# *The Mechanism and Applications of CRISPR-Cas9*

Paul Scherz

*Abstract.* The recently developed CRISPR-Cas9 gene editing technology is transforming basic biomedical research, but it also may have therapeutic applications. This essay examines how the technology works, its possible applications in somatic and germline cell therapy, and the use of gene drives to control disease vectors like mosquito-borne illnesses. While potentially valuable, all of these applications present ethical problems, including the specific risks of unintentional mutations; pre-existing concerns over the relationship between biomedical technology, power, and procreation; and CRISPR's unintended consequences for the environment. *National Catholic Bioethics Quarterly* 17.1 (Spring 2017): 29–36.

By allowing cheap, easy, precise, and efficient genetic modification, CRISPR-Cas9 is transforming biomedical research. CRISPR gives geneticists tremendous power. They can mutate or replace almost any gene in the genome and even alter multiple genes at the same time. Instead of depending on random mutations in whole organisms, they can use CRISPR to induce a large number of mutations in cell lines and identify genes that cause desired traits. CRISPR also enables researchers to induce chromosomal abnormalities, and they can use altered CRISPR proteins to turn genes on or off instead of changing the sequence of DNA.

Paul Scherz, PhD, MTS, has doctoral degrees in genetics and moral theology and is an assistant professor of moral theology and ethics in the School of Theology and Religious Studies at the Catholic University of America, in Washington, DC.



 

While most research applications pose few new ethical problems, even some of CRISPR's inventors are wary about using this technique in human therapies and enhancements, especially ones that alter the human germline. These fears have inspired calls for a moratorium not heard since the development of genetic recombination.[1] This essay describes the mechanism of CRISPR technology and its possible applications, highlighting three areas of concern for Catholic medical ethics: the risk associated with somatic cell gene therapy, techniques of germline manipulation, and the environmental consequences of preventing mosquito-borne diseases with gene drives. CRISPR has immense potential to fight both communicable and non-communicable diseases, but this very power leads to many ethical questions.

## CRISPR's Mechanism

CRISPR technology is based on naturally occurring mechanisms that many bacteria use as a sort of intracellular immune system to fight viral infection.[2] Instead of antibodies, which we use to defend ourselves against infectious agents, these bacteria use short sequences of about twenty to fifty nucleotides, called guide sequences, that match viral DNA.[3] These sequences can be used to make guide RNAs, which then associate with CRISPR-associated proteins (Cas).[4] These bacterial RNA-protein complexes bind to an attacking virus and cut its DNA, which matches the DNA of the guide sequence. These cuts cause the viral DNA to rapidly degrade, stopping the infection.

CRISPR elicits great excitement because geneticists have learned that they can efficiently modify genes if both strands of the DNA are cut at the target location. Earlier technologies required the difficult and time-consuming task of designing a new protein to cut each target site. With CRISPR, researchers can target almost any sequence in the genome by designing an appropriate guide RNA to match the target DNA. This is comparatively easy. In 2012, Emmanuelle Charpentier and Jennifer Doudna's laboratories at Umeå University in Sweden and at the University of California, Berkeley, respectively, showed that the *Streptococcus pyogenes* CRISPR system could be reconstituted and activated outside of a bacterial cell with just a single protein (Cas9) and a single guide RNA (sgRNA).[5] In 2013, Feng Zhang's

1. I do not address CRISPR's possible use in enhancement because these complex issues, such as the distinction of enhancement and therapy, the normativity of human nature, and even the possibility of enhancing complex traits, go beyond the scope of this essay.

2. See Addison V. Wright, James K. Nuñez, and Jennifer A. Doudna, "Biology and Applications of CRISPR Systems: Harnessing Nature's Toolbox for Genome Engineering," *Cell* 164.1 (January 14, 2016): 29–44.

3. These guide sequences occur in long arrays in the genome, where each guide sequence targeting a separate virus is separated from others by a repeated, short, spacer DNA sequence. Hence, they were named "clustered regularly interspaced short palindromic repeats" (CRISPR).

4. Another short RNA, called tracrRNA, is necessary in natural systems, but artificial systems combine tracrRNA and the guide sequence into a single RNA.

5. Martin Jinek et al., "A Programmable Dual-RNA-Guided DNA Endonuclease in Adaptive Bacterial Immunity," *Science* 6096.337 (August 17, 2012): 816–821. For a history, see Eric S. Lander, "The Heroes of CRISPR," *Cell* 164.1 (January 14, 2016): 18–28.

