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In Our Image: The Ethics of CRISPR Genome Editing

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Abstract: The advent of genome editing technology promises to transform human health, livestock and agriculture, and to eradicate pest species. This transformative power demands urgent scrutiny and resolution of the ethical conflicts attached to the creation and release of engineered genomes. Here, I discuss the ethics surrounding the transformative CRISPR/Cas9-mediated genome editing technology in the contexts of human genome editing to eradicate genetic disease and of gene drive technology to eradicate animal vectors of human disease.

Keywords: somatic cell editing; reproductive CRISPR; gene drive.

> "The power to control our species' genetic future is awesome and terrifying. Deciding how to handle it may be the biggest challenge we have ever faced."
>
> ~ Jennifer A. Doudna

Why genome editing?

The idea of genetic modification to pursue improvement in human lives and society is an old one. For centuries, humans have modified livestock and crops by selective breeding to dramatically improve yield, nutrition, disease resistance and other traits. These strategies, however, have always rested on the fortuitous appearance of natural variants.

With the discovery of the structure of the DNA double helix and the solution of the genetic code, the realistic prospect of genetic engineering started to take form. The subsequent invention of molecular cloning enabled the customization of DNA sequences and thus the first true genetic engineering. Techniques for efficient delivery of engineered DNA into eukaryotic cells eventually led to clinical trials to treat genetic disorders, with mixed results.

By the end of the last century, the challenge facing gene therapy in humans was the ability to deliver a therapeutic gene, in the cases of loss-of-function disorders, and how to inactivate a gain-of-function disorder (e.g., sickle cell disease). While engineered plasmids and viruses have been used successfully to install novel DNA elements in plant and animal genomes, the function of the transgene has often been variable and sometimes creates mutations at the sites of insertion [1].

The grail of genetic engineering in humans, other animals and plants, is genome editing: the modification of a gene either by targeted inactivation or targeted replacement with an altered gene product. The technical challenge is to edit a specific region in a huge genome to (a) yield a predictable outcome without (b) causing unintended secondary modifications and/or effects.

CRISPR-Cas9 and the revolution in genome editing

The first entrants into the genome editing sweepstakes were the zinc finger nucleases and the transcription activator-like effector nucleases (TALENs) [2]. Both technologies rely on delivering a DNA-cleaving endonuclease to a specific genome target based on the recognition of a specific nucleotide sequence target in the major groove of B-form DNA by the iterative use of protein folds. Each targeting peptide sequence is fused to a half of a dimeric endonuclease, so as to improve targeting specificity of the endonuclease cleavage. The double-strand break products at the targeted site can either generate mutations that inactivate a gene function or catalyze the replacement of a sequence at the targeted site by a related sequence (see discussion below and Figure 1).

The identification and biochemical characterization of the Clustered Regularly Interspaced Short Pandromic Repeats (CRISPR)/CRISPR-associated (Cas) bacterial defense system transformed the genome editing field. CRISPR/Cas editing achieves its specificity by using RNA to target a bound protein endonuclease. The specificity

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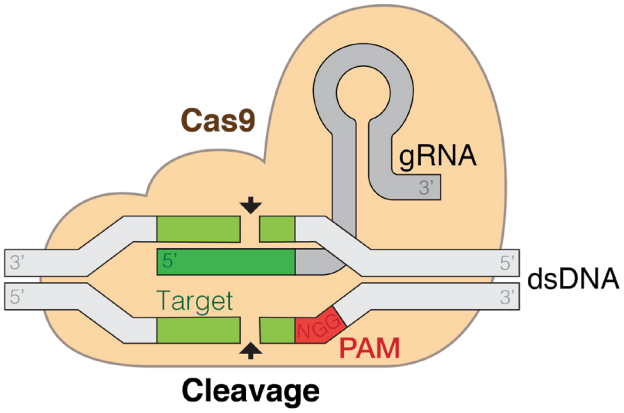

