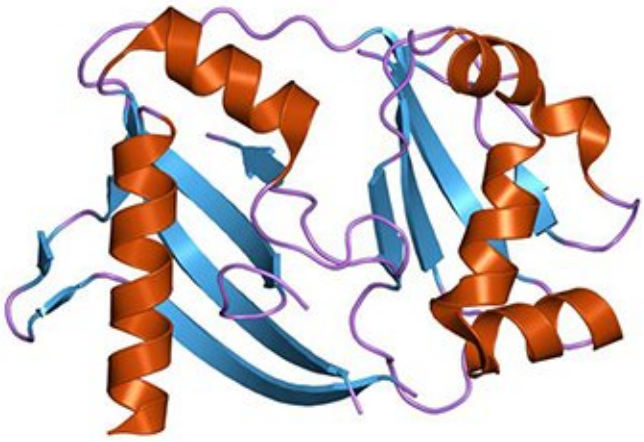
The cutting edge of DNA editing: translating CRISPR to improve human health



Structure of a CRISPR-associated protein, a pair of enzymatic scissors, used to cut and edit DNA

ACTG: A single letter can change our fate. These four letters represent the molecules that make up our DNA, which is subject to potentially deadly or disabling mutations from the moment of conception. For over sixty years, we have known the structure of DNA thanks to Watson and Crick, and for over a decade we have known every letter in our DNA thanks to the Human Genome Project. But despite discovering genetic changes associated with many cancers, Alzheimer's Disease, and thousands of other diseases with deleterious genetic mutations, we have only just discovered how to directly edit DNA. Coupling this fundamental discovery with further clinical exploration has the potential to transform human health, vastly increasing our scientific knowledge and leading to new therapies for previously incurable illnesses.

The bacteria E. coli is perhaps the most well studied organism on our planet, the workhorse of laboratories around the world. In the late 1980s, Japanese scientists discovered strange repeating segments of DNA in the E. coli genome (1). Throughout the 1990s, similar repeating segments were found in the DNA of many other bacteria. As the function of this DNA was unknown, it was named for how it appeared—"clustered regularly interspaced short palindromic repeats"—

CRISPR, for short. In the early 2000s, a Spanish researcher realized that these DNA segments were remarkably similar to viral DNA, and he published a theory that CRISPR functions as a primitive immune system for bacteria (2). Bacteria, like other living organisms, are susceptible to viral infection and CRISPR is theorized to help remember past viral infections by recording them in the bacterial cell's DNA. A special protein called a CRISPR-associated enzyme (Cas, for short) functions essentially as a pair of molecular scissors, dispatched to cut up viral DNA before the virus can kill the bacterial cell.

The simplicity of this system—DNA-based memory with molecular scissors to attack invading viruses—attracted tremendous interest from basic scientists in how CRISPR-Cas might be manipulated to further research. Numerous alterations to the system have been made, changing it from a defensive bacterial weapon to a precise scientific tool, allowing scientists to directly edit DNA and study genetic mutations with an accuracy and speed heretofore only imagined.

Before CRISPR, attempts at genetic modification and treatments for genetic diseases were complicated, slow, and often ineffective. Humans have been genetic engineers long before we knew it, choosing traits in animals and agriculture through selective breeding. With discoveries in basic science, we progressed to utilizing the machinery of the cell to interfere with the production of dysfunctional proteins. We attempted to reprogram viruses to infect diseased cells and fix harmful genetic mutations. But these methods were unpredictably imprecise, producing unintended genetic mutations. CRISPR has become a versatile and accurate tool, able to add or delete genes and activate or dial down gene activity.

Translating basic science to improve human health requires studying diseases in animal models —fruit flies, mice, and eventually primates. Previously, creating a mouse or a monkey with a particular genetic modification required generations of crossbreeding. CRISPR can be used in a one cell embryo to precisely alter a gene of interest, permitting scientists to create a study animal for a particular disease instantly and humanely, without needing to crossbreed intermediate generations (3).

