

Anticipating emerging biotechnology threats

A case study of CRISPR

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ABSTRACT. This article discusses the contingencies and complexities of CRISPR. It outlines key problems regarding off-target effects and replication of experimental work that are important to consider in light of CRISPR's touted ease of use and diffusion. In light of literature on the sociotechnical dimensions of the life sciences and biotechnology and literature on former bioweapons programs, this article argues that we need more detailed empirical case studies of the social and technical factors shaping CRISPR and related gene-editing techniques in order to better understand how they may be different from other advances in biotechnology — or whether similar features remain. This information will be critical to better inform intelligence practitioners and policymakers about the security implications of new gene-editing techniques.

Key words: CRISPR, gene editing, biosecurity, biological weapons, intelligence, off-target effects, technical challenges, diffusion, replication

In a 1955 issue of *Ladies' Home Journal*, it was touted that in the near future, nuclear energy would create a world “in which there is no disease... where hunger is unknown... where food never rots and crops never spoil... and routine household tasks are just a matter of pushing a few buttons... a world where no one stokes a furnace or curses the smog.” “Imagine,” the article continued, “the world of the future... the world that nuclear energy can create for us.”¹ As historian of technology Stephen Del Sesto writes, these thoughts were not those of an overzealous journalist but of Harold E. Stassen, President Dwight D. Eisenhower's special assistant on disarmament.² Other prominent experts believed that “atomic batteries would power automobiles, washing machines, and even tiny wrist-watch radios.”³ At that time, optimism about nuclear energy was shared by many Americans after decades of technological enthusiasm inculcated by a variety of American popular culture and press accounts and scientific, academic, and government pronouncements — these technological dreams about nuclear energy captured the U.S. popular imagination for nearly 30 years.^{4,5} Del Sesto writes, “these forces

reinforced one another making the dreams appear more plausible and closer at hand than they really were.”² What was left out of these utopian imaginaries was any consideration of how a variety of social, economic, organizational, and political realities might shape the development, adoption, and use of nuclear energy that would hinder these dreams from becoming reality. It is quite easy to dream up a large number of fanciful imaginaries for any given technological innovation — the critical question, then, is how any of these imaginaries connect with on-the-ground reality as a technology develops?

In a related but somewhat different vein than the historical nuclear energy example, the development of genetic engineering techniques since the 1980s has resulted in dominant security imaginaries — these being more dystopian in nature. In 1986, Douglas Feith, then deputy assistant secretary of defense in the Ronald Reagan administration, told the House Select Committee on Intelligence that “the stunning advances over the last five to 10 years in the field of biotechnology... mean new and better biological weapons for any country willing to violate the international norm against the possession of such weapons.”⁶ Feith worried about the development and production of offensive biological weapons that could become potent “designer biological weapons,” which he said were made possible by new biotechnologies using techniques that were simple and

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quick to use. These dystopian imaginaries about the creation of designer bioweapons for terrorist or state-level bioweapons attacks have continued for more than 30 years across academia, government, nongovernment entities, and the media.⁷ Monica Schoch-Spana writes that these kinds of bioweapons narratives are wrought in the U.S. apocalypticist tradition.⁸

More recently, a new gene-editing technique called CRISPR (clustered regularly interspaced short palindromic repeats) has emerged as a new focus of these dystopian imaginaries. CRISPR uses small strands of RNA and an associated protein (e.g., Cas9: CRISPR-associated protein) to cut recipient DNA at a precise spot.^{9,10} CRISPR is heralded as an important new innovation because it seemingly produces mutations much faster, with greater precision, and at a lower cost than previous gene-editing techniques.¹¹ Additionally, scientists have recently used CRISPR to create and “drive” edited genes through organisms.¹² These so-called gene drives ensure that, through natural reproduction, edited genes are passed on to the offspring of genetically altered organisms, thus ensuring that the edited genes are spread from one generation to the next.

These developments prompted policymakers and security analysts to begin pondering the security implications of CRISPR and evaluate its potential use for bioweapons developments.^{13,14,15,16,17,18} CRISPR scientists have themselves advanced several scenarios for bioterrorism, including the use of gene drives to create more lethal or infectious pathogens.¹⁹ Some scientists have argued that the expertise required to develop gene drives can be acquired relatively easily¹¹ and that an amateur scientist could set up a lab in his garage and develop a gene drive undetected.²⁰ Additional questions that have been raised include the following: Could CRISPR be used to introduce antibiotic resistance into a bioweapons agent? Will CRISPR allow the development of chimeric bioweapons — weapons that cause the symptoms of one disease but ravage the body with a different, undetected disease?

Echoing these concerns, Director of National Intelligence James Clapper included gene editing in his 2016 worldwide threat assessment report to Congress. Clapper warned, “Given the broad distribution, low cost, and accelerated pace of development of this dual-use technology, its deliberate or unintentional misuse might lead to far-reaching economic and national security implications.”²¹ In the fall of 2016, the President’s Council of Advisors on Science and Technology called for a new biodefense strategy that would address

emerging biotechnology threats like CRISPR that open up new possibilities for misuse.²² At the international level, United Nations entities are also struggling with how to regulate and anticipate nefarious international developments in gene-editing technologies.²³

To date, however, most policy and public discussions about CRISPR have focused on the technology — how it will drive new kinds of security threats — as if it existed in a vacuum. This kind of technological determinism — the belief that technology is the primary driver of history and contemporary life — has underpinned many popular narratives regarding American technological innovation since the Industrial Revolution.⁵ This way of understanding technology, however, oversimplifies what it takes, and how long it takes, to develop, use, and diffuse technology for beneficial *or* nefarious applications. Technology never develops and diffuses in isolation — it is always shaped by a variety of social forces — and understanding the complex interconnecting set of push-pull factors on technology is important to getting threat assessments right.