Figure 1: CRISPR/Cas9 targeting and cleavage mechanism. Guide RNA ("gRNA") pairs with one strand of DNA in a region (highlighted in green) of sequence containing the cleavage site. The Cas9 endonuclease (yellow) binds to the RNA-DNA complex and cleaves both strands of DNA at a position indicated by the arrows, creating a double-strand break. Image created by Marius Walter and licensed under Wikimedia Creative Commons [https://commons.wikimedia.org/wiki/File:GRNA-Cas9.png#metadata]

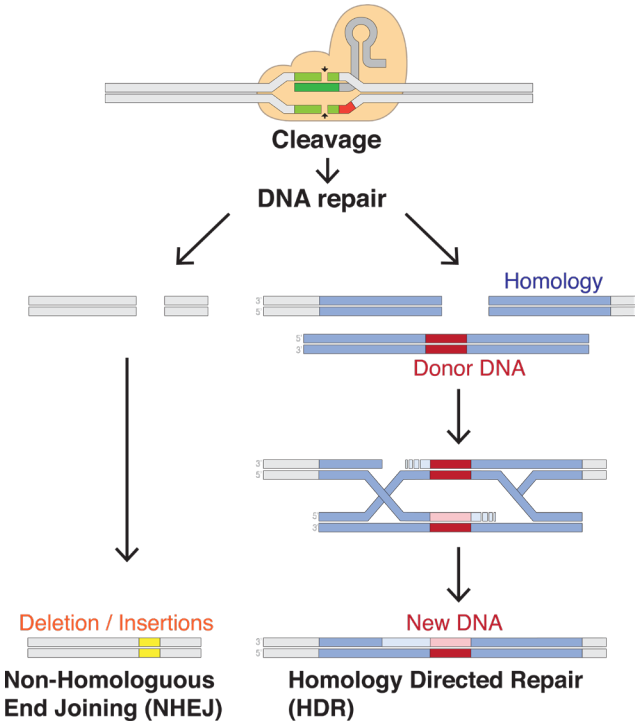

Figure 2: Two fates of double strand breaks created by CRISPR/Cas9. On the left, the break is directly re-sealed by a mechanism called "non-homologous end joining." The sequences at the re-joined ends may differ by short additions or deletions of nucleotides from the original sequence before cleavage. On the right, the break is repaired by aligning the flanking sequences (blue) with a homologous DNA sequence, either from a homologous chromsome or from an engineered DNA containing additional DNA (red) between the homology tracts. Using the standard recombination mechanism, the break is repaired (new DNA) with possible addition of novel DNA sequence at the site of the break. Image created by Marius Walter and licensed under Wikimedia Creative Commons [https://commons.wikimedia.org/wiki/File:DNA_Repair.png]

of nucleic acid macromolecular recognition, together with the relative simplicity of generating targeting RNA compared to targeting DNA-binding peptides, has made the CRISPR/Cas strategy the method of choice for most applications.

As with the zinc finger endonucleases and TALENs, CRISPR/Cas generates a double-strand DNA break at targeted sites. For genome editing, a CRISPR guide RNA is engineered to base-pair with a target site in a chromosome. The Cas endonuclease, bound to the guide RNA, then cleaves both strands of DNA at a site targeted by the guide RNA (Figure 1). The default response of the cell is to repair the break by non-homologous end-joining, an error-prone mechanism that often introduces deletions or insertions of nucleotides at the break site, and thus creates mutations (Figure 2). However, if an intact homologous DNA molecule is available, the double-strand break site can become a substrate for homologous recombination, creating the possibility to introduce a different, albeit related DNA sequence at the repair site (Figure 2). This versatile system has been engineered from the natural components into a simpler system that has found wide application in fungi, animals and human cells. For most purposes, the Cas component is a protein called Cas9 from the bacterium *Streptococcus pyogenes*.

The versatility and specificity of CRISPR/Cas genome editing has generated new toolkits for disease therapies in humans [3], crop and livestock improvement [45], and control of animal and plant pest species [6,7]. With this new power to remake ourselves and our planet in our own image comes new responsibilities. Here, I discuss some of the myriad applications of CRISPR/Cas and the ethical ramifications of these applications.

