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Genome editing with therapeutic purposes

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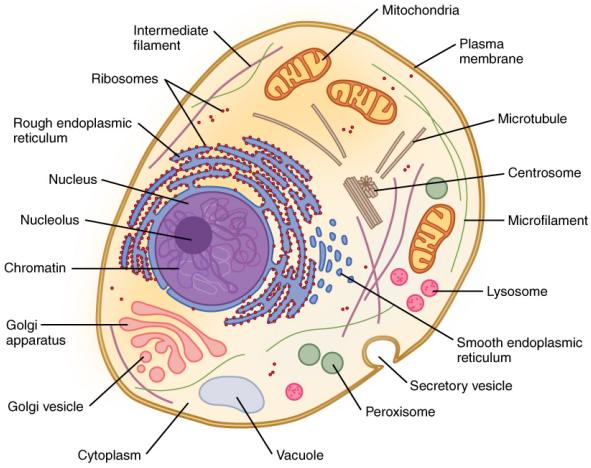
Humans, as every living organism, are formed by cells, in our case billions of cells. All the cells of a single person contain the same information in a molecule called DNA, since all of them come from a single cell, the zygote, the result of the fertilization of the oocyte by the sperm.



Drawing of an animal cell containing the primary organelles and internal structures. The nucleus is a cell's central organelle, which contains the cell's DNA. Download for free at http://cnx.org/contents/14fb4ad7-39a1-4eee-ab6e-3ef2482e3e22@8.25.

Each cell has a nucleus, were all genetic information is encoded just in four different types of molecules called nucleotides, and is this information the one which controls the function, the life and the death of the cell. Human cells have around 20,000 genes, which are small sequences of DNA (or not so small) with different functions; some of them extremely important, others less so. The set of all these genes is called the genome. For this reason, is very important that cells, during division, which is the process where a parent cell divides into two daughter cells, there is no any error copying the information encoded in the genome. But unfortunately, as nothing, the machinery in charge of this process is not perfect, and sometimes it introduces errors (also called mutations), meaning that one daughter cell will not have the initial information. These mutations are not as the ones we can see in movies or TV series that confer superpowers to the person who gets them. Many times, they are innocuous, but others, they can mean the beginning of a disease, called genetic diseases, but also the predisposition to suffer from a certain disease. There are a large amount of examples that come just from a single mutation in the genome (called monogenic diseases), and a larger amount of diseases caused by many of these mutations in the genome. There are some types of diabetes, Parkinson's and Alzheimer diseases caused by just one of these mutations, and then is cancer, which accumulates thousands of them. For many years, the treatment of these diseases tried to supply exogenously the substances that cells cannot produce, inhibit all that is abnormal, and in the case of cancer to kill the cells with very harmful substances. But, all these treatments have side effects that have implications in the quality of life of the patient. In 1972 showed up a new branch of medicine called gene therapy which is designed to introduce genes into cells, in order to compensate for abnormal genes.

By the end of the twentieth century, some researchers discovered one method to manipulate the genome of cells in the laboratory, or as it is commonly known, genome editing. Genome

