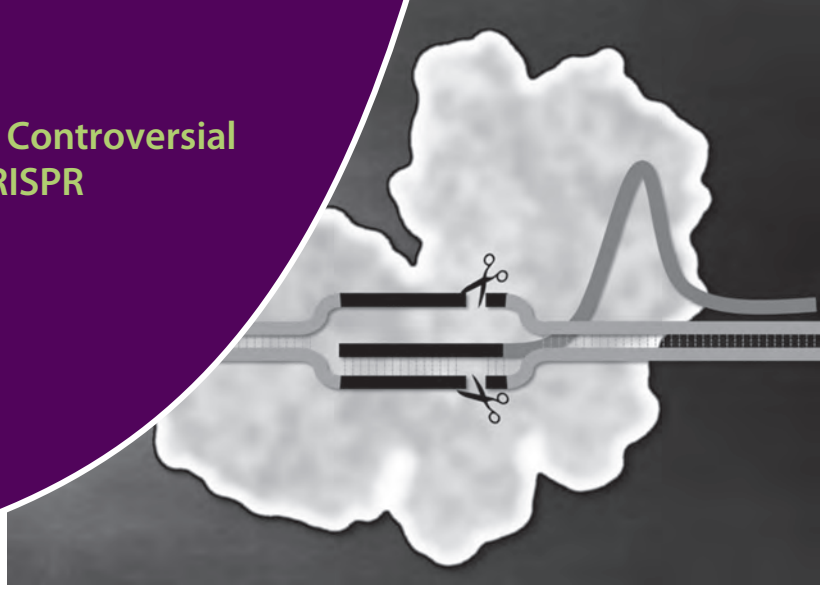
FEATURE ARTICLE

The Fascinating & Controversial New Science of CRISPR



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Abstract

CRISPR (also known as CRISPR-Cas9) is a powerful biotechnology tool that gives scientists unprecedented access to the genetic makeup of all living organisms, including humans. It originally evolved as an adaptive immune system in bacteria to defend against viruses. When artificially harnessed in the laboratory it allows scientists to accurately and precisely edit genes, almost as if using a word processor. In mice, CRISPR has already been used to treat diabetes, muscular dystrophy, cancer, and blindness. CRISPR has made cultured human cells immune to HIV, and a variety of CRISPR experiments involving human embryos are well under way. But CRISPR is not limited to biomedical applications. It is also revolutionizing the food industry and many areas of biological research. This article provides science educators a broad and up-to-date overview of CRISPR, including its discovery, application, and bioethical challenges. It is imperative that science educators help prepare students, both majors and nonmajors, for this compelling new era of biology.

Key Words: *CRISPR; gene editing; molecular genetics; bioethics.*

Introduction

You have probably already *heard* of CRISPR; it has been in the news quite a bit lately. But if you are not *teaching* CRISPR in the classroom, it is definitely time that you did. CRISPR is a powerful biotechnology tool giving scientists unprecedented access to the genetic makeup of all living organisms, including humans. CRISPR originally evolved as an adaptive immune system in bacteria to defend against viruses. When artificially harnessed in the laboratory it allows scientists to accurately and precisely edit genes, almost as if using a word processor. The potential applications are limitless, but they also open an enormous range of bioethical questions regarding how and when the technology should be used. We will explore some of these applications later, but first let's see how CRISPR was discovered.

"The potential applications are limitless, but they also open an enormous range of bioethical questions regarding how and when the technology should be used."

Putting the Pieces Together

The story of CRISPR began in 1987 when Japanese biologist Yoshizumi Ishino was studying DNA in the common gut bacterium *Escherichia coli*. While sequencing part of the *E. coli* chromosome, he discovered several short repetitive sequences separated by short random spacer sequences. These short repeat sequences are referred to as CRISPR (clustered regularly interspaced short palindromic repeats). Ishino's paper famously concludes, "The biological significance of these sequences is not known" (Ishino et al., 1987, p. 5432).

CRISPR repeats were soon discovered in many other types of bacteria. The sequences of the repeats were consistent and predictable, but the spacer sequences always appeared to be random – until 2005, that is, when Francisco Mojica, working in Spain, discovered that the spacer sequences were identical to sequences of bacteriophage DNA (Mojica et al., 2005).

Bacteriophages are viruses that infect bacterial cells. It seemed that bacteria were storing DNA fragments of various strains of virus in between the CRISPR repeats (Figure 1). Mojica speculated that the CRISPR system might represent some type of bacterial defense against the viruses, an idea that would later be proved correct.

The proof came from two microbiologists studying yogurt bacteria while working for Danisco. Rudolphe Barrangou and Philippe Horvath incubated bacteria in the presence of a specific bacteriophage. They then sequenced the genomes of surviving bacteria and found that the

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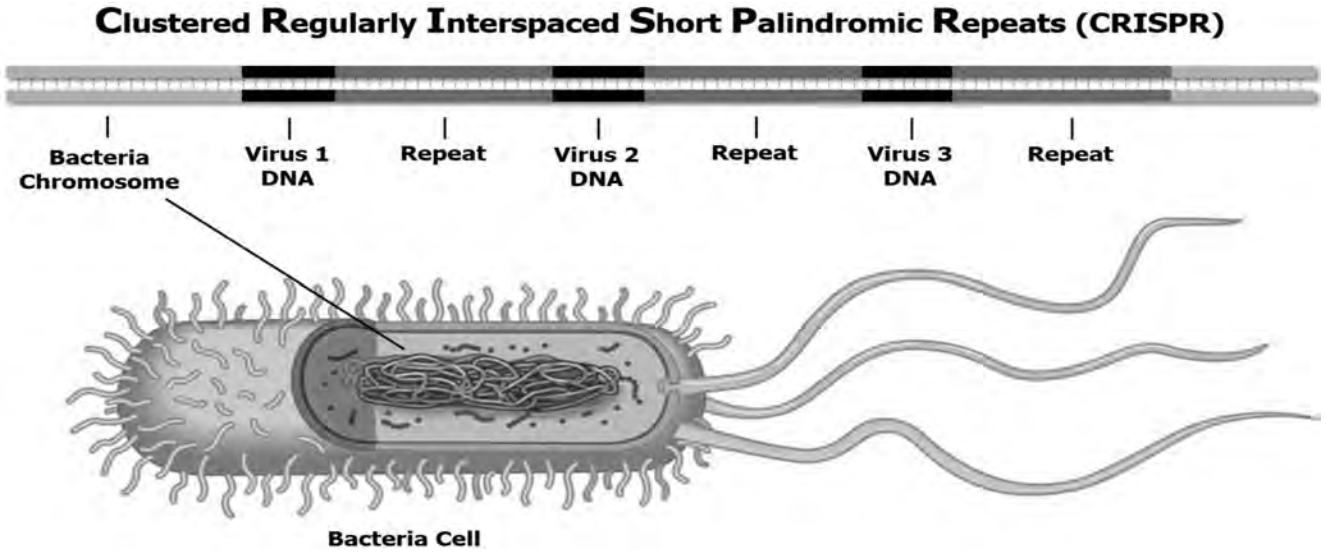

Figure 1. Arrangement of palindromic repeats and viral spacer sequences in the CRISPR region of a bacterial chromosome.

cells had incorporated portions of the bacteriophage genome into the CRISPR region of their chromosome. CRISPR clearly represented some form of adaptable (i.e., customizable) immunity (Barrangou et al., 2007).