Since the 1980s, there have a variety of actors “crying wolf” about how states and terrorists will adopt and use genetic engineering techniques for harm. Yet we have little empirical data over the past 30 years that show a specific state or terrorist group using any of these new biotechnological innovations to create biological weapons. And, even those who are known to have dabbled in applying genetic engineering to produce bioweapons (e.g., the Soviet Union) encountered a series of technological, social, political, organizational, and economic hurdles that prevented the development of viable weapons. Thus, existing data suggest a very mixed picture on the ability of individuals, groups, or countries to use new biotechnologies for harm. What the existing data do make clear, however, is that it is not just access to, or emergence of, science and technology that leads to a threat but how a variety of social, economic, organizational, and political factors shape the development and use of science and technology. This latter point is left out of existing dystopian bio-imaginaries, but it is critical to assess the threat posed by CRISPR or any emerging biotechnology.

This article explores how we can better assess the security threat of emerging biotechnologies such as CRISPR by highlighting what we currently know about the challenges of using CRISPR, what we do not know, and what empirical studies that examine the sociotechnical character of the life sciences and bioweapons development can teach us about making more accurate

threat assessments for emerging biotechnologies. Specifically, the article underscores key challenges in CRISPR research, namely, off-target effects (i.e., unexpected insertions and/or deletions in genetic sequences) and the problem of research replication. These two problems, and the current difficulty in finding solutions that can work in different contexts and for different organisms, question the touted ease of use and low cost of CRISPR. The article concludes by proposing new sets of studies and policy recommendations in order to better elucidate and anticipate the type of biosecurity threat(s) posed by CRISPR.

CRISPR as an enabling technology

Although developments in gene editing can be traced to the late 1980s, an important milestone was achieved in 2012, when an international team led by Jennifer Doudna and Emmanuelle Charpentier demonstrated that the CRISPR system could be repurposed to edit genes outside of bacteria. They also showed that the Cas9 protein is directed not by one RNA, as previously thought, but by two short RNAs (crRNA and tracrRNA), which their team reprogrammed into a single RNA guide to cut specific DNA sites, thus triggering the cells' natural repair mechanism. In other words, the team had created a simpler, more precise tool that could be used to disrupt, insert, or repair specific genes in any organism.^{24,25,26}

A year later, three teams — Doudna's at the University of California, Berkeley, Andrew Church's at Harvard University, and Feng Zhang's at the Massachusetts Institute of Technology (MIT) and Harvard — published research findings the same month, demonstrating the efficiency of the CRISPR-Cas9 system: it could be used to edit not only mouse cells but also human cells.²⁷ Since these publications, scientists worldwide have started using CRISPR-Cas9 in a variety of studies for medical, scientific, and agricultural purposes, and have created companies and start-ups to exploit their discoveries. Current CRISPR-related work ranges from investigating genetic diseases, finding cures for hard-to-treat diseases such as cancer and HIV, developing more efficient ways to generate biofuels, conferring disease resistance to animals and plants, and preventing the spread of insect-borne diseases such as malaria. Although the majority of research is limited to the laboratory, some projects have already advanced to clinical trials. The U.S. National Library of Medicine's Clinical Trials database shows several CRISPR-related

human trials are either underway or pending in China to treat various types of cancers.²⁸ Several other U.S. teams are awaiting approval for CRISPR-related human trials.^{29,30,31} Therefore, there is a lot of excitement over how CRISPR-related gene-editing systems may usher in the ability to more quickly and easily modify genes and transfer this technology in order to create a whole new set of beneficial applications.

Hype versus reality in CRISPR

Most policy and security discussion around CRISPR focuses on its achievements — what CRISPR enables scientists to do in biological work. However, these discussions rarely address problems that characterize the current development and use of this technique.³² To expand on this discussion, we present here two issues in CRISPR research that are worthy of noting in the context of misuse: (1) off-target effects and (2) problems with replication of CRISPR-related research and diffusion of the technology. New techniques and technologies are often riddled with challenges when they are being initially developed and implemented, so the fact that these two problems are present is not surprising. However, the extent to which these problems can be solved is important to consider in the application and diffusion of CRISPR for either beneficial use or for harm.

Off-target effects/unexpected mutations

In contrast to a lot of the hype about the precise targeting of CRISPR, there remain persistent problems with off-target editing.^{33,34} Ma, Zhang, and Huang³⁵ have speculated that these off-target effects with CRISPR may be the result of a natural and beneficial phenomenon for bacteria in the wild — these cleavage systems could work to protect bacteria against invading DNA contaminants or plasmid DNA, serving as an adaptive immune response. This benefit, however, is not of value for scientists wishing to control these effects in order to produce precise gene modification in biological research or for biological applications. To date, scientists do not fully understand how the various CRISPR systems actually work or how they can control them with 100% reliability. For example, CRISPR-Cas9 has proved to be a good tool for disabling genes, but it has been less successful with editing DNA. This is because Cas9 only cleaves the DNA; the editing is carried out by the cells' natural repair pathways, which

the technology (and the scientist) does not control.²⁶ In addition, Cas9 cuts blunt ends — both strands of the DNA are cut at the same position and are of the same length.³⁶ As a result, the cell's repair mechanism is more likely to stick the two ends back together than to insert a new DNA sequence, which can result in single or multiple errors.^{33,37}

In May 2017, a paper by Schaefer et al. published in *Nature Methods* indicated that a high number of unexpected — and some potentially deleterious — mutations had occurred in the use of CRISPR in mice studies.³⁸ Using whole genome sequencing, the researchers found that CRISPR had successfully corrected a gene that caused blindness in mice, but there were nearly 1,400 single-nucleotide mutations and more than 100 larger deletions and insertions, which were unexpected. None of these DNA mutations was predicted by the computer algorithms widely used by researchers to look for off-target effects.^{9,39,40,41,42} At a July 2016 American Society of Hematology workshop on genome editing, CRISPR scientist J. Keith Joung said, “In the early days of this field, algorithms were generated to predict off-target effects and [made] available on the web miss a fair number of off-target effects.” He added, “These tools are used in a lot of papers, but they really aren't very good at predicting where there will be off-target effects.”⁴³ Therefore, although computer algorithms are used, there are expectations that they will not catch all off-target problems.

