A spider's bit(e): how far are we from becoming Spidermen?

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I am a biotechnologist who has recently got a PhD degree in Molecular Biology and Genetics. Although I have always worked with bacteria, I believe knowledge is transversal to life sciences, through every level of complexity with the due scalability, never underestimating the power of learning from the small things. My research has always been focused on finding new alternatives to overcome some of today's health challenges. Every day, I get the chance to observe that fiction and science are more alike than we might think. It is often only a matter of time.

A Superhero's tale

Ever since we were kids, there wasn't anyone in the world who had never wished to be a superhero. And even as adults, we sometimes wonder how much easier some aspects of our life would be if we had super strength or X-ray vision. A superhero that has some incredible – and rather odd – superpowers is Spiderman. We all know the story of Spiderman: a super smart, thin and clumsy kid got bitten by a radioactive spider and became extra agile, extra strong, extra athletic, and able to crawl on walls and ceilings, besides gaining the "spider-sense". None of those "powers" is new to nature. For all of those who have already seen a spider running away, have witnessed the speed of average-sized regular spiders. Studies have also demonstrated that some spider species can carry several times their weight, even when hanging from the ceiling. And the ability to crawl on totally flat surfaces, like ceilings and walls, is well known from all of us. Now, imagine that amplified by radiation, and then, somehow transmitted to a human-being, with its due proportions, and you get Spiderman's powers. Even the Spiderman spider-sense probably results from an over-amplified capacity to feel vibrations, that in the case of spiders is due to tiny hairs and slits distributed all over their bodies. Web-slingers apart, all those extra-human abilities were given to Peter by the spider. But how?

The genetics of Spiderman

We can only infer the mechanisms involved in this strange transference of traits, as no information was provided by the creators of Spiderman. By biting Peter Parker, the radioactive spider injected some of its radioactive poison in Peter's blood stream. Luckily, he did not suffer any anaphylactic shock, and his body seemed to adapt well to the presence of the spider's poison. So well, that he even evolved to something new. The radioactive poison induced Peter's cells to change their metabolism within the whole body – skin, muscles and even brain. By the alteration of the genetic code it is possible, although improbable at that extent. Somehow, the poison could induce specific mutations in Peter's DNA. Alternatively, the poison itself could contain the spider's DNA which was recombined with Peter's human DNA, allowing the expression of spider traits. DNA recombination is a natural process that involves the exchange of genetic material between multiple chromosomes or between different regions of the same chromosome in a cell. It is responsible for increasing the variability within eukaryotic species. Here, something different happens – the production of recombinant DNA, which may include sequences from distinct organisms. Although this does not occur naturally in eukaryotes, it happens in bacteria which accommodate exogenous DNA into their genomes under specific circumstances. Also, it can be artificially induced in laboratory.