CRISPR soon captured the interest of University of California biologist Jennifer Doudna. Doudna suspected that a role might be played by some genes located near the CRISPR region. These CRISPR-associated genes (Cas genes) encode protein enzymes and had been discovered by Ruud Jansen back in 2002 (Jansen et al., 2002). Doudna's lab was able to work out the roles of two of the enzymes, but they still lacked an explanation of how the entire system worked. That would change, however, following a chance encounter between Doudna and Umeå University biologist Emmanuelle Charpentier in 2011. Charpentier was studying yet another Cas enzyme, called Cas9, which had been found in *Streptococcus pyogenes*, the bacteria responsible for strep throat. Cas9 is a nuclease with the ability to cut DNA like a pair of scissors.

In some respects, Cas9 is similar to the restriction enzymes discovered decades earlier. Restriction enzymes, however, are predestined to cut DNA at a short specific sequence. *EcoRI*, for example, always cuts at GAATTC. Cas9, on the other hand, is a *programmable* enzyme with the capacity to cut DNA at *any* sequence of nucleotides. Indeed, the enzyme must be told *where* to cut. Interestingly, these instructions come from an RNA molecule that attaches itself to the Cas9 enzyme (Figure 2). Charpentier and Doudna eventually put all of the pieces together and demonstrated how the CRISPR system operates in bacteria as an adaptive immune system against viruses.

When a virus infects bacteria, it first injects its DNA genome into the cell. The viral DNA instructs the bacteria on how to make more copies of the virus. The cell follows the instructions, produces hundreds of new viruses, and eventually ruptures, allowing the viruses to escape. However, if the bacterium somehow survives the infection process and does not rupture, it will take a fragment of the viral DNA and splice it into its own chromosome. It then adds CRISPR repeat sequences on either side. In fact, whenever a bacterial cell survives a viral infection it may perform this task. The bacterium is creating a database of known viruses, much like

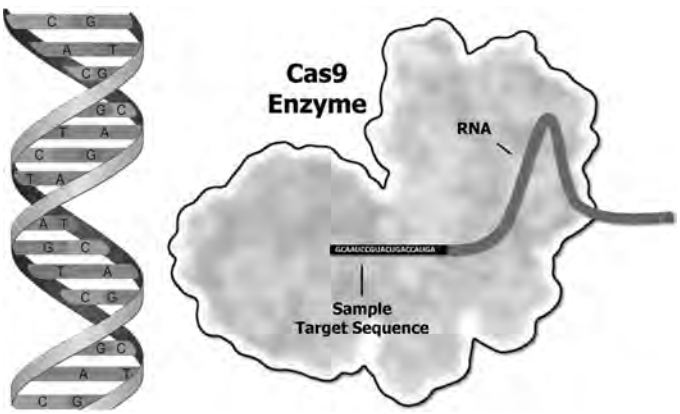

Figure 2. Nuclease capacity of the Cas9 enzyme. Cas9 will cut dsDNA at a location complementary to the 20-nucleotide target sequence specified by the attached RNA.

an FBI Most Wanted list. This Most Wanted list gets passed on genetically to all descendant cells so that subsequent generations will recognize the viruses even before being attacked.

But how does the CRISPR system actually defend against the viruses? Bacteria transcribe the entire CRISPR region into RNA, which is then cleaved into separate pieces of crisprRNA (Brouns et al., 2008). Each crisprRNA (also called crRNA) contains a CRISPR repeat along with a short 20-nucleotide stretch of viral DNA (Figure 3).

The crisprRNA is then loaded into the Cas9 enzyme by anchoring it to another piece of RNA called tracrRNA (Figure 4A). Charpentier had already discovered tracrRNA in conjunction with Cas9, but she had not been able to determine its function. It was now found to serve as a universal attachment site for crisprRNAs. Thus, the Cas9 enzyme can be programmed by the cell to cut at whichever target sequence is specified at the end of the crisprRNA. The cell is now ready and waiting, should that targeted strain of virus attempt to enter the bacterial cell again.

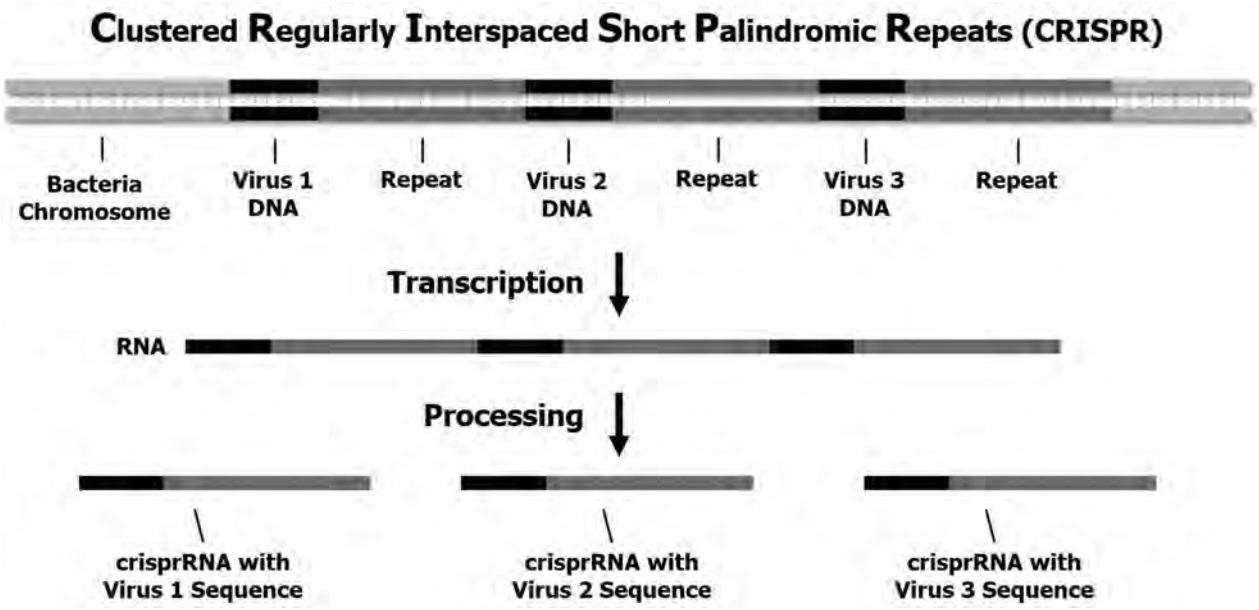

Figure 3. Transcription of CRISPR region of a bacterial chromosome. The CRISPR region of the chromosome is transcribed into RNA, which is then cleaved into separate crisprRNA molecules (also called crRNA). Each crisprRNA contains a repeat sequence and a short viral sequence specific to a particular strain of bacteriophage.