According to Schaefer et al., it was not clear where the problem resided with their unexpected mutations — whether better-designed RNA guides or the use of higher-fidelity Cas may reduce the off-target mutations, or whether *in vivo* off-targets are a general problem with CRISPR. According to one of the researchers, “We feel it's critical that the scientific community consider the potential hazards of all off-target mutations caused by CRISPR, including single-nucleotide mutations and mutations in non-coding regions of the genome.”⁴⁴ The study's authors recommend using whole genome sequencing to determine the presence of off-target mutations for those employing new CRISPR methods and reagents. Shortly after the announcement of this scientific publication, share prices on the biotech stock market dropped nearly 15% in one day for the two largest companies pursuing CRISPR therapies: Editas Medicine and Intellia Therapeutics.⁴⁵

Not unexpectedly, the Schaefer et al. paper quickly received criticism from CRISPR academic and industry scientists arguing that the published research was

flawed.^{46,47,48,49} In their rebuttal letters, scientists affiliated with Editas and Intellia, two companies that aim to commercialize CRISPR-based therapies, suggested that Schaefer et al. chose improper controls and should have performed sequencing of the control mice to make sure that the genetic variation they observed was actually due to CRISPR's off-target editing and not inherited. They also pointed to the small study size — two mice, one control — as a shortcoming. Other scientists claimed that unusual methods or outdated versions of CRISPR were used in the experiment. Because of these factors, critics of the experiment say that there is not enough information to rule out reasons other than CRISPR for the resulting mutations.^{46,48,50,51,52,53} Some of these scientists called for *Nature Methods* to retract the paper.

Others, however, were unfazed by the results. According to Gaetan Burgio, a scientist using CRISPR at Australian National University, “The claims over this paper are unsurprising as Cas9 enzyme could remain in the cells for days and create random indels [insertions or deletions] in the genome.” Burgio, however, goes on to note that his main concern with the Schaefer et al. paper “resides in the overestimation of the number of off-target effects due to the lack of rigor in the experimental design to detect these unexpected mutations.”⁵⁴ Stephen Floor, a CRISPR researcher at the University of California, San Francisco, emphasized that the Schaefer et al. study underscores the need for more focused inquiry on off-target problems:

This study highlights the importance of examining regions near to and far from the targeted site for genomic alterations. It will be interesting to repeat this study using other guide RNAs, repair templates, and editing protocols to determine if something about the conditions used in this work induces promiscuous editing. The ongoing use of CRISPR-Cas9 editing in many organisms suggests off-target editing is generally lower than is reported in this work. Determining the origin of this difference will inform *in vivo* editing protocols and associated analysis.⁵⁴

Burgio's and Floor's statements reveal that off-target effects are expected in using CRISPR and that there needs to be more careful research *in vitro* and *in vivo* to get at the factors shaping off-target effects in the Schaefer et al. study, as well as other CRISPR studies.

In light of the mounting criticisms of the Schaefer et al. paper, *Nature Methods* issued “an editorial

expression of concern” as an addendum to the paper in July 2017.⁵⁵ The journal stated that multiple individuals and groups had questioned the author’s interpretation that the single-nucleotide changes seen in the whole genome sequences of the two CRISPR-treated mice are due to CRISPR treatment. According to the journal, these changes could also be due to normal genetic variation. Schaefer et al. disagreed with the journal’s decision to issue this concern and with these criticisms. In response to their critics, they conducted additional analysis of their data and reported that they found no evidence to support the notion that the problem lies with the mice.⁵⁶

We found that a deeper look at our already publicly available whole genome sequencing data, combined with Sanger sequencing, demonstrates many alleles (more than two in each case we examined), including multiple [single-nucleotide variants], at CRISPR/Cas9 off-target mutation sites we originally reported. These multiple mutant alleles cannot be simply explained by parental inheritance.⁵⁷

Schaefer et al. provide some additional alternative interpretations of the data beyond CRISPR-induced causes but state that these would require additional research in order to further clarify the origins of the mutations. Therefore, as of this writing, questions remain about the cause of the mutations in the original Schaefer et al. 2017 paper, as well as larger questions about how off-target effects from using CRISPR *in vitro* might differ from *in vivo*.

Nevertheless, in April 2018, *Nature Methods* issued an editorial explaining that it had made the decision to retract the Schaefer et al. paper because of the lack of key controls that would enable a clear determination of the observed genomic variation to CRISPR; more data were needed to make this determination.⁵⁸ The editors also cautioned that more research is needed to determine off-target effects occurring *in vivo*:

There is relatively little published data on genome-wide effects of *in vivo* CRISPR treatment. Most studies of off-target changes in CRISPR-treated organisms are not agnostic; they examine genomic sites that are algorithmically predicted to harbor off-target sequences. While this is in keeping with the known mechanism of Cas9, the enzyme could, at least in principle, have unpredicted effects on the *in vivo* genome.⁵⁸

For all the controversy caused by the Schaefer paper, many publications before and since this one have called attention to missed off-target effects. The backlash caused by the Schaefer paper might be due more to the market effect of the paper on biotech companies that try to develop and eventually commercialize CRISPR-based treatments and drugs than to the actual revelations, which did not come as a surprise to many. For example, at an October 2017 meeting devoted to analyzing the security implications of CRISPR — organized by the Volkswagen Foundation in Hanover, Germany — scientists from all over the world noted that there remain significant problems with off-target effects in all fields of research, from human cell work to animal and plant research.⁵⁹ In a more recent development, three papers published by *Nature Medicine* and *Nature Biotechnology* in June and July 2018 point to the continued challenge of off-target effects. Indeed, the papers demonstrated that CRISPR can induce a wide range of unwanted deletions and complex genomic rearrangements that can lead to “pathogenic consequences” such as silencing genes that should be active and activating genes that should be silent, including those genes responsible for triggering the formation of cancers in the body.^{60,61,62}

As in the case of the Schaefer paper, the publication of the three recent studies caused a sharp fall in the stock value of U.S. companies that are attempting to commercialize CRISPR therapies.⁶³ But the papers did not cause the same pushback by the scientific and academic community, including by CRISPR biotech companies, as the Schaefer paper. It is possible that the latest papers have received a better reception because they illustrated the challenge of off-target using different types of cells, and at least in the case of the paper published in *Nature Biotechnology*, the journal’s editor worked with the authors for about a year to confirm their methods and results prior to publication. Be that as it may, these and previous papers indicate that the problem of off-target effects is significant and should not be dismissed. As a matter of fact, it has become a major research effort, as described next.

