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Human germline editing: a historical perspective

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Abstract The development of the genome editing system called CRISPR–Cas9 has opened a huge debate on the possibility of modifying the human germline. But the types of changes that could and/or ought to be made have not been discussed. To cast some light on this debate, I will describe the story of the CRISPR–Cas9 system. Then, I will briefly review the projects for modification of the human species that were discussed by biologists throughout the twentieth century. Lastly, I will show that for plenty of reasons, both scientific and societal, germline modification is no longer a priority for our societies.

Keywords CRISPR–Cas9 · Eugenics · Genome editing · Germline modification · Somatic gene therapy

The recent development of a new molecular technology that can cut DNA at precise positions opens the way to targeted modifications of the genome. This led in 2013 to what a journalist at *Science* called the "CRISPR craze" (Pennisi 2013). The possibility of addressing genes at the right position in the genome, avoiding insertional mutagenesis and cancer, was seen as a plus for somatic gene therapy, in which there are no modifications of the germ cells, and no transmission to the progeny. In contrast, modification of the germline was one possibility offered by the new system, which focused the discussions (Bosley et al. 2015): the risk for future generations was contrasted with the hope of eradicating genetic diseases.

These recent events and the subsequent debates cannot be understood independently of previous attempts to improve human reproduction. The discontinuity created by the discovery of the CRISPR–Cas9 system occurred in a continuity of

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efforts aimed at mastering human reproduction and improving the human genome. Positioning recent events in the context of this past work can cast some light on the ethical and societal issues raised by the use of the new technology.

Some difficulties stem from the fact that the new technology can be used for highly different purposes—to facilitate fundamental research in genetics or to produce transgenic organisms—which raise no ethical issues or ethical issues that are very different from those associated with modification of the human germline. Although I will focus the present contribution on this issue, it is necessary to remember that the new technology will in any case find its place in biology.

I will successively give a rapid description of the way this new technology emerged (Morange 2015a, b) and of the expression "genome editing", which is now commonly used to designate the results of the action of the CRISPR–Cas9 system (Morange 2016). I will then discuss how the new projects dovetail (or not) with previous projects designed to improve the human species. The latter evolved after the rise of molecular biology and the discovery that the genetic material was made of DNA. After 1960, there were plans to alter the sequence of DNA or to introduce new DNA sequences. I will argue that there is a partial or complete independence between the fact that a project is considered as more or less urgent and the availability of tools that enable it. Now that the tools are available, the need to pursue such projects seems far less obvious than it did in the 1960s when such a technology did not yet exist. The existence of alternative ways of solving the same problems and the absence in our societies of clearly shared objectives on the future of humankind stand in opposition to an immediate and unproblematic modification of the human germline.

1 The discovery of CRISPR–Cas9, its use for modifying the genome, and the new project of genome editing

CRISPR–Cas9 resulted from the convergence of two totally independent lines of research. The first emerged from the study of particular genome structures present in bacterial (and archaeal) genomes and the demonstration that they constitute an immune system. The second was a plan to design site-specific nucleases for different purposes. The encounter between the two research programmes was explosive. This story shows that a combination of goal-oriented research and fundamental research, guided only by the desire to explain natural phenomena, is an excellent recipe for productive scientific research!

The design of site-specific nucleases is a long-term project that originated in the discovery of restriction enzymes in the 1960s—endonucleases that cut DNA at specific sequences. Restriction enzymes were an essential component of the genetic engineering tools that rapidly spread in biological laboratories from the mid-1970s onwards and enabled molecular biologists to study the genes of eukaryotes and higher organisms.

Restriction enzymes were designed by evolution to degrade foreign DNA and for this reason they cut DNA at multiple positions in the genome. It was realized very early that endonucleases able to cut DNA at one position in the genome would be an

extraordinary tool, both to study the functions of genes (by selectively inactivating them) and to modify the genome. The hope of producing such endonucleases from the rare, high-specificity restriction enzymes occuring naturally rapidly evaporated, however. This objective could only be reached through de novo design.