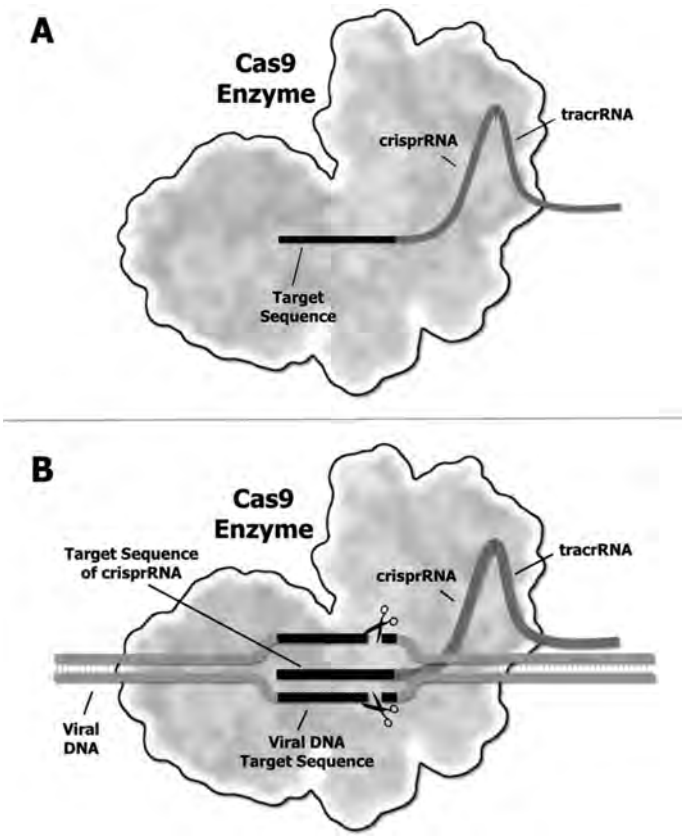

Figure 4. Cas9 enzyme programmed with crisprRNA and tracrRNA. (**A**) crisprRNA is loaded into the Cas9 enzyme by attaching to tracrRNA. (**B**) The programmed CRISPR-Cas9 complex binds to viral DNA that is complementary to the target sequence in the crisprRNA. The enzyme complex then creates a double-strand break, which inactivates the viral DNA.

If the viral DNA does enter the cell, the CRISPR-Cas9 complex will recognize the DNA by complementary base-pairing, open it up, and cut it (Figure 4B). Once cut, the viral DNA is no longer able to harm the cell. It is a remarkable system with the ability to learn and adapt. It is not unlike our own adaptive immune system, which learns to recognize harmful germs and produce highly specific antibodies to fight them off.

With the CRISPR mechanism worked out, Doudna wondered if it might be possible to customize the CRISPR system. Could researchers create their own crisprRNA and load it into the Cas9 enzyme? The first step was to simplify the crisprRNA/tracrRNA complex. Doudna and Charpentier essentially fused the two RNAs together to produce what they called guideRNA. The next step was to synthesize a guideRNA with a specific target sequence of their choosing to see if they could successfully program the Cas9 enzyme. Doudna and her colleague Martin Jinek set their sights on using CRISPR-Cas9 to specifically cut the green fluorescent protein (GFP) gene in a strain of *E. coli*. Bacteria possessing the intact gene express a green protein that fluoresces under ultraviolet light. Their goal was to design a custom CRISPR-Cas9 complex that could recognize and cut the GFP gene at a specified location.

Evidence that the cut had taken place would be as simple as observing a phenotypic change in the bacteria (i.e., successfully edited cells would no longer produce GFP). However, to truly demonstrate that the CRISPR-Cas9 complex cut the gene exactly where Doudna and Jinek intended, they chose to look for the cut DNA fragments using gel electrophoresis. If the complex cut at the desired location, it would generate fragments of a precise and predictable size. Sure enough, they found the expected fragments and published their groundbreaking work in June 2012 (Jinek et al., 2012). A new gene-editing technology had arrived with the added benefit of being fast, accurate, and inexpensive.

It is worth noting that Lithuanian researcher Virginijus Šikšnys worked out the functioning of CRISPR-Cas9 around the same time as Doudna and Charpentier (Gasiunas et al., 2012). Publication of Šikšnys's research was delayed, however, by extensive peer review and, unfortunately, his work is often overlooked.

Unlocking the Potential of CRISPR

The next important research question was to determine if a CRISPR-Cas9 system was present in more complex eukaryotic cells, such as human cells. The quick answer is no. However, Doudna and Jinek were able to *artificially* introduce a custom CRISPR-Cas9 complex into cultured human cells, and the technology worked as intended. They demonstrated their success by inactivating (knocking-out) the CLTA gene in human embryonic kidney cells (Jinek et al., 2013). Thus, CRISPR-Cas9 could be used to edit both prokaryotic and eukaryotic cells. The implications for new types of genetic research were profound. Researchers have long been interested in the prospects of knocking-out genes, as this provides a means of revealing a gene's actual function.

The same month that Doudna's group published their work on the CLTA gene, Feng Jhang at the Broad Institute and George Church at Harvard published similar studies involving the use of CRISPR to edit human cells (Cong et al., 2013; Mali et al., 2013). Jhang and Church, however, took the method one step further. They used CRISPR-Cas9 to introduce (knock-in) a *new* gene.

It turns out that when DNA is cut in a eukaryotic cell, the cell will attempt to repair the break using one of two repair mechanisms. The first option is called non-homologous end joining (NHEJ), in which the cell uses random nucleotides as molecular glue to join the cut ends back together. The incorporation of random nucleotides, however, constitutes a mutation. If this occurs within a gene, it will likely create a frame-shift error and inactivate the gene (Figure 5). Thus, despite NHEJ repair, the gene is knocked-out. This is what Doudna and Jinek had accomplished by targeting the CLTA gene in kidney cells.