Applications of CRISPR/Cas9 in humans

Somatic cell genome editing

A major therapeutic goal for genome editing in treatment of genetic diseases is to edit the genome of stem cells for the affected tissue of a patient. As an example, consider patients with sickle cell disease. These individuals carry

on both chromosome 11 homologs a specific missense mutation in codon 6 of the adult beta globin gene. This mutation results in abnormal polymerization of the hemoglobin in their red blood cells under conditions of low oxygen tension. The polymerization results in distorted, sickle-shaped erythrocytes that clog capillaries, causing local pain and tissue infarcts. Damaged erythrocytes result in anemia and repeated sickling crises cause long-term organ damage and premature death.

Since sickle cell disease is caused by a known single nucleotide change, it is a highly attractive target for genome editing. Furthermore, individuals who carry one sickle cell allele and one normal adult beta globin allele are essentially asymptomatic, so even editing one allele would be therapeutic. To accomplish this, it is necessary to deliver not only a CRISPR/Cas9 complex targeted to the 6^{th} codon mutation, but also a DNA molecule containing the normal sequence to be used as a repair template. While straightforward in concept, the challenge of recovering, editing and regrafting autologous stem cells from the patient is further complicated by the observation that stem cells do homologous recombination inefficiently and seem to prefer to repair double-strand breaks by non-homologous end joining.

Alternative strategies for sickle cell gene editing therapies build on an observation that some patients who are homozygous for the sickle cell allele have milder symptoms because of hereditary persistence of fetal hemoglobin gene expression, a gene normally silenced before birth. Thus, current clinical trials are focused on either mutating the silencer binding site upstream of the fetal globin genes or mutating the *BCL11A* gene encoding the repressor protein. In these cases, the mutation doesn't have to be precise and can be accomplished by the non-homologous end-joining mechanism.

A major technical challenge of somatic cell genome editing as a therapy is targeting the stem cells of the affected tissue. A single cycle of *in situ* genome editing targeted to stem cells would be ideal, since repeated cycles of genome editing risks triggering an immune response to the editing machinery. In the case of hematopoietic disorders, access, *ex vivo* manipulation and re-grafting modified bone marrow stem cells is relatively straightforward [8]. For solid tissues, targeting stem cells will pose the major challenge to stem cell genome editing. Promising results have been achieved in a mouse model of Duchenne's muscular dystrophy using an adeno-associated virus delivery system for CRISPR/Cas9 [9]. Proof-of-principle genome editing for several mouse models of inherited diseases has been performed with varying degrees of success [10,11]. A growing number of pioneering clinical trials are underway for CRISPR/Cas9-based therapies for certain inherited diseases and for cancer.

From an ethical standpoint, somatic cell genome editing is no more problematic than transgenic therapies. That isn't to say that somatic cell genetic engineering technology is risk-free [see, e.g., the case of Jesse Gelsinger (https://www.sciencehistory.org/distillations/the-death-of-jesse-gelsinger-20-years-later)], only that (1) the subjects of such technology can give informed consent and (2) the resulting modified human cells die with the subject and cannot contribute to the human gene pool.

Germline genome editing

An obvious way to bypass the technical hurdle of somatic stem cell access is to perform the editing using gametes or cleavage stage cells, so-called "germline genome editing." These strategies produce engineered cells that are totipotent, giving rise to all somatic cell lineages, as well as the germ line. Genome editing using embryos has successfully been performed in a variety of animal models [12], and notoriously in humans by Dr. He Jiankui in China [13]. Other strategies could include genomic editing of sperm or egg cells prior to fertilization.

It is critical to emphasize the distinction between somatic cell editing and germline editing. Rulli [14] underscores this distinction for CRISPR-based editing by rebranding it as "reproductive CRISPR," or rCRISPR. With somatic cell CRISPR editing, the goal is to treat an individual with a disease or condition to prevent premature death and/or relieve suffering. With rCRISPR, the goal is to create a healthy person whose existence was not inevitable.

With somatic cell editing, the promise is to cure disease, or at least mitigate the suffering in the treated individual. Does rCRISPR cure disease in an individual who would otherwise suffer from it? The answer is "no," since in the case of rCRISPR, the existence of an individual with an untreated condition is not inevitable [14]. In the case of the rCRISPR babies created by He Jiankui, the ostensible therapeutic goal was to prevent HIV infection from their HIV-positive father. This goal can already be achieved by washing sperm free of virus.

