Clustered Regularly Interspaced Short Palindromic Repeats, also known as CRISPR, is a gene-editing technology found naturally in archaea and bacteria. As the name suggests, CRISPR contains repeating genetic sequences that are palindromic, meaning they are read the same left to right, and right to left (e.g GATCTAG). Additionally, they are separated by unique sequences called spacer DNA.

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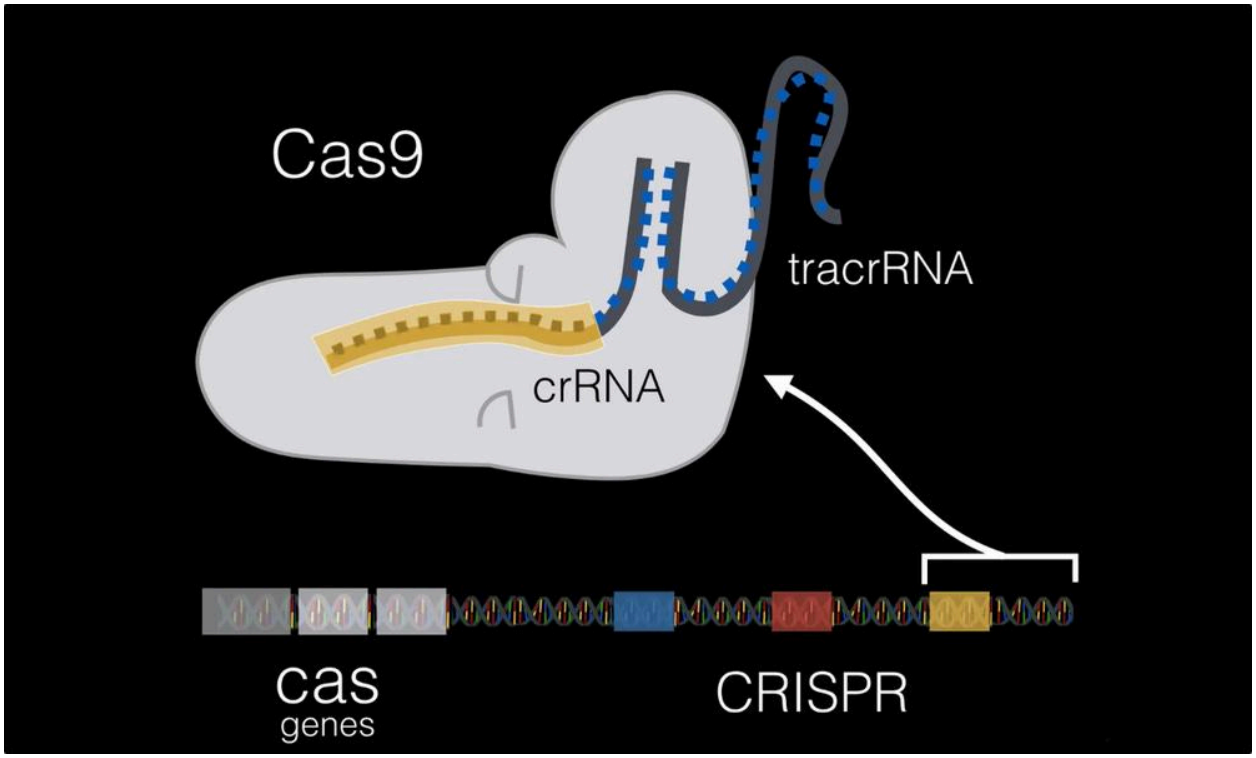
During its discovery in 1987, spacer DNA puzzled scientists, as they did not understand its function. However, in the early 2000s scientists discovered that **the DNA matched up perfectly with viral, more specifically bacteriophages1, DNA.** Furthermore, they found genes that were often found near CRISPR sequences, and they called them CAS genes, otherwise known as CRISPR-associated-genes. After analyzing them, they discovered that the CAS genes developed special CAS proteins, that included helicases2 and nucleases.3 The scientists hypothesized that this entire CRISPR-CAS structure was used by bacteria and archaea as an immune system to fight against bacteriophages. They determined that if a foreign bacteriophage injects their DNA into the organisms, the CAS genes will activate, forming a class one CAS protein. The protein will then bind to the viral DNA, and break it down, rendering it useless. More importantly, it takes that DNA and copies it into the CRISPR sequence. The **newly-added DNA becomes one of the aforementioned spacer DNA sequences**, explaining why other sequences matched up perfectly with viral DNA. If the bacteriophage decides to return, the CAS genes will activate once again; however, this time they will synthesize multiple proteins, forming a protein complex. The stored spacer DNA, which is identical to the viral DNA, will be transcribed into RNA, known as CRISPR RNA (crRNA), and will bind to the CAS complex. The