The second repair option, called homology-directed repair (HDR), is quite different. Most eukaryotic cells are diploid (i.e., every chromosome is part of a homologous pair of chromosomes containing similar genetic information). If one member of the pair is damaged, the other chromosome can serve as a template for repair. The cell simply copies the appropriate region of the intact chromosome into the defective region of the damaged chromosome.

In Doudna and Jinek's experiment, the human cell was not able to utilize HDR because the CRISPR-Cas9 complex would have likely cut both homologous chromosomes. Thus, the cell relied on NHEJ. Church and Jhang hypothesized that if they could somehow provide a piece of "donor" DNA with ends that matched (i.e., were homologous to) the cut ends of the original DNA, then the cell might operate as if the donor DNA was *completely* homologous and utilize HDR (Figure 6).

The donor DNA, which could be delivered via a plasmid, need only be homologous to the cut DNA at the *ends* matching the cut (see Figure 6). The *middle* of the donor DNA could be any sequence they desired, thus allowing them to "Trojan horse" a new sequence into the repair site (gene knock-in). Jhang demonstrated the technique by knocking the GFP gene into human embryonic kidney cells. With the proven ability to knock genes in and out of cells, the prospect of using CRISPR to treat human genetic disease became a very real possibility.

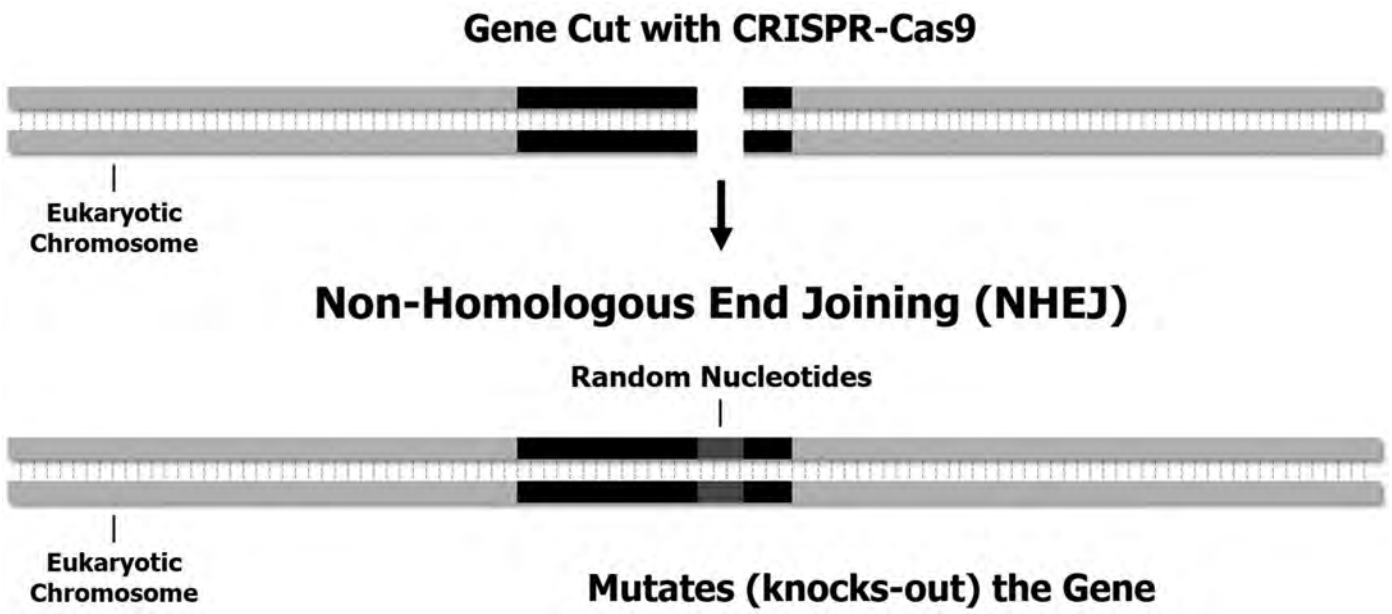

Figure 5. DNA repair by non-homologous end joining (NHEJ). The cut DNA is repaired by incorporating random nucleotides at the site of the break. These additional nucleotides, however, introduce a mutation into the gene, which disrupts the gene's function.

