) committee declared that plants modified with ZFN-1 and TALEN are not GMOs under the act (Box1), despite repeated statements from New Zealand EPA staff that the resultant organisms are GMOs [9]. The Sustainability Council, an independent council that undertakes research into genetic engineering issues, believes that the New Zealand EPA misinterpreted the act and is currently appealing the decision in the High Court [10].

EU

In 2010, the EU carried out a study of the New Plant Breeding Techniques (NBTs) in which genetic and epigenetic changes in the plant genome as well as the possibility of detection of these changes were evaluated [11] (Box1). In 2012, the European Food Safety Authority (EFSA) GMO panel issued a scientific report concluding that “breeding” with ZFN-3 may minimize hazards of food and feed products derived from plants with the induced disruption of a gene, as ZFN-3 facilitates DNA insertion into a predefined region of the genome, compared with traditional transgenesis or cisgenesis [12]. Additionally, they stated that ZFN-3 may be assessed under the EC regulations (Box1). At present, they express no opinions regarding regulations on ZFN-1 and 2.

USA

In 2012, the US Department of Agriculture informed a private enterprise that a genetically modified (GM) plant developed using ZFN with no exogenous DNA insertion would fall outside of the regulations [13] (Box1).

This appears to indicate a possible exemption for ZFN-1 in the product-based regulations.

Blurring of regulatory boundaries

Under either product- or process-based regulations, ZFN-1 and 2 consequently blur current regulatory boundaries (Figure 1). However, on a closer examination, the positions of ZFN-1 and 2 differ significantly in the product-based versus the process-based regulations. ZFN-1 is outside of the scope of the product-based regulations, but partly within the scope of the process-based regulations. This implies that the regulatory position of ZFN-1 depends on whether a country adopts product-based or process-based regulations. In contrast, ZFN-2 has both regulated and unregulated positions although the existence of, or the use of a short repair template is reflected in the map (Figure 1).

Although the regulatory response to genome editing becomes difficult, the current regulatory landscape suggests some directions. By definition, the use of ZFN-3 is regarded as a conventional transgenesis and/or cisgenesis. In the product-based regulations, an efficient assessment method should be required to verify that a product generated using ZFN-1 is outside of the regulatory scope. Further scientific and regulatory efforts are needed to minimize the frequency of case-by case responses to ZFN-1 use in the process-based regulations and ZFN-2 in the both types of the regulations.

Importantly, in the both regulatory systems, it is more significant to confirm the actual mutations caused by genome editing, and whether the mutations do or do not cause a functional change that can affect human health or the environment. To explain it differently, the emergence of genome editing technology may provide an important opportunity to form a new global consensus for future regulations in the field of genetic engineering.

Societal implications

Although regulatory preparations are delayed, the efficiency and effectiveness of genome editing in higher organisms does not authorize researchers to advance application of this technology without caution. The careless use of genome editing would raise social issues and/or rejection in agricultural and environmental applications. In the conventional genetic

engineering, the detection of exogenous DNA facilitates the characterization of the resultant organisms. Conversely, some organisms modified with genome editing appear to almost identical to naturally occurring organisms, implying difficulty in genetically characterizing these organisms. However, such organisms require scientific scrutiny prior to being released in the market and/or the environment.

Agricultural use

If genome editing results in unforeseen immunogenicity or toxicity in agricultural products, the consequences of widespread consumption of such products will be problematic. Although persuasive evidence of the safety of GM crops is available [14], careful food risk assessments would also be required for the agricultural use of genome editing technologies. At a minimum, the sudden discovery of an unintentional mutation in agricultural products would jeopardize the reliability of food labeling in various markets.

Environmental use

Some genetic mutations may cause a loss of function in modified organisms, likely resulting in the extinction in the environment even if they are released. However, other mutations may lead to a gain of function [15]. If organisms modified with genome editing in which a gain of function unintentionally arises are released without rigorous risk assessments, they may rapidly affect the local ecosystem by seriously threatening native species. Even if they do not pose a serious threat to native species, the released organisms may negatively impact the environment due to crossbreeding. Notably, a plant with a new trait that occurred in the wild due to the crossbreeding of different GM canola with herbicide resistance was recently discovered in the US [16].

In order to achieve a better relationship between biotechnology and society, researchers must act with caution, and establish a scientifically valid assessment method for evaluating organisms modified with genome editing. In particular, with regard to the off-target effect, whole genome sequencing is available to ensure that no off-target mutations develop following genome editing. If the sequencing is time-consuming, researchers must develop a novel, efficient method based on genetic or epigenetic vestiges which are associated with genome editing technology. For instance, in a recent report

on a primate modified via CRISPR/Cas-mediated gene targeting, the potential off-target sites were defined and comprehensively investigated in the primate genome [17]. Such an approach can be effective if a scientific and regulatory consensus is reached.