There are several known mutagenic agents, meaning, agents that can induce changes in a DNA sequence, including chemical compounds, biological agents – perhaps spider poison – and radiation. In most cases, humans can repair these mutations, that are usually identified as errors in the DNA sequence, and cells keep their regular functions. Otherwise, cells can undergo apoptosis, which is also referred to as "programmed cell death". This is the mechanism used by the body to get rid of cells that are damaged beyond repair, namely, due to the accumulation of errors in the DNA. This accumulation triggers a cascade of reactions starting with the signaling of the abnormal cell, going through shrinking and DNA fragmentation processes, and elimination of the cell. If the errors cannot be repaired and start accumulating, and the apoptosis process is somehow impaired, that leads to uncontrolled cell division and the subsequent development of cancer. However, some mutations can be tolerable, as they can be i) silent, which means that the final product of that gene will not be affected; ii) in a region that does not encode relevant information; or iii) simply ignored by the activation of DNA damage tolerance pathways that allow the DNA replication to continue, bypassing the damaged region. Some mutations can thus be irreversible and heritable. However, mutations are not necessarily bad. The existence of a low rate of mutations introduces some variability within the genome of a given species and, consequently, on their characteristics. And that is particularly important to increase the adaptability of the species as a whole. On this regard, Charles Darwin published "On the Origin of Species" (1859), where he proposed the theory of evolution by natural selection. Briefly, this theory postulates that evolution is driven by the selection and survival of the fittest organisms, meaning those that are most suited for the environment where they live in. These will be more likely to reproduce and, thus, to pass those traits to the next generation. This also means that, if the environment changes, the selected traits will also gradually change – or evolve. Thus, specific environmental conditions can sometimes favour a specific characteristic that initially was only present in a small part of the population. A common example is the sickle cell anemia. This is a genetic disorder characterized by the production of abnormal red blood cells, which are less efficient in transporting oxygen and flowing within the blood stream. However, this condition brings about an unexpected advantage to people infected with malaria. It seems that people carrying one copy of the mutated gene that is responsible for the sickle cell anemia are highly resistant to malaria. This vector-borne infectious disease is caused by a parasite, *Plasmodium falciparum*, which is transmitted by a mosquito. When sickle cells are infected with this parasite, they collapse, which prevents the parasite from interfering with other relevant proteins within the cell and, thus, protecting the host against malaria. Thus, this gene is particularly recurrent in areas with high incidence of malaria, such as central Africa and central America, as people carrying it are more likely to survive on those areas.

Back to our superhero, in the case of Peter Parker, mutations induced by the poison – by means of whatever mechanism – made him Spiderman. But one main question emerges: is that possible, scientifically speaking? And if so, how far are we from being able to do it? To start with, scientists would definitely not use spiders to inject whatever they decided it would be required. Lots of people are afraid of needles too, but they are still preferable to spiders or any other biting, stinging or touching animal. However, the delivery method is the last part of this intricate puzzle. The real deal is to develop a mechanism for the introduction of the mutations, *per se*, within the genome. And the truth is, this is already being done in many laboratories around the world. Just not in humans. Yet. Or, at least, not permanently.

Manipulating genes for the greater good

First of all, scientists have indeed been inducing mutations for a long time. To understand this, we have to remember that humans are trying to manipulate evolution since the very beginning. This is particularly obvious when we look, for instance, to crops like maize, wheat and rice, that are used for feeding nowadays and compare them to those from several years ago. We selected the biggest ones, the ones that produced more grain and kept their seeds, hoping that, the following year, they would grow even bigger and more productive. At some point, some variants were lost and the ones remaining will be best fitted to the climate we are living in. We also try to get the best out of our animals, by choosing the ones with some desirable characteristics. And we keep doing that in the following generations. However, all these attempts to manipulate the evolution raise ethical issues, concerning how far we should go and if the end justifies all the means. The problem of selecting specific traits is that, when something in the environment changes, the selected characteristics are not the desirable ones anymore. Until not long ago, we would have to go out there again, look for other variants and repeat the process. But, at some point, scientists realized that there should be a faster and more efficient way.