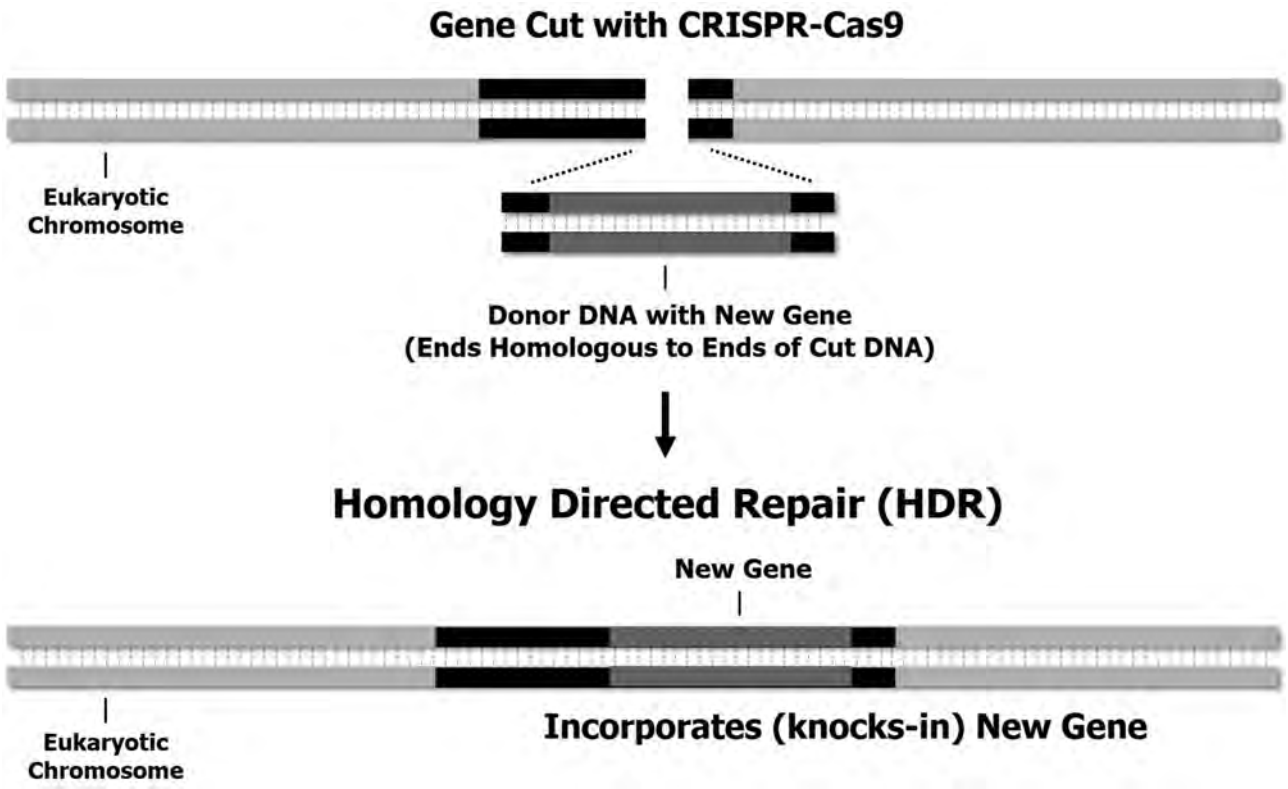

Figure 6. Homology-directed repair (HDR) of DNA. The cut DNA is repaired by copying the template DNA molecule (donor DNA) into the repair site. The ends of the donor DNA are homologous to the ends of the cut DNA (a requirement for HDR), but the donor DNA also contains a new gene that is smuggled into the repair site.

○ Biomedical Applications

Consider cystic fibrosis, for example. Cystic fibrosis is characterized by an excessive accumulation of mucus, notably in the respiratory tract. The problem ultimately stems from a genetic error. The CFTR gene, which encodes a protein used to transport ions across the cell membrane, is faulty. As such, chloride ions are not successfully transported, which leads to the formation of thick heavy mucus, chronic respiratory disability, and reduced life expectancy. CRISPR could potentially be used to correct this genetic mistake.

Scientists could create a CRISPR-Cas9 complex to target and cut the CFTR gene. This alone would not solve the problem, as the gene was already not functioning correctly. But if a piece of donor DNA containing the correct version of the CFTR gene were also provided, then this new correct version would be edited into the chromosome via HDR. In 2013, Hans Clevers used this exact approach to correct the cystic fibrosis mutation in human cells cultured in the laboratory (Schwank et al., 2013).

Although Clevers's experiment was groundbreaking, there is one obvious problem. Every cell in an adult cystic fibrosis patient possesses the faulty CFTR gene. As such, researchers would have to find a way to introduce the CRISPR repair mechanism into every adult cell, or at least into those of the respiratory tract. This would be extremely difficult to do. The ideal scenario would be to introduce CRISPR-Cas9 into the fertilized egg or early embryonic stage of someone with cystic fibrosis so that all future body cells of the adult organism would contain the corrected gene.

Many scientists, however, have expressed concern with performing such an experiment. It is one thing to treat the existing cells of an adult. It is quite another to genetically alter a human embryo, as this opens up additional ethical concerns. Indeed, CRISPR was opening a veritable Pandora's box of bioethical questions. In January 2015, Jennifer Doudna organized an international meeting of scientists and policymakers to discuss the pros and cons of the potential uses of CRISPR, including the editing of human embryos. The attendees agreed that editing human embryos crossed a bioethical line and began authoring a self-imposed ban on such a procedure. Little did they know that Chinese scientist Junjiu Huang had already crossed that line.

Huang had injected 86 embryos with CRISPR-Cas9 (Liang et al., 2015). His goal was to correct a gene associated with a blood disorder called beta thalassemia. Only four embryos were edited successfully, and many were found to have unintended (off-target) mutations. The experiment was so controversial and arguably unsuccessful that Huang's paper was rejected by both *Science* and *Nature*, the world's premier scientific journals.

In 2016, Chinese scientist Lu You reported the first use of CRISPR to treat an *adult* patient (Cyranoski, 2016). The patient had a form of lung cancer and, unfortunately, the patient's T-cells were unable to recognize and destroy the tumor. Lu removed the patient's T-cells and then used CRISPR to disable the PD-1 gene. This alteration would allow the T-cells to attack the cancer cells. The T-cells were then injected back into the patient with the hope that they would fight off the tumor. Lu has treated additional patients

with the CRISPR-based therapy and the results appear promising. Similar trials are now under way in the United States.

The potential biomedical applications of CRISPR are far-reaching. Thousands of individuals die each year in need of an organ transplant (heart, lung, liver, kidney, etc.). Patients must wait until an appropriate donor is available, often the result of a tragic accident. Even then, the donated organ must be a very close genetic match to ensure that the organ is not rejected. Jun Wu, a research scientist now at the University of Texas Southwestern Medical Center, is pioneering a solution.

Wu's goal is to develop interspecies organ donation. As part of his research, he and colleagues at the Salk Institute used CRISPR to grow rat organs inside the body of a mouse (Wu et al., 2017). First, they used CRISPR to turn off the organ-producing genes inside a mouse blastocyst. This step alone demonstrates the power of CRISPR technology, as it required the simultaneous modification of multiple genes, a nearly impossible task with existing methods of gene editing. They then inserted rat stem cells into the mouse blastocyst in hopes that the rat cells would generate the missing organs. The experiment worked, resulting in a hybrid organism called a chimera (Figure 7A). Wu and his colleagues managed to produce a mouse with rat organs (heart, eyes, pancreas, etc.). These organs could theoretically be harvested from the chimeric mouse and donated to a normal rat without risk of rejection.

Wu would like to use this technology to produce human organs inside another species of animal. Obviously rats and mice are too small, but it turns out that pig organs are remarkably similar in size and structure to human organs. Using the same approach, Wu inserted human stem cells into a pig blastocyst and the experiment worked. Figure 7B is a picture of the first pig/human embryo – a chimera. The embryo was removed for study after 28 days (first trimester of pig pregnancy), which allowed enough time for sufficient cell growth without raising ethical concerns.

The potential applications of CRISPR are limitless. Table 1 provides a partial list of CRISPR applications along with a reference to jump-start a student's interest in the topic.

