Ethical and safety issues with recent developments in genomics and biotechnology

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Abstract
The discovery of the DNA structure in the middle of the last century revolutionized the way we understand life and promised to change the practice in modern society. The following discovery of a recombinant DNA technique opened the gateway for recombinant DNA technology. Genetic engineering offered a window into the previously impossible - the mixing of traits between totally dissimilar organisms. Creation and use of genetically modified organisms has sparked significant controversy in many areas, including possible safety issues, ecological concerns, and economic concerns. However, to date not a single instance of harm to human health or the environment has been documented and benefits of gene technology have been widely accepted. The sequencing of the human genome which was followed by announcement of the draft sequence in 2000 was the next scientific milestone, with a major impact on research across the life sciences and application in numerous medical and commercial developments over the last decade. As the cost of DNA sequencing is constantly falling, and new full genome sequencing technologies are emerging, more and more genome sequences continue to be generated. In parallel, machines for the synthesis of DNA sequences are continuously improving and by 2010 DNA synthesis reached the length of 1 million bp, while up to 2015 generation of 100 million or more bp sequences is expected. In 2010 a creation of bacterium was published that has an artificial genome which meant creation of a living organism with no physical ancestor. The way to design an entire genome and get it to work was open, from abstract information to physical, living DNA design. Synthetic biology is starting to be big technological deal, with promises of new crops, new fuels, new ways of investigating diseases and new drugs to treat them. However, like all technologies it can be used for bad as well as for good. The world's databases are filling up with genomic information from every part of the tree of life. Parts of genomic sequences can be synthesized and pasted together with greater and greater ease. Is it possible that by analogy to software viruses, hackers of the future may turn to synthetic biology and turn out real viruses? And "garage biotechnology", can it appear and provoke big disturbance in what nature evolved in billions of years? Is it true that creating life is no longer the prerogativeof gods?

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Genomics and biotechnology - historical background: Biotechnology in its purest form involves (micro) biology, biochemistry and engineering to the use of living organisms, their parts or their products to modify human health and the environment. However, modern biotechnology in the end of the last century specified having its roots in the use of genetic engineering as well as cell- and tissue culture technologies. In contrast to traditional biotechnology which harnesses the potential of processes performed by living organisms, such as fermentation, modern biotechnology manipulates the genes of organisms and inserts
them into other organisms to acquire the desired trait. At the beginning and in its narrow sense, it referred to industrial production, but later-on the definition was upgraded with the use of modified organisms, their

products as well as the new methods and tools in agriculture, environment and medicine (Thieman and Palladino, 2008).

In principle, roots of modern biotechnology were established already in 1944 when Avery, MacLeod, and McCarty demonstrated that DNA is the genetic material (Avery *et al.*,1944). However, modern history started in 1953 when James Watson and Francis Crick determined the structure of DNA (Watson and Crick,1953). In 1966, the entire genetic code was deciphered (Sulek, 1969), in 1970 the first restriction enzyme was isolated which proved to be efficient "molecular scissors" for cutting DNA (see Roberts, 2005), and in 1973 Boyer and Cohen performed the first experiment of molecular cloning a DNA fragment and established recombinant DNA technology (Cohen *et al.*, 1973). In 1976 in San Francisco in California, USA, Genetech, first company for commercial production of recombinant products
was established, and in 1978 first recombinant protein, human insulin was produced in *E.coli* (Genentech, 1978). In the following years A. M. Chakrabarty, working for General Electric, had developed a recombinant Pseudomonas bacterium capable of breaking down crude oil, and he requested a U. S. patent for the bacterium but was refused, because the law dictated that living things were not patentable. However, in 1980 U. S. legal authorities overturned the decision following the idea a live, human-made micro-organism is patentable subject including "anything under the sun that is made by man" (Diamond and Chakrabarty, 1980). First functional gene transfer between mammalian species was performed in 1981 at Ohio University in Athens when researchers successfully produced rabbit genes in mice (Heartland Science, 1981). Since the term transgenic was first used, there has been rapid development in the use of genetically engineered animals with an increasing number of applications for the technology. In 1982 when the first recombinant DNA pharmaceutical, Genentech's recombinant human insulin was approved for sale in the USA and United Kingdom, also first animal vaccine produced by recombinant DNA technology was approved for use in Europe (see Sasson, 1998). Since then, there has been an exponential increase in the number of marketable recombinant products, a trend that continues in the twenty-first century. A major breakthrough came in 1983 when Kary Mullis developed the polymerase chain reaction (PCR), a scientific technique in molecular biology to amplify a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence (Mullis *et al.*, 1986). PCR became indispensable technique used in genomic and related medical research and application. In biology it enabled easier and fast cloning for DNA sequencing, DNA-based phylogeny, and, with further progress, also functional analysis of genes. In medicine it widely opened the door for fast advance in molecular medicine including diagnosis of hereditary diseases and genetic malformations, identification of genetic fingerprints in forensic medicine, and detection and diagnosis of infectious diseases. In 1984 Robert Sinsheimer at the University of California in Santa Cruz launched the idea of maping the unexplored territory of the human genome and in 1989 the interinstitutional academic Human Genome Project was established by US NIH and DOE. It formally started in October 1990, as the international 13-year effort with the aim to determine the complete sequence of the 3 billion DNA subunits and discover all the estimated 20,000-25,000 human genes and make them accessible for further biological study (The Human Genome Project, 1994). A parallel project was conducted outside of government by the Celera Corporation, which was formally launched in 1998. Most of the government-sponsored sequencing was performed in universities and research centers from the United States, the United Kingdom, Japan, France, Germany, and China. Maping and sequencing studies were also carried out on selected model organisms such as the bacterium *E.coli* (Blattner *et al.*, 1997), baker's yeast *S. cerevisiae* (Goffeau *et al.*, 1996), small flowering plant *A. thaliana* (AGI, 2000), nematode *C. elegans* (The *C. elegans* Sequencing Consortium, 1998), fruit fly *D. melanogaster* (Adams *et al.*, 2000), zebrafish *D. rerio* (Broughton *et al.*, 2001) and mouse *M. musculus* (Mouse Genome Sequencing Consortium, 2000), *which* helped in developing the technology and interpreting human gene function. First bacterial genome (*H. influenzae*) was