In the absence of rCRISPR, couples can still parent healthy children through a number of alternative mechanisms that do not risk transmitting engineered human genomes to future generations:

1. Pre-implantation genetic diagnosis can be used to screen for embryos free of a specific genetic defect during *in vitro* fertilization [15];

2. In rare cases where both parents are homozygous for the genetic trait, a sperm or egg donor can be used to contribute a normal allele to generate asymptomatic heterozygous embryos that still carries the genetic endowment of one of the parents;
3. Of course, couples may also adopt and raise genetically unrelated children.

Thus, rCRISPR is not morally urgent. No person who is sick will be made well by rCRISPR. The case against rCRISPR includes technical concerns as well. The human genome is large, creating the potential for off-target modifications. Thus, double-strand breaks could be created at unintended sites with unknown consequences. Since most of the human genome is comprised of DNA with no known function, local DNA sequence modifications may well have no effect, but this cannot be guaranteed. However, these concerns were made more urgent recently by a set of studies that uncovered off-target large-scale genome modifications in human embryos resulting from germline CRISPR editing. These include large sequence deletions and rearrangements that can be missed by standard testing methods [16]. The mechanism(s) underlying these changes are poorly understood.

Nevertheless, any future solution to off-target germline edits still fails to provide a compelling ethical case for rCRISPR in humans. Moreover, the opportunity cost of rCRISPR research should be considered. What urgent biomedical research will be shortchanged by the diversion of funds to support a technology that, at best, will benefit very few people because of the expense?

A further ethical objection to rCRISPR is that it represents a slippery slope to germline editing for enhancement. If parents can be allowed to edit the genomes of their progeny to correct inborn errors, why not to increase their height at maturity, their capacity to build muscle mass or their intelligence? Indeed, from an ethical standpoint, what is enhancement but correction of perceived limitations or deficiencies? In her book "Altered Inheritance" [17], Françoise Baylis distinguishes between "fast science" and "slow science." In this lexicon, "fast science" refers to the privatization of science and the drive for fast results that sometimes ignore science in the public interest. "Slow science," in contrast, requires negotiating with the broader public, respecting the big questions like how the science improves the human condition. Baylis doesn't offer a prescription for how to choose between fast and slow science, only an appeal to conscious and proactive ethical deliberation. There is currently no international consensus on how rCRISPR technology should be regulated and how those regulations should be enforced.

Non-human applications of CRISPR/Cas9

Genome editing in livestock, agriculture

The genetic manipulation of livestock and plants has been an ongoing human project for centuries. A superficial comparison between modern maize and teosinte, its ancestor, provides dramatic testimony to the power of genome modification through selective breeding. Of course, such transformations take hundreds of generations. The advent of genomics, paired with advances in cell biology and physiology, has identified key genes that can be edited to drive to fixation the desired traits in a couple of generations.

Transgenic plants and animals have been around for decades. In some cases, transgenic modifications—such as the expression of Bt toxin in transgenic plants to program insect pest resistance or the expression of provitamin A in transgenic rice—have been controversial, in part because of the introduction of foreign genetic elements in the genomes of edible plant products. A virtue of genome editing for crop and livestock improvement is that no foreign DNA sequences are involved.

An ethical challenge for any genetically modified domesticated organism is the escape of the germ plasm into unmodified domesticated or feral members of the same species. In the case of plants, for example, preventing modified pollination of unmodified plant flowers by nearby modified plants is difficult to guarantee. In the case of, for example, genetically modified salmon, the concern is that farmed modified salmon might escape the breed with unmodified (and sometimes endangered) wild salmon, potentially compromising the fitness of feral conspecifics.

Genome editing and the control of pest species

Various animal species are vectors for human diseases. From ground-dwelling rodents that carry the plague bacillus, to mosquitoes that carry malaria and yellow fever, large-scale plagues and smaller epidemics are transmitted to humans by specific animal species. To eradicate these disease vectors would block disease transmission, saving countless human lives. Various weed species cause significant crop damage and lower yields.

