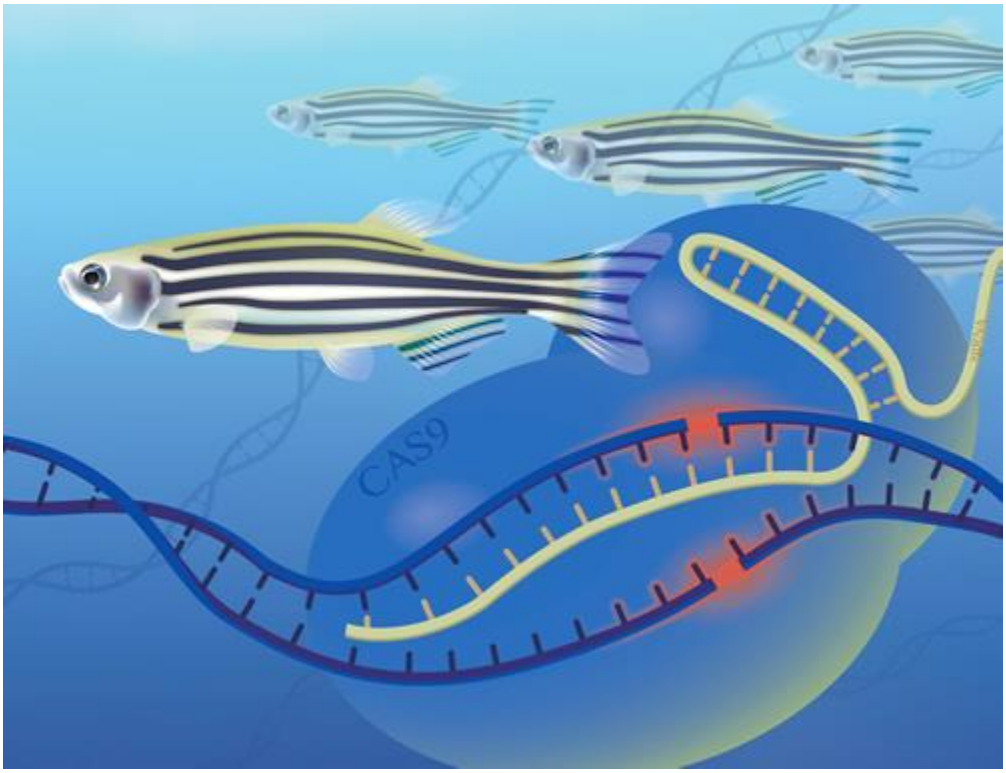
Genome editing: is a method that lets scientists change the DNA of many organisms, including plants, bacteria, and animals. Editing DNA can lead to changes in physical traits, like eye color, and disease risk. Scientists use different technologies to do this.

Overview

Genome editing technologies enable scientists to make changes to DNA, leading to changes in physical traits, like eye color, and disease risk. Scientists use different technologies to do this. These technologies act like scissors, cutting the DNA at a specific spot. Then scientists can remove, add, or replace the DNA where it was cut.

The first genome editing technologies were developed in the late 1900s. More recently, a new genome editing tool called CRISPR, invented in 2009, has made it easier than ever to edit DNA. CRISPR is simpler, faster, cheaper, and more accurate than older genome editing methods. Many scientists who perform genome editing now use CRISPR.

In the Laboratory



One way that scientists use genome editing is to investigate different diseases that affect humans. They edit the genomes of animals, like mice and zebrafish, because animals have many of the same **genes** as humans. For example, mice and humans share about 85 percent of their genes! By changing a single gene or multiple genes in a mouse, scientists can observe how these changes affect the mouse's health and predict how similar changes in human genomes might affect human health.

Scientists at the National Human Genome Research Institute (NHGRI) are doing just this. The **Burgess lab**, for example, is studying zebrafish genomes. Scientists in this lab delete different genes in zebrafish one at a time using CRISPR to see how the deletion impacts the fish. The Burgess lab focuses on 50 zebrafish genes which are similar to the genes that cause human deafness so that they can better understand the genomic basis of deafness.

