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Review

Biotechnology and genetic engineering in the new drug development. Part I. DNA technology and recombinant proteins

Agnieszka Stryjewska[1], Katarzyna Kiepura[1], Tadeusz Librowski[2], Stanisław Lochyński[3]

[1]Department of Bioorganic Chemistry, Faculty of Chemistry, Wrocław University of Technology, Wyb. Wyspiańskiego 27, PL 50-370 Wrocław, Poland

[2]Department of Radioligands, Medical College, Faculty of Pharmacy, Jagiellonian University, Medyczna 9, PL 30-688 Kraków, Poland

[3]Institute of Cosmetology, Wrocław College of Physiotherapy, Kościuszki 4, PL 50-038 Wrocław, Poland

Correspondence: Stanisław Lochyński, e-mail: s.lochynski@wsf.wroc.pl; Tadeusz Librowski, e-mail: mflibrow@cyf-kr.edu.pl

Abstract:
Pharmaceutical biotechnology has a long tradition and is rooted in the last century, first exemplified by penicillin and streptomycin as low molecular weight biosynthetic compounds. Today, pharmaceutical biotechnology still has its fundamentals in fermentation and bioprocessing, but the paradigmatic change affected by biotechnology and pharmaceutical sciences has led to an updated definition. The biotechnology revolution redrew the research, development, production and even marketing processes of drugs. Powerful new instruments and biotechnology related scientific disciplines (genomics, proteomics) make it possible to examine and exploit the behavior of proteins and molecules.
Recombinant DNA (rDNA) technologies (genetic, protein, and metabolic engineering) allow the production of a wide range of peptides, proteins, and biochemicals from naturally nonproducing cells. This technology, now approximately 25 years old, is becoming one of the most important technologies developed in the 20th century.
Pharmaceutical products and industrial enzymes were the first biotech products on the world market made by means of rDNA. Despite important advances regarding rDNA applications in mammalian cells, yeasts still represent attractive hosts for the production of heterologous proteins. In this review we describe these processes.

Key words:
biotechnology, recombinant DNA technology, directed mutagenesis, aranesp, activase

Introduction

Biotechnology is being defined as organisms or biological molecules, which are used in the industrial production. This term includes processes used for centuries, such as the production of alcohol, as well as the discovery in more recent years of genetic engineering [30, 40, 46]. Biotechnology in the pharmacology is being called biotechnology pharmaceutical, which is producing biopharmaceuticals. These are proteins with therapeutic meaning and recently nu-

cleic acid used in the gene therapy, which are being produced due to genetic engineering or traditional biotechnology.

The biotechnology industry has been developing most dynamically for the last 30 years of the 20th century. Today, there exist over 10,000 pharmaceutical companies producing in total about 5000 biopharmaceuticals. However, only 100 of these companies have a prominence and meaning in the industry. The medical success of sulfonamides and insulin gave new emphasis to the industry, which was boosted further by the commencement of industrial-scale penicillin manufacture in the early 1940s [32, 46, 48].

In the years 1971–1973 a new technology was brought into effect, which then became a great scientific turning point. It was recombinant DNA technology, otherwise known as genetic engineering, which until today is a base of many biotechnological processes. PCR (Polymerase Chain Reaction) is one of elements of the technology behind recombined DNA. The technique discovered in 1985 by Mullis enables researchers to produce millions of copies of a specific DNA sequence in approximately two hours. This automated process bypasses the need to use bacteria for amplifying DNA [11].

The basic biotechnological processes used most widely in the pharmaceutical industry apart from recombined DNA technology, and including within this directed mutagenesis, are biocatalysts, technology of monoclonal antibodies, technology of vaccines, metabolic engineering, and only recently also gene therapy. However, these processes are largely based on the tools of gene engineering. Biotechnology has also had a major impact on the pharmaceutical industry.

Recombinant DNA technology

The idea for recombinant DNA was first proposed by Peter Lobban, a graduate student of Prof. Dale Kaiser in the Biochemistry Department at Stanford University Medical School. Recombinant DNA technology is the technique, which allows DNA to be produced *via* artificial means. The procedure has been used to change DNA in living organisms and may have even more practical uses in the future. It is an area of medicine, which is at present in its initial phase of the overall concerted effort [32].

