

## CRISPR/Cas Gene Editing Borrowing Tools from Bacteria

AUTHOR »

Jacqueline Jaeger Houtman, PhD

SCIENTIFIC ADVISOR »

Sidi Chen, PhD

MANAGING EDITOR »

Anne M. Deschamps, PhD

**CRISPR/CAS GENE EDITING** has been called the biggest biotechnology discovery of the century, even though the 21st century is less than two decades old. Laboratories around the world have adopted this technique to edit genes simply, quickly, and inexpensively. It is already revealing new insights in basic science and may eventually be used to fight infectious diseases, genetic disorders, and cancer.

### BACTERIAL IMMUNITY

Only a small part of the human genome (and the genomes of most other organisms) are genes that directly code for the production of proteins. The rest is so-called non-coding DNA. Since the 1960s, non-coding regions were sometimes referred to as *junk*, out of ignorance of what these stretches of the genome were doing. Eventually, it became apparent that much of the non-coding DNA was involved in regulation—switching the coding genes on and off.

In 1987, a group of scientists led by Atsuo Nakata (Osaka University) looked for regulatory regions in *E. coli* bacteria and reported an unusual pattern of non-coding DNA. Five identical stretches of 29 bases were repeated and interspersed with unique sequences of 32 bases (spacers). It

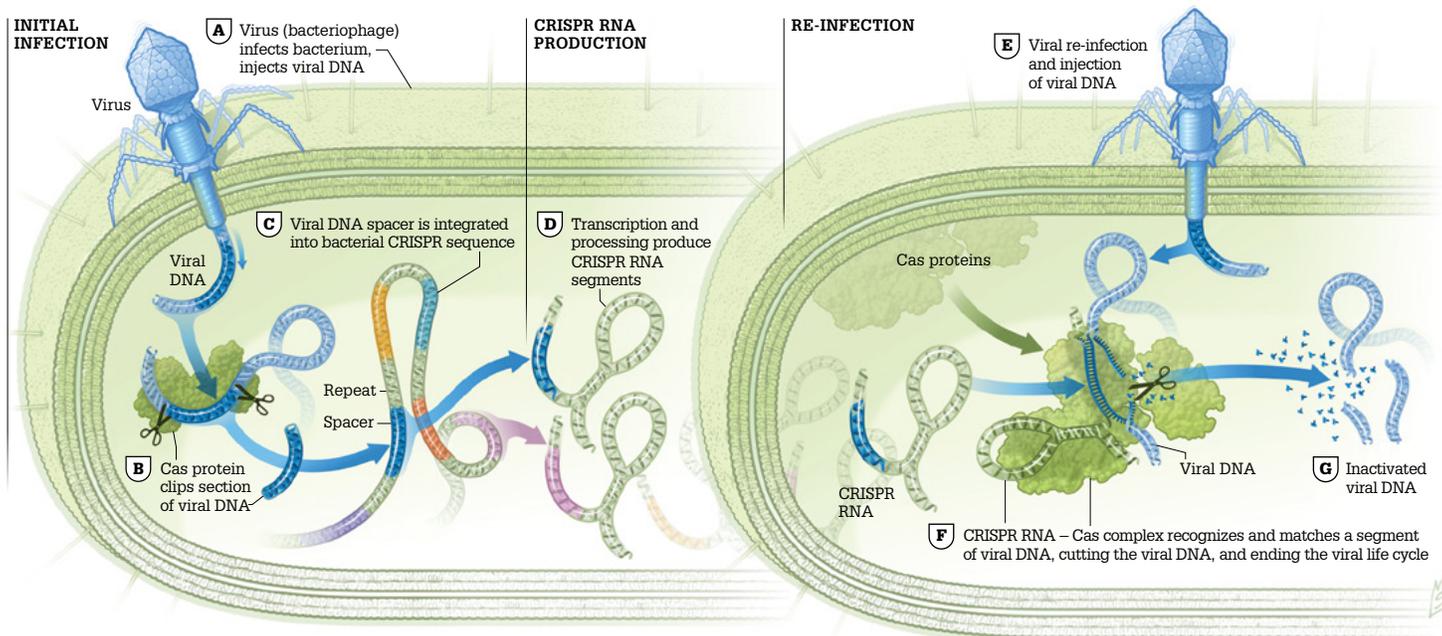
was an intriguing discovery at the time, but, as the authors put it, “the biological significance of these sequences is not known.” Similar patterns were found in many other species of bacteria. By 2002, this pattern had a name, CRISPR (clustered regularly interspaced short palindromic repeats). Nearby Cas (CRISPR-associated) genes were also described, but CRISPR/Cas was still a curiosity, with no known function.