Techniques to modify DNA in the genome have existed for several decades, but the conversation about the science and ethics of genome editing has grown louder due to faster, cheaper, and more efficient technologies.

While the popular media tends to focus on the potential use of genome editing in humans, the main application of this technology has been in basic research. Editing the genomes of yeast, bacteria, mice, zebrafish, and other organisms that scientists commonly study has led to countless discoveries about how the genome is connected to physical traits, like eye color, and disease.

Researchers funded by the National Human Genome Research Institute (NHGRI) and other research institutes at the National Institutes of Health (NIH) are adopting newer techniques, such as CRISPR, to conduct their investigations. A robust understanding of how the genome gives rise to health and disease will aid the development of new treatments, including **gene therapy**.

Genome Editing Methods

Scientists have had the knowledge and ability to edit genomes for many years, but CRISPR technology has brought major improvements to the speed, cost, accuracy, and efficiency of

genome editing. The history of genome editing technologies shows the remarkable progress in this field and also relays the critical role that basic science research plays in the development of research tools and potential disease treatments.

Homologous recombination

The earliest method scientists used to edit genomes in living cells was **homologous recombination**. Homologous recombination is the exchange (recombination) of genetic information between two similar (homologous) strands of DNA.Scientists began developing this technique in the late 1970s following observations that yeast, like other organisms, can carry out homologous recombination naturally.

To perform homologous recombination in the laboratory, one must generate and isolate DNA fragments bearing genome sequences similar to the portion of the genome that is to be edited. These isolated fragments can be injected into individual cells or taken up by cells using special chemicals. Once inside a cell, these DNA fragments can then recombine with the cell's DNA to replace the targeted portion of the genome.

This type of homologous recombination is limited by the fact that it is extremely inefficient in most cell types. This technique can have as low as a one-in-a-million probability of successful editing. Another weakness of homologous recombination is that it is inaccurate and has a high rate of error when the injected DNA fragments insert into an unintended part of the genome, causing what are known as off-target edits.

Zinc-finger nucleases (ZFN)

In the 1990s researchers started using **zinc-finger nucleases (ZFN)** to improve the specificity of genome editing and reduce off-target edits. The structures of ZFNs are engineered from naturally-occurring **proteins** that were discovered in **eukaryotic** organisms. Scientists can engineer these proteins to bind to specific DNA sequences in the genome and cut DNA. Once bound to their target DNA sequence, the ZFNs cut the genome at the specified location, allowing scientists to either delete the target DNA sequence or replace it with a new DNA sequence via homologous recombination.

Although ZFNs improved the success rate of genome editing to about 10 percent, it is difficult and time-consuming to design, construct, and produce successful zinc finger proteins, and a new ZFN must be engineered for each new target DNA sequence.

Transcription activator-like effector nucleases (TALENs)

In 2009, a new class of proteins called **Transcription Activator-Like Effector Nucleases (TALENs)** arrived to the genome editing scene. Similar to ZFNs, transcription activator-like effector nucleases (TALENs) are engineered from proteins found in nature and are capable of binding to specific DNA sequences.

While TALENs and ZFNs are comparable in terms of how efficiently they can create edits to the genome, TALENs bear the advantage of greater simplicity. It is much easier to engineer TALENs than it is to synthesize ZFNs.

Clustered regularly interspaced short palindromic repeats (CRISPR)

Though ZFN and TALEN technology increase the specificity and efficiency of genome editing, they are relatively expensive and complicated to use in the lab. Each edit would require the construction of a new ZFN or TALEN protein, and engineering proteins can be a difficult process that is prone to error. This is one reason why CRISPR is a game-changing technology; unlike its predecessors, CRISPR is a simple technology with little assembly required. CRISPR associated DNA sequences were first observed in bacteria in the early 1990s, but it was not until the 2000s that the scientific community understood its ability to recognize specific genome sequences and cut them via the Cas9 protein, a protein that works with CRISPR and that has DNA-cutting abilities. In nature, CRISPR is used by bacteria as an immune system to kill invading viruses, but it has now been adapted for use in the lab.