The cornerstone of most molecular biology technologies is the gene. To facilitate the study of genes, they can be isolated and amplified. One method of isolation and amplification of a gene of interest is to clone the gene by inserting it into another DNA molecule that serves as a vehicle or vector that can be replicated in living cells. When these two DNAs of different origin are combined, the result is a recombinant DNA molecule [39].

Currently, recombinant DNA technology has attracted headlines when it has been used on animals, either to create identical copies of the same animal or to create entirely new species. One of these new species is the GloFish™, a type of fish that seems to glow with a bright fluorescent coloring. While they have become a popular aquarium fish, they have other uses as well. Scientists hope to use them to help detect polluted waterways, for example.

Recombinant DNA technology is not accepted in certain quarters, especially social conservatives, who feel the technology is a slippery slope to devaluing the uniqueness of life. Furthermore, because some DNA work involves the use and destruction of embryos, there is more controversy created. Still, proponents of the technology say the ultimate goal is to benefit human life, not destroy it.

How to obtain genes?

Two major categories of enzymes are important tools in the isolation of DNA and the preparation of recombinant DNA: restriction endonucleases and DNA ligases. Restriction enzymes are DNA-cutting enzymes found in bacteria. Because they cut within the molecule, they are often called restriction endonucleases. In order to be able to sequence DNA, it is first necessary to cut it into smaller fragments. Many DNA-digesting enzymes can do this, but most of them are of no use for sequence work because they cut each molecule randomly. This produces a heterogeneous collection of fragments of varying sizes. What is needed is a way that cleaves the DNA molecule at a few precisely located sites so that a small set of homogeneous fragments are produced. The tools for this are the restriction endonucleases. The rarer the site it recognizes, the smaller the number of pieces produced by a given restriction endonuclease [7, 11].

By using the reverse transcriptase of an enzyme of retroviruses, the produced hybrid RNA-cDNA and the following RNAsa H digests the partly RNA thread

[11]. The cDNA incurred is deprived of introns and control sequences [1]. The remaining RNA fragments serve as primers for polymerase DNA I, which synthesizes the other cDNA chain [11]. Further proceedings are the same as in the formation of the genomic library; although the clones incurred contain the set of only these genes, which surrendered to the expression in the used cell.

Creating false fragments based on the known amino acid sequence of the protein requires fitting codons that correspond with particular amino acid. For short peptides it will be sufficient to create single strand DNA and hybridize with a second complementary thread. For long peptides, a lot of chemically encapsulated oligonucleotides undergo hybridization; polymerase (a polymerase is an enzyme whose central biological function is the synthesis of polymers of nucleic acids. DNA polymerase and RNA polymerase are used to assemble DNA and RNA molecules, respectively, by generally copying a DNA or RNA template strand using base-pairing interactions) and ligase (ligase is an enzyme that can catalyze the joining of two large molecules by forming a new chemical bond, usually with the accompanying hydrolysis of a small chemical group that is dependent on one of the larger molecules or the enzyme catalyzing the linking together of two compounds) fill the gaps incurred in the helix [24, 42].

Selection of the corresponding gene from the library occurs because of the use of the probe that is complementary to the given fragment of a marked thread of DNA [1]. The clones are transferred to a nitrocellulose or nylon membrane and are brought to denature the DNA through temperature (80°C for nitrocellulose) or UV (for nylon). After adding the marked probe, hybridization occurs with the denatured DNA strands. Unbound probes are then scoured and the sample is dried and subjected to autoradiography. The visible stripe corresponds to the sought gene [8]. The probe can be homologous, which is perfectly complementary to the given DNA fragment, or the probe can be heterologous when the sequence is only very similar to the studied particle [5, 38].