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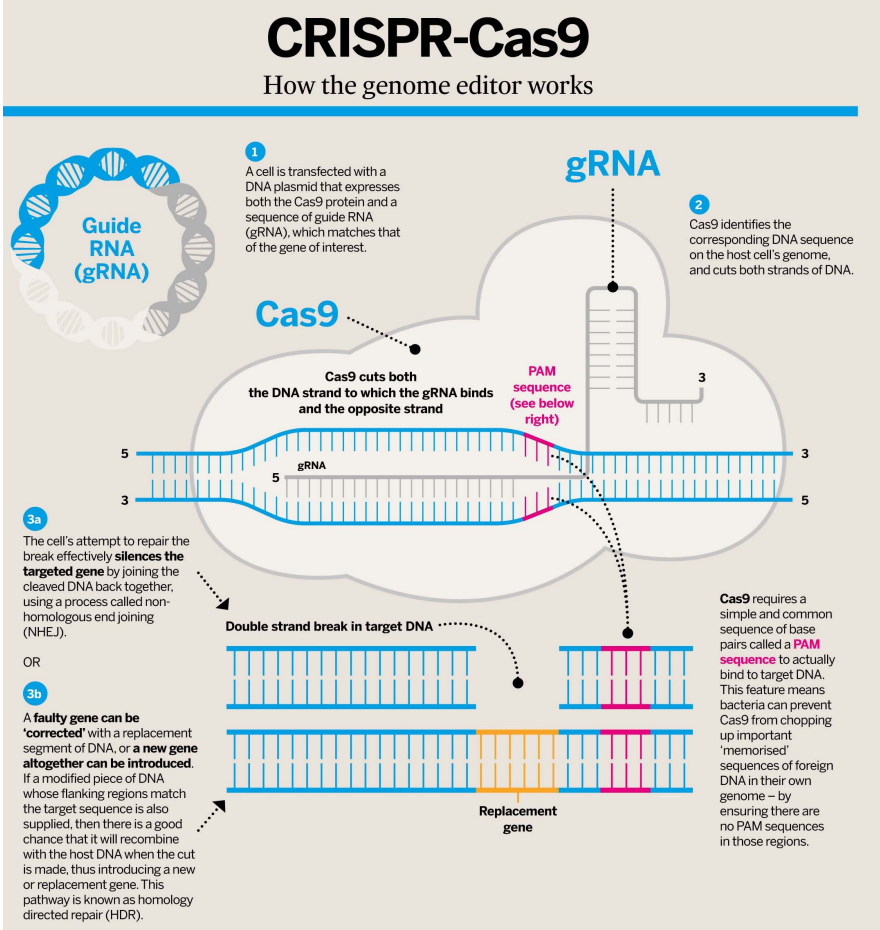
editing refers to the ability to modify with precision the information encoded in the genome, and one of the possible applications is to correct the wrong information which causes a disease, solving the original problem. Therefore, this approach is on example of gene therapy approaches. This method, called Zinc Finger Nucleases (ZFN), was the first in its class, and opened the door to the treatment of those diseases where just a single gene is mutated. The main problems are its prize (it costs around $5,000), and the difficulty to engineer them. A few years after, researchers discovered the second technique to perform gene editing, that was called TALEN (Transcription activator-like effector nuclease), which increased the specificity and the efficiency of the previous technique. There are currently some genome editing approaches using these strategies undergoing clinical trials in order to be approved to treat real patients with some specific diseases such as haemophilia.



Mechanism of action of CRISPR/Cas9, a method of genome editing which may offer a tool for curing genetic diseases. Edited from: Focus on Gene Editing. The Biologist 63: 32-33.

Those discoveries were of especial interest, but, thanks to a discovery done in bacteria, an easier, but also more effective and cheaper technique revolutionized the scientific world, that was CRISPR/Cas9. It was found as a mechanism of immunity that some bacteria have to fight against virus. When a virus attacks a bacterium, the virus introduces its genome, and makes more copies of the virus using the machinery of the bacteria, in a way that ends up killing the microorganism. But, bacteria have its own way to avoid viral destruction, that is thanks to this