With CRISPR, researchers create a short **RNA** template that matches a target DNA sequence in the genome. Creating synthetic RNA sequences is much easier than engineering proteins as is those required for ZFNs and TALENs. Strands of RNA and DNA can bind to each other when they have matching sequences. The RNA portion of the CRISPR, called a guide RNA, directs Cas9 enzyme to the targeted DNA sequence. Cas9 cuts the genome at this location to make the edit. CRISPR can make deletions in the genome and/or be engineered to insert new DNA

sequences. One group of scientists found that CRISPR is six times more efficient than ZFNs or TALENs in creating targeted mutations to the genome. Large-scale genomics projects that once took many years and tens of thousands of dollars can now be completed at a small fraction of time and price.

In the United States and around the world, scientific institutions are developing recommendations to facilitate decision-making for the responsible use of human genome-editing research.

QUESTIONS/ANSWERS :

Q: What is "CRISPR"?

A: "CRISPR" (pronounced "crisper") stands for Clustered Regularly Interspaced Short Palindromic Repeats, which are the hallmark of a bacterial defense system that forms the basis for CRISPR-Cas9 genome editing technology. In the field of genome engineering, the term "CRISPR" or "CRISPR-Cas9" is often used loosely to refer to the various CRISPR-Cas9 and -CPF1, (and other) systems that can be programmed to target specific stretches of genetic code and to edit DNA at precise locations, as well as for other purposes, such as for new diagnostic tools. With these systems, researchers can permanently modify genes in living cells and organisms and, in the future, may make it possible to correct mutations at precise locations in the human genome in order to treat genetic causes of disease. Other systems are now available, such as CRISPR-Cas13's, that target RNA provide alternate avenues for use, and with unique characteristics that have been leveraged for sensitive diagnostic tools, such as SHERLOCK.

Q: Where do CRISPRs come from?

A: CRISPRs were first discovered in archaea (and later in bacteria) by Francisco Mojica, a scientist at the University of Alicante in Spain. He proposed that CRISPRs serve as part of the bacterial immune system, defending against invading viruses. They consist of repeating sequences of genetic code, interrupted by "spacer" sequences – remnants of genetic code from

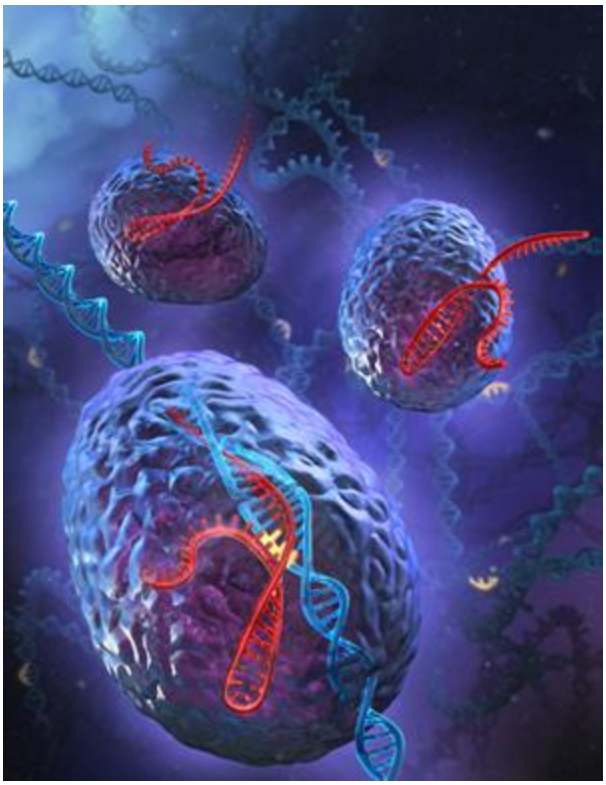
past invaders. The system serves as a genetic memory that helps the cell detect and destroy invaders (called “bacteriophage”) when they return. Mojica’s theory was experimentally demonstrated in 2007 by a team of scientists led by Philippe Horvath.

