Understanding CRISPR

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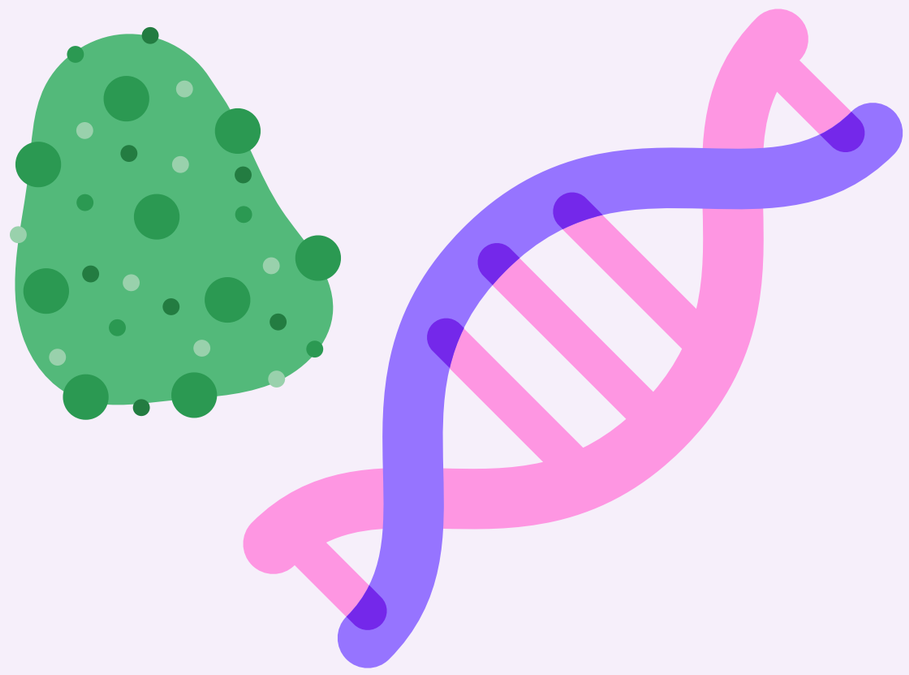
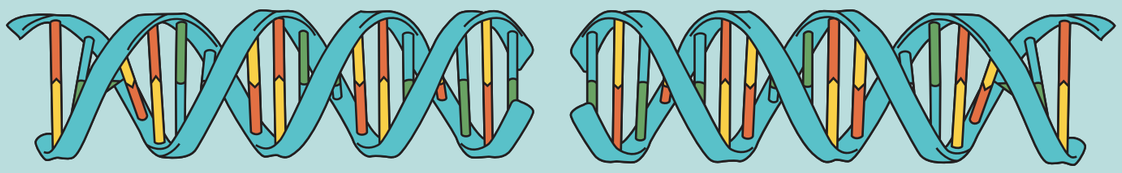


Defining **CRISPR** + History

Clustered Regularly Interspaced Short Palindromic Repeats

Changing your genome? How is that possible? Where is it needed? Should it be done? Every living organism is determined by their genes. What if we could alter that code to fix a mistake, or change a characteristic? The idea of genetic engineering first arose in 1973, when biochemists Herbert Boyer and Stanley Cohen, invented a method of cloning genetically engineered molecules in foreign cells (1). This was aided by the work of Paul Berg, in 1971, where he conducted a gene-splicing experiment, allowing foreign DNA to replicate naturally in bacteria. These experiments were possible due to prior discoveries (2), such as the discovery of alleles by Gregor Mendel in 1866, and the development of the DNA model by James Watson and Francis Crick with the help of Rosalind Franklins discoveries in the 1950s. CRISPR was first discovered in 1987 in Osaka University by Ishino (3), however, pioneers Jennifer Doudna (professor and biochemist at the University of California, Berkley) and Emmanuelle Charpentier (a microbiologist and director of Max Planck Unit for the Science of Pathogens) further discovered the CRISPR-Cas9 gene editing system, winning the Nobel Prize in Chemistry in 2020 (4). Charpentier discovered tracrRNA, while Doudna mapped the Cas proteins, where their collaboration enabled them to discover and develop the system of gene editing. The scientists became one of the few women to earn the Nobel prize, following 5 other since 1901. Angela Zhou, an information scientist, mentioned how CRISPR has been used to “modify immune cells to make them more effective at destroying cancer cells and to remove the HIV virus when it has integrated itself into the human genome. And CRISPR-based drugs are being developed to treat heart disease, blood disorders and blindness”.



How does it **WORK**

CRISPR sequences are crucial for single-celled bacteria and archaea, since they function as their immune system. When a mobile genetic element, particularly a virus (small infectious agents), invades, action is taken to eliminate the threat immediately, protecting the prokaryote (5). Cas proteins cut out a segment of the viral DNA (acting like molecular scissors) to stitch into the bacterium CRISPR region, capturing a ‘chemical snapshot’ of the infection (6). The viral codes are transcribed into short pieces of RNA, which bind to the Cas9 protein (in the CRISPR region). This results in complexes which latch onto free floating genetic material, searching for a match to the virus, resulting in the destruction of the viral DNA. When the immune system has attacked the virus, short CRISPR sequences, called ‘spacers’ are derived (adaption), allowing there to be a ‘genetic memory’, comparable with lymphocytes in the human immune system. This allows CRISPR RNA to be produced within the bacteria (targeting), which will guide the bacterial molecular machinery to destroy the viral material.