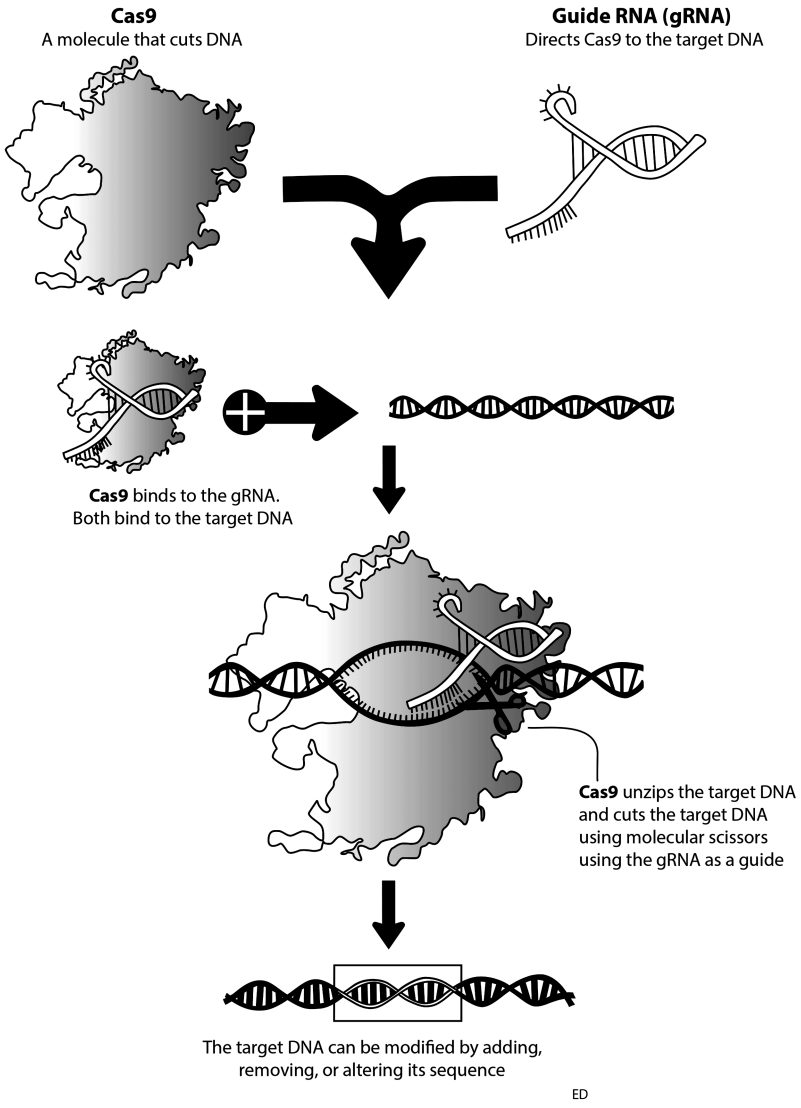


**Figure 1.** The mechanism of action of CRISPR-Cas9.

laboratory at MIT used a modified system to edit the genomes in mouse and human cells, and the system has developed rapidly since.[6]

The first step of CRISPR-mediated genetic modification is getting the system's components into cells.[7] The modification process is summarized in Figure 1.[8] Once in the cell, say a mouse cell, the Cas9–sgRNA complex scans the genome for targeted

6. Le Cong et al., "Multiplex Genome Engineering Using CRISPR/Cas Systems," *Science* 339.6121 (January 3, 2013): 819–823.

7. See Patrick D. Hsu et al., "Development and Applications of CRISPR-Cas9 for Genome Engineering," *Cell* 157.6 (June 5, 2014): 1262–1278.

8. Figure 1 was drawn by Evan Daigle, Providence College, Rhode Island.

sites.[9] After Cas9 binds to a DNA site in the mouse genome, the artificial sgRNA pairs with the mouse DNA. If the genomic DNA is complementary to the sequence of the artificial sgRNA, Cas9 cuts both strands of the targeted double helix. The mouse cell then tries to repair its DNA because breaks are inherently dangerous. There are two mechanisms for repairing a double-stranded DNA break in most cells—one error-prone and one more precise. The more common one, called non-homologous end joining (NHEJ), sutures the ends of the DNA back together, filling any gaps with random nucleotides. This process frequently results in mutations by inserting or deleting a base pair in the target sequence. This extra DNA piece changes the sequence of the gene, which can make the protein encoded by that gene nonfunctional. CRISPR largely acts by mutating a gene and making it not work.