newly formed complex will attach to the viral DNA and break it apart. The CRISPR-CAS complex shares many similarities with the human immune system, and the antibody system. As CRISPR displayed the ability to inactivate and perhaps imbed new genes quickly, it became a trending topic of research in the following years.

The most notable example of this was the identification and manipulation of the CRISPR-CAS9 system found by Emmanuelle Charpentier and Jennifer Doudna, who later received Nobel Prizes as a result. They were analyzing the CRISPR system of streptococcus pyogenes, and discovered that there was only one CAS protein being formed, which they labeled CAS9, containing two nucleases. They found that not only would a crRNA bind to the protein, but a new strand of RNA, called trans-activating CRISPR RNA (tracrRNA), would hold the spacer RNA into place. Moreover, they found that the tracrRNA was not a singular strand, but actually two strands with a small gap in the middle that binded to the CAS9 complex. The two scientists experimented putting a DNA strand of their choosing in place of the crRNA, and attempted to bind the two strands of tracrRNA.



Ultimately, they created the tracrRNA-crRNA chimera CAS9 complex, composed of the CAS9 protein and the chimera strand, which was the two binded parts of the tracrRNA containing a designated DNA sequence, also known as guide RNA (gRNA). The new complex now had the ability to splice genes and remove certain sequences. For example, in a DNA strand, if a genetic engineer wanted to remove a certain sequence from one of the helices,5 they would be able to use the gRNA from the complex to identify the sequence amongst the larger strand, and the two nucleases in the protein would cut the phosphodiester bonds of both helices and extract the chosen DNA sequence. The larger DNA strand would then be rendered useless. The discovery was ground-breaking as scientists now had the ability to deactivate dangerous mutated DNA strands, such as the BRCA1 and BRCA2 tumor-suppressing strands, which were known to increase the risk of cancer when mutated. However, that was not the end of the discovery. In fact, the two scientists discovered that if they used the CAS9 and gRNA to splice out a certain sequence, they could insert a host DNA that replaces the removed DNA. As there are many nitrogenous bases moving around in the internal environment of a bacteria, they would bind to the correspondent pairs of the host DNA, and ultimately, form a perfect strand of DNA. Essentially, scientists also now had the ability to insert selected DNA sequences. The discovery broadened the spectrum of genetic engineering, and hinted at the possibility of engineering living organisms.

Photo by Sangharsh Lohakare on Unsplash

Although the discovery was groundbreaking, it was met with controversy. Many people resented the idea of the genetic information of babies being altered to make them “perfect.”

In fact, the fear of this new age of genetic engineering is excellently portrayed in the film GATTACA, starring Ethan Hawke and Uma Thurman. Although it was made in 1997, before the major discoveries surrounding CRISPR, it depicts a world where embryos that are formed through in-vitro fertilization are genetically engineered to create “the perfect child.” As a result, the destiny of people is controlled by how perfect their genes are, rather than how they choose to live their lives.

Controversy around CRISPR still continues to recent years, as in 2019 a Chinese scientist was arrested for genetically engineering two embryos using CRISPR to humans that were resistant to HIV. As is evident, although CRISPR may allow for breakthroughs in the medical field, the controversy that follows it inhibits much of its usage.

1

Viruses that infect and replicate in DNA.

2

Proteins that unwind DNA through the middle by breaking hydrogen bonds.

3

Proteins that cut the phosphodiester bonds in DNA.

4

Proteins that recognize familiar intruders and help the immune system have a quicker and more effective response.

5

Plural form of helix as DNA has a double-helix structure