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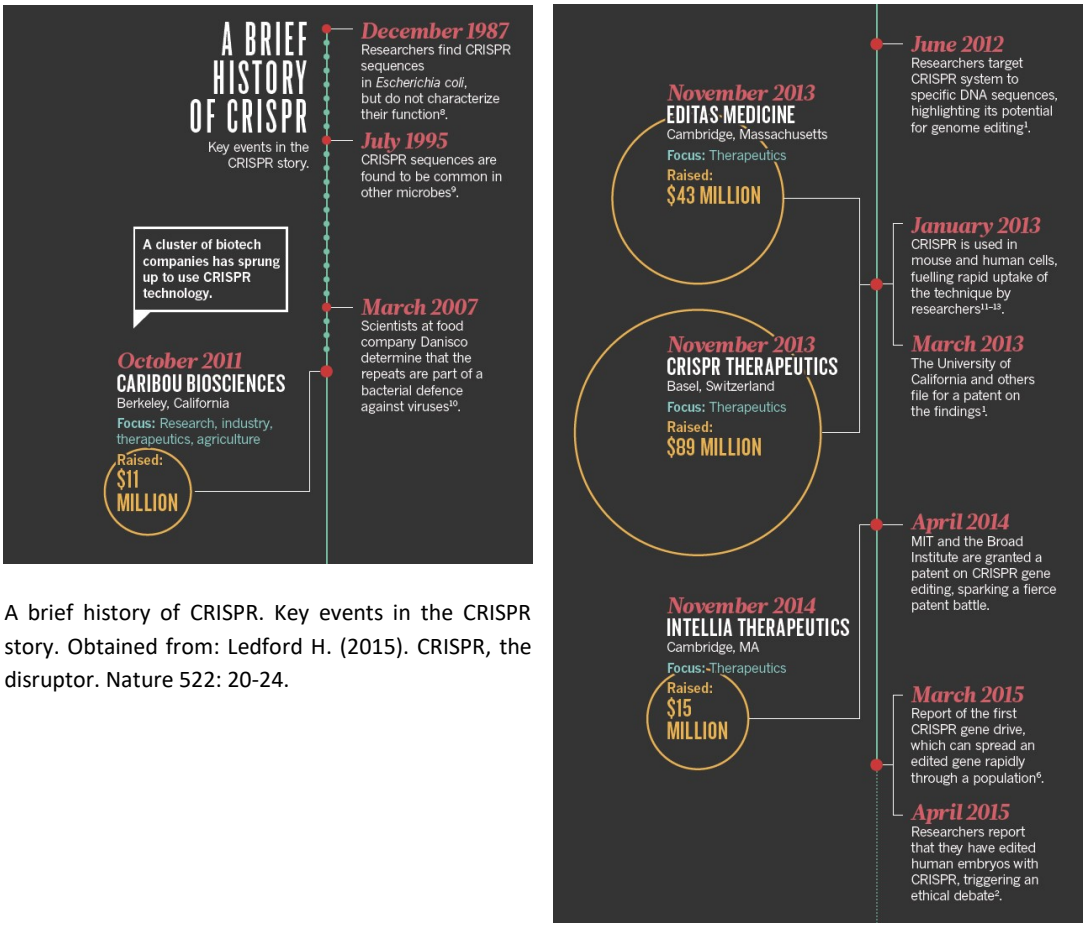
CRISPR/Cas9 system, that recognizes the viral genome with an RNA molecule, and Cas9 cuts it, inactivating the viral genome, so the virus cannot replicate anymore. Besides its function as a mechanism of immunity in bacteria, researchers rapidly found a clinical application to this system as a gene editing tool. Editing genes at will is based on its ability to cut the DNA in specific places, so the cell tries to repair the molecule of DNA as soon as possible. The repair can be random, where the machinery of the cell introduces or removes nucleotides randomly, or can use an external DNA molecule as a template to copy that information. This template can be added exogenously, and must have identical extremes to the sequence that is being repaired, but can have a different internal sequence. Thus, genome editing has the potential to cure diseases by disrupting endogenous disease-causing genes, correcting disease causing mutations or inserting new genes with protective functions. It would be easy and very useful treating monogenic diseases (such as cystic fibrosis) and sterility, and compared to the first tool to perform gene editing, CRISPR has a total cost around $30. Additionally, CRISPR can be modified in order to activate or silence genes that are altered in diseases. Compared to both other approaches, this method is extremely specific, with high cleavage efficiency and versatility. Although CRISPR can accelerate the gene therapy field, it is a bit far from being sold it in the market.

