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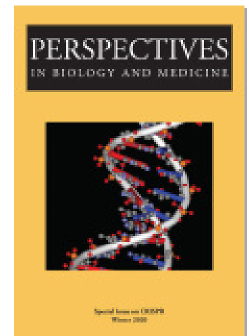
CRISPR Cautions: Biosecurity Implications of Gene Editing

Rachel M. West, Gigi Kwik Gronvall



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CRISPR Cautions

biosecurity implications of gene editing

RACHEL M. WEST AND GIGI KWIK GRONVALL

ABSTRACT CRISPR, a powerful gene-editing technology, is revolutionizing the life sciences and medical research. The technology has also become democratized. Costs to use CRISPR are low and decreasing, kits are available to make the use of CRISPR straightforward, and there is a rapidly growing scientific literature describing CRISPR methodologies and novel applications. However, like other powerful advances in the life sciences, CRISPR raises biosecurity concerns: it could be misused for harm, and it lowers technical barriers to biological weapons development. This essay describes the history and dissemination of CRISPR as genome-editing techniques have become widespread, outlines potential biosecurity concerns, and recommends actions governments and scientists may take to reduce biosecurity risks. While it is not possible to eliminate biosecurity risks from the misuse of biotechnologies, including CRISPR, steps can be taken to increase security while allowing this powerful technology to remain widely available for beneficent purposes.

CRISPR, A RECENTLY DEVELOPED GENE-EDITING TOOL, has become synonymous with rapid biological advancement. While gene editing had been performed in life sciences research for decades, genetic engineering with CRISPR is much more straightforward, faster, and less expensive—and thus, the technology

Johns Hopkins Center for Health Security, Department of Environmental Health and Engineering, Johns Hopkins Bloomberg School of Public Health, Baltimore.

Correspondence: Gigi Kwik Gronvall, Johns Hopkins Center for Health Security, 621 E. Pratt Street, #210, Baltimore, MD 21202.

Email: ggronvall@jhu.edu; revans46@jhmi.edu.

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has been rapidly democratized. CRISPR was built on a natural mechanism, the method by which bacteria resist infections from viruses called bacteriophage. Once infected, bacteria may recognize specific genetic sequences of the invading bacteriophage virus and chop its genetic material into pieces. This bacterial immune response, discovered through basic research, has been exploited by scientists to develop a gene-editing tool that can selectively find, cut, and replace specific sections of DNA. The work began in 2011–2013 in advanced research laboratories at the University of Vienna, Vilnius University of Lithuania, University of California–Berkeley, and MIT, but the use and refinement of CRISPR has since expanded to university research laboratories, start-up biotechnology companies, community laboratories, and even DIY (do-it-yourself) bio personal science kits all over the world.

As with many other powerful biotechnology advances, CRISPR raises dual-use concerns, in that in addition to all its numerous benefits, it also has the potential to be misused for harm and could lower technical barriers to biological weapons development. Specifically, CRISPR could allow a nefarious actor to edit an existing pathogen to make it more damaging, edit a non-pathogenic organism to incorporate pathogen genes and traits, and even, theoretically, to synthesize a novel pathogen. Given CRISPR's affordability, ease of use, and widespread availability, the potential for misuse likely increases, not only by a malicious actor but also through accident.

This essay describes the expansion and refinement of CRISPR as a genetic engineering tool, outlines potential biosecurity concerns, and recommends steps governments may take to reduce biosecurity risks while technology developments proceed. It is not possible to fully eliminate biosecurity risks from the misuse of biotechnologies, including CRISPR, but steps can be taken to increase safety and security while allowing this powerful technology to remain widely available for beneficent purposes.

How CRISPR Evolved as a Gene-Editing Tool

CRISPR is now a simple, robust, and efficient tool to perform genetic engineering used in laboratories all over the world, but it started out as a natural immune mechanism found in bacteria. It helped bacteria to resist the incorporation of foreign DNA from either viral bacteriophage threats or conjugation, a process in which one bacterium transfers genetic material to another through direct contact. CRISPR stands for Clustered Regularly Interspaced Short Palindromic Repeats, the repeating genetic sequences found in bacteria discovered to be pieces of foreign DNA. Though the system requires multiple components, it will be referred to as CRISPR, for simplicity, throughout this discussion.

This bacterial "immune mechanism" is not as intricate and complex as human immune systems; it is more accurate to think of it as bacterial "memory."

Prior exposure to foreign DNA arms bacteria to use CRISPR to prevent similar foreign DNA incorporation in the future. The short palindromic repeats have spacers between them, which are unique sequences that can be shuffled by the bacteria over time. These sequences are often a small piece of foreign DNA that the microbe would have previously encountered. Together with the Cas enzymes of the CRISPR system, these spacers allow the bacteria to recognize non-self DNA and remove it before it is incorporated into the genome. Hence, the bacteria can have "memory" of a previous infiltration, which helps it respond to any future attempts.

