Jennifer Doudna and Emmanuelle Charpentier’s Experiment About the CRISPR/cas 9 System’s Role in Adaptive Bacterial Immunity (2012) [1]

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In 2012, Jennifer Doudna, Emmanuelle Charpentier from the University of California, Berkeley, in Berkeley, California, and Umeå University in Umeå, Sweden, along with their colleagues discovered how bacteria use the CRISPR/cas 9 system to protect themselves from viruses. The researchers also proposed the idea of using the CRISPR/cas 9 system as a genome [3] editing tool.

In bacteria and archaea, researchers had found that CRISPR, which stands for clustered regularly interspaced short palindromic repeats, and CRISPR associated proteins, or cas, helped organisms recognize and silence the genetic material of viruses that have infected the cell before. In their experiment, Doudna, Charpentier, and their colleagues found how the specific molecules in bacteria can recognize and cut specific DNA sequences of invading viruses. Doudna, Charpentier, and their colleagues’ discovery of the CRISPR/cas 9 mechanism and proposal of using CRISPR/cas 9 for genetic editing led to the successful engineering of CRISPR/cas 9 as a novel method of editing genomes.

DNA is a molecule that contains genetic information for cells. RNA transfers genetic information from DNA to proteins. Cells use the genetic information in DNA and RNA as directions to make the proteins. When a virus infects a bacterium, the virus copies its DNA into the bacterium’s genome [3] and tricks the bacterium into making viral proteins, which can harm the bacterium. To prevent against a second infection from the same virus, bacteria have a system called CRISPR/cas. In bacteria, CRISPR regions of the genome [3] have spacer sequences of DNA that sit between segments of repeated DNA. The spacer DNA sequences match the DNA of viruses. Bacteria use those sequences to identify invading viruses. When a bacterium recognizes an invading virus, it copies the spacer DNA into a special kind of RNA, called CRISPR RNA, or crRNA. The crRNA molecule, with the help of trans-activating crRNA, or tracrRNA, guides a cas 9 protein to recognize and cut the virus’s DNA that matches the crRNA sequence. Cas 9 is a special protein that cuts double-stranded DNA. When the bacterium cuts the viral DNA, the virus is inactivated and can no longer affect the bacterium. Doudna, Charpentier, and their colleagues uncovered those mechanisms in their experiments.

The researchers working on the experiment came from different institutions, but they shared a research focus in molecular biology. Doudna received her PhD from Harvard University [4] in Cambridge, Massachusetts. She studied ribozymes, or RNA molecules that help biochemical reactions happen in the cell. Charpentier received her PhD from the Pasteur Institute in Paris, France. For her thesis, she studied how bacteria develop resistance to antibiotics. While at Umeå University, Charpentier discovered tracrRNA, an RNA molecule that helps guide cas 9 and plays a role in how CRISPR/cas 9 cuts DNA. Martin Jinek and Michael Hauer were postdoctoral researchers working with Doudna at the University of Berkeley, while Krzysztof Chylinski and Ines Fonfara were researchers working with Charpentier at Umeå University. Chylinski also worked at the University of Vienna in Vienna, Austria.

Before Doudna, Charpentier, and their colleagues discovered the mechanism of CRISPR/cas 9, scientists had reported that some bacteria and archaea used the CRISPR/cas 9 to defend themselves against viruses. Scientists speculated that crRNA, or CRISPR RNA, was involved in facilitating that defense through its involvement with the cas9 protein, though the mechanism was unclear.

In 2011, Doudna, Charpentier, and their colleagues began experimenting with the bacteria Streptococcus pyogenes [5] to understand the mechanisms of the CRISPR/cas 9 system. They aimed to accomplish several goals with their experiments, including identifying the molecules and mechanisms involved in protecting bacteria from viruses and demonstrating that CRISPR/cas 9 can be programmed as a gene-editing tool. First, Doudna, Charpentier, and their colleagues aimed to confirm that crRNA, and tracrRNA, or trans-activating CRISPR RNA, are necessary for cas 9 to recognize and cut double-stranded DNA from viruses. They also aimed to discover exactly how each molecule functioned in the CRISPR/cas 9 system. Lastly, they worked to find a novel method of editing DNA by using the CRISPR/cas 9 system.

To confirm that both crRNA and tracrRNA molecules guide cas 9 to recognize and cut DNA, Doudna, Charpentier, and their colleagues tested how well cas 9 cut DNA when either crRNA or tracrRNA molecules were present. The researchers performed
their experiments with target DNA in the form of a short linear double-strand, or a single, free piece of DNA, and plasmid, which is a circular loop of double-stranded DNA. First, the researchers attempted to use cas9 and crRNA to cut the linear DNA and the plasmid. The cas9 protein and crRNA worked together to target the DNA sequences the researchers wanted to cut. However, the researchers found that in both cases, with linear DNA and plasmid DNA, cas9 and crRNA could not cut DNA without tracrRNA. That confirmed that cas9 needs both crRNA and tracrRNA to guide it to cut DNA.