The hidden challenges of CRISPR

The scientific literature is filled with articles discussing the occurrence of off-target effects, and much of the current work on CRISPR aims to identify the sources of and solutions needed to reduce such events. This research also demonstrates that there are many

hidden challenges that make CRISPR more difficult to use than is generally described in the literature and media. In addition, the solutions to these challenges offered in the scientific literature are typically specific to an organism, and they require the use of technologies and specialized expertise, which not only increase the cost of the technology but also reduce its ease of diffusion.

One of the key challenges in using CRISPR is *designing the RNA guide*. Research has shown that some RNA guides are less efficient than others, some are inactive while others are promiscuous, and in the absence of a good RNA guide, multiple off-target effects can occur. In addition, several potential RNA guides can perform the same editing task, but each has different off-target outcomes. As a result, selecting the right RNA for the task at hand adds to the challenge of achieving proper design. In other words, the specificity and precision of CRISPR is largely conditioned by the type and specificity of the RNA guide.⁶⁴

Several web-based tools are available to help scientists select and design RNA guides by ranking them according to their potential off-target effects. However, these tools differ in their sophistication and do not always provide a full picture of potential off-target events. In addition, they require their users to know enough about the tools available to select the one that ideally fits the user's purpose. For example, some tools rank RNA guides solely on the basis of their sequence similarity to the predicted off-target sites, while others provide "specificity scores" by using additional factors, such as the number and location of potential mismatches with the off-target sites. Generally, off-target effects are more likely if the RNA guide and the predicted off-target site have less than a three base-pair difference.⁶⁵ In addition, these tools usually search for mismatches rather than insertions or deletions ("indels"). Yet research has shown that "SgRNA with a few indels may induce cleavage," thereby potentially causing off-target events.⁶⁶ Finally, these tools have been designed on the basis of data gathered in human and mouse studies. Consequently, it is not clear whether they can be applied to different types of cells and species.⁶⁷

Site selection is another challenge for CRISPR users. Indeed, selecting a target site that has few matching sites throughout the genome is important to minimize off-target events. Here, too, a variety of web-based computational tools can help users predict potential off-target events. Nevertheless, all of them have advantages and disadvantages, requiring their users to select

the one most appropriate for the work at hand.^{66,68} Importantly, none of these tools can predict off-target events with accuracy. Indeed, recent studies comparing the performance of various available tools have shown that they yield different predictions and miss a lot of mutations when their predictions are compared to results obtained from whole genome sequencing.^{67,69,70} Therefore, there is currently no easy solution to avoid off-target effects using CRISPR. This problem underscores the importance of using "unbiased" means of identifying off-target events (such as whole genome sequencing [WGS]), rather than relying on sequencing only the off-target sites predicted by algorithms.

Interestingly, other studies have shown that different methods of WGS can also produce varying results. In one study, WGS with multiplex Degenome-seq identified more than 700 mutations missed by another method called "GUIDE-seq"; at the same time, for some genes, Guide-seq identified more than 100 off-target sites missed by Degenome-seq. Therefore, the authors suggest using at least two WGS methods to achieve a more complete picture of off-target effects.⁷¹ This, of course, increases the cost, time, and expertise required for CRISPR research. It is worth noting that GUIDE-seq and Degenome-seq have been used in human studies but not in plants. In plants, CRISPR/Cas9 potentially produces more off-target effects than other gene-editing techniques, such as TALENS.⁷²

It is worth noting that although the cost of sequencing has been decreasing over time, the process is still expensive — costing up to \$10,000, depending on the number of samples and level of resolution.⁷³ Other methods, such as biochemically labeling the double-stranded breaks, can identify the places where CRISPR has cut the DNA, but this approach does not identify earlier cuts that may already have been repaired.⁷³ Using any of the foregoing approaches would also require working with bioinformatics experts, using customized software to analyze the resulting data. The need for additional expertise, tools, and costs is not factored in when people tout the "cheap and easy" characteristics of CRISPR.

Some of the solutions to these hidden challenges offered in the literature include (but are not limited to) using slightly shorter or slightly longer RNA guides, modifying the Cas9 enzyme to increase accuracy, using Cas9 inhibitors to reduce cleavage activity, and using novel Cas9 ortholog or other Cas enzymes.^{64,67,65,74,75,76,77,78} But as demonstrated in experimental work, these solutions work well in some cases but not in others.

This underscores the need for users to have sufficient knowledge to identify the source of the problem, use the appropriate solution for the type of cells she is working with, and devote significant troubleshooting to adapt a protocol to a new experiment or system. Just because one experiment is published showing how to minimize off-target effects does not mean that it can be readily adopted by others to work in a variety of systems. Oftentimes there is a need to translate and adapt the protocol, and in some cases the published experiment just may not work for a different system.⁷⁹

Fundamentally, off-target effects are important to consider when evaluating the security threat posed by CRISPR, because they show that the technology is not as easy, quick, and cheap as many media and scientific articles make it out to be. A detailed review of the type of off-target effects and the current solutions available indicates that (1) there are still many unknowns regarding how CRISPR works, and (2) the variety of problems and solutions available require the users to have expertise that is not necessarily widespread or easily acquired. For example, a recent study comparing the number of CRISPR reagents requested from Addgene and the number of papers published on CRISPR-related research shows that although a lot of researchers experiment with CRISPR, a very small number of them actually obtain results worth publishing.⁸⁰ In addition, the paper shows that proximity to the two American centers at the forefront of CRISPR research (Berkeley, Broad Institute/MIT) leads to a greater level of success. This indicates that a certain level of tacit knowledge is required to achieve positive results.⁸⁰ These factors have important implications for the ease with which a state or nonstate actor might choose to develop CRISPR for bioweapons purposes.