sequenced in 1995 (Fleischmann *et al.,* 1995), genetic maps for human and mouse were completed in 1996 (Dib *et al.,* 1996; Dietrich *et al.,* 1996), furthermore the first eukaryotic genome (the yeast; *Saccharomyces cerevisiae*) was sequenced in 1996 (Goffeau *et al.,* 1996), and, after draft genome sequences published in 2001 (Venter *et al.,* 2001; Lander *et al.,* 2001), in 2003 the human genome sequencing was reported to be completed, the sequence of the human genome was released, and the postgenomic era »officially« began.

Imapact and consequences of the genomic era on society: Immediately after the first gene cloning trials in the early 1970s it came clear that research on molecular basis of genetics will fully advance just by using gene technology. However, in July 1974, Paul Berg published in *Science* a letter claiming voluntary arrest of experiments on recombinant DNA technology until science will be able to assess the risk of technology and its application (Berg *et al.,* 1974). His fear was based on using viral DNA fragments in his cloning experiments and that cloned SV40 DNA might escape into the environment and infect laboratory workers who could then

become cancer victims. Berg was one of the key organizers of the international forum on recombinant DNA technology, the Asilomar Conference, which took place in February of 1975 at the Asilomar Conference Center on California's Monterey Peninsula. One hundred leading scientists met at the conference to discuss the potential risks of gene-splicing experiments. The conclusion was that most rDNA work should continue, but appropriate safeguards in the form of physical and biological containment procedures should be put in place (Berg *et al.,* 1975). To limit the spread of recombinant DNA, biological barriers should include bacterial hosts unable to survive in natural environments as well as nontransmissible vectors (plasmids, bacteriophages, or other viruses) able to grow in only specified hosts. Additional safety factor as proposed was physical containment, e.g. the use of hoods, limited access or negative pressure laboratories etc. Important safety factor was also the strict adherence to good microbiological practices, which would limit the escape of organisms from the experimental situation. Certain types of experiments were forbiden which included the cloning of recombinant DNA from highly pathogenic organisms or DNA containing toxin genes. Also forbidden were experiments that involved the production of more than 10 liters of culture using recombinant DNA molecules that might render the products potentially harmful to humans, animals, or plants. Additionally, the education and training of all personnel involved in the experiments was included as essential to effective containment measures (Berg *et al.,* 1975). The Asilomar Conference resulted in the NIH Guidelines for Research Involving Recombinant DNA Molecules which were published a year later (NIH, 1976). A Recombinant DNA Advisory Committee (RAC) was set up in order to exercise the control over a U. S. federally funded research on rDNA. At the beginning guidelines were stricter than those set out at Asilomar. However, critics still contended that they did not go far enough. Important questions arose regarding the ethics of a group of individuals regulating themselves, the role and competences of the boards in controlling other than federal funded research (e.g. private industry) etc. Therefore original RAC membership of scientists from molecular biology, genetics, virology and microbiology, was expanded to members from other scientific disciplines as well the general public (McClean, 1997). Guidelines for research involving recombinant DNA represented a milestone of responsible self-regulation in science, and served as a template for national and international regulation and further legislation in this field. There were several long-term impacts of the fact that governments became involved in regulating the research and that the policy was arising from both private and public debate. The very positive outcome was that other scientists than those directly involved in rDNA technology and also non-scientists are now on local, national and international review boards. This non-scientist representation is a direct result of public debate. It is also important to note that Asilomar was the first time that research was halted by scientists themselves until the potential hazards could be assessed, and the debate at this level was later continued also through the human genome project, and is nowdays in place with the development of extremely potent new technologies related to stem cell research and synthetic biology.

