

Human germline manipulation: technical limits and future perspectives

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It has been estimated that an average adult human body is constituted by 50 trillions (5×10^{13}) cells. Although some cells like adult neurons, do not usually multiply, many others (e.g. blood cells) can and do divide in response to different challenges; most tissues are continually renewed thanks to the balance between programmed cell death and cell proliferation and differentiation. To generate and maintain such a complex and dynamic structure, a very tight and precise control system has to be active at any moment and in any individual cell. These regulations are assured by the "Genome", the full set of genes in an individual, either haploid or diploid. In a human, the haploid set contains more than 3 billion base pairs of DNA and about 50,000 genes.

The human genome is contained in a set of 23 chromosomes which can be regarded as instructions books which we have inherited from our parents and that contain all the information needed to generate our body and maintain it in good health. Each cell of our body (also called somatic cell) receives two copies of the genome and uses this information to determine its exact role and destiny. The reproductive cells of our organism, called "Germ Cells", are an exception as they only receive one copy of the genome. The genes contained into these cells are those that will be passed to all the cells of the offspring. The different set of genes which we inherit from our parents specify not only our morphology, but also other more subtle characters such as our individual predisposition to diseases or our estimated life span. When mutations are present in germ cells they can be passed from one generation to another, they are at the origin of individual variations, but also of familial genetic diseases. When mutations occur in somatic cells, diseases of genetic origin, such as cancer can develop; in this case, however, the genetic lesion can not be transmitted from a generation to another.

Since the origin of life on earth, more than 3.5 millions years ago, the spontaneous appearance of mutations in inherited genes has been at the basis of the mechanism of selection and evolution. Never was the

phenotype capable to modify or improve the genotype of his offspring except by looking for a "better" potential marriage partner.

We are witnessing, in the last decades, a remarkable progress in our knowledge of the nature and function of the genome which is paralleled by a tremendous technical progress in our capacities to manipulate the early human embryo. The combination of these new advances might soon open the possibility of curing genetic disorder and even designing the genetic profile of future individuals. This suggests that we might have the power, early in the twenty-first century, of determining what sort of people there should be.

The aim of this paper is to evaluate which are the technical possibilities and limits to the manipulation of the human germ line which we can envisage for the next future.

The main technological breakthroughs which occurred in "genomic" science in the last decades are:

1. The genome of several organisms, including *Drosophila* has been completely sequenced and we are very close to know the complete sequence of the human genome. In other terms we have now opened the "books of life" written in our chromosomes and we know all the letters composing the text. This does not mean that we can yet understand what this text means.

2. We have developed sophisticated techniques to analyse the function and regulation of genes in vivo. This means that we will be able soon to know the relation between the expression of a gene or of a set of genes and a phenotype and we will know how to activate or repress their expression. In other words we have the tools to understand the meaning of the text written in our chromosomes.

3. The availability of very powerful computers and of new screening technologies gives us the possibility to know the genetic "profile" of individuals in large populations. This means that we will be able to associate characters such as predisposition to diseases with individual genomes.

4. We have learned how to modify the genome of individuals and of their progeny. This can be done in two ways either by adding a new gene into a genome, creating a "transgenic" organism, or by creating specific "targeted" mutation in a genome. This last possibility opens the way to modify at will the genetic nature of an organism, for technical reasons (see below) this approach can only be applied for the moment to mice and man.

5. We have developed very powerful technologies to manipulate and

analyse the early human embryo. Some of these technologies are already used routinely; for example it is possible to remove one of the cells composing a very early embryo to study its genetic composition. This method, called "blastomer biopsy" is used to assure that couples at high risk of transmitting a genetic disease have a healthy child.

6. In some case it has been possible to "clone" vertebrates. Genetically identical individuals could be obtained transferring a somatic nucleus to an egg. The efficiency of this technique is high only if the nucleus comes from an embryo, if the nucleus comes from a somatic cell of an adult animal to obtain one "normal" cloned individual hundreds of defective embryos were obtained.

If in a couple is present a version of a gene that could predispose their offspring to inherit a condition they want to avoid, genetic manipulation may seem like a way to prevent the undesired outcome. One use proposed for germline modification has been to 'cleanse' the gene pool of 'deleterious' genes. For example, Daniel E. Koshland, Jr., a molecular biologist and the former editor-in-chief of Science, has written, "keeping diabetics alive with insulin, which increases the propagation of an inherited disease, seems justified only if one ultimately is willing to do genetic engineering to remove diabetes from the germ line and thus save the anguish and cost to millions of diabetics' (1). Some people also suggest that germline modification would enable couples to 'enhance' certain characteristics of their offspring (2). In the article referred to above, Koshland raises the possibility that germline alterations could meet future 'needs' to design individuals "better at computers, better as musicians, better physically."

