Review article

GENETIC ENGINEERING: FROM A CLONE TO A PROTEIN

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October 15, 1980, was a date when the highest increase of the share price of one company was recorded on the world most powerful New York change - rates of shares of the company Genetech jumped by more than 2.5 times in only 20 minutes. This event was a grand entrance of a new technology into the global economy and it irreversibly established a brand new status of biology within the development of civilization. Genetech is one of the first companies within the field of molecular biotechnology that accomplished such an enormous commercial success no more than seven years upon the last discoveries in the series of scientific findings that had provided formulation of a new technology designated as genetic engineering or a recombinant DNA technology. Today, 20 years later, it can be rightfully claimed that expectations of the new technology are fulfilled: even if the qualitative shift it provided to fundamental research is disregarded, its direct commercial effects are very convincing - the total value of sold products produced by this technology exceeded the sum of 60 billions dollars in 2000. Let us cite market parameters of only some products manufactured by the application of genetic engineering: annual global consumption of recombinant human insulin amounts to approximately 4.6 tons only in the industrialized part of the world, while the annual sale of recombinant human erythropoietin, interferon's and a human growth hormone amount to 2, i.e. 1.5 billions dollars, respectively.

Key words: genetic engineering, clone, protein

COMMENCEMENTS

Genetic engineering developed as a direct result of the discoveries by molecular biology. Only 30 years, from the late 1940's, from the experiments performed by Oswald Avery, Colin MacLeod and Maclyn McCarty, that are considered the beginning of molecular biology as a scientific branch of biology, to the early 1970's, to the trials of Stanley Cohen and Herbert Boyer, were sufficient to start with studies within molecular biology that provided the discovery and comprehension of principal molecular mechanisms of life. Molecular structures that permit transfer and implementation of hereditary, genetic information, then mechanisms and participants in these processes were discovered. The operative image of physical and chemical interactions that are the essence of the phenomenon of life was developed. The apprehension of the "plan" of nature, that is a base of a structure and a function of all living creatures, led towards a subsequent step, to the application of the observed regularities in the construction of biological production systems towards new, molecular biotechnology.

SEVERAL DEFINITIONS

Genetic engineering (recombinant DNA, gene transplantation, molecular cloning, cloning) is a set of methods that provide the introduction of genes or some other their parts and more of less stable maintenance of such entity in the host cell. According to this definition genetically engineered organisms are developed by (1) the methodology of recombinant DNA that employs vectors, (2) techniques that permit a direct introduction of the genetic material from one into another organism (micro-initiation and micro-encapsulation) and (3) a technique of fusing cells or a technique of hybridomas that are used to form living cells with a new combination of the genetic material in a way that two or more cells are fused in a manner which is not possible in nature.

Molecular (new) biotechnology is the application of genetic engineering and agents developed by it, as well as, other scientific and engineering (technological) principles in material production.

Clone. Clone is a batch of cells developed by division of one cell. In the absence of spontaneous or generated mutations during clone propagation, all clone cells have identical genetic material.

Molecular cloning or DNA cloning. A fragment isolation of any heterologous DNA and its covalent binding to a replicon (plasmid, phage, etc.) in order to obtain a homogeneous population (i.e. clone) of the DNA molecule of a progeny by the introduction of such a developed molecule into a cell.

TOOLS AND PROCEDURES

Restriction enzyme. Restriction enzymes are fundamental elements of a restriction and modification system of bacteria. The aim of this system is to protect a genetic material of a bacterial cell against modifications that can occur by the introduction of foreign DNA gotten into the bacterial cell by some of natural