The issue of off-target effects will likely remain controversial and a pressing research issue in CRISPR; however, it is a critical question that needs to be addressed and not shut down merely because it creates complications for stock shares, advocates of the technology, and advocates of the impending threat that CRISPR poses. These off-target effects have been discussed in the context of ethical concerns about the use of CRISPR to treat a variety of medical conditions in humans and animals. At the same time, however, there has been little discussion of how these off-target effects can also cause problems for those wishing to develop a bioweapons capability using CRISPR. If one cannot control these off-target problems (or cannot set up a gene-editing experiment correctly), then would-be bioweaponeers would face the

same mutation challenges as has been seen with the use of classical genetic engineering techniques and in synthetic genomics approaches.⁷ For a historical example on this point, the Soviet bioweapons program is instructive. Igor Domaraskij, a leading bioweapons scientist at the Obolensk Institute in Russia, noted, “We needed to develop a strain of tularemia that was resistant to a large number of antibiotics. We came up with several, but during tests on animals the strains turned out to be less virulent than the original strains. The strains had lost their virulence. . . . It did not work out.”⁸¹ Thus, although the Soviets had used genetic engineering techniques to develop a novel antibiotic-resistant strain of tularemia, it proved worthless as a biological weapon. Recent discussions that one of us (Vogel) has had with a group of senior U.S. intelligence analysts indicate that they are also skeptical of the near-term bioweapons threat posed by CRISPR — particularly by nonstate actors.⁸²

Synthetic genomics and synthetic biology also provide a cautionary note to those jumping to quick conclusions about the potential for bioterrorism with advances in biology. Although synthetic genomics and synthetic biology were touted in the early 2000s as harbingers of the creation of new types of biological weapons, after more than 15 years, this yet remains to be seen.⁸³ This is because oligo synthesis and gene assembly techniques are prone to errors;⁸⁴ therefore, this requires that gene-length fragments be cloned and verified by sequencing, which can add to the final cost and time needed to do the work. Some gene synthesis work remains a challenge even to expert gene synthesis researchers and gene synthesis companies. Devin Leaks, vice president of research and development at Gen9, a gene synthesis company in Cambridge, Massachusetts, notes that “[t]his has resulted in a bottleneck that has prevented gene synthesis technology from reaching the Moore’s Law-like exponential growth curve observed in DNA sequencing over the years.”⁸⁵ Computational biologist Jack Schonbrun summarizes the problem: “The synthetic part is easy, it’s the biology part that’s confounding.”⁸⁶ This is related to the inherent problem of biological complexity that experts have raised cautions about over the years.⁸⁷ As Ernesto Andrianantoandro comments, “Despite the increasing impact of synthetic biology across research areas, many issues need to be resolved. . . . Fundamental challenges in engineering and design also remain. Modularity, context-dependence, reliability, and robustness to evolution are essential conceptual building

blocks for the guiding principles of synthetic biology.”⁸⁸ Bijan Zakari and Peter Carr note, “The inconstancy of engineered living systems is one factor that threatens to limit what we can achieve, leading us to speculate on the ultimate limits of synthetic biology.”⁸⁹

Just because the construction of synthetic genomes is not error- or problem-free does not mean that genomes cannot be made. From the synthesis of the first bacterial genome in 2008 to the creation of a variety of subsequent synthetic genomes and genome-size fragments, to the current laboratory consortium to create the first synthetic yeast genome, all of these research efforts show that it is possible to synthesize different kinds of genomes now, compared with the first artificial synthesis of the polio virus in 2002.^{90,91} However, examining these synthetic approaches does not indicate that creating synthetic genomes has become as easy and carefree as was touted in the early years of synthetic biology/synthetic genomics. The current consortium effort to create the first synthetic yeast genome involves a dozen laboratories from five highly developed countries: the United States, the United Kingdom, Australia, China, and Singapore. Therefore, synthetic genomics has not become trivial over time, but still requires teams of researchers, specialized know-how, significant funding, and infrastructure.

CRISPR is still a young technology, and how its off-target problems will turn out remains to be seen: will they be solved quickly and cheaply, with minimal expertise required? Or will CRISPR follow the path of synthetic biology and synthetic genomics, in which error generation in genetic sequences has remained a complex problem for more than 15 years. In the latter case, we would expect to see a lot of scientific focus on problem-solving around the off-target effects problem, and likely less progress on the applications — which would have implications regarding who might be able to use CRISPR for nefarious purposes and what funding, infrastructure, troubleshooting, expertise, and other resources would be required. It would also suggest that the current focus on lone-wolf terrorists’ use of CRISPR is misguided, and that the potential use of CRISPR by states needs to be analyzed in light of these technical challenges.

Problems with replication and diffusion

Another issue that comes up with CRISPR — particularly related to its misuse — is how easily this technology could be adopted and used by those wishing

to cause harm; essentially, this is a question of technological diffusion and technology transfer. Similar to the off-target problem, CRISPR diffusion currently shows a mixed bag of results based on empirical data and requires that one be careful to specify which aspects of CRISPR are able to diffuse and be replicated by other labs and what has proved more problematic. As the literature reveals, not all CRISPR research demonstrates the same ability to be replicated and transferred to other labs.