Several groups found that the CRISPR spacer sequences were not random; they were identical to sequences in bacteriophages—viruses that infect bacteria. In 2007, Philippe Horvath and his colleagues at the food ingredient company Danisco (now DuPont) described how CRISPR/Cas is a sort of bacterial immune system. (Figure 1) Working with *Streptococcus ther-*

*mophilus* (one type of bacteria used to make yogurt and cheese), Horvath’s group showed that bacteria with a particular viral sequence as a CRISPR spacer were resistant to that virus.

### BACTERIA TO BIOTECH

The bacterial CRISPR system intrigued Jennifer Doudna (University of California, Berkeley). At a conference in Puerto Rico in 2011, she met Emmanuelle Charpentier (Umeå University, Sweden), who was working on a Cas protein, later called Cas9 (a type of enzyme called a nuclease that cuts DNA). Together they studied CRISPR and Cas9 in bacteria and showed how Cas9 cuts DNA at a specific site, guided by the RNA it carries with it. They soon realized that the CRISPR/Cas system could be a powerful tool adaptable to any genome. In their seminal 2012 paper, they



**FIGURE 1 / HOW CRISPR AND CAS PROTECT BACTERIA FROM VIRUSES »** When infected by a virus, a bacterial cell clips a bit of viral genetic material and saves it as a spacer between the repeats in CRISPR. Transcription and processing of the DNA spacers produces CRISPR RNA segments. When another virus comes along that matches one of these RNAs, the Cas protein cuts the viral gene, ending the virus’s life cycle. This bacterial defense mechanism provides immunity against viral invaders. Illustration: © Michael Linkhoker, Link Studio, LLC.

## LIMITS AND LIMITATIONS

Scientists always weigh the risks and benefits of their research, so the April 2015 report that a group of scientists in China had used CRISPR/Cas to modify a gene in nonviable human embryos sent a wave of concern around the world. Was the technology outpacing policy? Was it safe? Was it ethical to tinker with the human germline? If gene editing could cure genetic diseases, who would be cured? The scientific community continues to consider the ethical implications of CRISPR technology and to formulate research guidelines, especially as they concern human embryos. “NIH will not fund any use of gene-editing technologies in human embryos,” stated Francis Collins, Director of NIH. “The concept of altering the human germline in embryos for clinical purposes ... has been viewed almost universally as a line that should not be crossed.”

In addition to important ethical concerns, there are technical limitations of CRISPR/Cas editing. One of the most prominent issues is

off-target effects, which are unintended changes in genes elsewhere in the genome. Another limitation is mosaicism: even when CRISPR is applied to a one-cell mouse embryo, some cells in the adult possess the edited gene and some do not. This technology still requires improvements in order to edit the DNA at one-hundred percent efficiency and avoid the problem of mosaicism. There may also be other unintended and unanticipated effects of gene editing that have not been observed in the few years since CRISPR/Cas was introduced.