With the increasing knowledge on genetics, and the evolution of molecular biology techniques and other disciplines in life sciences, scientists learned how to read the information contained within the DNA. Furthermore, they learned that it could be "copied and pasted". One can imagine how difficult it is to read the human DNA. Its whole extent was estimated to be around 2-meters long and to carry the equivalent to 1.5 Gb of information (considering the 4-letter code in which it is written) in a single cell. It is possible to literally "read" a genome, by sequencing technologies, particularly, the whole genome sequencing. In simple words, DNA sequencing is used to determine the exact sequence of a DNA molecule, using the above mentioned 4-letter code. After finding the sequence, this must be "translated", i.e., the sequence is divided into "words" and "sentences", that will give origin to amino acids and proteins that build up a body. Then, it is possible to compare the sequences of different organisms and observe the singularities between individuals from the same species. The whole genome sequencing refers to a comprehensive method to analyze the complete DNA sequence of an individual organism. DNA sequencing methodologies have been evolving since the 1970s to become faster, more automated and cheaper. The first organism whose genome was sequenced was the bacterial species *Haemophilus influenzae*. However, the Human Genome Project was the biggest booster of these technologies, with the first human whole genome sequencing announced in 2003, being continuously updated until today. It was not just a coincidence that the first ever sequenced genome has been a bacterial one. Scientists have started with far simpler organisms: bacteria. And "simpler", in this context, only means that their genomes are far smaller than those from eukaryotes and thus, easier to read. Genetic engineering was born in the second half of the XXth century, with the discovery of restriction enzymes (natural proteins with the ability to cut DNA in specific sequences) which provided tools that allow DNA manipulation. A few years later, in 1973, two biochemists, Stanley N. Cohen and Herbert W. Boyer, were able to cut DNA fragments, merge and insert them within a bacterial genome. And then, the proteins corresponding to the inserted DNA were produced by those bacteria. Although some requirements must be met and optimizations are often required, this approach has been successfully applied to create bacterial factories, used to synthesize human insulin, human growth hormone, alpha interferon, a hepatitis B vaccine, among other medically useful substances. Bacteria and yeasts are currently used to express and study the effect of specific mutations in proteins from diverse origins, contributing to improve the understanding of several practical and theoretical aspects of gene function and organization.

Knowledge increases exponentially. Regarding more complex organisms, plants can also be genetically modified. The most wanted characteristics are mostly related to: i) resistance to genetic diseases, plagues or drought; ii) the enrichment of their nutritional value, mainly to be cultivated in poor countries; iii) enable nitrogen fixation. Nevertheless, special attention must be given to possible unwanted side-effects: the more the complexity of the organism increases, the more intricate the crosstalk between several genes becomes. Manipulating a gene involved in a certain trait can have an unexpected impact on another cascade, which may result in unwanted, and even harmful traits. It should come as no surprise that animals have also been genetically engineered. Known and controversial examples are, for instance, salmons, which have been engineered to grow larger and faster; cattle that was enhanced to become resistant to the mad cow disease in the United States; and animals for laboratorial applications, such as mice. Naturally, as our ability to precisely engineer and edit animals' genomes increases, the public concern and ethical issues rise in the same extent, despite the intended potential benefits. Regarding both animals and plants, there is a major drawback that was absent when dealing with bacteria: they are multicellular. In bacteria, one can add the desired DNA to a bacterial suspension and, with a rather simple protocol, ensure the insertion of such DNA in a significant number of bacterial cells that can be further propagated. In more complex organisms, scientists deal with several aspects when trying to induce the required mutations, their complexity the main of them being. Altering the genome of a multicellular organism is not a precise science, since unexpected outcomes may arise depending on the targeted cells. Multicellular organisms are built up by several types of differentiated cells that, although sharing the same genetic code, express different genes and have the most diverse and complementary functions. It is important to distinguish between two groups of cells: germ and somatic lines. Germ cells give rise to the gametes of an organism and are originated from the primitive streak of the embryo; the somatic cells are basically all the other cells that are not from the germline and constitute the whole body. An important aspect is that mutations in the somatic cells will be only effective in the individual where the gene manipulation is conducted and thus, will not pass-through generations, not affecting the evolution of the species. Quite the contrary, mutated germ cells will give origin mutated gametes that will pass the mutations to the following generation via sexual reproduction. Although genetic manipulation of somatic cells is currently used and more easily accepted in therapies associated with several diseases, namely some types of cancer, manipulation of the germline is highly controversial and is only allowed for research purposes.