Less frequently, cells use a more complicated repair mechanism called homology-directed repair (HDR) that allows geneticists to insert new sequences into the genome, basically putting any gene they want into a chromosomal location targeted by CRISPR. This process is similar to the way each pair of chromosomes in the precursors of eggs and sperm rearrange themselves during sexual reproduction by exchanging stretches of DNA. Most animal cells contain two copies of every chromosome, one from the organism's father and the other from its mother. A cell repairs the broken section of its DNA by copying a similar DNA sequence from the matching chromosome within the cell. Thus, to fix a broken maternal chromosome III, the mouse cell looks in the paternal copy of the chromosome for non-damaged DNA sequences, which match the region around the break. Using this undamaged DNA as a template, the cell repairs the region around the break by pairing the broken strands from the maternal copy with the complete paternal chromosome.

Geneticists can trick the cell's error-correcting machinery by inserting a piece of DNA containing long sections of similar nucleotides into the cell around the region targeted by CRISPR, basically making the cell think there is another matching chromosome nearby. Using this method, geneticists can replace a diseased gene with a healthy one or put in an entirely new gene into a genomic location. While more powerful, HDR occurs more rarely than NHEJ, so CRISPR usually induces mutations that deactivate a gene.

## Somatic Cell Gene Therapy

The most obvious application for CRISPR is somatic cell gene therapy, in which doctors treat a disease by editing the genomes of adult cells that will not pass their modified DNA to the next generation. Using the patient's adult stem cells, induced pluripotent stem cells, or donor stem cells, doctors could mutate or replace a diseased gene in the lab, grow and multiply the stem cells, and return the edited stem cells back into patients.[10] The most straightforward approach would be to manipulate hematopoietic stem cells, which generate most of the cell types in blood. HSCs are

9. Cas9 grabs DNA at short, 2–5 nucleotide sequences found frequently in the genome, called the protospacer adjacent motif (PAM). All targeted sequences must be next to a PAM site.

10. While biologists could use embryonic stem cells, few researchers today propose using them because of technical and, to a lesser extent, moral reasons.

already targeted in gene replacement therapies for severe combined immunodeficiency. Researchers could also treat non-genetic diseases, for example, by mutating CCR5, the protein that helps HIV enter cells. In principle, patients receiving these modified cells would be resistant to HIV/AIDS. Although HSCs will probably be the first type of cells to be modified, one could make similar changes in any stem cell. Viruses, which have the advantage of infecting a wider range of cell types, could also be used to deliver new genes to target cells. However, Cas9 cannot fit in viruses because of its large size, although researchers are creating a smaller protein to circumvent this obstacle. These techniques could be the foundation for many other therapeutic modifications.

*Dignitas personae* states that somatic cell gene therapy is licit in principle, as long as it meets basic bioethical requirements like informed consent and a proportionate ratio of risks to benefits.[11] However, using CRISPR to modify somatic cells involves many risks. Though it is more accurate than other forms of genetic modification, it still can cut at non-targeted sites in the genome, a problem known as off-target effects, which might lead to another genetic disease or a carcinogenic mutation.[12] These off-target effects occur because CRISPR can tolerate a four base-pair discrepancy and bulges between the sgRNA and target sequence, although this seems to depend on the design of the sgRNA. In one study, an sgRNA led to mutations at over 150 non-targeted sites in the genome, although most sgRNAs lead to far fewer off-target mutations.[13]

Researchers are working on solutions to these problems by designing more specific sgRNAs, but the principles that distinguish a specific from a nonspecific sgRNA are still obscure. Geneticists are also modifying Cas9 to make it more accurate and are evaluating Cas proteins from other bacteria to see if they are more specific. Moreover, researchers have discovered a class of "anti-CRISPR" proteins that inhibit the ability of Cas9 to bind to and cleave DNA. These anti-CRISPR proteins are used by viruses to protect themselves from these bacterial defense systems.[14] Geneticists could use these proteins to modulate the length of time Cas9 is active in cells or the cells in which it acts, to decrease off-target effects. Most simply, geneticists could sequence the entire genome of a modified stem cell population, screening for deleterious mutations before using them in patients. None of these techniques has been standardized yet, and there is debate over the seriousness of risks posed by off-target effects. Some researchers argue that they occur at a similar rate to background

11. Congregation for the Doctrine of the Faith, Instruction *Dignitas personae*, On Certain Bioethics Questions (2008), n. 26.

