

“Life Science” field**Achievement : Elucidation of the genome editing mechanism by the CRISPR-Cas****Prof. Emmanuelle Charpentier** (France)

Born: December 11, 1968 (Age 48)

Director, Max Planck Institute for Infection Biology (Berlin)

Dr. Jennifer A. Doudna (USA)

Born: February 19, 1964 (Age 52)

Professor, University of California, Berkeley

Summary

Genome editing using the CRISPR-Cas system, announced by Prof. Emmanuelle Charpentier and Dr. Jennifer Doudna in 2012, is a revolutionary new technology in genetic engineering. It was adopted at an explosive rate as a useful tool for research in the life sciences. Today, it continues to be applied to research in a wide range of fields, such as breeding, drug development and medicine. This technology was developed in the process of elucidating the bacterial defense mechanism against such threats as viral infections. Bacteria can remember the DNA of intruding viruses by absorbing their DNA into their own. Upon the next infection, bacteria recognize the intruder's DNA and snips it with the RNA-guided CAS protein, thereby destroying intruding viruses. Genome editing by the CRISPR-Cas system takes advantage of this mechanism, and enables one to cut the DNA of any organism at arbitrary locations to edit freely by means of removing, replacing or insertion.

Meeting of two female scientists results in a long distance research collaboration

CRISPR is an abbreviation for Clustered Regularly Interspaced Short Palindromic Repeats. Bacteria genomes frequently contain repeating structures of palindrome-like identical sequences, made up of 20 to 50 bases, in between short sequences (spacers).

This repeating structure is CRISPR. It is sometimes called the CRISPR locus including the genes that code the string of adjoining Cas (CRISPR associated) proteins.

This repeating sequence came to be recognized as genome sequencing of many bacteria and archaeobacteria advanced, but its role was initially a mystery. Later on, it was discovered that spacer sequences are not inherent in bacteria, but are derived from foreign viruses and plasmids. It was then hypothesized that CRISPR's role is to provide bacteria with adaptive immunity against intruders, which was later proven to be the case.

The fact that small unicellular bacteria possess an ingenious immune system was received with great surprise and spurred research on how bacteria fight off invaders. This is the mechanism that was elucidated by Prof. Charpentier and Dr. Doudna. The two met in Puerto Rico in March 2011 at the conference on “Regulating with RNA in Bacteria” hosted by the American Society for Microbiology, and began their joint research immediately thereafter.

Prof. Charpentier, born in France, is a microbiologist with a PhD from Institut Pasteur. At the time, she was working at Umea University in Sweden, near the arctic region.

In the early 2000s, when she headed a small laboratory at the University of Vienna, she became interested in CRISPR, which had not yet received much attention. By 2009, she had discovered that two RNA and Cas9 protein in the streptococcus pyogenes genome play an important role in a bacterium's immune system.

Already at this point, Prof. Charpentier believed in its potential as a new technology for genetic engineering.

Dr. Doudna, on the other hand, graduated with a PhD from Harvard University where she studied RNA enzymes (ribozymes). As a structural biologist, she has since consistently researched the various functions

of RNA, including the elucidation of the three-dimensional structure of ribozyme crystals.

From 2002, she has worked as a professor at the University of California, Berkeley. Since she became aware of the hypothesis about CRISPR's potential role in the adaptive immunity of bacteria around 2005, she had been conducting research to elucidate the role of RNA in the defense mechanism of cells.

Looking back, Dr. Doudna feels that upon meeting Prof. Charpentier, intuition told her they could complement each other through joint research. Thus began a long distance research collaboration bridging Northern Europe and the West Coast of the United States, which soon produced results that would astonish the world.

The birth of a genome editing technology that enables us to freely rewrite DNA

Already in June of the year following their first meeting, the joint research group used the DNA of streptococcus pyogenes provided by Prof. Charpentier to elucidate the detailed mechanism of how the two RNA and the bacteria's Cas protein (Cas9) cut foreign DNA. Along with the discovery, they also demonstrated that this knowledge can be converted into a revolutionary genome editing technology. It was an achievement that truly took the world by storm.

When a bacterium is invaded by a foreign virus, the invader's DNA is fragmented by the Cas enzyme and stored in the CRISPR locus as spacer sequences. If the bacterium detects the same invader again, short-chain CRISPR RNA (crRNA) is produced using the stored spacers as the template.

crRNA then forms a compound with trans-activating crRNA (tracrRNA) which provides the base for the Cas protein. This compound (gRNA) guides the Cas enzyme to its complementary location in the invader's DNA.

The Cas9 enzyme that snips the DNA strand has two DNA incision sections, with one section cutting one of the strand and the other section cutting the opposite strand. The markers used for incision are short sequences approximately three-base-long called PAM (Proto-spacer Adjacent Motif) located throughout the intruder's DNA. The Cas9 enzyme coming in contact with PAM triggers incision.