In the late 1980s it came clear that the research was less dangerous than originally thought. Gene technology was recognized as save, provided being performed in accordance with good laboratory practice. Safety rules were considerably relaxed and as the result of easing the containment requirements for routine experiments, the use of rDNA technology became more prevalent and flourished. In countries where public opinion reflects good education system and well informed, balanced and objective public media, it was widely recognized the gene technology fulfilled the expectations. Namely, the development of the recombinant DNA technology has not just opened up research areas that previously were not possible, but also enabled big breakthrough of modern biotechnology which produced a number of pharmaceutical drugs and other natural compounds becoming thus accessible, cheaper, more pure and safer for general use (Marx, 1989; Luck, 2003). In 1982 first transgenic plant was produced, using an agrobacterium transformation system (Herrera-Estrella *et al.*, 1983), and in 1983 first successful transfer of a plant gene from one species to another was realized (Murai *et al.,* 1983). US Patent Office extended protection to genetically engineered plants in 1985 (see Lesser, 2009) and there were first field trials of transgenic plants (tobacco plants engineered to be resistant to herbicides) in 1986 in France and USA (Clive, 1996). However, first commercial cultivation of genetically modified plants came in 1992 in China (a virus resistant tobacco) (see Clive, 2006), and in 2001 there were already 52 million of hectares of land planted with genetically modified crops in 13 countries (James, 2002). As concerning animal biotechnology, first successful transfer of a gene from one animal species to another was performed in 1982 (Palmiter *et al.*, 1982), in 1986 transgenic pigs carrying the gene for human growth hormone were produced (Hammer *et al.*, 1986), and in

1988 US patent Office extended patent protection to genetically engineered animals (O'Connor, 1993). In 1996 Dolly, the sheep appeared which represented first successful cloning of a mammalian species (Wilmut *et al.*, 1997). Paradoxically, in contrast to novel pharmaceutical drugs and therapies that have revolutionized the treatment of serious diseases there were and still are much more doubts and concerns about the use of gene technology in the development of genetically modified plants and animals used as food. Concerns are also persisting about their possible impact on the ecosystem, because of their putative advantage over naturally existing concurrents. It is to note, that one of the long-term ramifications of Asilomar and later public debate on the potential biohazards of rDNA was that public discussion was often focused on "worst case scenarios" of recombinant DNA research. When any issue related to rDNA was discussed many "disasters" were proposed as reasons to prevent the research and following application from occurring. Some nations have even accepted legislation that prohibits genetically-modified plants and animals from entering into their food supply (Clive, 2002; The Center for Food Safety, 2006). However, there were more and more in power the views that also in the field of transgenic plants and animals science should go ahead, respecting the rule that prior to ever transgenic release into the environment long-term and systematic research of all possible impact on the nature should be done, respecting ethic principles which require responsible handling of the animals and plants world, and ecosystems (Hill and Sendashonga, 2006; Richmond, 2008; Freese and Schubert, 2004). In order to achieve international harmonization regarding research and application on recombinant DNA technology, Organization of Economic Cooperation and Development (OECD) in 1986 issued Recombinant DNA Safety Considerations including a number of recommendations (OECD, 1986; O'Sulivan, 1986) which were largely based on the former NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH, 1976) and served as template for developing a number of national legislations. In Europe the field was basically regulated through the EC directive 91/356/EEC on GMP (good manufacturing practice), Council Directive 90/219/EEC, of 23 April 1990 on the deliberate release into the environment of genetically modified organisms, and Council Directive 90/220/EEC, of 23 April 1990 on the contained use of genetically modified organisms. The main purpose of both directives on genetically modified organisms (219 and 220) was the protection of the population and the environment from infectious genetically modified organisms (WHO, 2002).