The first strategy was to start from a class of proteins that interact with DNA, the so-called zinc-finger proteins, and to combine them with an endonuclease that cuts DNA independently of its sequence. The zinc-finger motif recognizes a short sequence of DNA: combination of different motifs increases the specificity of recognition. The rules of recognition of DNA sequences by these motifs were progressively described, zinc-finger nucleases were produced, and their efficiency and specificity were demonstrated at the beginning of the 2000s. Their first therapeutic uses were initiated in the mid-2000s (Carroll 2008). One such use was to cut (and delete) the gene coding for the CCR5 cell receptor for HIV in T-lymphocytes of patients suffering from AIDS (Perez et al. 2008). This prevented the infection and death of these cells after their reintroduction into the organism. Another family of nucleases called TALENs was developed (Christian et al. 2010) and shown to be superior to the zinc-finger nucleases.

Meanwhile, observations were made that broadened the prospective uses of these specific nucleases. Initially, they were seen as tools to cut DNA, to inactivate genes or to permit the insertion of transgenes at specific positions in the genome. Insertion of a transgene at a precise place is an efficient way of enabling its stable expression. It also avoids the side effects that might result from insertion of the transgene close to genes whose expression might be perturbed by this insertion. Replacement of gene sequences by other sequences was achieved by means of a very different and inefficient process called homologous recombination.

It was discovered in the 1990s that the efficiency of homologous recombination is increased dramatically by cutting the genome at the place where homologous recombination has to occur (Puchta et al. 1993; Rouet et al. 1994). So, specific endonucleases became the required tool to replace one copy of a gene by another copy, for instance an inactive copy by an active, “normal” one. It is within this re-designed plan to produce specific endonucleases that recently discovered enzymes involved in bacterial immunity found a new function.

The presence in bacterial and archaeal genomes of DNA arrays of clustered regularly interspaced short palindromic repeats (whence the acronym CRISPR given later), separated by different spacer sequences, was progressively described in the 1990s. At the beginning of the 2000s, it was discovered that these arrays were linked to a certain number of genes called Cas (for CRISPR-associated). The role of the proteins coded by these genes and of the DNA arrays remained totally unknown. These functions began to emerge in 2005 when three groups showed that the spacer sequences were derived from infecting viruses (called bacteriophages or simply phages; see, for instance, Pourcel et al. 2005). In 2007, an experiment confirmed this hypothesis, by showing that new phage sequences were added to the CRISPR arrays after infection by a new bacteriophage, and demonstrated that deletion of spacer sequences specifically reduced or abolished immunity against the type of bacteriophages from which these sequences were derived (Barrangou et al. 2007).

It was concluded that the different CRISPR–Cas systems were immune systems protecting bacteria and archaea against invading viruses.

Therefore, in 2007, the physiological significance of these CRISPR–Cas systems was understood, but neither the role of the Cas proteins nor the precise mechanisms ensuring bacterial protection were known. As early as 2006, a parallel had been established between the CRISPR–Cas bacterial systems and the RNA interference mechanism found in plants and animals some years before (Makarova et al. 2006). Up to 2011, it was more or less admitted that the transcription of the CRISPR arrays generated short RNAs derived from the spacer sequences, similar to the siRNAs active in RNA interference, which interfered with the RNAs of invading bacteriophages.

However, evidence progressively accumulated that the bacterial immune system was different from the RNA interference system of eukaryotes, and that the DNA of the invading phages was the target of the response, cut by an endonuclease belonging to the Cas proteins. In 2012, a precise mechanism was proposed involving a general nuclease called Cas9 associated with two small RNAs derived by transcription of the CRISPR array (Jinek et al. 2012). Both RNAs hybridized with the DNA of the invading phages, guiding the nuclease activity of Cas9 to precise sequences of these phages. The choice of the sites to be cleaved was not determined by a protein recognizing a specific DNA sequence associated with a non-specific nuclease as in the case of zinc-finger and TALEN systems, but by RNAs associated with a universal nuclease. Targeting of a new site, it was found, only required replacement of the RNAs.

Cas9, with its associated guiding RNAs, appeared as the perfect tool sought by biologists and hitherto only imperfectly represented by the zinc-finger and TALEN systems. It is difficult to admit that the perfect solution for cutting DNA at precise positions, elaborated by bacteria and archaea, had never been imagined by biologists. There are probably two reasons for this failure. The first is that RNA interference acted as a decoy, preventing immediate consideration of other possible mechanisms. The second is that biologists have, since the discovery of DNA-RNA hybridization in the 1950s, used this phenomenon for very different purposes: isolation of RNAs, characterization of their abundance, etc. But targeting DNA with RNAs, for instance to prevent gene expression, gave mixed results. In addition, it seemed that organisms themselves had not exploited the possibilities of regulating gene expression offered by DNA-RNA hybridization.