From the laboratories of Doudna (UC Berkeley), Charpentier (Max Planck Institute), Zhang (MIT), and Church (Harvard), CRISPR quickly grew from its natural role in bacteria to a tool for specifically nicking double stranded DNA, and then directing repair via a guide RNA that is encoded within the plasmid system. CRISPR as a gene-editing tool uses a predesigned spacer sequence of DNA and an endonuclease enzyme. While a variety of enzymes have been used, the most developed is Cas (Broad Institute 2019). The system works as follows. A CRISPR sequence (the designed spacer sequence) is transcribed, forming a guide RNA (gRNA) that directs the Cas enzyme to the site of interest in the DNA where there is homology. Then, Cas makes a cut in the DNA. From there, a known DNA sequence can be inserted as directed by the gRNA, an additional sequence can be included, or a process called non-homologous end joining will simply insert random mutations at the cut site. Both uses give a scientist the option of using the CRISPR system to produce a simple mutation, perhaps to disrupt a gene, or a directed mutation, where the scientist provides the desired sequence to be inserted. These mutations can be used in sequence to slowly insert an entire gene into an organism's genome, or a hybrid version of a gene, if the scientist desires. Put more simply, the CRISPR system is like a construction crew sent out to a specific site. The gRNA sequence is the address typed into the GPS; the more specific the address, the more likely the correct site will be identified. The Cas enzyme can be thought of as the construction crew; the crew needs to be directed to the correct construction site for their work to be effective. Once the crew arrives, they may work on the site at the given address. Too vague of an address may result in the crew going to the wrong site, which could be disastrous.

As a genetic engineering tool, CRISPR has been used in laboratory research on many varieties of microorganisms, plants, and animals, including mice, goats, and pigs (Wang et al. 2015; Zheng et al. 2017). Not only has this easily adaptable tool allowed for studies to determine gene functions, such as gene knockout or knock-in studies (where a gene is added to a genome), but it has also allowed scientists to tailor model organisms, such as mice, to better represent human diseases.

Funding for CRISPR-related work has rapidly escalated. In 2011, the US National Institutes of Health (NIH) awarded about $5 million for CRISPR-related

projects. Today, that funding has increased to over $1 billion, with over 12,000 related publications in 2018 (Gallo et al.,2018). Such investments are not limited to the public sector, and companies such as Editas have raised millions of dollars from private donors. The ease of use and investment in CRISPR technologies ensures its role as a key genetic engineering tool in the academic and private sectors for years to come.

CRISPR's Expansion to Eukaryotic Cells

Though the Charpentier, Doudna, and Siksnys laboratories worked extensively on biomolecular characterization of CRISPR, in 2013 the Zhang laboratory at the Broad Institute of MIT and the Church lab of Harvard University were the first to demonstrate its utility in organisms other than bacteria (Broad Institute 2019). Their innovation centered on using eukaryotic cells, such as cells found in plants and animals, rather than strictly using bacteria or fungi such as yeast. Before CRISPR, gene editing in eukaryotic cells could be time-intensive and complicated. For instance, with zinc-finger nuclease (ZFN) gene editing in human cells, gene-editing efficiencies could be as low as 30%. In addition, to add smaller regions of DNA (less than 1,000 base pairs), the efficiency is reduced to 10% (Chou, Leng, and Mixson 2012). Targeting DNA in non-replicating cells, like stem cells, is also difficult with ZFNs and requires a virus "carrier" to deliver the ZFN into the cell. ZFNs remain valuable and specific gene-editing tools, but the limitations of efficiency and complexity make CRISPR more attractive.

The variety of endonucleases available has also advanced as CRISPR has been refined as a gene-editing tool. There are now two main classes of CRISPR: Class 1, which is more varied and typically found in archaeal systems such as organisms that live near hot ocean floor vents, and Class 2, which is simpler and mostly found in bacterial systems (Wang, La Russa, and Qi 2016). The most commonly used system, CRISPR-Cas9, is a Class 2. When combined with modification enzymes, this system may be useful to treat rare genetic diseases. Work is now underway in a mouse model with Fragile X syndrome (Liu et al. 2018). In Fragile X syndrome, expression of the gene *FMR1* is too low because of DNA methylation modifications. Using CRISPR, DNA methylation can be reduced, making the gene more accessible for transcription and translation and thus reducing symptoms of Fragile X syndrome.

CRISPR may also be combined with other elements, such as non-pathogenic viruses, to help "carry" the elements to the right place. This is underway in early research to treat neurodegenerative diseases (Gaj and Perez-Pinera 2018). CRISPR-Cas9 may also be pooled with multiple guide RNAs, allowing the editing of multiple genes in one step. This pool of guide RNAs allows the Cas enzyme, which cuts the DNA, to be guided to many different parts of the genome. Using pooled guide RNAs to target many genes at once can also be valuable to un-

derstand systemic effects, such as impacts on metabolism or response to therapy (Wang, La Russa, and Qi 2016). Returning to the construction analogy, this method would be like giving a construction crew a list of addresses all at once, with one crew responsible for all the work. Further, the CRISPR-Cas9 system has been modified to become inducible by miRNA (micro RNA), which are small forms of RNA that can serve as "on/off" signals for gene expression. Combinations with miRNA could allow for temporal or tissue specific activity that may be useful for some therapies (Hirosawa et al. 2017).