The mapping of human genes was an important step in the development of medicines and other aspects of health care which rapidly advanced with the progress of the human genome sequencing. Huge progress in molecular biology has made diagnosis of some of the diseases (not just at the clinical but also prenatal and neonatal level) very sensitive, accurate
and rapid. With the discovery of genes involved in the etiology of various diseases it became possible to identify those which confer susceptibility to the disease. Ethical concerns and questions appeared such as whether the knowledge that one is carrying the susceptibility gene could lead to more problems than benefits? Will it lead to discrimination in employment,
insurance policies, social life? As concerning genetic screening questions arose such as who should be tested and under what circumstances, should a sample be taken from every new born baby to type the child genetically, and who should have access to this information? (Sequeiros and Guimarães, 2008; Genetic Testing – Issues, 2009). It is generally recognized that information on genetic status of a person has to be kept strictly confidential, and that informed consent has to be obtained from the participants in genetic testing, after explaining the risks and benefits of the research or treatment (Bowles and Marteau, 1999; Singer *et al.*, 2008).

In 1990 Anderson, Blaese, and Culver reported the first approved gene therapy case which was performed at the National Institute of Health in USA, on a four year old girl to treat a genetic defect that left her with an immune system deficiency. The effects were only temporary, but successful (Anderson *et al.*, 1990). Since then scientists have carried out hundreds of trials on thousands of patients. But only a few dozen of them have involved diseases caused by defects in single genes. Most researchers are working on multigene disorders such as cancers and also several nonfatal conditions. Some ethicists fear that multi-gene research will eventually lead to the genetic manipulation of these traits (New Scientist, 97). Here the question arose about what traits should be treated. What are ethics correcting a recessive and possible fatal gene, should it be done or we resign ourselves to biological fate? And the major question, should treatment only involve somatic cells and not germ cells? It is clear that with somatic cell gene therapy therapeutic DNA is not passed on to the offspring. However, it may significantly enhance the proportion of abnormal genes in the population, because individuals with defective genes will survive and pass these genes to their offspring. This can be avoided by introducing the therapeutic DNA into the germline cells. However, there are two important reasons for delaying decision for this approach which may represent chang-

ing the genetic make-up of a human being. On one side the technology needs to be improved to be sure that abnormal foetuses are not formed due to the germline manipulations. On the other side, there is not consessus about changing broader genetic make-up of a human being, and germline manipulation may lead to selecting ‘good’ genes for physical appearance, intelligence etc. Who will stop attempts to change the human race with these powerful tools?

Similar ethical issues as in somatic and germline gene therapy in humans came with cloning of a sheep from an adult cell (Wilmut *et al.*, 1997) followed in the following years by cloning other mammal species and acompaigned by mass media sound predictions of potential cloning of a human being (BBC News, 2004; Eigen, 2010). On one side, technology of nuclear transfer will surely contribute to our understanding of genetics and reproduction, and will be highly supportive in improving the process of breeding where farm animals are considered. On the other hand, serious ethical, moral and spiritual concerns appear when human cloning is in question. Whereas there is general agreement in the scientific community and society that making numerous copies of a human individual is abnormal way of reproduction which from the technical and ethical point of view should not be permitted (UN Ad Hoc Committee, 2005; Bill Text, 2009; Council of Europe, 1999; Islam QA, 1997), opinions concerning stem cell research and therapeutic cloning are not unianimous (Kohrs, 1999; BBC News, 2003).

Genetic testing, gene therapy, stem cell research and cloning are regulated by most national and international legislations. In US where a great part of new technologies arose, there are several acts concerning the use of modern biotechnology in human research and medicine (e.g. Executive Office of the President, 2007; Administration News. 2008). In Europe national legislations in general

are following the conclusions and recommendations of the Convention for the protection of Human Rights and dignity of the human being with regard to the application of biology and medicine: Convention on Human Rights and Biomedicine (ETS 164) from April 1997 and signed by most European countries, which sets out the fundamental principles applicable in day-to-day medicine as well as those applicable to new technologies in human biology and medicine (Council of Europe, 1999). In the proceedings of its Tenth Conference hold in Jeddah, Saudi Arabia, in July 1997, The Islamic Fiqh Academy issued a Fatwa stating that human cloning is prohibited by the faith (Islam QA, 1997).

In conclusion, the advances in biotechnology of the genomic era present both benefits and risks. It has revolutionised the process of drug manufacture, and diagnosis and treatment of human diseases. However, it raises important ethical issues in the society and its social structures including families, preventive medicine, employment, health insurance etc. Therefore interaction of science with the general public is of high priority, in order to educate them and prepare them better for the impact of biotechnology. The scientific and medical communities and the whole society have great responsibility to use the powerful tools of genomic science for the maximum benefit of mankind.