Next, to show that crRNA is sequence specific, meaning that it only targets a specific sequence of DNA, the researchers attempted to cleave DNA with cas9, tracrRNA, and crRNA. However, for those trials, the crRNA sequence did not match the target DNA sequence. Because of that, the researchers hypothesized that cas9 could not successfully recognize and cut the target DNA. When cas9 failed to cut the DNA, the researchers confirmed their hypothesis that crRNA is sequence specific. Thus, Doudna, Charpentier, and their colleagues confirmed that both crRNA and tracrRNA were necessary for guiding cas9 in recognizing and cleaving target DNA.

In addition, Doudna, Charpentier, and their colleagues showed that a recognition site on the target DNA was also necessary for cas9 to cut the DNA. The recognition site is called PAM. In bacteria, PAM is a short DNA sequence in the CRISPR region that comes right after the targeted DNA sequence. To evaluate the importance of that recognition site, the scientists altered the sequence of PAM in the target DNA and attempted to use cas9, crRNA, and tracrRNA to cut the DNA. They hypothesized that with an altered recognition site, the cas9 would not be able to recognize or cut the DNA. Cas9 did not cut the DNA, showing their hypothesis to be correct.

To further determine the mechanism of the CRISPR/cas9 system, Doudna, Charpentier, and their colleagues tested how each molecule played a role in recognizing target DNA and cutting it. To explain how cas9 recognized target DNA, the researchers tested how well cas9 bound to target DNA with and without the presence of tracrRNA and crRNA. If the cas9 protein bound well to the DNA without its guiding molecules, then that would mean cas9 does not need crRNA or tracrRNA for activation and sequence recognition. The research team found that cas9 on its own and cas9 with only crRNA did not bind well to the target DNA. The result suggested that tracrRNA helps activate or orient crRNA so that crRNA can assist cas9 in binding with DNA.

The researchers also tested the impact of shortened tracrRNA sequences on recognizing the target DNA. They hypothesized that shortened tracrRNA molecules would still be able to activate the cas9 protein. They found that to an extent, shortened tracrRNA molecules could still be used with crRNA and cas9 to recognize target DNA. That result showed that a modified tracrRNA molecule could potentially still work with cas9 to cut DNA. Furthermore, they tested cas9 proteins from other species of bacteria in S. pyogenes. They found that cas9 proteins from closely related bacteria could be used successfully to recognize and cut DNA with crRNA and tracrRNA. That result indicated that DNA, crRNA, and tracrRNA most likely evolved at the same time, because cas9 from closely related types of bacteria worked with DNA and RNA in S. pyogenes.

As part of their experiment, Doudna, Charpentier, and their colleagues found how the cas9 protein cuts double stranded DNA. They reported that cas9 has two regions that were similar to other proteins known to cut genetic material. To test how each region impacted the function of cas9, Doudna, Charpentier, and their colleagues made different versions of a cas9 protein, disabling their function. First, the researchers attempted to cut DNA in a plasmid form with tracrRNA, crRNA, and the modified cas9 protein with one region disabled. They found that the plasmid was nicked but still in a full loop formation, meaning that the cas9 protein was only able to cut one of the two strands of the DNA. The research team repeated the experiment with a cas9 protein disabled in the other region. They found the same result as they had found in the first modified region. The results suggested that each region of cas9 protein cut one strand of the DNA. Therefore, a modification in one region caused cas9 to only cut one strand of the double-stranded DNA. Unmodified cas9 with no mutations in those regions cut both strands of DNA.

Finally, Doudna, Charpentier, and their colleagues proposed that scientists could engineer the CRISPR/cas9 system to cut DNA in a specific location and modify the DNA sequence in the lab. They demonstrated that cas9 protein can be programmed to cut DNA at certain sites with a hybrid RNA molecule consisting of crRNA and tracrRNA. The research team designed a hybrid RNA molecule that combined the crRNA and tracrRNA and tested it with cas9 to cut DNA. They found that the hybrid RNA and cas9 recognized and cut DNA. To test whether that method was applicable for any DNA sequence, they engineered five different hybrid RNA molecules to cut a specific DNA sequence. In all five cases, they found that cas9 cut the DNA sequence at the correct locations. The demonstration of programming cas9 with a hybrid RNA molecule introduced the possibility of using CRISPR/cas9 as a simple method to edit DNA.

In 2013, Feng Zhang at the Broad Institute at Massachusetts Institute of Technology in Cambridge, Massachusetts, furthered Doudna, Charpentier, and their colleagues’ research and demonstrated the use of CRISPR/cas9 for editing DNA in mouse[6] and human cells. Doudna and Charpentier received numerous awards for their co-discovery of the CRISPR/cas9 system, including the Jacob Heskel Gabbay Award, later renamed the Gabbay Award, the Canada Gairdner International Award in 2016, and Japan Prize in 2017. Both women were also named in the top hundred most influential people by Time magazine in 2015.
Since Doudna, Charpentier, and their colleagues’ discovery, scientists have used CRISPR/cas 9 to edit the genomes of mammalian cells and animal and plant embryo cells, including human zygotes. Human zygotes are sperm [7]-fertilized egg [8] cells. While notable scientists including David Baltimore and Paul Berg have expressed ethical concerns of genomic editing in humans [9], CRISPR/cas 9 gene editing shows the potential to treat genetic diseases and cancer as scientists learn how to engineer the CRISPR/cas 9 system to change DNA in human cells.

Sources


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