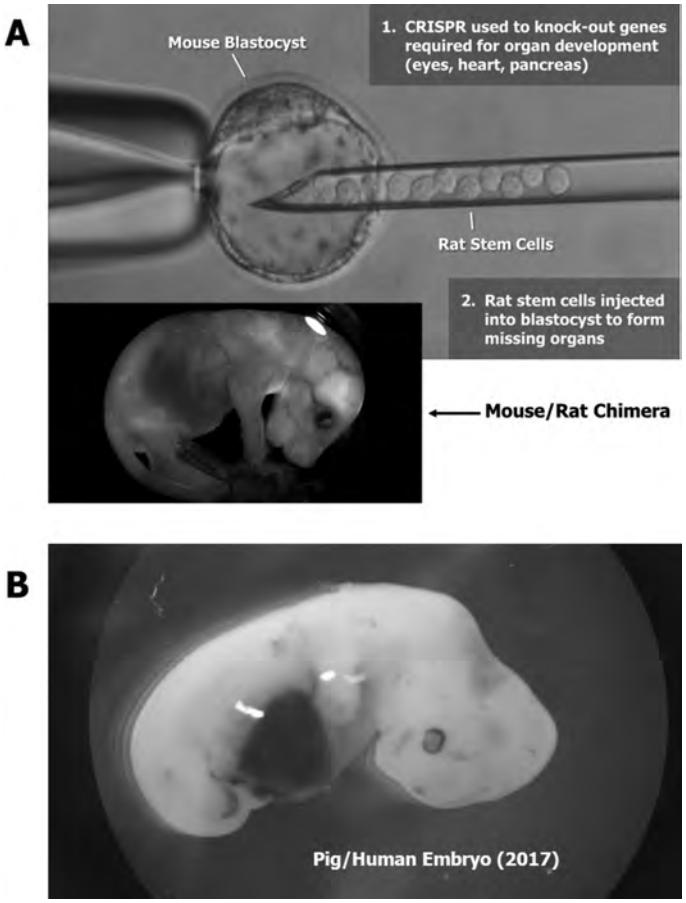

Figure 7. Creation of a chimera using CRISPR-edited blastocyst and stem cells (Wu et al., 2017). (**A**) CRISPR was used on a mouse blastocyst to knock-out the genes associated with organ development. The blastocyst was then injected with rat stem cells capable of producing organs. The procedure resulted in the formation of a mouse/rat chimera. (**B**) A procedure similar to that described in A was used to create a pig/human chimera.

Table 1. Various applications of CRISPR technology.

Application	Reference to Explore
Biomedical	
Diabetes	https://www.healthline.com/diabetesmine/could-gene-editing-be-used-cure-diabetes#1
Cancer	https://www.statnews.com/2019/05/02/crispr-targeting-cancer-seeking-go-ahead/
HIV	https://www.sciencemag.org/news/2019/03/curing-hiv-just-got-more-complicated-can-crispr-help
Food biotechnology	
Mushrooms	https://www.foodsafetynews.com/2018/12/the-little-mushroom-that-could-with-a-little-help-from-its-friends/
Soybean oil	https://www.the-scientist.com/news-opinion/gene-edited-soybean-oil-makes-restaurant-debut-65590
Cattle	https://www.wired.com/story/crispr-gene-editing-humane-livestock/
Basic research	
Gene drives	https://www.synthego.com/blog/gene-drive-crispr
Cellular barcoding	https://www.nature.com/articles/d41586-018-05934-z
Gene targeting with deactivated Cas9	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4922510/

○ CRISPR-Edited Foods

In addition to its many biomedical applications, CRISPR has the potential to revolutionize the food industry. In 2016, the U.S. Department of Agriculture (USDA) approved the first CRISPR-edited food item for human consumption – white button mushrooms. Yinong Yang, a plant pathologist at Pennsylvania State University, used CRISPR to disable an enzyme that normally causes the mushrooms to brown, thereby extending their shelf life (Waltz, 2016).

Interestingly, in 2018 the USDA noted a distinction between gene modification and gene editing, at least with regard to plants. Whereas *gene modification* refers to the introduction of foreign DNA (i.e., from another species), *gene editing* refers to changes that theoretically could be produced and propagated naturally through mutation and selective breeding, even if the changes are actually accomplished using biotechnology tools such as CRISPR. Thus, many CRISPR-edited foods will not be subject to regulations or labeled as genetically modified organisms (GMOs). Decisions regarding livestock – which will come from the Food and Drug Administration rather than the USDA – have yet to be finalized. Nevertheless, scientists are already using CRISPR to edit a wide variety of domesticated animals, including beefier and hornless cattle (Barber, 2019).

○ Gene Drives

Yet another fascinating application of CRISPR is a tool called a gene drive. Originally conceived by Harvard biologist Kevin Esvelt, a gene drive is a synthetic segment of DNA that includes the Cas9 gene and a guideRNA gene, along with a particular gene of interest (also called payload DNA), all in one self-functioning unit (Esvelt et al., 2014). The payload DNA can be a new gene or a modified version of an existing gene.

Once a gene drive is introduced into the chromosome of a diploid organism, the drive will generate a Cas9/guideRNA complex that will cut the homologous chromosome and then copy the gene drive into the break via HDR. With the gene drive now present in both chromosomes, the organism is homozygous for the drive, including the payload DNA (Figure 8).

Gene drives are capable of knocking-in or knocking-out genes in an organism. More importantly, gene drives can be propagated into an entire population of organisms via sexual reproduction, thus allowing for genetic modification at the population level.

Consider Figure 9A, which shows how a gene is propagated in a population via normal inheritance – that is, with no gene drives involved. If one of the original parents is heterozygous for a gene of interest, then the gene should statistically be transmitted to half of the offspring. This pattern of inheritance would continue in subsequent generations with the gene never accumulating to an appreciable level within the population.

Now look at how the gene could be propagated using a gene drive (Figure 9B). The original parent would initially be heterozygous for the gene drive (including the gene of interest), but the drive itself would ensure that it is copied into the homologous chromosome, thus making the parent homozygous for the drive and payload gene. Zygotes in the first generation of offspring would initially be heterozygous for the gene drive, but once again, the drive would