Using CRISPR in **LABS**

This immune system can become a precise gene editing tool, targeting DNA in almost any organism, helping alter the DNA and change specific genes (6):

1. A guide RNA is designed in the lab to match the gene they want to edit
2. This RNA strand is attached to CAS9
3. Guide RNA directs the CAS9 to the target gene
4. The proteins molecular scissors snip the DNA
5. Once it is cut, the cell will try to repair it

Typically, ‘nucleases’ trim and join the ends back together in the process of ‘nonhomologous end joining’. However, this process is prone to mistakes, which can lead to extra or missing bases, often resulting in unusable or turned off genes. If scientists could add an extra template DNA to CRISPR, cellular proteins could perform a different repair process ‘homology directed repair’, where temporary DNA is used as a blueprint to guide the repairing process (this allows scientists to repair a defective gene or insert a new one). Overall, this produces rDNA (recombinant, meaning it contains a new, different genetic combination).

Applications of **CRISPR**

This primitive, yet elegant defence system can be used in labs, farms, hospitals and more. CRISPR can lead to critical advances in patient care by drastically changing the trajectory of a disease as scientists learn the effects of turning on and off or changing genes within an organism. The first human clinical trial using CRISPR-Cas9 happened in China in 2016 using lung cancer patients (7) where the PD-1 gene (Programmed Cell Death Protein 1) was inactivated ex-vivo (outside the person), to keep lung cancer in check. This gene normally down-regulates the immune system allowing cancer to multiply, thus the inactivated gene enhances the body's ability to fight the cancer. However, the success of this trial was kept confidential to the families. Later in July 2019, ex-vivo therapy using CRISPR-Cas9 was performed for a patient with sickle cell anaemia in the US (3), which significantly improved the patient's condition for months, providing promising results, however the cost has limited further trials, which are estimated to be 0.5-1.5 million USD. Nevertheless, CRISPR can be used as a fast genetic editing system, providing advantageous outcomes.

Currently many clinical trials are taking place for the treatment of diseases such as: hearing loss (Hunter Syndrome), Huntington's disease (stops part of the brain from working properly over time), Angelman's syndrome (delayed development, problems with speech and balance), Parkinson's disease, spinocerebellar ataxia (affects the cerebellum), cystic fibrosis (sticky mucus), turner syndrome (one of the X chromosome is missing or partially missing), muscular dystrophy, sickle cell anaemia, Gaucher disease (missing an enzyme to break down lipids). Promising future applications for CRISPR-Cas9 can be see in HIV and human papillomavirus.

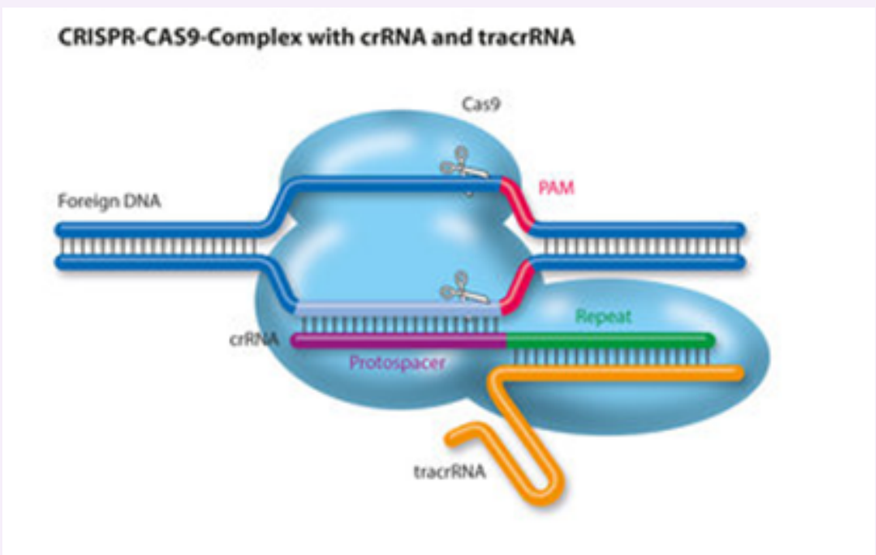
In other settings, gene editing can result in plants that yield larger fruit, improve drought tolerance and drug resistance, and mosquitos that can't transmit malaria as well as so much more!

Considering **ETHICS**

CRISPR is not a perfect process yet, meaning errors can occur and people may misuse it. One of the largest controversies is in the use of the human embryos, since it may lead to unfair enhancement purposes, but also is affecting the unborn baby who is given no autonomy as to what happens to them. It is believed these concerns should be managed through policy and regulation (8). Ethical discussions are centred around the human germline because these changes will be passed down to future generations, where it is difficult to predict the long-term changes. Gene editing may also lead to off-target effects (where more cells are affected) or mosaicism (when some cells carry the edit, but others do not). This can lead to an array of unknown consequences. Ethical questions are also raised about genetically edited microbes which may be released into the environment, causing unforeseen changes to the world.

Recent Study on **HEART DISEASE (9, 10)**

Hypertrophic cardiomyopathy (HCM) is the most prevalent genetic heart disease, where the left ventricle heart muscles thicken, leading to stiffness, causing arrhythmia, heart failure or sudden cardiac death. Professor Eric Olsen is conducting a promising study to create in vivo therapy for the treatment of HCM. By editing the gene, he believes this will cause a lifetime fix, as heart cells turnover very slowly, stating it should be a "one-and-done therapy". A test in mice revealed the editing efficiencies were over 30%, preventing the onset of HCM, showing similar cardiac function to healthy mice. Furthermore, researchers found injecting gene editing reagents to edit the CaMKIIδ gene may lead to protection from ischemia/reperfusion (IRI, cellular dysfunction and death as blood no longer flows to the muscles). Further research is being conducted to repair permanently damaged tissues following a heart attack, which may lead to many exciting opportunities in the advancements of CRISPR-Cas9 gene editing!



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