As noted earlier, the challenges related to guide RNA design are compounded by the fact that design rules do not translate easily from one type of cell to another. As a result, a malevolent actor trying to replicate published work done with a specific guide RNA on a specific cell may not be able to apply it to the organism they are interested in. Additionally, rapid diffusion of a technology does not necessarily mean successful use. The Thompson and Zyontz study, cited earlier, shows that although a lot of scientists experiment with CRISPR, only a few achieve results. Additionally, the variety of troubleshooting solutions cited in the literature would require even a trained researcher to have enough familiarity with the options and technologies to select the right one that might work for her experiment, test it, and solve additional problems that may arise. Such uncertainties would pose potentially insurmountable challenges to an untrained individual unfamiliar with the intricacies of each option and technology. Finally, it is also important to weigh in on the role of the sociotechnical environment, and particularly the role of tacit knowledge, in an individual’s/group’s ability to replicate CRISPR work.

It is important to note that reproducibility challenges are not uncommon in science. According to a 2016 poll of 1,500 scientists reported in the journal *Nature*, 70% of them had failed to reproduce at least one other scientist’s experiment (50% had failed to reproduce one of their own experiments).⁹² Among those surveyed, although 52% agreed that there is a significant crisis of reproducibility, less than 31% thought that failure to reproduce published results means that the result is probably wrong.⁹² Researchers are also becoming more and more aware of the various factors that may explain the inability of their colleagues to replicate their work. For example, in two recent cases of scientific replication — one related to cancer research⁹³ and another related to worm aging⁹⁴ — the original authors of the research and the researchers who failed to replicate their work decided to cooperate in order to identify the

sources of problem in replication. Although they agreed to standardize their methods, using the same equipment, reagent, and techniques, problems persisted. Email exchanges and phone conversations could not solve these problems. It was only when the scientists from both teams worked side by side in the same lab that they could identify seemingly unimportant practices that had vexed replication. In the cancer study, small differences in how scientists isolated the cells produced radically different results. In one lab, the scientists stirred the cells gently; in the other, the cells were shaken vigorously.⁹³ In the worm study, research found that the way the scientists picked up the worms to place them in a different agar plate produced different results. But they also found that other variables that cannot necessarily be controlled, such as small variations in temperature in incubators and lab benches — or food quality — continued to generate differences in results between the different teams *and* within the same lab.⁹⁴

The CRISPR literature is filled with examples of “simple tinkering” of a process to achieve better results. So it would not be surprising to see cases of irreproducibility in CRISPR work as well. Therefore, it is important to understand the variability in lab procedures, protocols, and practices, as well as differences in their environment, to identify challenges in reproducibility. In this respect, the case of the NgAgo study is revealing, as it illustrates both the problem of reproducibility and the challenges of finding solutions to the off-target effect of CRISPR/Cas9 (NgAgo was presented as an alternative to CRISPR/Cas9).

In May 2016, Gao et al. (subsequently referred to as the Han lab, in China) published a paper in *Nature Biotechnology* reporting that the enzyme NgAgo can be used to edit mammalian genes — this would serve as a gene-editing technique alternative to the CRISPR-Cas9 system.⁹⁵ In the paper, the scientists used a wide variety of genetic sequences to guide NgAgo to edit eight different genes in human cells and to insert genes at specific points on chromosomes. This technique was seen as an improvement over the CRISPR-Cas9 system, because NgAgo cuts only the target genes, whereas CRISPR-Cas9 can have off-target effects; also, CRISPR-Cas9 requires certain genetic sequences to be near the cutting site in order to start the editing. In spite of limited efficiency (21% to 45% at various targets) in cleaving mammalian genomes, NgAgo was suggested as a more accurate, versatile, and cheaper gene editor than CRISPR-Cas9.

Starting in July 2016, scientists in China and other countries began raising concerns that they could not replicate the results of the experiment. An online survey by molecular biologist Pooran Dewari of the MRC Centre for Regenerative Medicine in Edinburgh, Scotland, found that only 9 out of 105 researchers could get NgAgo to work.^{96,97} Chunyu Han, the principal investigator of the NgAgo experiment, indicated that he has only been able to get the system to work on cells cultured in his laboratory. The experiment failed when using cells purchased from an outside source, which were later found to be contaminated with bacteria. Other scientists have said that the NgAgo system worked but was less efficient than CRISPR-Cas9. In an online biology forum, Han outlined a series of details and the need for “superb experimental skills” to avoid bacterial contamination and other problems.⁹⁸ Although visiting researchers went to Han’s laboratory, they were not allowed to perform genome-editing experiments involving mammalian cells when they were there, and therefore they were unable to learn the use of the technique from Han’s lab.⁹⁹

With these results, Burgio has stated that the NgAgo system “might work. . . but if so, it’s so challenging that it’s not worth pursuing.”⁹⁶ There have been a set of papers published indicating failed replications of NgAgo; some of these have involved the use of genetic materials provided by the Han lab.^{69,99,100,101} In addition, groups who had earlier reported their initial success in reproducing the results have not been able to bolster their preliminary data to a publishable level.¹⁰² Similar to the controversial Schaefer et al. 2017 off-targets paper, the editors at *Nature Biotechnology* published in November 2017 an “expression of concern” to accompany the original Han et al. paper. Internal and external review of the paper by *Nature Biotechnology*, however, found no obvious flaws or errors in the experimental work.¹⁰²

In August 2017, Han and colleagues retracted their NgAgo paper because “of the continued inability of the research community to replicate the key results.” However, Han argued that the NgAgo system is only effective when “key requirements are met.” According to *Nature*, Han has revealed that he had identified a contaminant that explains why he and others have been unable to reproduce the NgAgo results.¹⁰³ Although there have been accusations of fraud in the Han et al. study, the problem of replication may be due to factors that can be revealed only if the author works side by side with others in the same lab, as was the case for the cancer and worm studies discussed earlier.