mechanisms such as virus (bacteriophagous) infection or transformation. The restriction and modification system is capable to distinguish the host cell DNA molecule from a DNA molecule of a foreign origin and to cut the foreign DNA by restriction enzymes endonucleases at specific code sequences. Many of such enzymes were isolated and designated by abbreviations derived from the name of bacterial species and strain they were isolated from and usually from the temporal order of their isolation. Hence, the designation *Eco*RI means that this restriction enzyme was isolated from the bacterium *Escherichia coli* (*Eco*), of the strain *RY 13*(R) and that it was a first restriction enzyme (I) isolated from this bacterial species. The principal property of restriction endonucleases is to hydrolyze phosphodiester bonds in both strands of a double stranded DNA molecule at a specific site of the DNA molecule for each enzyme. The site specifically recognized by the enzyme is designated as a restriction site for a given enzyme. Each of these sites has a specific primary structure, i.e. a specific sequence of nucleotides. It generally consists of 4 to 6 nucleotides while the nucleotide sequence is of a palindromic structure. Such a structure designates the nucleotide sequence that is identical in both strands readable from 5' to 3' (Figure 1). The restriction enzyme hydrolyses (in the laboratory jargon "cuts") either opposite phosphodiester bonds or breaking bonds are slipped off as in the example of the enzyme *Eco*RI presented in Figure 1. In the case of restriction enzymes producing breaks on opposite phosphodiester bonds of the double stranded DNA molecule we are talking about restriction enzymes that produce DNA fragments with blunt ends, while in the other case, DNA fragments with sticky ends are produced, i.e. with ends that carry single stranded elongations with several nucleotides (there are four nucleotides in the majority of these enzymes).

Restriction enzymes in genetic engineering are used as scissors in tailoring - they provide a precise cut of a DNA molecule fragment that carries certain genetic information. Namely, a possibility that a particular nucleotide sequence making a palindromic restriction site for one restriction enzyme within one gene will be repeated several times is relatively small. In the simplest case, we can imagine that one gene is located at a DNA fragment flanked by two restriction sites for a same enzyme. The processing of the total DNA of a given organism by this restriction enzyme will produce a certain amount of fragments of the given lengths at which a gene regulating a given trait, i.e. coding a certain gene product -protein or RNA - is located. In a more complicated case, one gene can be found at a fragment flanked by restriction sites for two restriction enzymes or it has to be resorted to a partial processing of the total DNA by a certain enzyme as its repeated restriction sites are also located within the gene itself. The partial processing means such conditions of enzymic reaction under which DNA will not be totally cut out at all restriction sites, but only to a certain extent. Such a restriction mixture at the end of reaction contains a set of fragments of different lengths of which some also include an intact gene of interest.

Ligase. Enzymes of ligase participate in the DNA replication, as well as, in molecular mechanisms of correcting faults and lesions on the genetic material.

They were discovered in the analysis of the replication process of a lagged strand as enzymes covalently binding adjacent Okazaaki fragments by forming phosphodiester bonds. In the recombinant DNA technology they serve as glue (or thread) by which restrictively processed DNA molecules bound into a unique DNA molecule. At that, the DNA fragment ends have to be treated with the same restriction enzyme or restriction enzymes that produce blunt ends so that ligase can form a covalent bond. Hence, ends of two DNA fragments can be bound ("ligated" in the jargon) if fragments were previously treated with the same restriction enzymes or if they have blunt ends.

Cloning vector is any genetic element that permits the introduction of genetic material into a particular cell. The task of the vector is to introduce a foreign DNA into the host cell and to provide its replication and transcription. The construction of the cloning vector was provided by studies on bacterial plasmids and different viruses - from bacteriophages to plant viruses. The plasmid is a natural extrachromosomal genetic element autonomously replicating DNA molecules and containing antibiotics resistant genes (Figure 2). Various plasmids with the most different properties necessary for diverse applications have been constructed in genetic engineering. Hence, for instance, the majority of plasmids presently applied in *E. coli* genetic engineering have a polylinker, a sequence containing a dozen of different restriction sites. Each of these sites is located in only one copy per plasmid and provides cloning, introduction of a restriction defined DNA fragment into the plasmid. Besides bacteria, plasmids exist in some yeasts, hence *in vitro* constructed plasmid vectors are used in genetic engineering of these lower eukaryotes. Virus vectors developed from bacterial viruses - bacteriophages. Bacteriophagous vectors in *E. coli* genetic engineering were derived by modification of natural *E. coli* phages. These vectors can accept DNA fragments longer than plasmids, but they are somewhat more complex for the use. In addition, there is a greater number of chymeric vectors (cosmid, phagemid, phasmid) formed by combining plasmids and phages. The development of genetic engineering provided the application of different, specifically modified animal and plant viruses that are used for the introduction of genetic material not only in *in vitro* culture but also into eukaryotic cells.