Having elucidated this mechanism, their research group demonstrated that by designing and synthesizing gRNA that corresponds to the target DNA locus, and introducing it into a cell together with Cas9, the target DNA can be discriminately cut at as many PAM positions as desired with pinpoint accuracy.

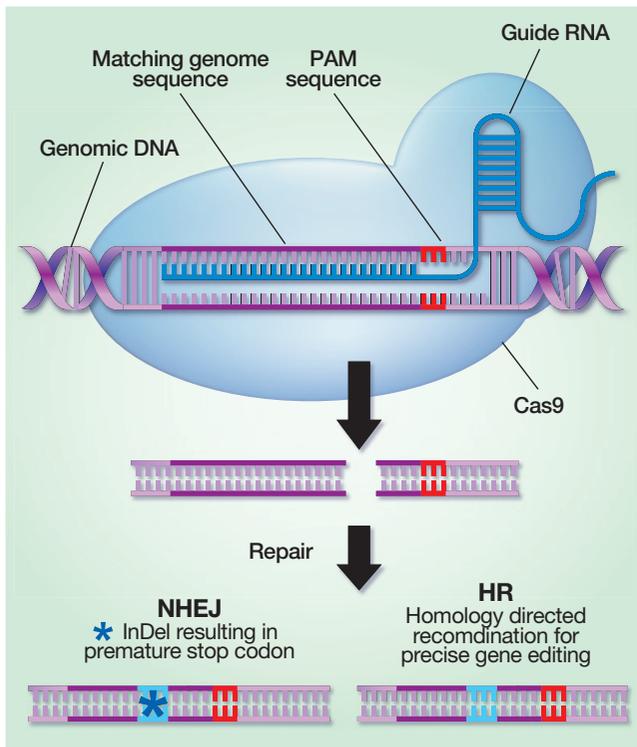
The incision site of target DNA is recombined by an intracellular repair mechanism, but due to the shift that occurs in the base sequence, the gene loses its function and becomes knocked out. Furthermore, introducing a base sequence into the incision site induces homologous recombination repair, resulting in the insertion of this base sequence into the DNA.

It is without a doubt that the whole world was excited by the discovery of a new genome editing technology that is highly versatile, precise and efficient. Thanks to its low cost and speediness compared to existing techniques such as ZFN and TALEN, as well as its overwhelming simplicity, CRISPR has attracted many researchers and spurred further trial and research.

25 years since the discovery of a mysterious sequence – The future of genome editing

The mysterious repeating sequence in the DNA of bacteria was first observed back in 1987. Dr. Yoshizumi Ishino of the Research Institute for Microbial Diseases at Osaka University reported the existence of a mysterious base sequence in *E. coli*, concluding his report with the words, “its biological significance is unknown”.

25 years on, this mysterious base sequence has been adapted



into the CRISPR-Cas9 technology which enables us to edit the genome of all living things, and is on the verge of bringing about unprecedented technological innovation across various disciplines of the life sciences.

The development and improvement of the CRISPR-Cas9 technology has since become very competitive, with researchers striving to improve precision and ease of use.

Its popularity has been so remarkable that tailored kits have now become available, and there is even a commercial genome editing service operating with success.

In early 2013, the first genome editing of a mammalian cell using CRISPR-Cas9 was performed. Today, genome editing of human cells, such as those in conjunction with iPS cells, has advanced to the stage where it is now aiming for clinical application. The fields of agriculture and livestock breeding are also seeing much activity with promising research efforts.

While genome editing technology has the potential to revolutionize the future of mankind, there are also growing concerns over the ethical issues it raises and its negative impact on the ecosystem, as exemplified by the genetic modification of reproductive cells and the eradication of specific organisms.

From early on, Dr. Doudna has been a vocal advocate on these issues and proposed discussion amongst the scientific community. At the 2015 "International Summit on Human Gene Editing" hosted by the National Academy of Sciences of the United States, a consensus for a certain level of restraint on human gene editing research was reached. Cautiously yet resolutely, scientists' efforts in genome editing are rapidly transforming the field of life science within just a few years from the innovative breakthrough achieved by Prof. Charpentier and Dr. Doudna's joint research.

After the remarkable achievement of their joint research, the two scientists are now pursuing new research in their respective fields. Prof. Charpentier is now the director of the Max Planck Institute for Infection Biology and has also founded a venture business on gene therapy. Similarly, Dr. Doudna has founded a venture business and is collaborating with biotech companies to apply her technology into a wide range of fields.

There is great anticipation in the future activity of Prof. Charpentier and Dr. Doudna.