PCR allows for obtaining a lot of chosen fragments of the polynucleotide chain. This sequence remains unknown. Knowledge of the flanking sequences, which are located on both sides of the section, intended for amplification is, however, sufficient. For the reaction mixture, aside from the DNA, large amounts of two types of short oligonucleotides, which are complementary to the flanking sequences and serve as primers, and heat resistant Taq polymerase are added. Heating the content of the tube to 94°C causes the denaturation of the double helix DNA. Cooling to 50–60°C makes the single strand oligonucleotides bind. After being reheated to 74°C the Taq polymerase begins acting and synthesizes the new chain. Multiple (25–30 times) repeats of these steps causes the creation of millions of copies of the amplified segment [11].

Introduction a gene into vector

A bacterial plasmid can be the vector – spherical autonomous particle DNA, bacteriophage (phage λ, e.g., M13), which is a virus infecting the cells bacterium and cosmid, a plasmid with *cosy* ends [11]. In the case of the long molecules of the DNA of mammals, created artificial chromosome vectors (YACi, Baci, MACS) are created that can fit large fragments [1, 31].

DNA fragments, incurred through endonucleases cuts, do not always have the sticky ends required to connect the two molecules of DNA. They are also missing in the case of cDNA. Subsequently, single strand, complementary homopolymers are formed that are then attached until the ends 3' and 5' of the second chain of the particle with blunt ends with the help of terminal transferase. The other method is to connect linkers, the sequence of double-stranded blunt ends that contain at least one restriction point. After ligation linkers to DNA, they are digested by the appropriate endonuclease, which creates sticky ends [8]. Adapters are used when the cloned DNA particle has the same restriction point as the linkers. Adapters have one blunt end and one cohesive end. End 5' is deprived of the phosphoric group, and the adapters therefore do not connect together in a mixture before connecting to the DNA. After the blunt ends of the adaptors and the fragment of the DNA bind, the polynucleotide kinase joins the phosphoric group to the recalled place and can then reach ligation of the examined DNA from the DNA of the vector [11].

Another example is a vector of the polymer. Polymer-based vectors are biologically safe, have low production costs, and are efficient tools for gene therapy. Although non-degradable polyplexes exhibit high gene expression levels, their application potential is limited due to their inability to be effectively eliminated, which results in cytotoxicity. The development of biodegradable polymers has allowed for high levels of transfection without cytotoxicity [24].

Transfer of recombinant vector into cell

In order to facilitate entering the vector into the bacterial cell, one should make the cell competent. Plunging the cell in $CaCl_2$ solution or some other salts and then heating for 2 min to 42°C causes it to become more susceptible to accepting the new DNA from the surroundings. Entering the recombined plasmid into the bacterium cell is known as a transformation.

Phage vectors are introduced in the process of transfection or packaging *in vitro*. The transfection also consists of creating competent bacterial cells and heating the solution, but introduces the RF (replicative form) or M13. During *in vitro* packing, it puts up with being in a mixture of recombinant material of λ phages in the protein border. It uses the phage genes that are responsible for the production of capsid proteins and tail.

After obtaining a sufficient amount of proteins required for packaging, complete recombined phages which bacteria are then formed, which attack through the natural way of infection and are entering their DNA sample into them [11].

It is possible to implement the vector in this way by putting it at first in leptosomes, and then by natural fusion of leptosomes with the cell or by precipitation of molecules in the calcium phosphate on the surface of the cell. Fungi, yeast and plants have cell walls that are destroyed with enzymes. Protoplasts are formed that can easily enter the DNA fragments. Treatment of the cell by electroporation, brief electrical pulses, increases the efficiency of the transformation. Leaching of enzymes destroying the cell wall causes the wall to begin to rebuild and the targeted recombinant arises.

Microinjection is a method that enables the new DNA to enter directly into the nucleus of the cell with the micropipette. This applies to animal and plant cells. Biolistics is based on pelting the cell with slices of tungsten or gold associated with the DNA [11].

Selection of transformants and the identification of recombinants

After implementing the vector, bacteria is being cultured to a peculiar base, known as a selective one. The composition depends on what the factor is that allows for the diversity of organisms in which there has been a vector implanted. Often this factor is at least a one-gene plasmid that encodes a protein, excluding the antibiotic. Plating transformants for the fuel containing antibiotics in its composition, which is a sensitive, vaccinate wild strain. It allows the identification of mutant cells because it will grow despite the earlier presence of harmful substances. Before planting, there is an incubation period at 37°C for an hour from the beginning of the transformation, set in order to create the largest possible number of recombinants.