Genomic library comprises a collection of clones that represent the entire genome of an organism. The fundamental prerequisite necessary to prepare the genomic library is to provide its representativeness, i.e. to provide that the total sum of DNA fragments located in all clones overlaps the entire DNA length of one genome. The size of cloned DNA fragments that has to be produced by the restriction enzyme depends, first of all, on the selection of the applied vector. Each of potential vectors has its own capacity, a maximum length of DNA fragments that could be introduced into it. Artificial chromosomes that can contain fragments of genomic DNA of the order of magnitude of 10^6 bp are the most powerful vectors used to establish genomic libraries. Cosmid libraries with separate clones containing fragments of introduced DNA of approximately 45,000 bp in length are mainly in use. **Gene library or gene bank** is a collection of clones that represent genes

that are active in studied cells. Such a library can be constructed by the use of different sources of the genetic material including genomic DNA. Thus, constructed **cDNA libraries** are often specific by the fact that the total iRNA is used as the initial material for the construction. Such an iRNA is transcribed into DNA by means of the enzyme of reverse transcriptase and then is cloned into a vector. Such banks represent banks of genes that are functional in a certain moment and are transcribed into iRNA. Besides, it is necessary to mention that due to existence of introns, i.e. complex genes in eukaryotes, only cDNA banks could be used for expression of eukaryotic genes in the recombinant DNA technology.

Electrophoresis is a movement of charged particles in a fluid medium under the influence of an electric field. Particle electrophoretic mobility (U) is a property of macromolecules by which they are electrophoretically distinguishable from one another, i.e. by which the specific velocity of their mobility is determined. The application of this property provides separation of molecules, i.e. their distinguishing. Electrophoresis is an unavoidable technique in studies of molecules of both, nucleic acids and proteins. At present, electrophoresis is mostly performed by agarose (almost always for double stranded molecules of nucleic acids) and polyacrilamide used for electrophoretic separations. These matrices provide improved separation and lessen diffusion in separated molecules.

Hybridization of nucleic acids (molecular hybridization) is a detection method of molecules of nucleic acids that are interrelated by nucleotide sequences. Molecular hybridization comprises a combining of single strands of probe and target DNA, whereby molecules of the probe are designated by either some of radioactive isotopes or some of non-radioactive markers. The following two types of double stranded molecules are produced under conditions that permit the formation of double stranded structures of molecules of nucleic acids: homoduplexes in which both strands originate ether from the target DNA or DNA probe and heteroduplexes in which each strand is from a different source.

The applications of nucleic acids hybridization are very numerous since the method provides detection of selected, specific, nucleotide sequences. Namely, let us imagine that a gene has been isolated from a eukaryotic species and that we are interested in the fragment in the fragment mixture, produced from the total genomic DNA by a restriction enzyme, this gene is located in. The first step in the hybridization analysis would be a preparation of a hybridization probe. In our, hypothetical, case a fragment of DNA that carries this isolated gene would be the probe. This fragment has to be labeled, radioactively or nonradioactivelly, in order to provide monitoring of its interactions with other molecules. In the subsequent step, all double stranded fragments of the target DNA, as well as, the probe would be isolated and denaturated. In the third step, the contact and pairing by spontaneous building of hydrogen bonds between complementary bases, denaturated molecule of the hybridization probe, and the target DNA would be allowed. The paired hybridous molecules of the probe and the complementary fragment of the target DNA are detected as a double stranded DNA molecule that is radioactively labeled. This type of analysis is today mostly performed on molecules of nucleic

acids, which were previously arranged in a certain way following their electrophoretic separation and fixation (southern and northern blotting), fixation to a carrier of nuclei of metaphase chromosomes and similar (*in situ* hybridization).

