CRISPR-Cas9 and Genome Editing

Facilitates precise, inexpensive, and easily programmable editing of genomic DNA.

Updated last May 17, 2017

WHAT IT DOES

CRISPR-Cas9, often abbreviated CRISPR (clustered, regularly interspersed short palindromic repeats), is a molecular tool that can edit DNA. CRISPR-Cas9 is naturally found in bacteria and other microbes, but scientists recently discovered that they could insert it into the cells of other organisms (like plants and animals) to edit their genomes (the collective DNA of a cell). This genome editing (sometimes called genome engineering) is akin to the more established field of genetic engineering (GE), wherein scientists alter the physical properties of an organism at the cellular level through manipulation of DNA. CRISPR-Cas9 is a significant improvement over other genome editing technologies, which are more challenging to design, more expensive, or less precise. Thus, this technology is accelerating the development of a number of GE projects, such as:

- **Gene drives**, which are projects that seek to alter the genomes of wild populations of organisms by breeding them with GE organisms. These GE organisms are designed to spread, or “drive”, a desired change into the wild population using “selfish” DNA (i.e., DNA that is inherited at higher rates than normal). For instance, a gene drive could be used to sterilize disease-carrying mosquitoes or decrease the population of invasive rodents.
- **Other genome-edited animals**, engineered for a food source, research, transplantable organs, disease control, or even hypoallergenic and novelty pets.
- **De-extinction efforts**, like the proposal to resurrect the woolly mammoth.
- **Genome-edited bacteria**, algae, and other microbes engineered for health, consumer, or environmental purposes.
- **Human gene therapy** used to correct genetic disorders. The most immediate applications will be in adult disorders where the affected cells are easy to extract and treat, (e.g., sickle cell disease). In the future, CRISPR-Cas9 might be delivered directly into the body to alter the DNA in a particular cell type. CRISPR-Cas9 also opens the door for making changes to germline DNA (DNA that is heritable) to prevent the passage of genetic disorders to future generations. Such changes would be accomplished by editing DNA in eggs, sperm, or early embryos.

RELEVANT SCIENCE

A cell’s genome contains instructions that tell the cell how to function and how to interact with other cells. These instructions are encoded by a linear sequence of basic building blocks, called nucleotides, strung end-to-end. The basic unit of instruction is called a gene: each gene encodes information used for the production of other molecules, such as RNA and proteins, which operate and structure the cell.

Sometimes a cell is manipulated by foreign genetic instructions, like DNA injected into the cell by invading viruses. This is also true for bacteria, which are engaged in their own perpetual struggle against viruses. To fight back, bacteria (and other prokaryotes) have developed an antiviral defense – the CRISPR system. CRISPR (clustered, regularly interspersed short palindromic repeats) is a term that refers to copies of viral DNA that bacteria collect in the wake of viral infections. These viral DNA copies are used to remember the virus from which they are derived, allowing the bacteria to quickly attack and neutralize any subsequent infections.

The CRISPR system is mediated by Cas (CRISPR-associated) proteins, like the DNA-cutting Cas9. Cas9 is targeted at a specific sequence of DNA by a guide RNA molecule, which matches the target DNA sequence. Once Cas9 is guided to a target, it completely severs the DNA at a precise location.
Scientists recently found that CRISPR-Cas9 could function in non-prokaryotic cells, such as human cells, and could be used to precisely edit DNA. Specifically, they found that:

- By changing the sequence of the guide RNA, CRISPR-Cas9 can be reprogrammed to target almost any location across a genome. For example, it can be used to cut and inactivate specific genes that researchers are interested in studying.
- After Cas9 cuts the target DNA, the cut can be rejoined by a cell’s DNA repair system. This repair process, called non-homologous end joining, is often imperfect, resulting in a random change (or mutation) in the DNA sequence such as an insertion, deletion, or substitution of nucleotides.
- Going a step further, scientists can control this repair by inserting into the cell a short piece of synthetic (lab-manufactured) DNA that is largely identical to the target DNA, except for those modifications the scientists want to make to the target DNA. After the target DNA is cut, the synthetic DNA (with the desired changes) can serve as a template for the repair and be copied into the target DNA.
- CRISPR-Cas9 can be targeted at multiple locations in the same genome (multiplex genome editing) with greater ease than other current genome editing systems.