In its simplest form, the application of CRISPR/Cas9 to drive a pest species to extinction would involve

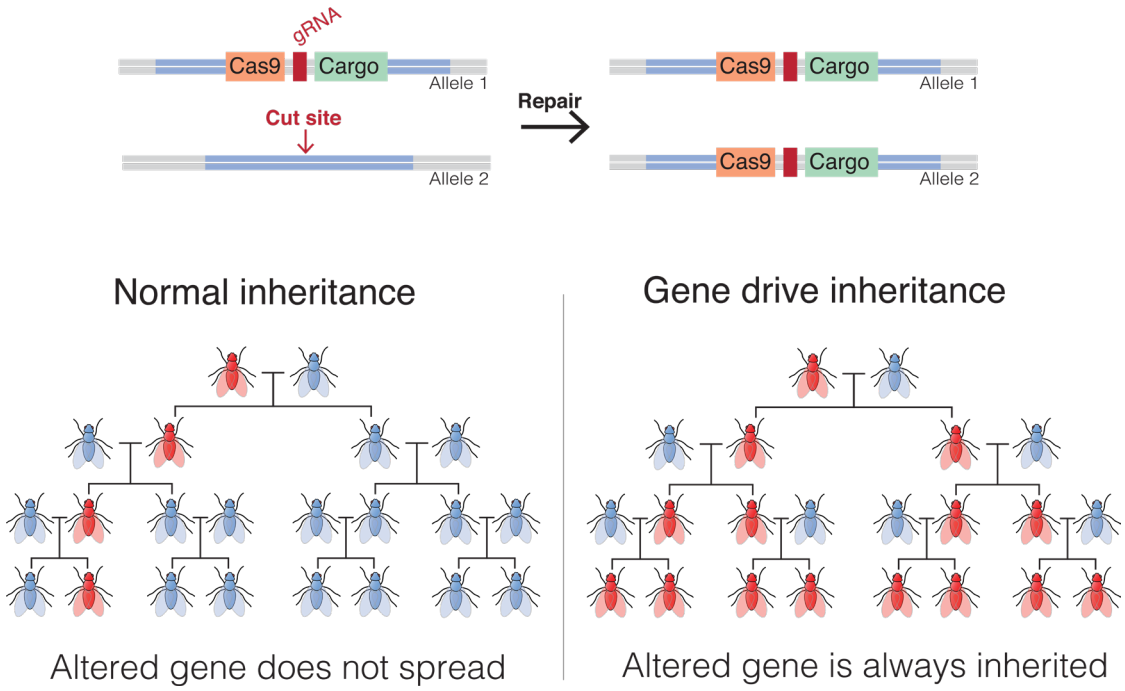

Figure 3:Gene drive mechanism. The upper cartoon shows the conversion of a diploid cell that is heterozygous for a gene drive CRISPR/Cas9 cassette (left) into a cell that is homozygous for the gene drive cassette. The cassette encodes the Cas9 endonuclease (orange box) and the guide RNA (red box), and may contain additional coding sequences (green box labeled "Cargo"), flanked by sequences homolous to those on either side of the cut site in the unmodified chromosome. The lower cartoon contrasts the fate of a transgene in conventional inheritance (left) with the fate of a CRISPR/Cas9 targeting transgene (right). In the absence of CRISPR/Cas9 (left), a fly bearing one copy of the transgene (red) will transmit that chromosome to half of its progeny. With gene drive (right), all progeny of the fly carrying the gene drive cassette (red) will inherit a copy of that cassette. With gene drive, the transgenic flies rapidly outnumber the non-transgenic flies. Image created by Marius Walter and licensed under Wikimedia Creative Commons [https://commons.wikimedia.org/wiki/File:Gene_Drive.png]