Determination of the DNA primary structure, sequencing. The determination of the nucleotide sequence of a gene is one of the standard procedures used in genetic engineering. The primary gene structure permits the identification of a gene structure, monitoring of spontaneous mutation occurring in that gene or the introduction of specific mutations at the chosen site into the gene. Based on the primary gene structure it is easy to derive the primary structure of a gene product, protein, to compare genes of different organisms and to determine phylogenetic relatedness of species. The perception of the primary structure is necessary for discovering the mode of gene expression control and for providing the conditions under which a certain gene can be regulated by the most different regulatory elements that are either isolated from other genes or are *in vitro* constructed (promoters). Several methods of DNA sequencing have been developed, but Sanger method implemented in 1977, for which the author won a Nobel prize, and its numerous modifications, are mainly applied. This method is based on the three principal solutions.

A mechanism of the DNA molecule replication is used in the determination of the primary structure: a single stranded matrix, a single stranded DNA molecule, for the synthesis of a new strand whose sequence is decoding, is used; (b) a synthesis of a new strand begins with a short piece of DNA added to one end of a strand of DNA (primer) that is complementary to the stranded matrix that is bound to it by spontaneous building of hydrogen bonds; (c) the enzyme DNA polymerase is involved in the synthesis of the new strand.

The DNA visualization is performed by the introduction of a radioactively labeled nucleotide.

The synthesis of a new DNA strand is ceased by the introduction of dideoxynucleotides (ddNTP).

In order to determine the location of each of four nucleotides (ATGC) one reaction mixture for each is used; there is only one dideoxynucleotides of a low concentration in this mixture. The reaction mixture with ddATP is used for the determination of the nucleotide A location. Hence, this mixture consists of all four "normal" deoxynucleotides (dNTP, where N is one of bases - A, G, T and C) out of which one of a low concentration is radioactively labeled (ddATP). The synthesis of a new DNA strand is permitted by the presence of the primer, DNA polymerase and all nucleotides, and it is proceeded until ddATP nucleotide is incorporated into the stranded matrix, opposite the T nucleotide. The further synthesis is prevented, because ddATP lacks the OH group at 3' de(s)oxyribose site, hence, the DNA polymerase is prevented from introducing a subsequent nucleotide because the hydroxyl group does not exist and the nucleotide cannot be bound. Since the reaction mixture is arranged in a way that it contains much less ddATP molecules than their "normal" de(s)oxy analogues (dATP), hence they, will be introduced with a lower concentration, and the reaction mixture will at the end contain newly synthesized

fragments of different lengths that are characterized by the mutual property - ddATP is the last incorporated nucleotide. The simultaneous electrophoretic analysis of all four reaction mixtures (mixtures with ddATP, ddGTP, ddCTP and ddTTP) at the same gel establishes a so-called scale of radioactive single stranded fragments of newly synthesized DNA that are electrophoretically separated into four adjacent lines on the gel and that are distinguishable from one another by their lengths; fragments are always present in one of four lines of separation that end with different ddNTP and that differ in the length by one nucleotide. A fragment of a certain length could have been formed only by the introduction of one of four dideoxynucleotides. The nucleotide to be introduced is determined by the restriction mixture, i.e. by the type of ddNTP that is added to the mixture. The nucleotide sequence is easily readable by a simple comparison of positions of electrophoretic bands in all four lines.