12. Henriette O'Geen et al., "How Specific Is CRISPR/Cas9 Really?," *Current Opinion in Chemical Biology* 29.1 (December 2015): 72–78.

13. Shengdar Q. Tsai et al., "GUIDE-seq Enables Genome-Wide Profiling of Off-Target Cleavage by CRISPR-Cas9 Nuclease," *Nature Biotechnology* 33.2 (February 2015): 187–198.

14. April Pawluk et al., "Naturally Occurring Off Switches for CRISPR-Cas9," *Cell* 167.7 (December 15, 2016): 1829–1838.

mutations in unmodified cells, and that improvements can eliminate the problem.[15] Others are less convinced that the issue is easily surmountable and believe its persistence would create difficulties for therapeutic applications.[16]

## Germline Gene Therapy

Other ethical concerns arise from using CRISPR to modify germline cells, in which changes to the genome of eggs and sperm are inherited by future generations. Previous technologies required very complicated techniques, but CRISPR can be injected into a single-celled embryo. This technique could transform the genome in every cell of a new human life and is already revolutionizing the production of transgenic animals. *Dignitas personae* n. 26 provides a nuanced ethical analysis of germline gene editing. While not ruling out this therapy in principle, the document cites three problems that make current techniques illicit: First, it creates risk for the child; second, it requires illicit techniques like IVF; finally, it creates risks for all of humanity by introducing new elements into the gene pool.

Off-target effects in a child's genome may play havoc with development or cause future diseases. Furthermore, if CRISPR is injected into single-celled embryos, there is a danger that the system will not start editing the genome until after a few cell divisions have occurred. Consequently, the genome of only some cells in the embryo might be edited, creating a mosaic embryo. We are all mosaics to some extent, since our cells mutate throughout our lives, but it is unclear what problems mosaicism might cause at the early stages of human development. Moreover, this condition may defeat the purpose of genetic modification because the target cells might not be changed. These problems are not merely theoretical: a Chinese laboratoryfound mosaicism and off-target effects in human embryos injected with CRISPR.[17]

Researchers could mitigate these risks by making genetic changes in either pluripotent stem cells or specific germline stem cells and sequencing their genomes to ensure the absence of deleterious off-target mutations.[18] These stem cells could then be differentiated into germ cells, probably sperm, and used in IVF. Such a procedure might obviate some of the risks to new life, but it divorces procreation from the conjugal act. However, a modification could bring the process more in line with magisterial teaching on sexuality. Instead of making sperm in the lab, scientists could alter spermatogonial stem cells and put them back into the father's seminiferous tubules, where they would hopefully generate mature sperm. The genetic changes could then be passed on through the conjugal act. This procedure is already

15. George Church, "Eight Questions to Ask before Human Genetic Engineering Goes Mainstream," *Washington Post*, February 25, 2016, https://www.washingtonpost.com/.

16. One researcher told me that he believes off-target effects will delay CRISPR's clinical use for a decade.

17. Puping Liang et al. "CRISPR/Cas9-Mediated Gene Editing in Human Tripronuclear Zygotes," *Protein and Cell* 6.5 (May 2015): 363–372. The embryos used in the Chinese experiment could not undergo full development because they came from eggs fertilized by two sperm.

18. Church, "Eight Questions."

possible in rats.[19] However, it is cumbersome, and not all the father's sperm would be transgenic, so researchers and fertility clinics are more likely to use IVF for the time being. Nonetheless, the availability of this approach suggests that CRISPR may be used without IVF and its attendant moral problems.

Even if techniques for germline editing were licit, prominent scientists have highlighted the unknown risks of tinkering with heritable genomes and the dangers of shaping our children at such a basic level.[20] The paper describing the Chinese experiment was rejected by major journals for ethical concerns.[21] It is heartening that the scientific community is addressing these issues, although it is unclear to what extent this debate will be affected by philosophical and religious voices, aside from a small group of principlist and utilitarian bioethicists.[22] Moreover, the low cost of CRISPR and the inconsistent regulation of biomedical research in much of the world make it unclear whether such discussions will halt the use of this technology.[23]

## Nonhuman Organisms

CRISPR has made the genetic modification of animals and plants cheaper and easier, but most of its applications in biomedical research and agriculture are extensions of previous genetic technologies.[24] There are two developments Catholic bioethicists should note, though. First, CRISPR allows precise changes to be made in a broader range of animals. For example, neuroscientists now can easily modify the genomes of primates like rhesus monkeys and chimpanzees. Pigs, a possible source of organs and tissues for human transplantation, can also now be modified. These capabilities raise questions about the ethics of conducting research on large mammals, especially those closely related to us.