> "We are able to have a molecular scalpel for genomes"
>
> Jennifer Douda



A brief history of CRISPR. Key events in the CRISPR story. Obtained from: Ledford H. (2015). CRISPR, the disruptor. Nature 522: 20-24.

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The CRISPR system has two components. The first one is a protein or endonuclease, called Cas9, that has the ability to produce a double cut in the DNA, the one which later needs to be repaired. The second component of the system is a guide RNA molecule that interacts with the sequence that we want to cut, but also binds the Cas9 protein, bringing together the endonuclease to the target DNA sequence.

The first use of CRISPR to fix a disease-causing mutation in and adult animal was performed in 2014 by the team led by Daniel Anderson of the Massachusetts Institute of Technology (MIT) in Cambridge, where they were able to correct a mutation associated with a human metabolic disease called tyrosinaemia. The problem was that they used a method that is not considered feasible in people, and just a few percentage of cells had corrected the mutation. Since then, other diseases have been tried to be treated with CRISPR in animals. For example, it has been demonstrated that CRISPR eliminates HIV in live animals, since this technology can target the virus to eliminate the integrated viral genome from the cells or preventing infection of the virus.

> "The primary uncertainty concerning gene editing was whether "reasonable alternatives" already exist"
> Sheldon Krimsky

As said before, this is a versatile system, and cannot only be applied in biomedicine, but it is also very useful in basic research, to improve plant breeding, to generate animal models, or to improve nutrition, just to name only a few examples. For example, other scientists are studying how to enhance crops and livestock using gene editing. Before the arrival of gene editing tools, this was generally done by inserting a gene into the genome at a random position, using sequences from bacteria or virus. The problem is that the process is not very efficient, and it has been criticized for mixing DNA from different species. In the past few years, researches using CRIPSR have been able to make disease-resistant crops, as well as disease-resistant goats and vitamin-enriched oranges.

Other researchers are studying how CRISPR could be used on organisms in the wild. The attention has been focused on a method called gene drive, which can sweep an edited gene through a population. This technique could be used to eradicate diseases from its reservoirs or herbicide resistance. Widely, a genetic change in the organism requires a long time to spread through a population because the mutation on one of a pair of chromosomes is inherited by only half the offspring. But a gene drive allows a mutation done by CRISPR on one chromosome to copy itself to its partner, so the offspring will rapidly have the mutation. For example, scientists from the University of California presented genetically-modified mosquitos incapable of transmitting malaria to humans. So, the idea would be to release these mosquitos that are able to pass on their resistance to malaria to the rest of mosquitos' population taking advantage of gene drive in order to eradicate the disease.

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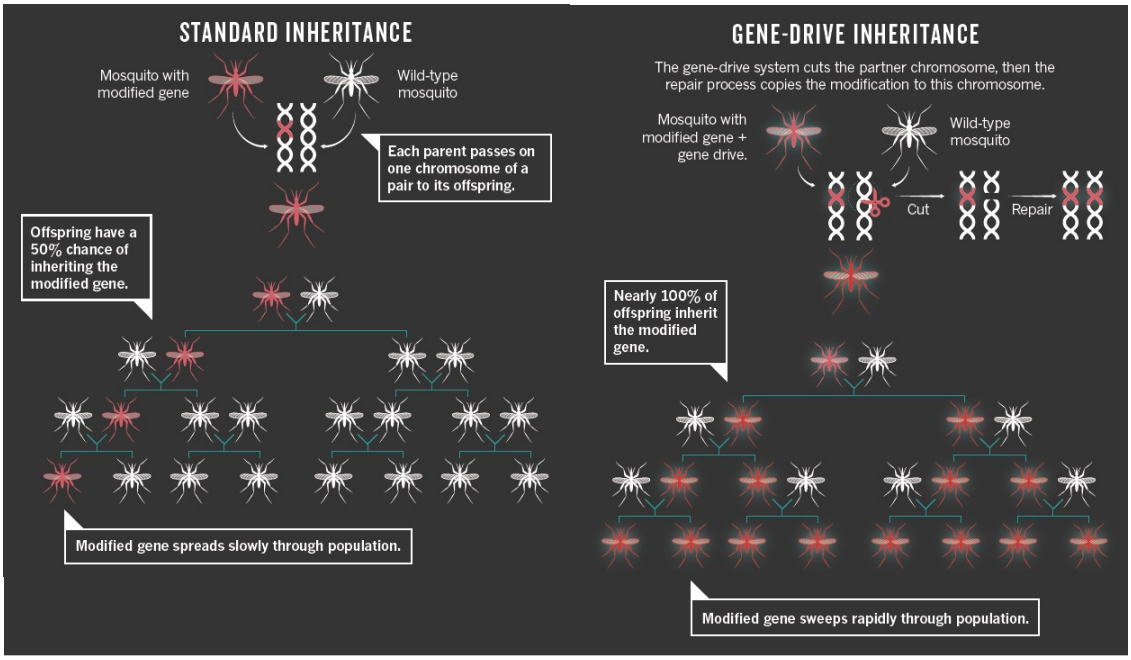