Postgenomic era and new technologies: A draft of the human genome sequence became available in April 2000 and was completed in 2003. Fifteeen years ago at the start of the sequencing estimates about the number of genes in the human genome reached up to as high as 2,000,000, whereas by the end of the project predictions varied from 30,000 to 40,000, and in 2004 the International Human Genome Sequencing Consortium announced a new estimate of 20,000 to 25,000 genes. Some say that in 2000 the »genomic era« was over and »post-genomic era« started. What dose it mean? First, in the last ten years, we have seen a dramatic increase in the amount of sequence data publicly available, including many whole genome sequences. Second, new interdisciplinary scientific disciplines appeared referred to as »functional genomics« and »systems biology«, which means biological science is not anymore looking at gene by gene (protein by protein) or a limited group of genes (proteins) in order to reveal function of a gene/protein in the body but is considering the cell being a system of networks of simultaneous presence of biomolecules and interplaying molecular events (at the transcriptomic, proteomic and metabolomic levels), and thus discovering biological functions of a cell, organ or organism as a whole. And third, all this progress was associated with a tremendous development of new powerfull technologies.

The Human Genome Project officially started in 1990 and estimations about the cost of sequencing the whole genome turned around 3 billion US dollars. When James Watson co-discoverer of the DNA double-helix – became the first individual to have his genome sequenced, in 2007, the cost was around $1 million. In November 2008 a US company Applied Biosystems announced that it had sequenced the genome of a Nigerian man for less than $60,000. In February 2009 a third-generation human genome sequencing company Complete Genomics, based in Mountain View, California, announced it can read entire human genomes at $5000 a shot (Aldhous, 2009), and in November the same company which in 2009

sequenced over than 50 genomes, lowered the price down to $1,700 (Drmanac *et al.*, 2010). Worldwide, ninety genomics centres and labs when asked by *Nature*, estimated that at least 2,700 human genomes would have been completed by the end of 2010, and that the total would rise to more than 30,000 by the end of 2011 (Nature News, 2010).

If in 2001 the number of base pairs stored in nucelotide sequence databases turned arround 10 billion, The International Nucleotide Sequence Database Collaboration published that by the end 2010 this number reached up to 1000 billion and the number of complete genomes sequenced (all species) with information stored at 4,300 (Cochrane *et al.*, 2011). In parallel to the advance in sequencing, also platforms for DNA synthesis developed, so that by 2010 it was possible with unique DNA synthesiser to construct a 1 million bp continuous DNA sequence, in contrast to the year 2004 when sequences up to 10,000 bp were accessible. Predictions for 2015 are going up to 100 million bp sequences. Suddenly, both technologies allow that (abstract) information (which is becoming the biggest

technology in the world) can be transformed to the physical and even living DNA design. In May 2010 a team of scientists at the J. Craig Venter Institute announced that they have successfully created a living organism (a bacterium) with a completely synthetic genome (Gibson *et al.*, 2010). They created the lifeform by synthesising a DNA code and injecting it into a single bacteria cell. The cell containing the man-made DNA then grew and divided, creating a hitherto unseen lifeform. New discipline referred to as »synthetic biology« turned science fiction into science fact, and it was proved that human is now capable to design an entire genome from information and get it to work. Material and information became interconvertible.

Dr. Venter said: 'We are entering a new era where we're limited mostly by our imaginations.' But this breakthrough which to some opinion had changed views on the definition of life, however, opens an ethical Pandora's box. Ethicists said he is 'creaking open the most profound door in humanity's history' – with unparalleled risks. Some say that by 'creating artificial life that could never have existed, he is even going towards the role of God' (Macrae, 2010). Of course, there are big promisses such as creation of microorganisms which can produce novel medical drugs and vaccines, or creation of synthetic bacteria designed to produce clean biofuels, to mine precious metals from rocks and industrial waste, to digest oil slicks and render toxic spills harmless, and even (stil in the fiction domain) creation of organisms engineered to live on Mars and other planets. However, there is a company of severe environmental and ethical concerns. What would mean for the biosphere the creation of living beings with capacities and a nature that could never have naturally evolved? It's important to remember that the wide range of diverse and complex organisms on Earth, and their interplay, represents the product of almost four billion years of evolution. Great danger also lies in the abuse these techniques to modify *in silico* existing viruses and make them *de novo* as highly contagious or virulent and thus the most powerful bioweapons imaginable.

In the coming years, we can expect an ongoing debate about what defines life, both "real" and artificial, and what would be the consequences of the use of new powerful post -genomic technologies on the human society and environment.

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