The challenge of replication is not merely a contemporary problem. As detailed studies by sociologist of science H. M. Collins and others have shown over the past 30 years, the question of what counts as replication is critically connected to what counts as the “same” equipment/materials/infrastructure/conditions and what is considered “competent” or “proper” technique.^{104,105,106} This is nontrivial, because scientific work is designed and implemented under very specific local conditions and practices and can involve particular materials and experimental setups, which often have tacit dimensions; therefore, it takes work to be able to adapt and transfer the tacit dimensions of laboratory work to successfully work in new contexts. The popular notion of the ease of scientific replication is contradicted by experimental practice and decades of published papers in the scientific and social science community illustrating how difficult and precarious replication can actually be, particularly in cutting-edge areas of science and technology.¹⁰⁴

For example, in certain kinds of biological work that uses fetal bovine serum, scientists have noticed problems in replication of experiments when the type or age of the cows was changed, or when they purchased the serum at different times of year (e.g., with the assumption that cows are eating different things during winter versus summer), or based on whether the animals received hormones or antibiotics.^{7,107} As a result of these problems, laboratories have informally adopted a set of disciplinary practices to mitigate some of this variability (e.g., buying a particular lot of serum in multiyear quantities). These kinds of informal laboratory practices, however, are not mentioned in traditional materials and methods sections and are learned within the community of practitioners that do this work. Cells’ behavior can also change with density, proliferation rates, growth media, the type of lab dish, light conditions, the presence of contaminants and time kept in culture, and personal scientist idiosyncrasies in lab work. Therefore, there are a lot of variables that can change from one lab to another, resulting in problems for replication. Sociologists of science Karin Knorr Cetina, Bruno Latour, and Steve Woolgar describe laboratory research that involves a tremendous amount of tinkering and skill to make recalcitrant laboratory material do what it is supposed to do.^{108,109}

This is not to say that scientists cannot reproduce laboratory methods or findings, but published methods and protocols do not necessarily produce a scientific practice that can be easily replicated in a new

environment by individuals with no prior exposure to how the practice was originally constituted. In addition, making a technology standardized, which is easy to diffuse, is not trivial — it often requires a significant amount of work to make the technology de-localized and translatable to work in a new context.¹⁰⁸ In addition, the world of controlled laboratory experiments is different from the real world — scientists purposefully construct systems in order to escape the messiness of nature, to study controlled, cleaned, and purified phenomena.^{108,110,111} Thus, scientists can make an experiment work under the highly controlled and ordered environment of a lab, but it can fail in the natural world or when conditions change. This is particularly the case in cutting-edge science and technology.

Therefore, to really understand how, when, and under what circumstances CRISPR can travel to new contexts and be reliably used, one has to understand the requirements for the social distribution of knowledge in various gene-editing systems — some may be easier to replicate and transfer than others. Currently, we do not know these requirements for CRISPR, because no studies have been done to compare and contrast the difficulty of replication of different CRISPR experiments. The problems in scientific replication also underscore the need to study failure in experiments. If one is able to understand why a particular experiment did not work as it should have, this can give indicators as to the “hardness” of the skill level required for the task or other local conditions and factors that have to be met in order to successfully use the technique. These factors also have implications for how easy or difficult it might be for a would-be bioterrorist to replicate a gene-editing experiment for harmful purposes.

Other challenges

Off-target and reproducibility challenges are only two of the many challenges that an individual or group might face in using CRISPR for malevolent purposes. There are also challenges in delivering the CRISPR system to the desired organ or tissue. Currently, human applications of CRISPR-based treatments of diseases such as cancer involve *ex vivo* manipulations: cells are extracted from the patient, edited, and re-injected into the individual. But there are no good ways to deliver the CRISPR system directly into a target organ, as would be necessary for a bioweapon. Scientists have explored the use of plasmids or viruses, but both options can trigger the immune system, thus deactivating CRISPR,

or they may persist in the individual's body and cause off-target effects. The CRISPR system is also too large to fit in one viral vector, so generally two viral vectors are needed to ferry different components of the CRISPR system. Others are exploring the use of lipid or gold nanoparticles to deliver CRISPR. But these options are still under study.¹¹²

The bigger challenge might just be that of nature itself. A recent study indicated that some individuals may carry immune proteins and cells that target the Cas9 protein, which might inactivate CRISPR or cause dangerous side effects.¹¹³ Another study showed that HIV can develop mutations near the site targeted by CRISPR, thus evading the immune system. More importantly, the editing performed by CRISPR introduced mutations that helped the virus escape CRISPR.¹¹⁴ Gene drives are not immune to such challenges: a recent study involving the use of gene drives to reduce the fertility of female *Anopheles gambiae* mosquitoes showed that the insects evolved a natural mutation that restored the animal's fertility.¹¹⁵ These examples show that the CRISPR-Cas9 system may not behave as scientists (or those with a nefarious purpose) might intend.

Technique versus system for bioweapons capability

With all of the foregoing said, however, let us assume that one is able to reliably and accurately mutate a pathogen using CRISPR or one of its gene-editing variants. This does not mean that one automatically has a biological weapon. Creating a mass-casualty biological weapon requires more than mere access to a pathogen, a particular technique, or a piece of biotech equipment — although all of these are important components. To create a potent bioweapons capability, there are a variety of technical issues that must be addressed. First, one must acquire access to a virulent pathogen or toxin — CRISPR may allow one to create a lethal, or more lethal, variant of a pathogen or toxin, but this is not without problems. As noted earlier, the Soviet Union learned the hard way that even though they created a highly antibiotic-resistant strain of tularemia bacteria, it was so environmentally sensitive that it could not survive in the environment. Therefore, merely being able to genetically engineer a pathogen did not ensure a viable bioweapons capability. Also, one still has to overcome other hurdles. Unless one creates a highly infectious agent, a would-be terrorist would have to

determine how to produce larger quantities of the agent. Production in larger batches is not as trivial as growth of an agent in a petri dish. The former Soviet Union experienced difficulties in scaling up its bioweapons agents in new facilities.¹¹⁶

Along with production, one also has to think about how to protect the agents to survive in the environment, as many biological agents and toxins can be environmentally sensitive. The processing of agents is a key challenge in developing a bioweapons capability — the United States, Russia, and Iraq all encountered problems in creating special formulations to stabilize their bioweapons agents.¹¹⁷ One then has to find an appropriate delivery form of the material (e.g., liquid or dry), which can also pose challenges for viability, and then employ an appropriate delivery device under the right conditions. Former U.S. bioweaponeer Bill Patrick has noted, “You can have the best agent in the world, but the physics of dissemination mean that unless you have good conditions and with a good delivery system to get that *improved* agent on target, you're going to fail.”¹¹⁸ Past state and nonstate bioweapons programs have shown that addressing these factors requires knowledge, specialized skills, and management and organizational expertise, as well as materials, infrastructure, and equipment. Therefore, the important takeaway here is that gene editing is only one small component of an entire process required to produce lethal biological weapons. Unless gene editing (or other science and technology developments) can be shown empirically to alter the other factors of bioweapons development, technical hurdles will remain. This also points to the need to look not only at gene manipulation but also at all of the other factors shaping production, processing, and delivery of bioweapons agents in threat assessments.