Polymerase Chain Reaction (PCR) is a contemporary technique that amplifies numerous copies of DNA fragments. The technique can be analogously presented as a molecular copier, which produces a great number of copies of a DNA fragment under *in vitro* conditions. The technique makes use of properties of the enzyme DNA polymerase isolated from bacteria living at increased temperatures (in thermal springs) and properties of the DNA molecule replication. For that purpose, short oligonucleotide strands (up to a few dozens nucleotides) are synthesized and they restrict the DNA fragment to be amplified and are complementary with the ends of its strands. These artificial short oligonucleotides represent primers for replication of both strands of the DNA molecule. The reaction mixture contains a double stranded DNA molecule, both oligonucleotides at a high concentration, the thermostable DNA polymerase and the mixture dNTP. The initial step starts with heating of the reaction mixture, which results in denaturation of a double stranded DNA molecule, i.e. in separation of complementary strands. The temperature is decreased in the next step, providing pairing of oligonucleotides with complementary nucleotides of both strands. Then, the DNA polymerase initiates the introduction of nucleotides by the elongation of complementary bound oligonucleotides. The reaction is proceeded until there is a matrix, i.e. as long as the end of molecules for which oligonucleoitdes are bound is not reached. At the end of this cycle there are two molecules in the reaction mixture that were produced from the initial double stranded molecule. In the succeeding cycle, four single stranded molecules are denaturated and paired with complementary oligonucleotides. At the end of DNA synthesis in the second cycle, there are four double stranded molecules. The initial DNA molecule shall be copied in thousands or millions of copies by a great number of repeated identical cycles. The DNA fragments amplification has a significant application in both, genetic engineering *per se* and separation of the most different diagnostic methods that require the employment of DNA molecules.

EXPRESSION OF HETEROLOGOUS GENES

Amounts of many biologically active substances (BAS) of the most diverse applications in daily life are limited in nature or it will be soon. Due to so many different reasons, other BAS are either unavailable or available with great difficulties. For instance, let us consider a group of related human proteins important for the cure of broad-spectrum viral infections - interferons - proteins that participate in antiviral, antiproliferative and immunomodulatory activities that occur as a bodily response to viral infections. First of all, let us consider a natural source of these proteins. There is only one - human blood. Interferons of animal species cannot be used as they are not identical to human interferons. At the same time, the total amounts of interferons that can be isolated from blood are very low as their concentration in blood of healthy people is extremely low. Hence, the natural source of interferons practically does not exist. A chemical synthesis of polypeptide chain of interferons (on the average, approximately 160 amino acids depending on the interferon type) could be an alternative method. A polypeptide chain of this length cannot be synthesized yet, not even qualitatively (in small amounts), let alone in a quantitative synthesis. Even if the organic synthesis succeeds in the synthesis of large proteins there is still a question of a price of such a synthesis. The experience gained in the organic synthesis of antibiotics, which is possible and experimentally demonstrated, indicates that organic synthesis is not a good source for the future: none of the antibiotics is completely chemically synthesized, because the price of such a synthesis is enormous. Which alternative is offered by genetic engineering? Principally, the concept is very simple: we should rely on both, knowledge gained within molecular biology and living systems for production, i.e. a cell/organism as a chemical factory. Living organisms daily produce enormous amounts of organic matter consuming, at the same time, inexpensive sources of energy and nutrition; they are capable to reproduce autonomously and to transmit a method of one substance production from one generation to another. The intervention is necessary only in one segment of the cell structure - in its genetic information. It is necessary to alter the initial genetic information of the cell that will be turned into a production system by means of the introduction of an additional genetic information, new for this cell, that originates from another organism; the introduction shall be performed in such a way that it results in a desirable product in a new host. This fundamental concept promises an epochal alteration of the production in many other human activities; the initial attempts fulfilled some expectations, but our ability to manipulate genetic information correctly is still limited by our lack of knowledge.

The production of proteins of interest can be at present achieved by genetic engineering in several types of cells: (1) bacterial cells. The production systems have already been developed in both Gram-positive and Gram-negative bacteria. *Escheria coli*, a Gram-negative bacterium, is an essential and today an unavoidable production system in genetic engineering. (2) Yeasts. These lower eukaryotic organisms are one of favorite model-systems as they are also monocellular micro-organisms, which make operations with them easier; moreover they are eu-

karyotes, thus their cell machinery is more similar to cells of mammals what is often of a great advantage. (3) *In vitro* cultured insect cells. This is a more recent model-system within genetic engineering which is more easily operated than other cultured cell systems. (4) *In vitro* cultured human and mammalian cells. Beside an apparent advantage in the production of therapeutic human proteins due to matching or at least a great relatedness of host cells with cells that naturally synthesize such molecules, this model-system suffers from imperfections caused by huge demands of cultured mammalian cells that are necessary to be fulfilled for their survival and reproduction what inevitably make operations more expensive and difficult. (5) Transgenic organisms; transgenic plants and animals. Only transgenic plants are used to a large scale, while experiments with animals are still in their infancy. The utilization of these systems is still relatively limited due to lack of information on them. Such a situation causes additional problems with public observation as people worry a lot, while the degree of their understanding is very low.