Other class 2 systems, Cas12 and Cas13, have been primarily used to screen for diseases in humans. Cas12 was used in a system to screen for human papillomavirus (HPV), and Cas13 was recently engineered to differentiate the closely related Zika and Dengue viruses (Chen et al. 2018; Gootenberg et al. 2018). This screen relies on the specificity of CRISPR guide RNAs and the nucleotide cleaving by Cas. Essentially, a library of guide RNAs specific to the pathogen are used against an unknown pathogen sequence of interest. If these guide RNAs bind, then Cas will cleave the DNA. Their designed construct allows this cleavage to release a signal, giving a positive "hit" if there is a match to the pathogen library.

CRISPR is relatively easy to use, particularly in comparison to previous methods of gene editing, and it has been demonstrated to cause few off-target effects— instances where edits are made in the wrong place— making it an attractive tool for genetic modification on a larger scale. Nevertheless, the few off-target effects can have major impacts on an organism. A recent review of CRISPR-Cas9 papers highlighted the variability of CRISPR's tendency to have off-target effects. While some systems, such as in stem cells, appear to be highly specific with few erroneous Cas9 nicks, other guide RNAs, such as those growth factor genes like VEGF, appear to have lower specificity (O'Geen, Yu, and Segal 2015). The authors suggest that using tools such as whole genome sequencing to scan for sequences that may match the guide RNA, or simply using more than one guide RNA to improve specificity, can reduce off-target effects. Schaefer and colleagues (2017) identified over 1,000 potential off-target effects in a mouse model that involved CRISPR targeting of the *Pde6b* gene, which would cure blindness in mice. There must be a careful balance between modifying the genome to remedy a disease state and inducing off-target mutations that may negatively impact the organism.

These off-target effects are not exactly like side effects, because they are potentially more damaging. As George Mason University scholars Ouagrham-Gormley and Popescu (2018) explain, these off-target mutations will remain even after CRISPR-Cas9 systems are removed; in contrast, if a patient stops taking a drug that produces negative side effects, the side effects typically cease. These off-target effects are also of concern in developing CRISPR tools for the biosecurity community, as researcher and past Doudna laboratory member Kyle Watters (2018) asserts, as these unintended mutations can theoretically lead to worsening

of disease or fatal outcomes. It can be difficult to measure the potential number of off-target mutations, though Watters explains that methods such as CIRCLE-seq can help. CIRCLE-seq relies on purifying out any Cas9-nicked DNA by treating the genome with Cas9, circularizing any sequences that are nicked, and then using high-throughput sequencing to identify these regions that the Cas9 cut. This allows for rapid identification of at-risk sequences of the genome, even without a reference genome available (Tsai et al. 2017). Using new tools to reduce the risk of off-target effects is promising, but more research is needed to shift the balance in favor of clean, efficient targeting of the gene of interest.

Applications for CRISPR

Applications for CRISPR are diverse and growing, from domestication of crops, to use as antibacterial therapeutics (Greene 2018; Khan et al. 2019). One reason CRISPR is so revolutionary is that it enables scientists to tailor the system to target genes of interest, regardless of the type of organism, quickly and efficiently. It is broadly useful. The gene of interest could be one that has positive impacts on an organism, which scientists may wish to transfer to other similar organisms, or one that has negative impacts, which should be mutated or deleted. CRISPR may be used in agricultural settings to add desirable traits for improved breeding or hardiness, or to help domesticate a potentially useful crop. Recent studies have used RNAi silencing to identify the major allergen gene of peanuts, Ara H2. Silencing this gene appears to reduce the allergenicity of the protein (Dodo et al. 2008). Such a gene could be targeted by CRISPR, and CRISPR plasmids targeting these proteins are currently for sale by Santa Cruz Biotechnologies (Santa Cruz Biotechnologies 2019). In bacteria, CRISPR could target antibiotic-resistant genes to render them resistant once again and improve treatment options and outcomes.