However, not all vectors contain a cloned gene. In order to check which cells are recombinants and have a ligated vector with the gene, insertional inactivation I is used. Cloning of the new DNA fragment can take place in the centre of the naturally present gene in the vector, when it has characteristic restrictive sequences. If the slit gene has encoded resistance proteins to some antibiotic, then cells of the host containing such a vector will not also be immune to it. This happens in the case of plasmid pBR322, which has resistance genes to tetracycline and ampicillin.

The gene of the resistance to tetracycline has a BamHI restriction point, which is then cut. Recombinants containing this vector are therefore insensitive to ampicillin, but sensitive to tetracycline. At first, bacteria are being pleated to the base with the ampicillin, where transformants survive. Next, a method of replicas is applied – transformants are transferred to the base with tetracycline. Non-recombinated survives only, and we know the position of the recombinants on the previous plate.

Restriction endonuclease *Bam*HI shows a striking resemblance to the structure of endonuclease *Eco*RI, despite the lack of sequence similarity between them. The active site of *Bam*HI is structurally similar to the active sites of *Eco*RI, but the mechanism through which *Bam*HI activates a water molecule for nucleophilic attack may be different [35].

In the case of the pUC8 plasmid resistance genes to the antibiotic aren't being used, although it has a resistance gene to the ampicillin. Present in its genome, the gene lacZ' coding part of the β-galactosidase (decomposing lactose and its analog X-gal), is the factor that allows for the determining of the presence of recombinants. Insertional inactivation of lacZ' causes the β-galactosidase does not create. Cells are planted at the base with the ampicillin and X-gallim, which decompose by enzyme and cause the growth of the blue products. Accordingly, non-recombinated bacteria will form blue colonies, recombinanted – white. This method is called the selection of Lac.

Many λ phages and all M13 also have a lacZ' gene. Recombinants can be identified by insertional inactivation as described above. Moreover λ phages are re-

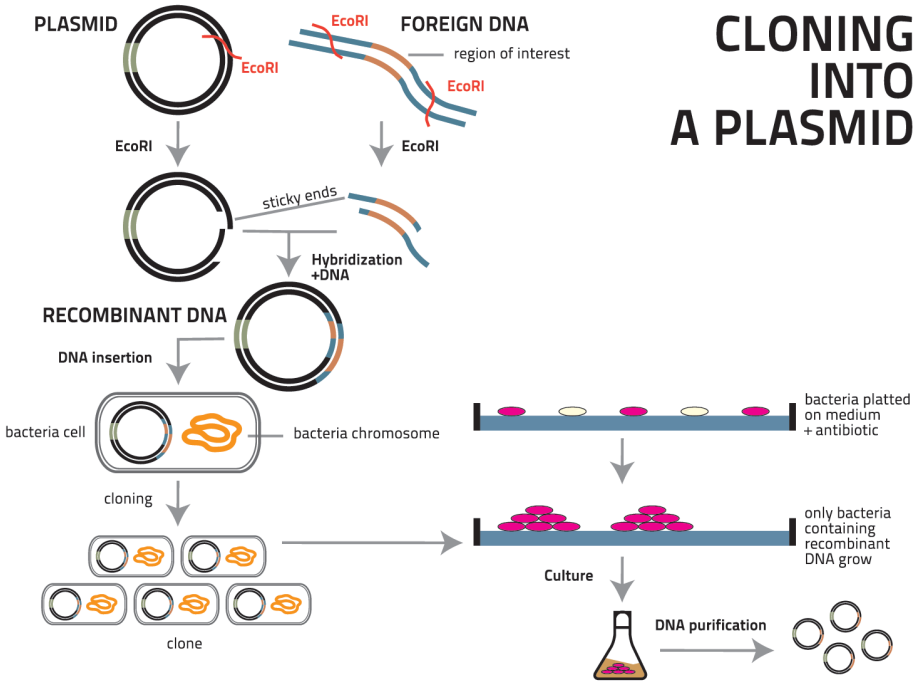

Fig. 1. Steps for recombinant DNA technology

strictive in the gene sequence of cI, and the insertion in this place of a new fragment of DNA causes the colonies grown on the plate will to be clean. On the other hand, when a recombination isn't occurring, the colonies are muddy. λ vectors are determined also as Spi+ when they cannot infest the cell which was already infected with the P2 phage. When λ phages are recombined, these become Spi– and are infecting bacteria from P2. Only such cells will now grow on the plate (Fig. 1) [11].