The introduction of heterologous, foreign genetic information into a cell is connected with a prerequisite for a technique that will permit a physical entry of a DNA molecule and its endurance in a new cell. The choice of the technique depends, first of all, on the type of both, host cell and used vector (plasmid or virus). Transformation is the simplest method for bacteria and yeasts - the use of the natural process by which introduced DNA penetrates the host cell. Although the process is natural, its inherent frequency is extremely low; hence under laboratory conditions it has to be elevated in various ways.

The introduced DNA, once incorporated into the cell, can endure in it due to either its own regulatory element designated as a site of the replication origin, or to mechanisms that provide its covalent binding with DNA molecules of the host-cell, i.e. provide the introduction into a genome. The maintenance and inheritance of newly introduced genetic information is a key prerequisite for the use of progenies of such a cell (clone) in the production.

The implementation of newly introduced genetic information means an expression of a foreign gene, i.e. its efficient and controlled transcription, then translation of transcribed iRNA, as well as, a correct performance of the post-translation production of a newly synthesized polypeptide chain, maturation of protein in a functional form and its directing into a certain cell compartment. A part of these complex processes of the genetic information implementation could be regulated, but other part is beyond our direct influence.

Promoters, DNA sequences, provide recognition of genes on a fragment of double stranded DNA and beginning of its truthful transcription. The promoters used in genetic engineering are mainly hybridous ones - constructed *in vitro* out of elements of different natural promoters. Such promoters are constructed to fulfill the requirement to be strong, i.e. to provide a high level of gene transcription into iRNA, and at the same time to be strictly controlled. The mode of a promoter regulation depends on each individual experiment. In case of the production of a protein synthesized in bacteria or yeasts in a fermentor, an inducible promoter is mainly selected; this promoter is mostly activated when a certain number of cells

per vessel volume unit are achieved by addition of a specific inductor. Prerequisites for the promoter selection in transgenic organisms can be far more complex - they have sometimes to provide a spatially limited expression in a tissue.

The process of iRNA translation is performed in the host cell, simplistically explained, without specific prerequisites for modifications of genetic information that was cloned due to the universality of the genetic code. However, the universality of the genetic code is not sufficient as certain organisms mostly opt for a certain codon of each amino acid. Due to this, iRNA, transcribed from a foreign gene, generally contains a message coded with "unfavorable" codons of the host cell, resulting in the decrease, faults in the synthesis, but also in termination of the synthesis of the polypeptide chain. Such a problem is solved by the exchange of codons in a foreign gene, i.e. the foreign gene is chemically synthesized, and thus the transmitted message remains identical, but is now transcribed by codons that are "favorable", comprehensible to the host cell.

Post-translation modifications of a newly synthesized polypeptide chain during its functional maturation are numerous; they encompass not only enzymic steps of the addition of certain groups (e.g. phosphorylation, methylation, glycosilation), as well as, various proteolytic reactions, but also processes in which protein possesses a functional spatial form. The primary structure of the polypeptide chain, an amino acids sequence, carries information that specifies these reactions and the result depends on the capability of a given host cell to interpret this information correctly.

A cell synthesized protein can be stored either in the cell itself in its different compartments or can be extracted of it. At present, the protein guidance into some of cell compartments can be only partially impacted. Hence, the extraction of recombinant proteins of the cell is achieved by the fusion of genes of interests with specific DNA sequences that code secretory sequences, short polypeptide chains that provide the direction of homologous proteins of the host cell into the environment. However, the existence of such a secretory sequence will not be extracted by every protein of the cell, because its guidance is affected also by the primary structure of a certain protein. In addition, the prospect of a protein synthesized in different cells can be dissimilar. For the time being, testing of a greater number of host cells, i.e. expression systems parallelly is the only reasonable experimental approach.