A better way forward

In order to better assess security threats related to CRISPR and other gene-editing technologies, we reiterate the need to engage in more complex sociotechnical assessments of gene-editing laboratory work. To date, apart from our own work, we are not aware of other studies that have examined these factors to understand gene-editing developments. This means that many opportunities exist to conduct a variety of in-depth micro- and macro-level studies that can further elucidate specific factors shaping development and diffusion of gene editing. As it is still an emerging technique, now is an

opportune time to begin case studies and track the development of the technique and its changes and outputs over time. The central set of issues that policymakers and biosecurity experts face is how to accurately identify and assess the risks and benefits posed by these new technologies; without better assessments, inadequate or counterproductive policy responses will follow.

One useful starting point for such analysis could be based on the following studies that have been done with synthetic biology. For example, the Woodrow Wilson Center's Synthetic Biology Project started in 2009 and keeps an annual inventory of companies, universities, research institutions, laboratories, and other centers across the globe that are active in synthetic biology.¹¹⁹ The project also tracks important changes and features in the field over time (e.g., rate of growth of the field in universities, rate of growth of industries in Europe versus the United States, types of applications of synthetic biology companies). Another report by the European Commission has provided a literature and statistical review of the synthetic biology sector in Europe and North America.^{120,121} This report focuses on specific kinds of research activities in the synthetic biology field and what laboratories and principal investigators are conducting this research; a 2014 report by the European synthetic biology community conducted detailed mapping activities of funding programs across Europe.¹²² Susan Molyneux-Hodgson and Morgan Meyer have traced the beginnings of a synthetic biology network and community making in Europe and the United Kingdom.¹²³ In addition, Paul Oldham, Stephen Hall, and Geoff Burton have conducted a scientometric analysis of scientific literature to identify the range of institutions, researchers, and funding agencies involved in synthetic biology worldwide to better understand the challenges to use and diffusion of the technology and to trace the networks and relations among them.¹²⁴ In doing so, these researchers are attempting to document the linkages and knowledge transfer between countries. These kinds of studies could be applied to study the diffusion of gene-editing research. Although the data counted and generated by these studies have been primarily quantitative, such data could be a starting point to generate and guide further in-depth qualitative studies of gene-editing work in specific companies, laboratories, nations, and regions. For example, the authors of this article have launched a new research project that looks at specific micro- and macro-level case studies of gene-editing research worldwide.

At the micro level, key laboratory research and the development of applied products from it could be probed to examine the role of tacit knowledge in this work and to what extent gene editing is eliminating the need for tacit knowledge in life science research — or is instead creating requirements for new types of tacit knowledge. At the moment, there are not enough fine-grained analyses of different kinds of gene-editing work to be able to parse these differences. Other studies could begin to examine what sociotechnical and socio-organizational bottlenecks have emerged in translating gene-editing research to more applied products. Finally, there remains a rich area of study that could be devoted to conducting in-depth national, regional, and transnational case studies of gene editing that look at the social, legal, regulatory, cultural, political, and economic factors that have shaped the development and diffusion of gene editing in each of these contexts.¹²⁵ We have begun this work, but there is much that remains to be done in this space.

Such research could produce more realistic assessments and help shape more informed and appropriate policy responses. For example, with better national and international understanding of the types of CRISPR-related research being conducted, we can begin to review corresponding national and international regulatory and policy frameworks to see whether there are gaps, as well as best practices, in biosafety and biosecurity measures in particular countries. This knowledge could also lead to new types of training programs (biosecurity, bioethics) for practicing scientists that emphasize responsible research practices. Examining the scientific literature with both qualitative and quantitative understandings of gene editing can identify particularly “risky research” and provide a focal point for policy deliberations and intervention in those settings — rather than gene-editing research as a whole. U.S. government funding agencies such as the National Institutes of Health, Department of Defense, Defense Advanced Research Projects Agency, and National Science Foundation, as well as private foundations, could play an important role in issuing funding calls for such research — the costs of which would be small compared with the cost of unnecessarily directing government attention and resources toward an imagined gene-editing threat.

Conclusion

This article has outlined the range of complexities and contingencies that remain in assessing the

biosecurity threat of CRISPR and related gene-editing technologies. Although there is still a lot to be evaluated in terms of how these technologies will develop in the near and distant future, it is clear that we need to start now in collecting more and varied types of empirical data about how these technologies are developing and diffusing. We also need to situate empirical data about CRISPR in conversation with what we know about past and present biosecurity concerns, rather than looking at CRISPR in isolation. In addition, there is a vast literature and set of case studies on the sociotechnical character of other types of life science and biotechnology work that can be used to create more informed understandings of what is happening with CRISPR — and whether, how, and to what extent CRISPR differs from other scientific developments in biology. We need this kind of data and analysis to better inform U.S. policymakers on whether to prioritize gene editing as a biosecurity threat, and if so, how this might create new types of regulatory or oversight actions that are deemed necessary. These need to be evidence-informed decisions — and right now (as has been the case with so many past biosecurity threats), there is very little evidence to inform decision-making.

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Anticipating emerging biotechnology threats

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