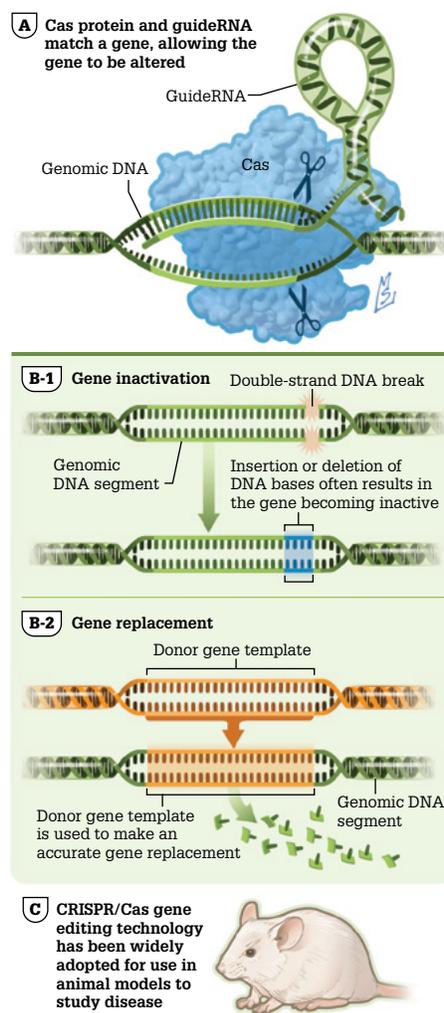
Groups of scientists around the world are working to optimize approaches while actively engaged in discussing the ethical implications of CRISPR technology and how this new discovery should be regulated. In December 2015, an international meeting on human gene editing released a set of guidelines, while recognizing the need to continue the discussion as the technology evolves.

showed that it could be engineered to cut out a specific DNA sequence and replace it with another sequence. This “alternative methodology based on RNA-programmed Cas9,” they wrote, “could offer considerable potential for gene-targeting and genome-editing applications.” (Figure 2)

It did not take long for that potential to be realized. Within months, genes in mouse and human cells were successfully altered in the laboratories of George Church (Harvard University) and Feng Zhang (Massachusetts Institute of Technology). Scientists around the world quickly adopted CRISPR for use in plants, fungi, fruit flies, roundworms, frogs, zebrafish, pigs, mice, monkeys, and disease-causing organisms. As Doudna and Charpentier had predicted, the system is “efficient, versatile, and programmable.”

Unlike other gene editing technologies, which require a new protein to be constructed for every gene of interest, the CRISPR/Cas system uses the same Cas nuclease for every gene, with a custom-made strand of RNA (called guideRNA) directing the nuclease to the desired spot in the genome. Mice with genetic alterations specifically designed to model human diseases can be produced in weeks instead of years. In contrast to some transgenic technology, CRISPR/Cas leaves the targeted gene in its original location, thus retaining nearby regulatory sequences. In addition, CRISPR/Cas methods can alter several different genes at the same time.

High-throughput genomic screening using the CRISPR/Cas system was demonstrated in 2014 by two groups of scientists at the Massachusetts Institute of Technology, one led by Feng Zhang and the other by David Sabatini and Eric Lander.



**FIGURE 2 / WHAT CRISPR CAN DO »** CRISPR/Cas technology can be used to modify, delete, and/or replace new genes. The use of this technology is rapidly expanding in the scientific community to develop animal models of disease. *Illustration:* © Michael Linkinhoker, Link Studio, LLC.

Both studies were supported with public funds from the National Institutes of Health (NIH). The investigators used thousands of guideRNA sequences to inactivate more than 18,000 genes in cultured cells. They could then screen the population of cells, each of which had one gene inactivated, to find which genes were necessary for tumor cell survival and resistance to chemotherapy drugs. Potentially, such screens can be used to identify targets for drug development or, in a clinical setting, provide personalized therapy with the most effective drug identified for an individual cancer patient.

## THE FUTURE OF CRISPR

Research continues at a rapid pace, refining the technique with new nucleases and new ways to deliver genes to cells. For example, a strain of mice engineered to express the Cas9 protein has been developed so that researchers can edit genes by simply introducing the guideRNA. Although permanent elimination of inherited diseases in the human germline may not be feasible or ethical, gene editing of affected cells in the body—in the blood, for example—could be used to treat some disorders.

The range of possible applications for CRISPR/Cas appears to be boundless. Basic research benefits from the simplicity and low cost, accelerating progress in a multitude of disciplines in industry, agriculture, pharmaceuticals, and medicine. Numerous scientific reports using CRISPR technology appear weekly, providing insights into our world and opportunities to improve lives. Thus, seemingly esoteric basic research on the bacterial immune system has, in just a few years, transformed the present field of biotechnology. Its future is yet to be imagined. 🌐