Several hundreds proteins of the most diverse origins have been synthesized in various expression systems. The majority of these recombinant proteins are used for the experimental purposes, while a relatively small number is commercially utilized. However, recombinant proteins such as erythropoietin, insulin, growth hormone, interferon, coagulation factor, different enzymes such as recombinant proteins that are used for the production of vaccines, show a high commercial value of the genetic engineering technology and point out to a further progress in this technology exploitation.

PROSPECTS

Nowadays, decoding of the entire genetic information of an organism, either bacterial or a mammalian, is a routine operation performed by specialized companies by means of automated procedures. Such a company is capable to interpret, sequence a genome of a bacteria in a month. Nevertheless, interpreted nucleotide sequences and their computer processing that predicts which segments of the DNA molecules represent functional genes and functions of their gene products are still, to the greatest extent, just a possibility, i.e. these data are sufficient for formulating a more or less statistical prediction. The determination of the actual functions of certain genes, i.e. of their gene products, as well as, the mode of the administration of the implementation of a particular piece of genetic information is enormous work yet to be done. Beside this direction of the research, it can be expected that the final gene products, proteins, shall have an important role in future studies. Their functions, the three-dimensional structure and its dependence on the primary structure, amino acid sequences, and probably the most challenging of all, mechanisms of interactions between proteins and DNA molecules and interactions among proteins will be studied. This direction of the research shall result in new information on fundamental molecular and biological processes and mechanisms. At present, it is not possible to perform any study on fundamental processes without the application of the recombinant DNA technology. On the other hand, new information, a profound insight into essential life processes will permit elimination of the majority of actual limitations within genetic engineering. In such a way, the genetic engineering technology will be able to aim its studies at more and more complex operations within principal cell functions, towards engineering of integrity of cell functions and modifications of its genetic information. The goal of these studies used to be, is and will be the cell modification in a way that the cell becomes a highly efficient production reactor for the most diverse substances without which modern civilization, modern mankind, cannot exist.

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GENETIČKO INŽENJERSTVO: OD KLONA DO PROTEINA

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I z v o d

Datum 15. oktobar 1980. zabeležen je na Njujorškoj berzi kao dan kada je ostvaren najveći rast cena akcija jedne kompanije u istoriji ove najmoćnije svetske berze - za samo dvadeset minuta cene akcija kompanije Genentech skočile su za više od 2,5 puta. Ovaj dogadaj je označio ulazak na velika vrata jedne nove tehnologije u svetsku ekonomiju i nepovratno utvrdio novo mesto bilogije u razvoju civilizacije. Jer Genentech je jedna od prvih kompanija u oblasti molekularne biotehnologije koja je ostvarila ovaj neverovatni komercijalni uspeh samo sedam godina posle poslednjih u nizu naučnih otkrića koja su omogućila formulisanje nove tehnologije nazvane genetičko inženjerstvo ili tehnologija rekombinantne DNK. Danas, dvadesetak godina kasnije, sa punim pravom se može tvrditi da su osnovna očekivanja od nove tehnologije ostvarena: ako i zanemarimo kvalitativan pomak koji je omogućila u fundamentalnim istraživanjima, pogled na njene neposredne komercijalne efekte u to nas uverava - ukupna vrednost prodatih proizoda koji su nastali ovom tehnologijom premašio je 2000. godine sumu od 60 milijardi dolara. Pobrojmo tržišne parametre samo nekih proizvoda nastalih korišćenjem genetičkog inženjerstva: godišnja svetska potrošnja rekombinantnog humanog insulina je samo u industrijalizovanom delu sveta oko 4,6 tona dok se godišnje proda rekombinantnog humanog eritropoetina u vrednosti od 2 milijarde dolara, - interferona i humanog hormona rasta u vrednosti od oko 1,5 miliardi dolara.

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