Another barrier to the manipulation of cells from more complex organisms is related to the required methodology to mutate their genomic information. And again, nature provides answers with the most suited tool to introduce DNA within a host cell and force it to produce proteins that were not coded there before. Viruses. They are not even considered “beings” as they cannot survive by themselves without a host to infect. However, they are perfectly equipped to deliver genetic information in a complex organism. Scientists started engineering viruses, making them not harmful, by impairing their replication, while maintaining their ability to deliver genetic material with the required information that would then translate into the desirable characteristics. For example, this approach was successfully applied in gene delivery in plants, to induce desirable agronomic traits or to produce valuable biotechnological compounds, including pigments or vaccines. Another well-known example is Dolly the sheep, the first ever cloned adult mammal, born in 1996. British developmental biologists from the Roslin Institute (Edinburgh, Scotland) cloned a somatic cell, a mammary gland, taken from an adult ewe, using electrical pulses to fuse it with an unfertilized egg cell, whose nucleus had been removed, which then began to divide. This constituted a milestone, since it proved that adult mammals could be successfully cloned using somatic cells. Following this success, the consequent debate concerning the many possible uses and misuses of mammalian cloning technology was ignited. Other approaches can be used to deliver foreign DNA to a new host and those will be discussed in the following section, with particular emphasis on potential human hosts.