The expression that is now exclusively used to designate this new method of genome modification is “genome editing”. It was progressively introduced by biologists in parallel with the development of these new specific endonucleases, the zinc-finger nucleases and TALENs. The reasons for using this expression were explained by Fyodor Urnov in 2010: the action of biologists on the genome has now become so precise that it has to be compared to the work of an editor on a text (Urnov et al. 2010). The use of this expression emphasizes that one of the major objectives for which this new technology will be used is to repair the errors of nature, to replace mutated genes by their normal copies. This objective is the one that is often put forward to justify a modification of the human germline.

2 The long road towards human genome modification

It is obvious that human germline modification is not discussed today as it was 70 years ago, when the chemical nature of genes was unknown. Improvement of human reproduction was considered more abstractly, and the solution was sought at a more global level, by preventing individuals harbouring "bad" genes from reproducing and thus transmitting them to future generations; or, conversely, by favouring the reproduction of individuals bearing "good" genes. While the methods are so different, some of the reasons to put them into practice are similar: to prevent the intergenerational transmission of hereditary diseases, and to develop the human characteristics that are considered the most valuable.

So it is not without interest to revisit past debates. I will focus on three periods that are particularly significant because they corresponded—as our present time—to fast scientific developments: the 1930s, with the rise of a new vision of evolution called the Modern Synthesis, the 1960s, with the rapid expansion of molecular biology, and the 2000s, with the completion of the human genome sequencing project and the rise of the post-genomics era.

It is fascinating to discover in the writings of the fathers of the Modern Synthesis not only how confident they were that they had discovered the mechanisms of evolution, allocating, in particular, a major role to natural selection, but also how this knowledge instilled in them an acute awareness of their responsibilities for the future evolution of human beings, and more generally of the living world (Delisle 2009). Two immediate objectives concerning human beings were clearly articulated. The first was to prevent the transmission of bad genes that were no longer eliminated by natural selection because of the social and medical care given to the individuals affected by the resulting diseases. The second was to increase human cognitive and social abilities, although the way forward was far from obvious in practice.

In the 1960s, the state of knowledge was very different. The chemical nature of the gene and the mechanisms by which genetic information was translated were known. There was now the theoretical possibility of modifying the sequence of the genome directly to "correct" genetic errors that lead to the production of non-functional proteins or to introduce new genetic information. But there were no practical ways of making these changes in organisms other than bacteria. More seriously, it was wholly unclear how to develop the tools needed for making such changes.

This situation probably explains the widely differing attitudes of molecular biologists, which ranged from enthusiasm to pessimism regarding the possibility of intervening in the human genome in the near future. One of the most enthusiastic was Rollin Hotchkiss, who had done important work on the possibility of transforming bacteria by addition of exogenous DNA. He imagined prospects extending far beyond the correction of genetic errors, aiming instead at improving "musical ability" or "skill in political oratory" and the aptitude to play polo or "to ride graceful and sure" (Hotchkiss 1965, p. 199). These examples might look like jokes, and maybe they were, but the whole article shows that the author was fully

convinced of the possibilities raised by the new knowledge and the future importance of genetic improvement. In an article entitled "Prospects for genetic intervention in man", Bernard Davis was more cautious, considering that these interventions would be difficult (Davis 1970). In particular, the fact that the human characteristics most slated for modification probably result from the action of an ensemble of genes, many of which were still unknown, made the operation difficult and clearly out of immediate reach. Despite the significance, scientific and philosophical, that he attributed to the molecular revolution, Jacques Monod was even more pessimistic. In *Chance and Necessity*, he considered that the modification of the human genome was, at that time, impossible (Monod 1971) and that the size of the genome, and its complexity, would perhaps forever prevent such intervention.