Concluding remarks

Although genome editing demonstrates efficient and effective genetic engineering, the new biotechnology is creating indistinct boundaries in the existing GMO regulations. Under the present conditions, researchers should act with more caution in research and development using genome editing technology compared to traditional genetic engineering technology for the accountability of science. Most importantly, an international harmony is required on this issue, as we experienced at the Asilomar Conference in 1975[18, 19]. In order to harness the potential of genome editing for future science and broad applications, researchers, private enterprises, and regulators should proactively discuss and establish appropriate regulations based on a scientific assessment.

Acknowledgements

This article does not necessarily reflect the views of our organizations. The authors take full responsibility for this opinion.

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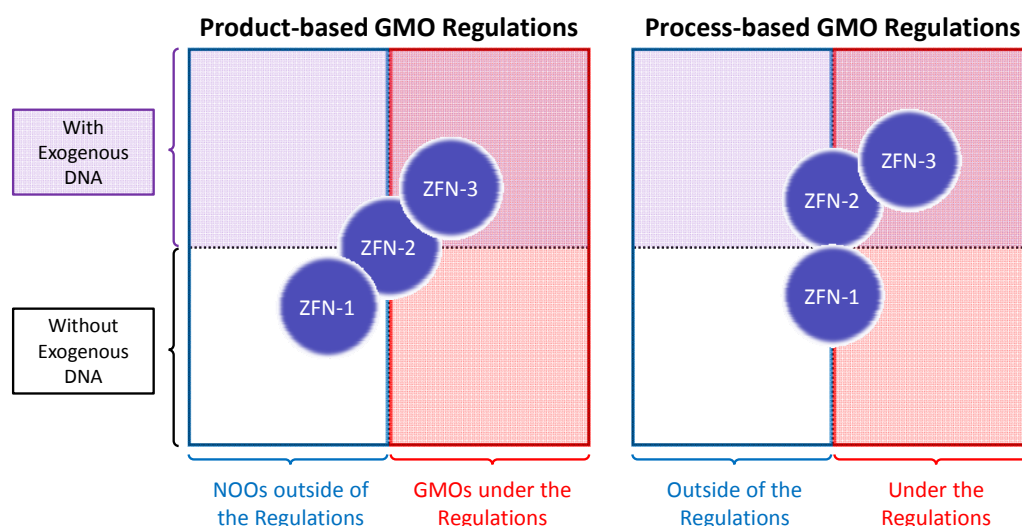


Figure 1. The presumed treatment of organisms modified with genome editing technology under genetically modified organism (GMO) regulations. The positions of zinc finger nuclease-1 (ZFN-1; site-specific random mutations involving one or a few base pairs without exogenous DNA), ZFN-2 (mutations and gene repair with short exogenous DNA), and ZFN-3 (transgenesis with long exogeneous DNA) (Box 1) are mapped in the product-based or the process-based regulations for GMOs or naturally occurring organisms (NOOs). In this analysis, the form of genome editing enzymes is presumed to be protein or RNA, not DNA.

Box 1. Genome Editing Technology and GMO regulations

DNA repair pathways used in genome editing [1]

NHEJ: nonhomologous end joining is a DSB repair pathway that ligates or joins two broken ends together without a homologous template for repair, thus leading to the introduction of small insertions and deletions at the site of the DSB.

HDR: homology-directed repair is a template-dependent pathway for DSB repair, using a homology-containing donor template along with a site specific nuclease, enabling the insertion of single or multiple transgenes, in addition to single nucleotide substitutions.

Zinc finger nuclease (ZFN) technologies used in plant breeding techniques [11]

ZFN-1: NHEJ is used to introduce site-specific random mutations (substitutions, deletions and insertions) involving one or a few base pairs

ZFN-2: HDR with a short repair template is used to generate site-specific desired mutations and the copying of the repair template

ZFN-3: HDR with a large stretch of DNA is used to cause site-specific transgenesis (targeted gene addition or replacement)

The GMO legislation or the equivalent closely relevant to “Responses by regulatory agencies”

Argentina: the National Biosafety Framework (Developed under the United Nations Environment Program - Global Environment Facility Biosafety Project)

Australia: the Gene Technology Act 2000

EU: the Regulation (EC) 1829/2003 on Genetically Modified Food and Feed

New Zealand: the Hazardous Substances and New Organisms Act 1998

USA: 7 CFR Part 340 – Introduction of organisms and products altered or produced through genetic engineering which are plant pests or which there is reason to believe are plant pests