In January 2013, the Zhang lab published the first method to engineer CRISPR to edit the genome in mouse and human cells.

Q: How does the system work?

A: CRISPR “spacer” sequences are transcribed into short RNA sequences (“CRISPR RNAs” or “crRNAs”) capable of guiding the system to matching sequences of DNA. When the target DNA is found, Cas9 – one of the enzymes produced by the CRISPR system – binds to the DNA and cuts it, shutting the targeted gene off. Using modified versions of Cas9, researchers can activate gene expression instead of cutting the DNA. These techniques allow researchers to study the gene’s function.

Research also suggests that CRISPR-Cas9 can be used to target and modify “typos” in the three-billion-letter sequence of the human genome in an effort to treat genetic disease.



An artist's depiction of the CRISPR system in action.

Illustration by Stephen Dixon

Q: How does CRISPR-Cas9 compare to other genome editing tools?

A: CRISPR-Cas9 is proving to be an efficient and customizable alternative to other existing genome editing tools. Since the CRISPR-Cas9 system itself is capable of cutting DNA strands, CRISPRs do not need to be paired with separate cleaving enzymes as other tools do. They can also easily be matched with tailor-made “guide” RNA (gRNA) sequences designed to lead them to their DNA targets. Tens of thousands of such gRNA sequences have already been created and are available to the research community. CRISPR-Cas9 can also be used to target multiple genes simultaneously, which is another advantage that sets it apart from other gene-editing tools.

Q: How does CRISPR-Cpf1 differ from CRISPR-Cas9?

CRISPR-Cpf1 differs in several important ways from the previously described Cas9, with significant implications for research and therapeutics.

First, in its natural form, the DNA-cutting enzyme Cas9 forms a complex with two small RNAs, both of which are required for the cutting activity. The Cpf1 system is simpler in that it requires only a single RNA. The Cpf1 enzyme is also smaller than the standard SpCas9, making it easier to deliver into cells and tissues.

Second, and perhaps most significantly, Cpf1 cuts DNA in a different manner than Cas9. When the Cas9 complex cuts DNA, it cuts both strands at the same place, leaving 'blunt ends' that often undergo mutations as they are rejoined. With the Cpf1 complex the cuts in the two strands are offset, leaving short overhangs on the exposed ends. This is expected to help with precise insertion, allowing researchers to integrate a piece of DNA more efficiently and accurately.

Third, Cpf1 cuts far away from the recognition site, meaning that even if the targeted gene becomes mutated at the cut site, it can likely still be re-cut, allowing multiple opportunities for correct editing to occur.

Fourth, the Cpf1 system provides new flexibility in choosing target sites. Like Cas9, the Cpf1 complex must first attach to a short sequence known as a PAM, and targets must be chosen that are adjacent to naturally occurring PAM sequences. The Cpf1 complex recognizes very different PAM sequences from those of Cas9. This could be an advantage in targeting, for example, the malaria parasite genome and even the human genome.

Q: What other scientific uses might CRISPR have beyond genome editing?

A: CRISPR genome editing allows scientists to quickly create cell and animal models, which researchers can use to accelerate research into diseases such as cancer and mental illness. In addition, CRISPR is now being developed as a rapid diagnostic. To help encourage this type of research worldwide, Feng Zhang and his team have trained thousands of researchers in the use of CRISPR genome editing technology through direct education and by sharing more than 40,000 CRISPR components with academic laboratories around the world.

REFERENCES :

https://ghr.nlm.nih.gov/primer/genomicresearch/genomeediting