Foreseeing is always problematic and those who play a major role in the accumulation of scientific knowledge are not always the best placed to imagine the consequences of the scientific revolutions to which they have contributed. In the 1970s, the new tools of genetic engineering were developed to isolate, identify, modify and transfer from one organism to another the genes of animals and plants, and rapidly found their way to lab benches. Transgenesis and gene therapy developed rapidly. The possibility of curing genetic diseases by the introduction of normal gene copies was seriously considered. It is beyond the scope of this article to describe the numerous obstacles that were encountered and which, with rare exceptions, delayed the development of gene therapy. For practical and ethical reasons, the emphasis was put on somatic gene therapy, and germline gene therapy was considered as both impracticable and dangerous. The low efficiency of gene insertion in the genome and the impossibility of controlling the site of insertion were obstacles that seemed unlikely to be overcome any time soon. These drawbacks were acceptable for seriously ill patients, but not for future members of the human species.

Progressively, however, new results challenged the hitherto unanimously approved ban on the modification of the germline. The possibility of replacing one copy of a gene by a slightly modified one through homologous recombination became a reality in mice through the work of Mario Capecchi and others at the end of the 1980s (Thomas and Capecchi 1987). This was immediately exploited by biologists to explore the functions of genes. More and more sophisticated transgenic animals were produced using the classic method of transgenesis or the new one. Some of the results obtained were puzzling: the performances of animals could be rather easily improved by the addition of new genetic information or even by the disruption of an endogenous gene. For instance, mice overexpressing a particular form of the receptor for the neuromediator glutamate performed better in learning and memorization than wild-type mice. It was always possible for evolutionary biologists to argue that these transgenic animals were "better" in the controlled environment of the laboratory, but would prove unfit in a natural environment. But the biologists who produced these results preferred to see in them "a promising strategy for the creation of other genetically modified mammals with enhanced intelligence and memory" (Tang et al. 1999, p. 68). No additional comments are needed, except maybe to notice the rapid shift from memory to intelligence!

These results led around 2000 to a renewed debate on the ban on germline modification, which in 2001 Jonathan Knight called “biology’s last taboo” (Knight 2001). A colloquium was held in Los Angeles in 1998, at the end of which Jim Watson and others called for the abolition of the legal obstacles to modification of the human germline (Wadman 1998). In 2002, Gregory Stock, the organizer of the Los Angeles meeting, published a book entitled “Redesigning Humans”, which had a huge influence in the United States (Stock 2002). The argument of the book was simple, and clearly expressed. All efforts made in biology for more than a century had been oriented towards one objective: to describe the mechanisms of reproduction and inheritance in order to control and orient them in humans. Now that this objective had finally been reached, it would be nonsense to renounce it.

The possibility of preventing mitochondrial genetic diseases by transferring the nuclear genetic material from a one-cell embryo into the cytoplasm of a recipient enucleated oocyte harbouring normal mitochondria, authorized in the United Kingdom in 2014, has been considered as the first example of germline modification in humans—even if the modification of the germline was limited to the genetic information present in mitochondria, a minute part of the total genetic information of the organism.

3 Is the door to human germline modification now open?

In 2014, an experiment was done on mice suffering from a form of muscular dystrophy analogous to Duchenne muscular dystrophy in humans. The endonuclease Cas9, a guide RNA containing the two RNA sequences required to orient the cleavage made by Cas9 in the affected gene, and a sequence of DNA permitting repair of the mutated gene by homologous recombination were injected into the one-cell zygote after fertilization (Long et al. 2014). Although the replacement of the mutated gene was not complete, it was nevertheless sufficient to decrease or even abolish the symptoms of the disease. Since the replacement of the gene occurred very early during development, it was probable, although not directly checked, that a correction of the mutated gene had occurred in a fraction of the cells forming the germline. Attempts have already been made on human embryos (Liang et al. 2015).

It appears now that the best strategy is to engineer directly human reproductive cells, or more precisely the germ stem cells, instead of the early embryo. This would permit verification of the correct replacement of the gene and of the absence of off-target mutations before fertilization and further development. What is new and opens the door to these projects is the efficiency and specificity of the cleavage made by Cas9, which increases the efficiency of homologous recombination to the point where a modification of the germline has become possible. The medical applications of the new technology thus seem obvious: to edit the mutated genes responsible for genetic diseases, and to erase from the medical landscape the diseases due to these mutations. However, what might appear to be an obvious objective is not, for a variety of reasons.