An exciting development is that CRISPR can also be used to rearrange a bacterial genome without inserting large or complex sequences that would impede gene expression. The Chin lab, of the Medical Research Council Laboratory of Molecular Biology at Cambridge, used Cas9, as well as gRNA, to target two sites within the *E. coli* genome, which normally has only one chromosome. These Cas9 cuts broke up the genome into two fragments. The researchers simultaneously introduced another circular piece of DNA, the bacterial artificial chromosome (BAC), that they designed to have linker sequences that would allow for selection of certain bacteria over others by providing a gene that allowed modified bacteria to survive in altered conditions, such as with antibiotics. The BAC was also cut by Cas9 into two linker fragments. These, in theory, would then fuse with the two fragments of the *E. coli* genome, making *two* chromosomes that maintained their functions. They further demonstrated this technique to create seven chromosomes, and they showed the process was reversible by using the

same techniques to fuse the genome back to its original form. Their manipulation of the genome using CRISPR-Cas9 showed a creative, novel method of modifying bacterial genomes (Wang et al. 2019). This demonstrates CRISPR's growth as a laboratory tool, from being used to alter single point mutations to allowing scientists to rearrange entire chromosomes.

CRISPR also appears to be an attractive tool for barcoding a set of cells, such as in a tumor, to better understand their development. These barcodes are analogous to trackers, in that any cell with a barcode can be sourced to that tumor, so that metastasis, the spread of cancer throughout the body, can be detected. While scientists know that spread and establishment of several tumors in different tissues depends on certain tumor cells escaping, until recently, it was difficult to understand how or why certain cells were spreading. CRISPR barcoding can help trace how cells from tumors travel throughout the body. However, a recent study showed that while the barcodes allowed tumor development to be tracked, the mouse model system also appeared to have a delayed tumorigenesis caused by off-target CRISPR effects (Rogers et al. 2017). In pluripotent stem cells, CRISPR use sometimes leads to p-53 stress responses, which often contribute to tumor formation (Ihry et al. 2018). Such risks must be addressed through additional research and refinement.

Beyond the system of CRISPR, the basic repertoire of nucleotides that make up the targeted genes are being expanded with novel, synthetic nucleotides beyond A, T, G, and C. A recent study demonstrated a genetic system that relied on not four but eight nucleotides. Dubbed Hachimoji nucleic acids, the system expands the repertoire of possible genetic code (Hoshika et al. 2019). Such a development could be revolutionary—not only in the field of genetics, but also with regard to gene-editing tools like CRISPR. As CRISPR can allow for the insertion of genes that are not found in the organism naturally, CRISPR could potentially allow insertion of novel nucleotides into an organism. This could have an effect on the biology of the organism, but also expand the tools and reagents of the CRISPR system. Several academic laboratories and companies, such Synthorx, are currently working on novel nucleic acids. Synthorx expanded the nucleic acid repertoire to 6, in the context of improving cancer therapies (Synthorx 2019). The ability to create, and then selectively modify, novel genetic code opens a new arena of genetic modification. Most of this research will likely be for the improvement of health and scientific knowledge, but the risks of misuse must also be considered. For instance, Synthorx is using these novel nucleotides to create novel protein sequences that are then used to make synthetic, therapeutic proteins (such as antibodies) with improved function over natural proteins. One such protein is THOR-707, currently in phase 1 clinical trials. THOR-707 is a synthetic variant of a human antibody, and it has antitumor activity that may improve current chemotherapy regimens (ClinicalTrials.gov 2019). This presents a legitimate use of CRISPR to improve cancer therapies.

On the other hand, the potential misuse of such technology lies in synthesis or modification of pathogens. If novel nucleotides can be introduced to modify virulence of a pathogen, or introduce pathogenicity to a common microorganism, this could be a biosafety issue.

Gene drives are another potential application for CRISPR. A gene drive propagates (or drives) genes into offspring at a higher inheritance rate than would be expected in nature, in what is referred to as "super-Mendelian inheritance." Offspring not only inherit the modified gene, but they inherit the CRISPR system as well (Synthego 2018). The use of a gene drive should lead to more organisms with the gene (or disrupted gene) of interest—instead of 50% of offspring as expected in Mendelian inheritance, close to 100% inheritance. Gene drive systems have been proposed for pest management, improvement of crop yields, as well as manipulation of vector populations to reduce the spread of diseases such as malaria.

Gene drives proposed for pest management, especially agricultural pests, reduce the fitness of an organism or give a fatal trait to the organism, such as the transformer (*tra)* gene that is essential for female development in the new world screwworm. This pest can be fatal in mammalian hosts, including agricultural livestock, and it remains a major agricultural issue, despite previous efforts to use sterile male insect release to reduce insect populations. According to mathematical models, using a gene drive to target a gene specific to females could suppress the population of new world screwworm in areas where sterile male release is not effective (Scott et al. 2018). The CRISPR gene drive inserts a mutation that has negative effects on an organism, a mutation that would typically not be advantageous and would not be passed along to future generations. However, with a gene drive, the mutation can persist and affect the population into the future, reducing the pest population as a whole (McFarlane, Whitelaw, and Lillico 2018).

For improvement of crop yields, theoretically one could insert a gene that would increase crop fitness. This could be by increasing resistance to agricultural pests and plant pathogens, as seen with *Nicotiana benthamiana,* a plant related to tobacco. Researchers created plants that over-expressed CRISPR-Cas9 specific to the beet severe curly top virus (BSCTV), which resulted in plants that had lower levels of virus overall (Ji et al. 2015). While *N. bethamamiana* is not necessarily a widespread crop, this use of CRISPR demonstrates its utility in increasing resistance to plant pathogens and understanding their biology.