A single genetic mutation producing a disease is the exception and not the rule. Sequencing technology has exposed the incredible genetic complexity of diseases including cancer, autism, and epilepsy, which have intricate networks of polygenic associations. CRISPR allows scientists to systematically test and catalogue these genetic networks. Elucidating which mutations are critical steps in the pathways to cancer and disease will identify new therapeutic targets and enable personalized therapy.

The ability to edit DNA and regulate gene expression may produce new therapies for illnesses like Alzheimer's Disease and HIV. Mutated amyloid precursor protein is associated with early-onset Alzheimer's. Researchers have been studying how to hijack its expression with CRISPR, so that instead of causing dementia it protects against cognitive decline (4). Attacking Alzheimer's from another avenue, CRISPR has been used to induce stem cells to grow into neurons, which may result in therapies for many degenerative neurologic diseases (5). CRISPR may also play a role in treating HIV infection, which has been incurable because the retrovirus incorporates itself directly into an infected person's DNA. Because the virus develops progressive resistance to antiretroviral drugs, a definitive therapy is essential. Recent scientific studies have shown that CRISPR may be able to delete HIV from the DNA of an infected person's cells, potentially curing the disease permanently (6).

CRISPR may transform not just research and human disease, but our entire world. Agricultural companies are interested in the technology's potential to edit crops to make them drought-resistant and faster-growing. Scientists have begun to explore how CRISPR can alter populations of mosquitoes to prevent transmission of Zika virus or malaria. This wide-reaching potential of CRISPR has ignited important ethical discussions. Bioethicists and scientific researchers must come together to develop consensus positions and moratoriums on particular applications. Broad public education and engagement regarding CRISPR's potential benefits and risks are needed before developing regulations to guide its use.

Significant improvements are being made to CRISPR and its associated enzymes. Researchers recently modified the Cas enzyme to make precise edits to DNA without breaking DNA strands, demonstrating the potential to specifically correct genetic mutations (7). Continued searches

through bacterial DNA have revealed new enzymes that improve CRISPR's performance (8). From its humble and accidental discovery in the genomes of simple bacteria, CRISPR is set to become one of the most fundamental basic science research tools with broad applications in science and medicine. Further research and public engagement are needed to fully translate CRISPR's immense potential to improve human health.

1. Ishino Y, Shinagawa H, Makino K, Amemura M, Nakata A. Nucleotide sequence of the iap gene, responsible for alkaline phosphatase isozyme conversion in Escherichia coli, and identification of the gene product. *J Bacteriol* 1987;169(12):5429–33.
2. Mojica FJM, DíezVillaseñor C, GarcíaMartínez J, Soria E. Intervening sequences of regularly spaced prokaryotic repeats derive from foreign genetic elements. *J Mol Evol* 2005;60(2):174–82.
3. Niu Y, Shen B, Cui Y, et al. Generation of gene-modified cynomolgus monkey via Cas9/RNA-mediated gene targeting in one-cell embryos. *Cell* 2014;156(4):836–43.
4. Jonsson T, Atwal JK, Steinberg S, et al. A mutation in APP protects against Alzheimer's disease and age-related cognitive decline. *Nature* 2012;488(7409):96–9.
5. Chavez A, Scheiman J, Vora S, et al. Highly efficient Cas9mediated transcriptional programming. *Nat Methods* 2015;12(4):326–8.
6. Hu W, Kaminski R, Yang F, et al. RNA-directed gene editing specifically eradicates latent and prevents new HIV1 infection. *Proc Natl Acad Sci* USA 2014;111(31):11461–6.
7. Komor AC, Kim YB, Packer MS, Zuris JA, Liu DR. Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. *Nature* 2016.
8. Zetsche B, Gootenberg JS, Abudayyeh OO, et al. Cpf1 is a single RNA-guided endonuclease of a class 2 CRISPR-Cas system. *Cell* 2015;163(3):759–71.