How can the human germline be modified ? Two possible paradigms are available to modify the genome in the germline. First of all it is possible to inject a segment of foreign DNA into an egg. In some cases (about 10% of the injected embryos) the injected fragment gets integrated into the genome. If the embryo then develops we will have obtained a "transgenic" animal. In mice and many other animals, the generation of transgenic individuals has actually proved technically quite easy. In the first widely-reported successful experiment using the germline technique, an extra gene that promoted the synthesis of growth hormone was introduced into fertilized mouse eggs and the unusually high levels of the hormone made the mice grow to twice their normal size. Transgenic techniques are also being used to modify farm animals in attempts to increase yields of meat or enhance its nutritional quality, to cause them to produce pharmaceuticals in their milk, and to make their organs more suitable for human transplantation.

Can we envisage to create, in the near future a transgenic human? Although, this techniques appears quite simple to apply, several technical problems are still unresolved. First of all, as mentioned above, only few of the injected embryos would actually become transgenic, one should therefore plan to inject many human eggs, let them develop in vitro and test them individually by blastomere biopsy to identify the few transgenics before re-implanting. A second problem consists in the fact that the foreign DNA integrates randomly into the genome, this can give rise to two different problems. First, the injected DNA could disrupt a normal gene and therefore cause the loss of its function (3) or, second, it might be integrated in a region of DNA which would induce its expression in undesired locations. This last problem (ectopic expression of the transgene) is quite common and almost unavoidable using classical transgenic technologies, when applied to humans it might cause undesirable and unpredictable effects. To avoid these last problems it has been suggested to inject into eggs "mini-chromosomes", long DNA fragments which would not integrate in the genome, but would add genetic material which would replicate and divide with the rest of the genome. If generating a classical transgenic organism corresponds to adding a page at random within the "book of life", this last solution would correspond to add one booklet of instructions to the set of 23 chromosomes.

However, adding an extra gene would not, in many cases, help to cure a genetic disorder. Very often it would be essential to remove the defective gene and substitute it with a normal or enhanced version. This type of genetic manipulation is routinely done on mice thanks to a technology known as "homologous recombination". The technique is based on the fact that complementary stretches of DNA tend to pair. If one injects into a cell a DNA fragment containing appropriate regions homologous to the genomic DNA then the fragment will integrate in a specific location of the genome and substitute an existing gene. With this technique is therefore possible to correct specific mutations without altering the rest of the DNA. Some technical problems are, however, present. First of all the appropriate insertion of the DNA fragment is a very rare event. It is therefore necessary to inject the fragment in many thousand cells in order to select a single cell with the expected mutation. The technique consists therefore in inserting the mutation in "Embryonic Stem Cells" (ES cells), cells derived from very early embryos which can be grown at will and that, when re-injected into another early embryo have the capacity to give rise to any tissue, including germ cells. ES cells can at the moment be obtained only from mouse and human embryos. The technique of homologous recombination has been widely used to reproduce in the mouse the genetic lesions which give rise to human genetic diseases. It has also been used to inactivate mouse genes (the so-called knock-out mice) to study their function in vivo.

Will homologous recombination be ever applied to correct human genetic disorders or to introduce new genes into the human species ? Technically it does not seem impossible. We already have all the instruments to conceive such an experiment. We should generate ES cells from an embryo, modify them in vitro by homologous recombination and then use a nucleus from the "cured" cell to clone a new individual using an egg derived from the same mother from which the ES cells were derived. This technical approach will permit for the first time to correct the stigma of a genetic disorder without having to suppress mutant individuals. This approach seems actually much more precise and safe than the creation of transgenic humans as we will have a complete control on the DNA modification which we are introducing.

In conclusion, although many technical problems remain still to be solved, it appears that germline manipulation in humans is certainly conceivable and that it will most probably be implemented in the near future. The ethical, social, economic implication of these new technical possibilities are being investigated by many thinkers and decision makers (see for example ref. 2). However, the most important implication remains that for the first time since the creation of life a new mechanism of evolution is getting into place. The speed of adaptation of our species to sudden environmental challenges could be greatly increased in the future giving us the hope to adapt and survive to sudden climatic and environmental changes.

A new destiny for the human species ? A new future for life ?

References

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