Second, with the continuing toll that malaria exacts on the world's poorest and the emerging threat that Zika poses to unborn life, public health experts are seeking to use CRISPR to suppress mosquito populations through what is called gene drive technology. Genetic technologies already target mosquito-borne illnesses through

19. Karen M. Chapman et al., "Targeted Germline Modifications in Rats Using CRISPR /Cas9 and Spermatogonial Stem Cells," *Cell Reports* 10.11 (March 24, 2015): 1828–1835.

20. Edward Lanphier et al., "Don't Edit the Human Germline," *Nature* 519.7544 (March 26, 2015): 410–411; See also David Baltimore et al., "A Prudent Path Forward for Genomic Engineering and Germline Gene Modification," *Science* 348.6230 (April 3, 2015): 36–38.

21. David Cyranoski and Sara Reardon, "Embryo Editing Sparks Epic Debate," *Nature* 520.7549 (April 30, 2015): 593–594.

22. Many proponents of a moratorium argue that germline editing is unnecessary because we could address genetic diseases through preimplantation genetic diagnosis or screening followed by abortion.

23. Many fear that Chinese researchers are conducting these experiments, and the United Kingdom's Human Fertilisation and Embryology Authority allows experimental modification of human embryos. Other countries are following suit.

24. For magisterial teaching on genetic modification of nonhuman organisms, see Francis, *Laudato si*' (May 24, 2015), nn. 130–136.

the release of modified mosquitos whose offspring are either sterile or resistant to diseased parasites. Such efforts may eradicate target populations or prevent them from spreading disease, but they tend to be short-term solutions since the altered mosquitoes are eventually outcompeted by wild ones. In contrast, a gene drive can make an altered gene inherited in higher-than-normal ratios, which helps it spread through the population faster.[25] Normally, each individual has both a maternal and a paternal version of a gene, and each of that individual's offspring has a 50 percent chance of receiving either copy. A gene drive changes one copy of the gene, in theory, giving offspring a nearly 100 percent chance of inheriting the altered gene.

Geneticists could generate mosquitoes with Cas9 and an sgRNA targeting the desired sequence in the genome. As Cas9 and the sgRNA are introduced into each new mosquito, both copies of the targeted gene will be knocked out in every generation, replaced by the Cas9 and sgRNA. Thus, the change will spread quickly through the population, which could either drive the species to extinction or create species-wide resistance to a diseased parasite, making it an incredible tool for public health. However, once released, the gene drive would be out of human control, possibly leading to irreversible, unintended consequences through mutations or transfer to other organisms. Although scientists are developing a shut-off mechanism, it is unclear whether such a strategy will work outside the lab.[26] Though promising, gene drives could pose grave environmental risks.

## Three Major Ethical Questions

CRISPR is an amazing resource for biomedical research and therapy. Yet as with any new technology, it raises ethical concerns. By focusing on technical aspects, I have set aside important questions regarding the justice of spending research funds in this area and debates over who is entitled to the rewards of this technology, which itself raises three major ethical questions: First, we must ask how much risk from off-target effects is acceptable in therapeutic uses, a concern that may bar the technology from clinics. Second, it reintroduces older questions about human power over the next generation and the relationship between technology and human sexuality. Third, gene drives crystallize new concerns about humans' relationship with the environment. These are just the first of many issues that will surface as scientists develop new applications for CRISPR.

25. Valentino M. Gantz et al., "Highly Efficient Cas9-Mediated Gene Drive for Population Modification of the Malaria Vector Mosquito *Anopheles stephensi,*" *Proceedings of the National Academies of Sciences* 112.49 (December 8, 2015): 6736–6743; Andrew Hammond et al., "A CRISPR-Cas9 Gene Drive System Targeting Female Reproduction in the Malaria Mosquito Vector *Anopheles gambiae,*" *Nature Biotechnology* 34 (2016): 78–83; and National Academies of Sciences, Engineering, and Medicine, *Gene Drives on the Horizon* (Washington: National Academies Press, 2016).

26. Examples of such strategies are use of the anti-CRISPR proteins discussed earlier or use of a second gene drive that targets or corrects the original gene drive, as in James E. Dicarlo et al., "Safeguarding CRISPR-Cas9 Gene Drives in Yeast," *Nature Biotechnology* 33 (2015): 1250–1255.