Comparison between standard inheritance and gene drive in a mosquito's population. Obtained from: Ledford H. (2015). CRISPR, the disruptor. Nature 522: 20-24.

Another example would be a group of scientists from the University of Harvard that have already managed to eliminate those pig's genes that turn on an immunogenic response in humans, with the aim of transplanting pigs' organs to humans without the complications associated with the rejection by the immune system of the patients. Also, CRISPR has been widely used to create animal models for research to mimic human diseases and to study development by mutating or silencing genes, or even to modify yeasts to produce biofuels.

"Sex is cheaper and it's more fun than IVF, so unless you've got a real need, you're not going to use it"

Alta Charo

But although CRISPR has much to offer, it also carries some ethical and safety concerns. The problem arose in 2015, when news broke that scientists used the CRISPR system to genetically modify human embryos, tough it was performed on defective embryos and without real success. Although these embryos were unable to result in a live birth, it generated a debate whether and how CRISPR should be used to make heritable changes to the human genome. But the issue comes because if disease-embryos with the repaired mutations were allowed to develop into babies, they would not only be healthy, but also would not transmit the disease to their descendants. Besides creating more healthy embryos for in vitro fertilization (IVF), it could be also used when screening embryos is not an option, or to reduce hard IVF cycles for women. Generally, an important issue in research is the balance between risks and benefits, where benefits must be greater than risks. Although CRISPR has more specificity than the other approaches, it has been seen some off-target effects. Thus, CRISPR may cut unintended sequences, causing new mutations. For this reason, researches have put a great deal of effort to further enhancing the specificity, and just two years ago, they found a synthetic variant of the system that has very few off-targets, which would be a key point in the translation of the technique to the clinics.

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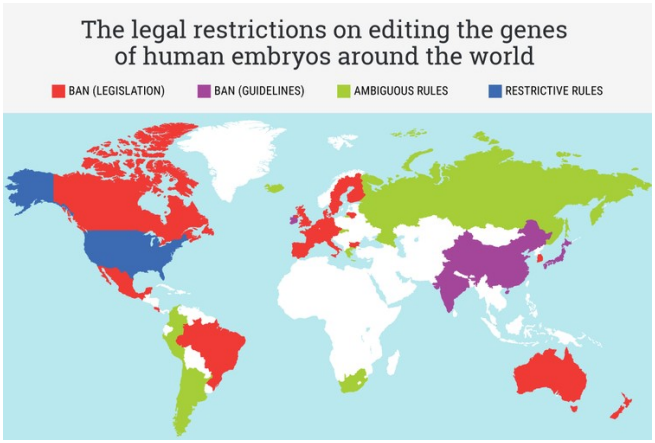
This discussion was reopened a few months ago when He Jiankui, a Chinese scientific, claimed that he had created the firsts babies using CRISPR. In November 2018, the researcher announced the birth of twins with their DNA modified, and in January 2019 he also noticed that a second woman was breeding another genetically modified baby. He modified the embryos prior the implantation in uterus entering a mutation in the CCR5 gene which confers resistance to HIV infection. The Chinese Government has demanded the cessation of his scientific activities, but this has not avoided He entering the list of "ten people who mattered this year" in 2018, published by the prestigious British magazine Nature.