Gene drives have also been proposed to reduce the burden of vector-borne diseases such as malaria, a disease which had 228 million cases, and over 405,000 deaths, in 2018 alone (WHO 2019b).While antimalarial drugs and insect control efforts have made a substantial impact in malaria burden over the past decade, the decline has stagnated in recent years. Consequently, novel control efforts are needed, and a gene drive may present another tool. A gene drive can be used either to suppress the mosquito population, specifically of *Anopheles gambiae*, or

to modify the vector to reduce its capacity to spread malaria. One such gene drive target is a gene that normally confers fertility in female mosquitoes, called *AGAP005958*. Identified by the lab of Crisanti in 2016, this gene is expressed in the ovaries, and when there is a complete homozygous deletion or disruption, the female mosquitoes fail to lay eggs. Targeting this gene led to sterility in female mosquitoes, which would result in population suppression in the wild (Hammond et al. 2016). This could be cost-effective and impact large mosquito populations (Hammond and Galizi 2017). Using a gene drive, in addition to other malarial control efforts, could greatly impact the public health of millions of people without extensive human interventions; once the gene drive begins to spread, mosquito mating will maintain the drive.

DIY Bio and Community Laboratories

CRISPR is not only limited to traditional laboratory contexts with academically trained scientists or in the field as a gene drive. Community laboratories and DIY bio enthusiasts (also called "biohackers") are using the technology, in many cases to make biological science more accessible for those not in traditional science careers. DIY bio, a citizen science movement that aims to put science in the hands of the public, is an excellent example of a novel market for CRISPR-Cas9. DIY bio laboratories traditionally operate under low biosafety containment requirements (biosafety level 1 or 2), primarily working with non-pathogenic organisms; most involve work that requires about the same levels of containment as would be seen in a high-school laboratory. CRISPR-Cas9 gives them a low-cost method to produce rapid genetic modifications and provide a rich learning experience about genetics. Kits available online appear to actively market to those in DIY bio spaces, sometimes disparaging the "traditional" science laboratories from which this technology was developed. One of the more well-known companies is Odin, founded by Josiah Zayner, a well-known and controversial proponent of citizen science. Zayner's biotechnology supply company has expanded in recent years to include kits for the genetic manipulation of a wide variety of organisms, including plants and animals. One kit sold on the site is for genetic modification of tree frogs, with a CRISPR insertion that increases expression of a growth hormone and consequently increases the size of the frogs (ODIN 2019).

While these kits and laboratories may aid in public education in science and provide rich learning experiences (and potentially, a space for entrepreneurs in biotechnology), there is potential for misuse—though at this time, largely misuse related to self-harm. In 2017, the FDA issued a warning against "self-administered gene therapy" after biohackers were attempting to use CRISPR kits on themselves. The creator of the Odin Kit, Josiah Zayner, attempted to inject himself with a CRISPR construct in order to increase his musculature (Lee 2017). Specifically, he was attempting to target and knock out his myostatin gene, which

typically would inhibit growth of myoblasts, or muscle cells (Zayner 2018). In goats, myostatin knock-outs have been demonstrated to alter metabolism and increase muscle mass (He et al. 2018). Another example of a biohacker using themselves to experiment is that of Aaron Traywick, who injected himself with CRISPR targeting herpesvirus at a Facebook-broadcast event (Mullin 2018). Traywick himself suffered from herpes infection, but was trying to demonstrate how valuable CRISPR could be as a treatment for common human diseases. Nevertheless, biohackers' attempts to use their own bodies to demonstrate efficacy of a product seems a dangerous precedent for developers in the future, and one which the FDA has publicly decried.

The Potential for Harm

As CRISPR tools become more widely available and applicable, it is possible for the technology to be misused by an individual actor or organization. CRISPR significantly reduces the cost and expertise barriers of earlier gene-editing methods. Previous methods, such as ZFN genome editing, could cost thousands of dollars and are currently only available through Sigma Aldrich (Perkel 2013). These ZFN kits require some laboratory expertise, as well as cell lines or isolated organisms on which to use the tool. They allow researchers to custom design ZFNs to genes of interest, transform them into a cell of interest, and within a few days the ZFN will begin to edit the gene. According to the manufacturer, only 1–20% of cells will be mutated, which is a lower percentage than with CRISPR. However, mutated cells can be harvested in a few weeks. ZFNs were used extensively for years, but the tool often required adding different enzymes or required complicated methods, such as cold culture conditions, for the ZFN expression to be effective (Gaj, Gersbach, and Barbas 2013). This differs from the current CRISPR kits, which may provide *E. coli* as an initial organism and require far fewer supplements.