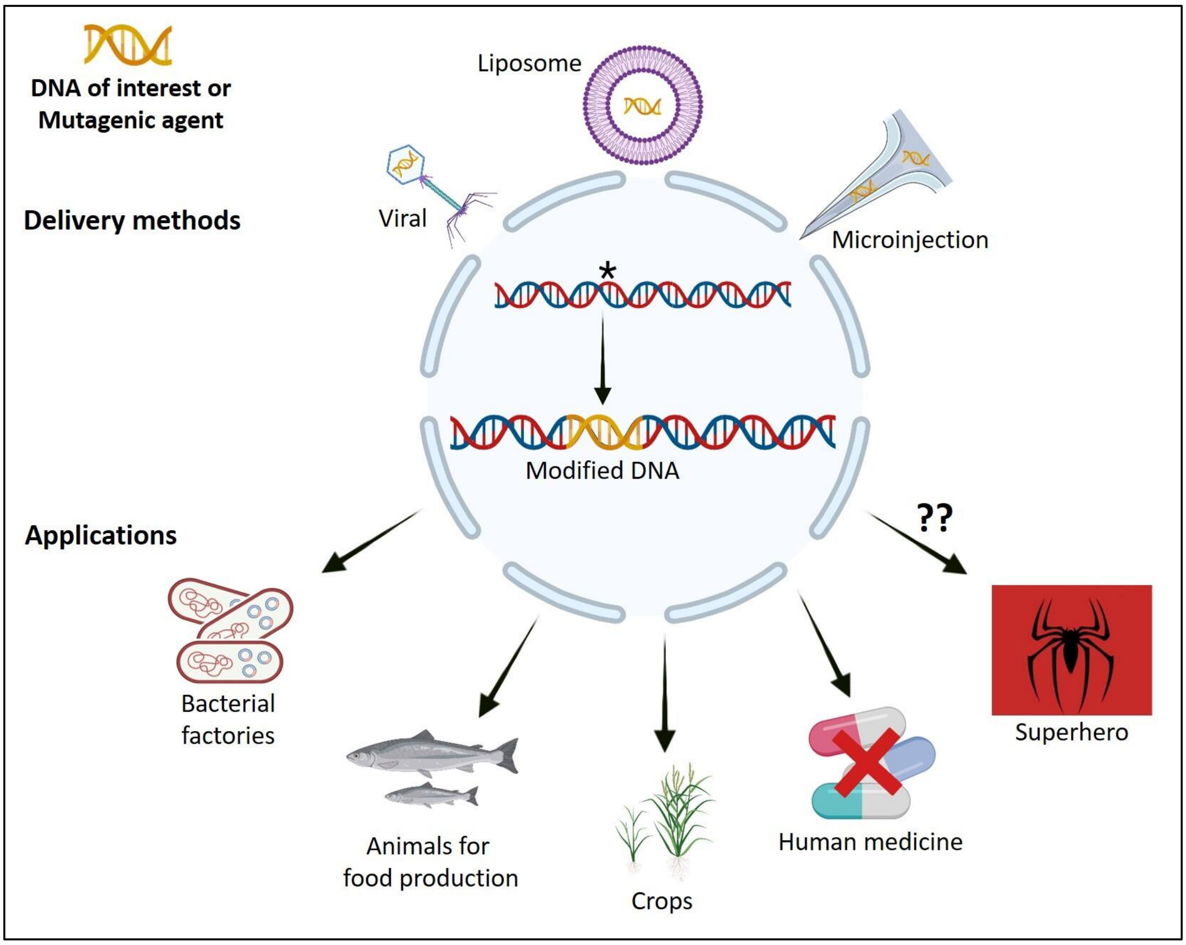

Figure 1. Genetic manipulation and gene therapy. A brief overview of the main techniques used to insert or induce changes in the DNA and the possible applications. Created with BioRender.com.

How about humans?

Can we actually "edit" humans? Reported successes in the correction of genetic errors, associated with disease in animals suggests a potential application of gene editing in gene therapy for humans. Currently, there are several trial studies aiming to apply gene therapy to overcome some disorders, which include several types of cancer, acquired immunodeficiency syndrome (AIDS), cystic fibrosis, hemophilia B, rheumatoid arthritis, among many others. However, a lot is still required to make this therapy on a regular basis, since the process is very complex and efficient techniques must be developed and optimized case-by-case – sometimes, patient-by-patient. Firstly, it is important to deeply understand the targeted diseases and their genetic basis as well as possible interactions with other cells/organs/systems. Then, it is equally essential to identify which cells are specifically affected and understand how it would be possible to reach them. Since all human cells carry the same genetic information, altering the whole genome often is not the right solution. Gene therapy can be designed to be applied either in stem cells or somatic cells. As mentioned, mutating the stem cells, such as the fertilized egg, would allow the substitution of a defective gene by integrating a functional gene into the genome. These alterations would be extended to all the individual's cells and, consequently, they are hereditary and transferable to subsequent generations, thus, mitigating genetic and hereditary diseases. As mentioned, germ cell editing is only allowed for research purposes, for ethical reasons. On the contrary, by applying gene therapy directly to somatic cells, only the specifically targeted cells would be affected. Those effects would be restricted to the patient and would not be inherited by the future generations.

The method by which the correct DNA sequence is delivered within the targeted cells is a critical step for a successful implementation of gene therapy. When dealing with human medicine several aspects must be considered, starting with the safety concerns, for the patient, the environment and the professionals who manipulate it. The vehicle – the so-called "vector" – must be highly specific while showing high efficacy to release the desired DNA. Additionally, it should not induce allergic or inflammatory responses in the patient's immune system. Upon delivery, the newly added DNA is expected to trigger one of the following: increase normal functions, correct deficiencies, or inhibit deleterious activities. The industrial feasibility of the production of large amounts of the vector is not of less importance. The techniques under consideration nowadays are divided into two major groups: virus-mediated or physical mechanisms, which include several approaches, from cationic polymers and liposomes to DNA microinjections and particle bombardment. The adequate mechanism must be chosen according to the nature of the DNA to be inserted and the specific application. In the last few years, several therapies have been approved for use in human medicine, after decades of efforts. These therapies are to be applied in the treatment of several clinical conditions including neuromuscular diseases, inherited blindness, and cancer, bringing a new hope to several patients whose diseases were considered uncurable or whose symptoms were difficult to stand and overcome.