The first is that bad genes, as eugenicists would have called them, are, in the most common cases, due to recessive mutations transmitted through generations by individuals who will never be affected by the disease. The disease results from the reproductive encounter of two parents who do not themselves suffer from the disease but who both carry one mutated copy of the gene. Would it be reasonable to do the complex experiments described previously on individuals bearing only one altered copy of the gene? Even if there might be a benefit for society, there would be no benefits at all for the individuals, only risks. There would also be risks for later generations if the modification were nonspecific and altered other genes.

If, for this reason, the modification of the germline was done only on individuals in whom both copies of the genes are mutated, the benefit for humankind, the decrease in the frequency of the disease, would require in the best case hundreds of generations to be observed. There are much simpler ways of decreasing the frequency of the disease: preimplantatory or prenatal diagnosis, and elimination of the embryos and foetuses in which the two genes are mutated. In addition, somatic gene therapy will also benefit from the use of the CRISPR–Cas9 system: the same group that attempted to cure muscular dystrophy in mice through injection at the one-cell stage, demonstrated 2 years later the efficiency of a postnatal somatic gene therapy in mice (Long et al. 2016). Similar strategies have been proposed for another common genetic disease, sickle cell anaemia (Tasan et al. 2016).

The rare genetic diseases in which gene editing might be the right answer are those caused by dominant mutations, such as Huntington's chorea. Correction of the disease in affected individuals and prevention of transmission of the gene forms responsible for the disease to future generations would coincide. But both objectives can also be reached through preimplantatory diagnosis and selection of embryos devoid of any mutation, without the risk of collateral alterations of the genome during its editing that might appear only in future generations.

Whatever the development of genome editing, it is an illusion to hope that genetic diseases will disappear. At each generation, new mutations appear. The extent of these de novo alterations of the genome varies from one disease to another. It is relatively frequent for some genetic diseases, as muscular dystrophies. In any case, genome editing will have to be repeated at each generation.

Another dimension of germline editing is the possibility of modifying the genome not to eliminate a disease, but to choose the future characteristics of a child and to enhance his or her capacities. I will not discuss the legal and ethical issues, which are addressed differently in different parts of the world. I will rather focus on the scientific aspects, their feasibility and consequences for the human species. It is already technically possible to choose some characteristics, sex, for instance, and others could easily become possible in the near future: eye colour, muscle strength, perfect vision, etc. The choice of other characteristics is presently impossible, and may remain inaccessible for a long time: a high IQ, a good temper, etc. The issues raised by Bernard Davis more than 40 years ago remain. These characteristics result from multiple genes, most of which still unknown, acting together during development, concomitantly with effects from the environment, for instance the behavior of other individuals. The existence of this complex genetic organization

prevents simple genetic determinism. Modifying such a process in a precise direction is an objective far beyond the present knowledge of biologists.

Projects that may emerge, not through a particular desire of parents, but from a societal design project, belong to the latter category and are currently beyond the reach of biologists: the production of long-lived humans, resistant to a large spectrum of diseases, immune to drug addiction, having a low risk of developing mental diseases and a lack of aggression, etc. But even if these genetic modifications were possible—we have seen that most of them are not—what would be the consequences for the human species? The specific requests of families will probably go in different directions, creating a genetic buzz rather than a trend. It is probably the same for what I have called the societal choices. Apart from some health issues, it is not obvious that humans share a common vision of what would be "a superior human being".

The contrast with the situation in the 1930s is striking: most evolutionary biologists in those days agreed on the directions of evolution that had to be favoured. The situation now is probably hopeless for those supporting an active policy of genome editing. It is not obvious that, in contrast with the 1930s, we see the future of humankind in its biological evolution. What threatens the future of human beings today is external to human biology: the degradation of our environment, the rapid disappearance of animal and plant species, climate change, etc. Improving human nature has become far less important than preserving an environment compatible with the survival of the human, and many other, species. This is why I am optimistic. We need not fear a genetic new world similar to that described in *Brave New World* (Huxley 1932) or in the movie *Gattaca*. It is not the genetic projects pursued by cranks that are frightening, but rather the inertia of human societies that might continue to degrade their environment to the point of no return.

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