Edits to embryos, eggs or sperm is also known as germline modification, and it is of particular concern because a person created using those cells would have had their genetic composition changed without consent, and would transfer that change to future generations. Some people are afraid that this technology, in the wrong hands, could open the field to a non-therapeutic intervention to perform eugenics or designer babies. This could mean that people with means could pay to have children with enhanced traits while those with disabilities would be devalued. Many characteristics have a genetic component besides the environment, which could be intervened for selfish purposes. Thus, gene editing has opened the door to both medical and non-medical ends. That's why the UNESCO called for a temporary ban on gene editing of the human germline (egg and sperm). Many countries have totally banned gene editing on human embryos, but in other cases, the legislation is not so clearly defined. Even when they are, those rules are rarely legally binding, while other countries only have guidelines. One example is China, which prohibits gene editing of embryos, but doesn't enforce similar laws to prosecute the use of ultrasounds for sex selection and to end up with unauthorized stem cell clinics.



International regulatory landscape on human embryo editing in 39 countries around the world. Modified from: Araki M. and Ishii T. (2014). International regulatory landscape and integration of corrective genome editing into in vitro fertilization. REPROD BIOL ENDOCRIN 12:108.

> "There are already a lot of dodgy fertility clinics around the world"
>
> Tetsuya Ishii

The scientific community has opposite thoughts in this topic: those who think that "we need a halt on anything that approaches germline editing in human embryos" as said by Edward Lanphier, chairman of the Alliance for Regenerative Medicine in Washington DC, and those who think that although there is a need to a wide discussion about safety and ethics of editing embryos, the potential to eliminate inherited diseases should tip the balance in favour. What they both agree is that with so many unanswered questions, it is important to keep expectations of CRISPR under control.

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Modifying human beings is not the only aspect which worries the society. Some people are afraid for an intended or accidental release into the environment of experimental modified organisms, which could cause an ecological disequilibrium, because could have severe and unknown consequences for an ecosystem. For example, it might mean the emergence of other pests, or even affect both predators above and preys below the food chain.

Among those who agree using this new technology there is also discrepancy. Since the discovery of the utility of the CRISPR system, there is a "patent war" to find out who must own the patent for the new discovery. The teams of Jennifer Doudna at Berkeley and Emmanuelle Charpentier at the University of Vienna stated how CRISPR/Cas9 could be used to precisely cut DNA, and filed for a patent. But, Feng Zhang at the Broad Institute of Harvard and his colleagues in the MIT showed how it could be adapted to edit DNA in other cells such as plants, animals and humans. Although the MIT filed for a patent seven months later than the University of Berkeley, the MIT get their patent granted first. It was because Zhang's patent was reviewed faster than Jennifer's, since patent applications do not become public until 18 months after they are filled. So, there is a conflict of interest, since the holder of the patent could make millions of dollars from CRISPR/Cas9 applications in the industry and from licensing the original patent. Although the patent applications were in 2012, today in 2019 there is not a final verdict, but hopefully, during this year, we will see an end to the war between Berkeley and MIT in one way or the other.

What is already a fact is that some small companies are already working using the CRISPR technology, and some of them have already launched a successful anticancer therapy using CRISPR such as Intellia Therapeutics and Editas Medicine. Big pharma companies such as Novartis and Bayer are cooperating with these small companies that own some of the patents of this technology. So not much time is left until gene editing is widespread as a clinical tool.

"CRISPR has the potential to open a new branch of medicine, editing the genome to cure disease"
Mark Fishman

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