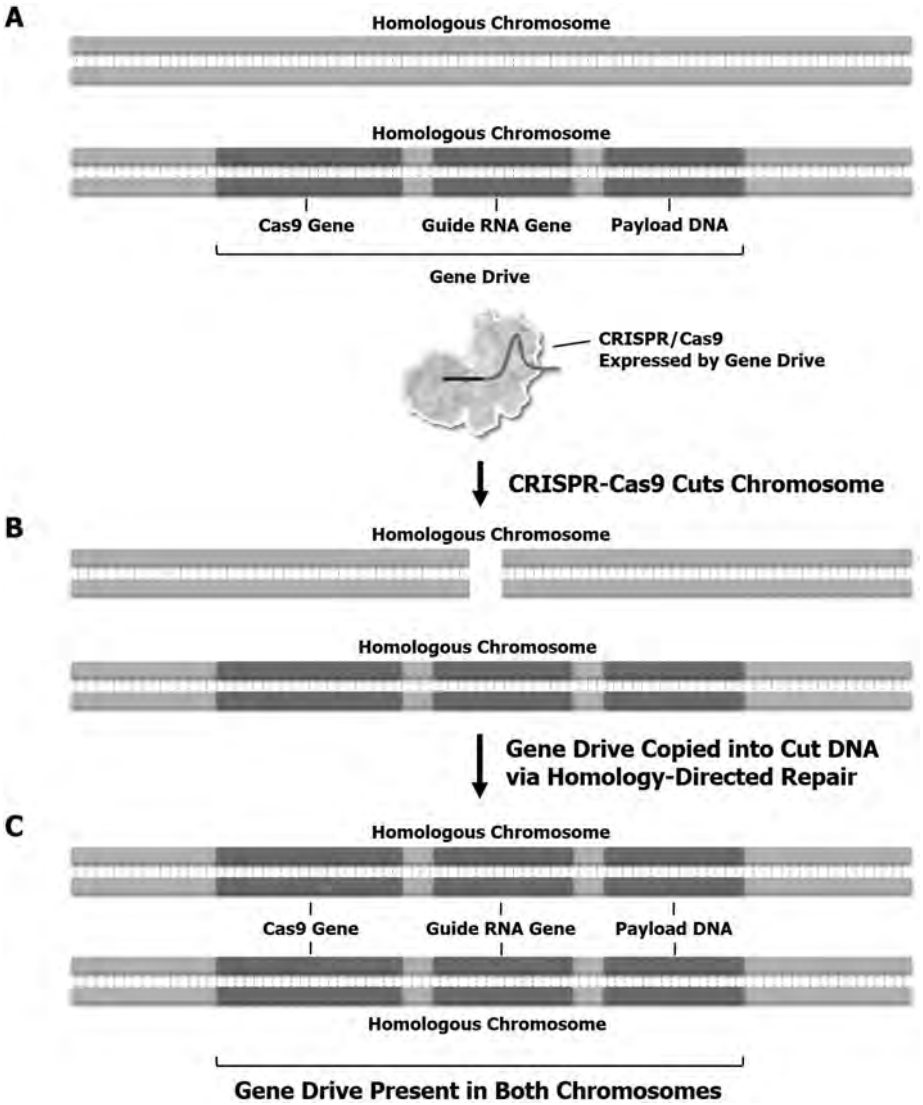

Figure 8. Structure of a CRISPR gene drive. (**A**) The drive is initially present in one of the homologous chromosomes. The drive expresses the Cas9 enzyme and guideRNA, which form a CRISPR-Cas9 complex. (**B**) The enzyme complex cuts the other homologous chromosome at the designated target sequence. The chromosome containing the gene drive can then serve as a template for homology-directed repair. (**C**) As such, the gene drive is copied into the cut chromosome at the site of repair, ensuring that both chromosomes have a copy of the drive.

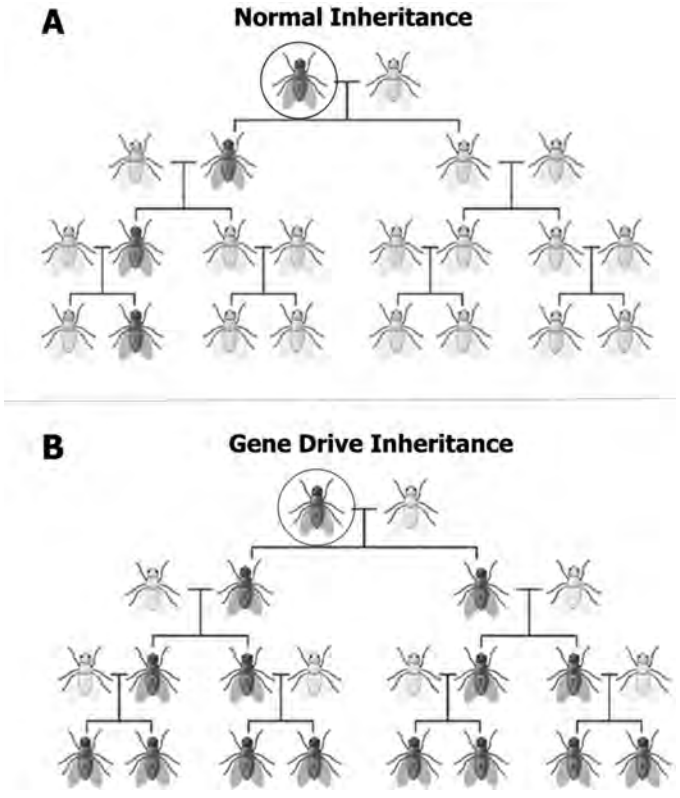

Figure 9. Normal vs. gene-drive-based inheritance. (**A**) One of the original parents is heterozygous for a gene of interest. With normal processes of sexual inheritance this gene will be continually passed to ~50% of the offspring but will never accumulate in the population at large. (**B**) One of the original parents is heterozygous for the gene of interest, which is located within a gene drive. The gene drive copies the drive and gene of interest into the other homologous chromosome, thus making the parent homozygous. As such, the gene drive is passed to 100% of the offspring. The offspring are initially heterozygous for the gene and drive but, like the parent, become homozygous. Thus, the gene of interest is rapidly propagated into the population.

copy itself into the homologous chromosome, ensuring that the offspring become homozygous. This pattern of inheritance would continue in subsequent generations with the drive and gene of interest becoming increasingly present within the population. So, how might this technology be used?

In one interesting application, scientists are hoping to use gene drives to eliminate malaria, a disease that continues to kill over a million people every year. The malarial parasite is transmitted by the *Anopholes* mosquito. Researchers created a gene drive that makes females of the species reproductively sterile (Hammond et al., 2015). Introducing the gene drive into the environment could conceivably drive the mosquito species to extinction and help eradicate the disease. In February 2019, researchers in Italy began a large-scale release of the CRISPR-edited mosquitos into a controlled high-security environment. If the technology works, gene drives could be used to address other insect-borne diseases, such as Zika virus. Moreover, gene drives could help eradicate invasive pests and create more efficient crops.

Gene drives represent an extremely powerful technology with the potential to alter entire populations of organisms. Indeed, the enormous power of gene drives has not gone unnoticed by the U.S. government. In 2016, the director of national intelligence added gene editing to a list of threats posed by "Weapons of Mass Destruction and Proliferation." Ironically, Esvelt's lab is already working on an antidote to gene drives: a gene drive programmed to remove another gene drive.

○ Patent Disputes

With so many uses of CRISPR under development, there are billions of dollars at stake in terms of profits, patents, and prizes. Recall that Feng Jhang was one of the first to use CRISPR on human cells. His success instigated an intense patent dispute between his research team at the Broad Institute of MIT and Harvard and Jennifer Doudna's team at the University of California. Although Doudna and Charpentier first published the CRISPR-Cas9 technology, the Broad Institute argued that Jhang modified the procedure in significant ways to exploit HDR. The Broad Institute also paid a fee to have their patent application expedited through the process. The courts have ruled in Jhang's favor, a decision with enormous financial implications.