CRISPR now has significant funding behind related research projects, proven success in a variety of organisms, and a relatively low cost. This has allowed the democratization of this gene-editing tool, which provides promise for basic research and public health. However, the wide availability could also make it an attractive tool for a nefarious actor. With many companies selling CRISPR reagents and a plethora of literature explaining CRISPR methods, the barriers to entry to research have been lowered for legitimate and illegitimate actors.

CRISPR as a Potential Biosecurity Hazard

The potential for CRISPR to revolutionize genetic engineering also raises concerns that it could increase biosecurity threats by lowering barriers for the development of biological weapons. The ability to rapidly modify a genome at relatively low cost compared to previous methods could make CRISPR sys-

tems attractive for nefarious actors at all levels, from individuals through nation states. In the realm of biosecurity threats, CRISPR may be misused to create increased-virulence pathogens, neurotoxins, and even de novo organisms (DiEuliis, Berger, and Gronvall 2017; DiEuliis and Giordano 2017). A de novo organism would be completely synthetic, although it may have the same genome as an existing pathogen like smallpox. Creating a completely novel organism using synthesis is theoretically possible, but it is likely to require extensive training, funding, and time for research and development, which is less possible for some types of actors (Gibson et al. 2010).

A recent National Academies of Sciences, Engineering, and Medicine study, *Biodefense in the Age of Synthetic Biology* (2018), was undertaken to develop guidance on evaluating biosecurity risks associated with new biotechnology. The authors categorized potential threats by level of concern and offered potential solutions or safety measures that could reduce the risk of a certain technology. The authors recommend that the US Department of Defense, who requested this study, continue to innovate and engage in biotechnology, but that an assessment framework should also be used to examine novel biotechnology and its potential broader applications in the scientific and public spheres. The authors also categorized potential risks by relative concern, identifying the re-creation of known pathogens, such as smallpox, as among the highest of concern, while rating the creation of a novel pathogen as a lower risk. CRISPR could allow for rapid, efficient editing of a pathogen to possess the virulence factors of another pathogen, or it could allow a researcher to recreate a known pathogen whose genome is published. Given these biotechnology areas of concern, the misuse of CRISPR warrants recognition as a potential biosecurity threat.

While not exactly a biosecurity risk, the accessibility of a powerful genetic engineering tool has already led to ethical challenges, with Chinese scientist He Jiankui's engineering of human embryonic genomes (Cyranoski 2018). He's work violated a longstanding norm prohibiting genetic modification of the human germline, where these modifications may be passed on to future generations. A Chinese court recently found He guilty of illegal medical practice and has sentenced him to three years in prison (Joseph 2019). An international effort to monitor efforts in human genome editing is the WHO Expert Advisory Committee on Developing Global Standards for Governance and Oversight of Human Genome Editing (WHO 2019a). This committee will proactively identify areas of human genome editing research that may require further governance.

CRISPR is already commonly used in animals and plants for research purposes, but some potential use scenarios, such as for gene drives, could have longer-term environmental and agricultural impacts. Current gene drive efforts are focused on pest management, fortifying crops, and reducing disease vector populations (Burt and Crisanti 2018). Gene drive efforts in species such as white footed mice serve to reduce a host of ticks that spread Lyme disease. By target-

ing these mice to make them resistant to the bacteria that causes Lyme disease, one can break the cycle of transmission to reduce Lyme disease transmission to humans (Rajewski 2019). However, ecologists are concerned about the spread and long-term ecological impacts of these mice, something that is very difficult to model (Snow 2019). Current research involving agricultural pathogens, such as the banana streak virus that integrates in banana genomes and makes breeding difficult, highlights the dual-use aspects of CRISPR (Tripathi et al. 2019). Researchers were able to use CRISPR to edit banana streak virus genome remnants from bananas to prevent proper virus replication and improve breeding techniques. Yet a nefarious actor could design a CRISPR system that enhances the virulence of a virus so that it is able to integrate itself with a crop in a similar way. This could potentially impact food sources and would be difficult to control. It is important to note that delivery and other weaponization issues would make this a much more difficult task than it may initially appear, but dual-use risks must be acknowledged when using CRISPR in agriculture.

Gene drives have raised public concern that the changes may be difficult to reverse and may have unintended consequences (Doebeli et al. 2018). Either by intentional or accidental misuse, CRISPR-based gene drives could have dramatic impacts on indigenous species in addition to crops. Further, CRISPR has been suggested as a method to control invasive species (Callaway 2018). Yet engineered organisms may, in themselves, present an invasive species if improperly introduced into the field. It is important to identify the knowledge gaps that may complicate efforts to control CRISPR-modified species, as there may be unintended effects such as altering gene flow within a population (Moro et al. 2018). While many studies have attempted to model gene drives within populations, this cannot completely encompass a complex, dynamic ecosystem (Hayes et al. 2018). For instance, models have indicated that eliminating *A. gambiae* mosquitoes would not have any large impacts on the ecosystem (Collins et al. 2019). However, it is very difficult to verify this model, as one cannot completely wipe out a species of mosquito to "test" the impact of eliminating that species.