Once we have enough knowledge regarding the specific function of each human gene and the interactions between genes, it would be definitely possible to manipulate virtually any trait that is encoded within our genome. Nowadays, genetic screening is used to select embryos generated via *in vitro* fertilization. A pre-implementation genetic diagnosis is run in a single cell from the eight-cell embryo, whose DNA is analyzed for the presence of diseases associated with genetic alterations, before the implantation in the mother. This screening used to be performed only to determine the sex of the embryos, to avoid the transmission of sex-linked diseases that could be identified in the families' medical stories. Since then, the genetic diagnosis of embryos has been applied to detect single-gene related diseases, such as Huntington's disease, and it is now used to diagnose more than 170 different conditions, including cystic fibrosis and hemoglobin disorders. This screening can also be applied to detect chromosomal abnormalities, in an attempt to improve the pregnancy rates and decrease the levels of miscarriage associated with *in vitro* fertilization. Recently, genetic screening was also employed to identify – and select – embryos not carrying genes associated with increased breast cancer risk, to irradicate breast cancer in families who have been suffering from this condition for several generations. However, each of these advances raises more and more controversy around the application of gene-based selection. Likewise, the application of gene editing in humans raises ethical concerns, particularly regarding its potential use to alter other traits that go beyond health issues. As all scientific fields, this is full of steps back and forward. Among unsuccessful trials, one can list the gene therapy trials conducted in France (2002) with children suffering from Severe Combined Immune Deficiency (SCID), a disease that is linked with the X chromosome. Although the first results seemed promising, with general improvement of the children's condition, after a few months, some of them started showing signs of cancer-like diseases, that were likely to be a direct consequence of the treatment. More recently, a scandal involving the birth of twin girls with allegedly edited genomes (2018) has brought this issue to the spotlight, with the World Health Organization proposing the formation of an international committee to establish strict guidelines for human gene editing. On the other hand, the first gene therapy successful story occurred in 1990, with a 4-year-old girl, who also suffered from SCID. Currently, there are nearly 400 active gene therapy trials around the world and an increasing number of gene therapy drugs are starting to enter the market.

Ethics in Genetics

"With great power comes great responsibility": while we may have – or may acquire – the skills to perform gene editing, shall we do it without limits? Once we can identify the right "switches", we should be able to create "super-plants" and "super-animals". Or even "super-humans", immune to a wide range of diseases, with increased immunity, stronger, more intelligent and maybe prettier – why not? At some point, parents would even decide the physical aspect and several traits of their children and make "the perfect baby". Sceptics ask if we should manipulate our "manual of instruction" indiscriminately, even if, or when, we have the expertise to do it. Perhaps, at some point, this kind of manipulation could be used for achieving specific external characteristics with no relation to health outcomes. Although most of these traits are often subjective and might depend on variable trends and fashion moods, we must consider the role of variability in the evolution. As discussed before, it is variability that provides a species with a full toolbox of possibilities to adapt to any upcoming event. As we select and refine specific characteristics, other traits will naturally be lost over time. And these might be useful one day, later in our evolution as a species.

In conclusion

To conclude with a short answer for the question that drove us here: is it possible for a human to become a Spiderman? Scientifically speaking, with the exponentially growing knowledge in genetic manipulation and the insights on the human genome, we can be sure that it would be possible to control genetically encoded traits towards any desired characteristic. However, we are not there yet. The mechanisms needed to induce mutations and substitute genes still require extensive investigation and the exact effects of each shift need to be assessed and deeply characterized. But science is surely on the right path to make it happen, perhaps in a near future. However, the main question that remains is: once we can do it, should we do it in any circumstance? And to what extent? Ethical concerns should be carefully analyzed as they raise valuable questions on the limits that should be imposed in the application of genetic manipulation approaches in humans in the future. Although gene therapy products and research are strictly regulated nowadays, as our knowledge increases, novel possibilities and problems may arise, requiring the solid establishment of new regulations to ensure that manipulation will not be used for less crucial, or even for deleterious, purposes. In the meantime, we should always try to be informed about the pros and cons of such technologies and draw our own informed conclusions as far as this matter is concerned.

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