establishing a transgene within a gene that is either essential for viability or fertility. The transgene would contain a sequence cassette that encodes guide RNA and endonuclease genes to target the insertion site of the transgene in the normal allele of that locus. Flanking the cassette are the DNA sequences that also flank the target site for the CRISPR/Cas9 double strand break. When the transgene is paired with a normal sequence homolog, the homolog is cleaved and the resulting double strand break can be repaired by homologous recombination using the transgene-bearing chromosome, thus creating a diploid cell homozygous for the transgene-disrupted allele (Figure 3). Thus, instead of a 50% probability of transmitting the transgene, the CRISPR/Cas9 transgene is transmitted to >90% of the progeny in each generation (Figure 3). In principle, a few transgenic flies released into the wild could drive the transgene into the entire population within 15-20 generations. In principle, a successful gene drive could be created for any organism that reproduces sexually, mates freely in the wild and has a short generation time.

When tested in the malaria host *Anopheles gambiae*, transgenes at three separate loci that result in recessive sterility were transmitted to progeny at rates of 91-99% [6].

A limitation with this strategy is that the programmed breaks can be resolved by nonhomologous end-joining, which can destroy the CRISPR-Cas9 target site. As long as this also destroys the gene function, the product still confers sterility. But if the repair results in a deletion or insertion that retains function, the resistant chromosome can spread with a strong selective advantage. Including two or more transgenes are included in the same stock should overcome this problem.

A major technical barrier to success in the implementation of CRISPR/Cas9 eradication of insect pest species is reduced fertility in organisms heterozygous for the transgene insertion. It is also feasible that compensatory mutations in the wild population will suppress the homologous repair mechanism and interfere with the gene drive mechanism. The application of gene drives to weed species, while theoretically feasible, is

additionally challenged by a variety of technical barriers including efficient delivery of the drive transgene, identification of suitable targets to be disabled, the mating systems of weed species, pollen dispersal efficiency, large target population size, seed dormancy and long generation time [7].

Ethics of gene drives

Several concerns have been raised about the ethics of releasing engineered gene drives in the wild [18]:

- Is it ethical to deliberately drive a pest species to extinction? In the case of mosquitoes, there are plenty of other mosquitoes that could potentially fill the Anopheles ecological niche but can't host the falciparum protozoan that causes malaria.
- Who decides whether someone can release a gene drive that can transgress national borders? How is this different from release of an invasive species? These ethical decisions will have to be adjudicated on a case-by-case basis by panels representing all the potentially affected nations. While there are "kill-switch" CRISPR-Cas9 inhibitors that could be introduced to limit or reverse gene drives [19], release of transgenic pest species capable of driving a CRISPR/Cas9 kill switch to fixation in a population would thwart future attempts at pest control using genome editing.

Counterbalancing these ethical concerns:

- Is it ethical to decide not to prevent diseases such as malaria and dengue that kill tens of millions of people every year? Where is the ethical boundary between driving a disease vector to extinction and killing off a species, like non-vector mosquitoes or poison ivy, that are a nuisance but not life-threatening?
- Is it an ethical imperative to replace the need for pesticides that have collateral effects on human health and/or the environment? How are alternative pest control measures, like sterile male release, to be compared to the risk/benefits of gene drives?

The National Academies of Science, Engineering and Medicine published an analysis of gene drives and their impact on human health in 2016 [20]. While it raises many pertinent issues, it offers no concrete ethical landmarks to guide public debate. While the potential benefits are obvious, the risks are speculative since the technology has yet to be applied in the field.

Summary

The breathtaking speed with which CRISPR/Cas has been adapted to genomic editing is outrunning the ability of the human community to question the many intended applications. While the potential to dramatically improve human health and well-being through the use of genomic editing strategies is certainly exciting and a deserving focus of resources, the application of this powerful toolkit must be constrained by deliberation and some form of consent by representatives of all stakeholders. In the case of the human genome, even when the day comes that all off-target modifications can be prevented, the ethics of introducing novel modifications to the gene pool must inform any use of reproductive CRISPR. Humanity now has the power to engineer the living world, including itself, in its own image. In the words of Venki Ramakrishnan,: "When considering what we can do with technology, we also need to consider what we should do."

Conflict of interest: The author states no conflict of interest

Data Availability Statement: Data sharing is not applicable to this article as no datasets were generated or analysed during the current study.

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