**WHY IT MATTERS**

CRISPR-Cas9 is having a revolutionary impact. Since 2012, it has spread across the globe to be used by thousands of researchers, many of whom did not previously conduct genome editing or engineering. Recent patent trials in the US and Europe have highlighted the importance of CRISPR-Cas9 as a commercial technology. Because of its ease and simplicity, CRISPR-Cas9 is crossing over into the under-regulated world of do-it-yourself biology, or biohacking. This widespread use promises to drive a significant increase in the number and scope of new genome-edited products.

The majority of US biotechnology products, such as those involving genome editing, are regulated by the Environmental Protection Agency (EPA), Food and Drug Administration (FDA), and the US Department of Agriculture (USDA) under the Coordinated Framework for the Regulation of Biotechnology, a system of regulations created in 1986.

The Coordinated Framework sought to balance protecting health and environment with enabling innovation, and focused on the safety of the end product rather than the process by which that product was created. Thus, it is unclear if limited changes to an organism’s DNA by genome editing (especially when the editing removes DNA rather than adds it) would be regulated by the current Coordinated Framework (genetic engineering). This has been the case for a number of plants made with genome-editing techniques, like mushrooms edited with CRISPR-Cas9; the USDA said these were not to be regulated.

In July 2015 the Obama White House initiated an effort to overhaul this system, which led to the 2017 Update to the Coordinated Framework. The goal of the update was to “clarify the current roles and responsibilities of the EPA, FDA, and USDA in the regulatory process” for products of biotechnology. Another part of the 2015 Obama White House initiative was the development of an “expert analysis of the future landscape of biotechnology products,” which was conducted by the National Academies of Sciences, Engineering, and Medicine (NASEM). The NASEM report was released shortly after the Coordinated Framework update. While it considered the update to be a good “starting point”, it stated that “the profusion of biotechnology products over the next 5-10 years has the potential to overwhelm the US regulatory system.”

The report went on to indicate that the relevant agencies responsible for the regulatory system should enhance their risk assessment mechanisms to account for new biotechnologies like CRISPR-Cas9 and their associated risks. For instance:

- Genetic changes, especially in animals, might have unintended negative effects on the organism or environment that are not immediately discernable, perhaps requiring more extensive risk assessment modeling and monitoring.
- Multiplex genome editing, as enabled by CRISPR-Cas9, might have unintended complications due to synergistic effects (the edits might interact in an unexpected way).
- One potential risk of releasing genome edited organisms into the wild, e.g., via gene drives, is the possibility that the genetic change (or the CRISPR-Cas9 system itself) might unintentionally jump to another species (a horizontal gene transfer) or become
unstable and nonfunctional.

- Biohacker communities, which are usually small and decentralized, represent a unique challenge to government oversight agencies, which often rely on other parties (like companies) to work towards compliance.
- Genome editing in humans, especially in relation to heritable, germline DNA, raises significant ethical questions about the boundaries of intervention.

RELATED POLICIES

The Coordinated Framework (and its recent update) was developed around the following pre-existing laws:

- The Federal Plant Pest Act (superseded by the Plant Protection Act) (USDA);
- The Federal Food, Drug, and Cosmetic Act (FDA);
- The Toxic Substances Control Act (EPA); and

Human Genome Editing: Science, Ethics, and Governance is a report from the National Academies of Sciences and Medicine that was published on February 14, 2017.

Preparing for Future Products of Biotechnology is a report from the National Academies of Sciences, Engineering, and Medicine that was published on March 9, 2017.

Regulation of Intentionally Altered Genomic DNA in Animals is a Draft Guidance that was released by the FDA on January 19, 2017. It was published to inform the US industry of the FDA's thoughts on genome editing in relation to animal products. Of note, the draft guidance indicated that the FDA believes that previously used terms, “genetic engineering” and “transgenic”, were inadequate for defining genome edited products.

ORGANIZATIONS

In 2012, two groups of scientists, one led by Jennifer Doudna and Emmanuelle Charpentier and the other by Virginijus Siksnys, found that they could harness CRISPR-Cas9 and reprogram it to target any DNA sequence in a genome. A year later, two other teams of scientists, one led by Feng Zhang and the other by George Church, showed that CRISPR-Cas9 could be transplanted into eukaryotic cells (like animal cells) and used to precisely edit their DNA.

Jennifer Doudna of the University of California, Berkeley, Department of Molecular & Cell Biology

Emmanuelle Charpentier of Umeå University, Department of Molecular Biology

Virginijus Siksnys of Vilnius University, Department of Protein – DNA Interactions

Feng Zhang of Broad Institute of MIT and Harvard, Department of Brain and Cognitive Sciences

George Church of Harvard Medical School, Department of Genetics

PRIMARY AUTHOR

Alex Robeson, PhD

EDITOR(S)