○ Bioethical Considerations

Artificial editing of genomes did not begin with CRISPR. Transgenic mice were developed in the 1970s, and genetic alterations of embryonic stem cells have been a common research practice since the 1980s. Genome editing was further enhanced with the development of zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) during the 2000s. These techniques, however, remained technically challenging and largely inefficient. CRISPR-Cas9, on the other hand, is inexpensive, precise, and easy to use. As such, it has pushed the field forward at a rapid pace, perhaps without sufficient discussion of the legal and ethical implications inherent to its use.

Bioethics generally operate within four frameworks: (1) rights and responsibilities, (2) consequentialism, (3) autonomy, and (4) virtue (Science Learning Hub, 2007). A brief explanation of these frameworks is provided in Table 2 and can serve as a framework for discussing the bioethical implications of CRISPR with students. It is not the purpose of this article to explore these parameters in depth, but it might be helpful to consider a few examples.

In January 2019, Chinese scientists edited macaque monkey embryos with CRISPR to induce symptoms of sleep disorders and then cloned the animal with the most extreme symptoms (Liu et al., 2019; Qiu et al., 2019). Signs of the disease included loss of sleep and changes in blood hormones, but also increases in anxiety, depression, and "schizophrenia-like" behaviors. The goal of their work was to produce genetically identical monkey models of disease for biomedical research. The question arises, however, whether the intentional creation of disease in higher primates via gene editing and cloning is ethically acceptable. For example, does it align with the virtues of human society? This might be an interesting question to discuss with students.

Some of the most significant bioethical questions raised by CRISPR regard the editing of human sperm and egg cells prior to

Table 2. Frameworks associated with bioethical decision making.

Bioethical Framework	Description	Pertinent Example
Rights and responsibilities	The rights of one imply the responsibilities (or duties) of another to ensure those rights.	The imperative to treat cancer patients with available, though perhaps not fully tested, gene-based therapies
Consequentialism	The benefits and harms resulting from an action must be weighed against each other.	Using gene drives to eradicate the *Anopholes* mosquito in an attempt to eliminate malaria
Autonomy	An individual's right to choose for oneself may or may not exceed the benefit of a single decision applicable to everyone.	Editing human embryos and germ cells without the consent of the embryo or future generations
Virtue	Decisions should be congruent with what the community accepts as "good," such as honesty and kindness.	Creating genetic models of human disease in primates

in vitro fertilization. If this application is pursued, parents could correct genetic problems prior to conception. Of course, they could also customize and enhance many other traits – producing true designer babies. Scientists, however, have traditionally been reluctant to allow editing of human germline cells, as the edits would be permanently passed on to future generations without consent and without full knowledge of long-term consequences.

Nonetheless, in 2018, Chinese researcher He Jiankui claimed to have performed the first CRISPR editing of human embryos (twin girls) that were subsequently implanted into the mother, carried to term, and delivered (Marchione, 2018). Jiankui inactivated the CCR5 gene in order to make the babies resistant to HIV. His work is extremely controversial. Indeed, Jiankui was fired by his university and faces charges of ethical violations. In response to Jiankui's reported experiments, an international group of scientists – including the National Academy of Sciences and the UK Royal Society – called for a moratorium on the editing of human germ cells intended for implantation. "There is wide agreement in the scientific community that, for clinical germline editing, the risk of failing to make the desired change or of introducing unintended mutations (off-target effects) is still unacceptably high" (Lander et al., 2019). Perhaps pressured by the advances being made in China, the National Academy of Sciences had recently updated its position on gene editing of human germ cells (Kaiser, 2017). In an abrupt and significant change, it supported gene editing of both embryos *and* germ cells in cases of serious disease, although such embryos could not be implanted. As of this writing, that remains its current position.

○ What's Next?

David Liu at the Broad Institute of MIT and Harvard recently introduced an ingenious new version of CRISPR called CRISPR-Prime (Anzalone et al., 2019). Liu combined modified versions of guideRNA and Cas9 with reverse transcriptase to create a true "search and replace" gene editor capable of extreme accuracy and precision.

Prime editing utilizes a modified version of guideRNA called prime editing guide RNA (pegRNA). The pegRNA contains a targeting sequence at one end and an RNA version of the desired DNA edit at the other end. In other words, pegRNA plays the role of both guideRNA and donor DNA. During prime editing the pegRNA initially targets a location in the genome. A modified version of Cas9 then cuts (nicks) one of the DNA strands at the target location. The Cas9-pegRNA complex is coupled to reverse transcriptase, an enzyme naturally found in retroviruses (such as HIV) and capable of transcribing RNA into DNA. Once the single strand of genome DNA genome has been cut, the distal end of the pegRNA serves as a template for reverse transcriptase to copy the desired edit into the DNA strand. Cas9 then nicks the unedited strand of DNA, which the cell subsequently repairs via HDR using the already edited strand as a template.

Prime editing is extremely versatile, accurate, and precise, significantly reducing the incidence of off-target changes. This new version of CRISPR may alleviate many of the concerns associated with gene editing moving forward.

○ Conclusion

The landscape of biological research is shifting beneath our feet. Although this article has focused primarily on biomedical applications, CRISPR is revolutionizing fields of basic research as well. For example, researchers have managed to deactivate the nuclease capacity of Cas9 (called dCas), which allows the guideRNA-dCas9 complex to serve purely as a gene-targeting device. By tethering it to other reagents, dCas9 can be used for such things as gene regulation, genomic screening, and cell fate engineering (Dominguez et al., 2016).

The current and potential applications of CRISPR are both exciting and hopeful. Nonetheless, CRISPR is not yet a perfect technology. The potential for off-target mutations remains a challenge for many CRISPR-based experiments. The guideRNA target sequence, although 20 nucleotides long, can tolerate one to three mismatches when binding with DNA, resulting in unintended cuts and mutations. Off-target mutations can result in elevated cancer risks. This problem can be reduced significantly, however, using the new Prime editing technology.

Opinions regarding CRISPR vary tremendously and can generate some interesting discussions with students. Many would cite

the law of unintended consequences and urge restraint if not outright prohibition of many CRISPR technologies and applications. But the temptation to control nature is ever present and provides an ongoing tension between that which we *can* do and that which we *should* do. This temptation was recognized soon after the genetic code was deciphered in the 1960s. According to Marshall Nirenberg, one of the code-crackers himself, "Decisions concerning the application of this knowledge must ultimately be made by society, and only an informed society can make such decisions wisely." The current generation of students will have to engage difficult and profound bioethical questions, because with CRISPR, the technology to alter the future direction of life has clearly arrived.

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