Attempts to use gene drives to decimate crops or impact local resources could present a biosecurity threat that could have a wide range of consequences. Keeping this in mind, the Defense Advanced Research Products Agency (DARPA) created the Safe Genes Project to not only address potential issues in gene drive technology and biosecurity, but to also promote defensive research to create countermeasures (DARPA 2017). Seven research teams are funded by the project, with each having an overall goal of (1) developing genetic tools to provide better control of gene drives; (2) creating drug-based treatments to reverse or prevent effects of gene drives; or (3) identifying ways to mediate gene drive impacts on ecosystems. This cooperative effort between government and scientists is an excellent example of forward-thinking research that helps support gene drive research by making it safer and more responsible.

Amateur and citizen scientist use of CRISPR also presents complications for biosecurity. While many biosecurity issues, such as de novo synthesis of a pathogenic virus, may require an experienced scientist with access to a variety of reagents, the widely available CRISPR kits have been used by individuals in (unsuccessful) attempts to modify their own genomes. Intentional, though inexperienced, actors may use CRISPR to alter existing microorganisms to increase their pathogenicity, or to create chimera organisms (Zhang 2018). The democratization of these kits may be an exciting advance for science education, and there is always the potential that biotech entrepreneurs will get their start this way, but it does present challenges to traditional biosafety training methods. Many DIY bio laboratories, including Baltimore Underground Science Space (BUGSS), have their own biosafety officers. This allows all members to readily ask questions and prepare their experiments safely, ensuring that all experiments are conducted at the correct biosafety level (BUGSS 2019). Further, specialized FBI agents—WMD Coordinators—often work with DIY bio laboratories and help explain and enforce laws regarding biosafety (Keulartz and van den Belt 2016). While there will always be a possibility that a malicious actor within the DIY bio space will attempt to misuse technologies such as CRISPR, the DIY bio community seeks to keep their work transparent and is proactive in their commitment to biosafety.

Fortifying Biosecurity in the Age of CRISPR

CRISPR can be considered a dual-use technology, in that while it has an array of benefits to science, medicine, and public health, it also has the potential to be used maliciously. It thus joins a long list of powerful biotechnology tools that lower barriers against biological weapons development. There are no total solutions that can be enacted that reduce the risks of misuse to zero. Even if a particular nation were to outlaw CRISPR, the process is already used all over the world. Work in biotechnology and genetic engineering will continue apace, and medical countermeasures such as vaccines and drugs will require the use of these technologies, as well as means to detect and attribute misuse. In addition, since the governance of cutting-edge technologies like CRISPR will be enacted by the scientific leaders of the technologies, countries whose scientists are not represented among that group may lack opportunities to shape the rules.

However, while there are no total solutions, partial solutions can help to deter nefarious actors, to increase the likelihood that misuse will be detected and attributed, and to limit accidental misuse. One example of a partial solution is the international governance effort led by the World Health Organization (WHO) on the testing and release of genetically modified mosquitoes, including the use of gene drives, for the purpose of malaria control (WHO/TDR and FNIH 2014). They are providing a framework for how gene drives should be responsibly used

and the safety testing required before GM mosquitoes may be released into the wild.

Another successful partial solution are the steps taken to prevent an ill-intended actor from buying the genetic material for a pathogen from a company. In 2010, the US Department of Health and Human Services (HHS) published the *Screening Framework Guidance for Providers of Synthetic Double-Stranded DNA*, which outlined for gene synthesis companies how they should screen customers and their orders for possible misuse, and what they should do if they get a "hit" on a regulated pathogen. Since that time, most gene synthesis companies internationally have adopted similar guidelines for their own screening algorithms to actively screen orders. These partial solutions cannot prevent all forms of bioterrorism—it is certainly possible for a potential bad actor to acquire pathogens from a variety of places, not just through ordering the genetic material from a gene synthesis company—but these actions raise barriers to misuse.

Some additional suggestions for enhancing biosecurity in the age of CRISPR follow.

Support Scientists' Ability to Self-Govern

Governments should work to support, and enhance, scientists' ability to self-govern, to give them the control and authority that can make necessary self-governance more effective. Many partial solutions that may increase biosecurity are technical, specific, and emerge as scientific research advances, so it is critical that scientists pursue self-governance and that governments support them to do so, and to give them the tools to develop rules of the road which may eventually lead to regulations. Scientists are the most familiar with the technological limits and possibilities of the biotechnologies they are developing, and biosecurity considerations should be added to their concerns. Of course, self-governance cannot prevent all misuse, but no other system of governance can, either.

Some examples of self-governance include the WHO gene-editing group that will determine what the rules for human gene editing, publishing requirements in scientific journals to use institutional review boards and institutional biosafety committees in protocols, as well as regulations that clearly outline what is not allowed. It is also important to consult with scientists who are at the leading edge of their fields to assess what controls may be necessary. Well before regulatory structures have been put into place, scientists working at the leading edge of research may need to make decisions about pursuing areas of research or deciding on whether a procedure is safe. These decisions are generally made by the scientists themselves, often in consensus groups of experts in related fields, and including ethicists.

The February 1975 Asilomar conference on recombinant DNA technologies is frequently cited as a prime example of scientist self-organization and governance to address the potential risks of emerging technologies, in that case, the advent of

recombinant DNA technology (Berg 2008). In response to the He experiments using CRISPR in germline editing, there has been a similar response from scientists. Leading scientists, including the creators of CRISPR technology, recently called for a moratorium on heritable CRISPR editing in humans (Lander et al. 2019). Unlike Asilomar, the act in question has allegedly been accomplished and not prevented, but its repetition has thus far been avoided. The widespread condemnation is good evidence that other scientists will not proceed down He's path until more comprehensive guidance as to how gene editing should proceed has been agreed upon. Although there is a long way to go to make sure that scientists understand their responsibilities, scientists are the "boots on the ground" regarding biotechnology, and they have the potential to be the best reporters of misuse—even if this failed in the He case (Cohen 2019).

Beyond CRISPR, there is an opportunity for governments to inform scientists about their responsibilities to protect their research and powerful biotechnology tools, and to increase their knowledge of biosecurity. Given that the most egregious examples of biological weapons development and use are increasingly historical, it is strongly suspected that many leading scientists today are broadly unfamiliar with the history, or unaware that the tools and technologies of their trade could be misused. The fact that there is a legally binding treaty prohibiting bioweapons development and use—the Convention on the Prohibition of the Development, Production and Stockpiling of Bacteriological and Toxin Weapons and on Their Destruction, also called the BWC—is not expressly taught to scientists. Since the majority of scientific research at US universities and federal institutions is funded by taxpayer money, through the National Science Foundation or the National Institutes of Health (LeMieux 2017), guidelines for laboratory training by a federal agency could be relevant to all those receiving funding. There is precedent for guidance to promote actions taken to increase biosecurity but which do not overstep into legal requirements, such as the guidance currently used with synthetic DNA screening (HHS 2010).

Provide Relevant Biosecurity Training

At the institutional level, biosecurity training may be provided.

Current PhD trainees often participate in research ethics courses, and these could be augmented with training for those working with CRISPR and other genetic engineering technologies. If scientists learn the risks and guidelines for biosecurity early in their careers, they will carry this awareness throughout their work in academia, industry, or other careers. Biosecurity training modules could be similar in scope and in time commitment to current training in topics such as chemical waste and bloodborne pathogens. The training could address current guidelines regarding CRISPR technology, legal limits of research (such as germline editing of human embryos), and suggested actions for those with concerns,

as well as providing information about where scientists should turn if they have concerns.

Promulgate CRISPR Biosafety Guidelines

Relevant CRISPR Biosafety guidelines should be made public to encourage nonacademic and DIY bio research safety. This could include promoting safe science techniques and discouraging activities such as storing bacterial samples close to food. Federal guidance may not extend to the DIY bio communities, or to nontraditional scientists who desire to use CRISPR outside of a federally funded source, or internationally. Since CRISPR technologies are already widely available, through traditional providers such as ThermoFisher Scientific and non-traditional providers such as Odin Technologies, it may be difficult to attempt to address every possible user of CRISPR technologies. But if institutions and federal funding sources were to institute relevant guidelines, DIY bio communities could use these as a model, something that is already being pursued by nongovernmental sources, funded by the Open Philanthropy Project.

Conclusion

The potential benefits of the democratization of CRISPR are significant. The technology makes genomics and gene editing more accessible for those who may not have access to science courses, or who want to challenge themselves beyond the classroom. While it may be less likely that amateur scientists will discover applied uses of CRISPR, which would require cell cultures and potential clinical trials, the learning opportunities are the main benefit. Keeping STEM accessible and diverse can give rise to new leaders in science and new discoveries and businesses focused on biotechnologies.

Education and training of scientists to properly identify and report potential security issues related to CRISPR would allow self-governance that would minimally impede scientific growth and innovation. Proper education would facilitate collaboration between scientists and policymakers, as scientists would better understand the legislative perspective regarding biosecurity (Minehata et al. 2013). Governments should create guidance and regulations that support scientists and give them tools for governance. This has been demonstrated by the NIH, with their *Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules,* which provides researchers with a clear framework. The potential for CRISPR technologies is enormous: it can allow for vast improvements in therapeutics and revolutionize gene-editing studies that will reveal even more about the intricacies of the genome. Further, the availability and accessibility of this technology can inspire amateur scientists and the DIY bio community to promote STEM education. With small, incremental partial solutions towards biosecurity, misuse of CRISPR and other genetic engineering tools could be minimized, so that we may all benefit from its enormous promise.

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