It is also possible to test the initiative of the enzyme directly, of which the gene was itself cloned, if it does not occur naturally in the cell or the use of antibodies to the protein [8] (Tab. 1).

Production of the human insulin

The early success of recombinant DNA technology relies heavily on the elucidation of the biological possesses at the molecular level in microbial systems. The first commercial application was realized in the microbial production of human insulin. The primary role of insulin is to control the absorption of glucose from the bloodstream into cells where glucose is utilized as an energy source or converted into glycogen for storage. Insulin's function is to regulate the level of glucose in blood [3, 6, 12, 14].

Insulin is produced and stored in the β cells of the Islets of Langerhans of the pancreas. Insulin is released from storage in the pancreas into the bloodstream [32]. Before the advent of biotechnology, the insulin used for the treatment of type I (insulin-dependent) diabetes mellitus was obtained by extracting the hormone from porcine or bovine pancreatic tissues. In the early eighties, human insulin produced by recombinant technology entered the pharmaceutical market. In one of the approaches the sequences for the A and B chains were synthesized chemically and inserted separately downstream of the δ-galactosidase structural gene controlled by the *X lac* promoter. The construction was such that the insulin chains would be made as fusion proteins joined by a methionine to the end of the P-galactosidase protein. The expression vector also contained an Amp' marker. The transformants were then screened by plating on a culture medium containing X-gal and ampicillin. The insulin A chain and B chain transformants were grown to harvest the cells in large quantity. The cells were lysed and the insulin A chain and B chain were purified separately. Because the insulin A gene was fused to the δ-galactosidase gene, therefore the insulin protein produced was therefore a δ-galactosidase-insulin hybrid (Fig. 2) [7, 12, 23].

Tab. 1. Examples of drugs produced by recombinant technology

Active molecule	Drug name	Disease
Insulin	Humalog, NovoRapid, Gensulin R, Humulin R, Actrapid HM	for diabetics
Factor VIII	Factor VIII from Bayer	for males suffering from hemophilia A
Factor IX	Factor IX from Bayer	for hemophilia B
Human growth hormone (HGH)	Genotropin, Humatrop, Serostin	for growth hormone deficiency
Erythropoietin (EPO)	Eprex, Epogen	for treating anemia
Interferon	Avonex, Rebif, Betaseron	the hepatitis B virus
α-L-iduronidase (rhIDU; laronidase)	Aldurazyme	mucopolysaccharidosis type I (MPS I; deficiency of α-L-iduronidase) for the treatment of non-neurological symptoms
N-acetylgalactosamine-4-sulfatase (rhASB; galsulfase)	Naglazyme	mucopolysaccharidosis VI (MPS VI 4-sulfatase deficiency of N-acetylgalactosamine, Maroteaux-Lamy syndrome)
Granulocyte colony-stimulating factor (G-CSF)	Neupogen	stimulating neutrophil production (e.g., after chemotherapy) and for mobilizing hematopoietic stem cells from the bone marrow into the blood
Tissue plasminogen activator (TPA)	Activase	for dissolving blood clots
Adenosine deaminase (ADA)		for treating some forms of severe combined immunodeficiency (SCID)
Dornase alfa	Pulmozyme	for cystic fibrosis
Glucocerebrosidase	Ceredase	for type 1 Gaucher's disease
Hepatitis B surface antigen (HBsAg)	Engerix B	to vaccinate against the hepatitis B virus
C1 inhibitor (C1INH)		used to treat hereditary angioedema
Follicle-stimulating hormone (FSH)	Pergonal	to treat fertility issues in women, especially women who are anovular and oligoovular

A more effective method, also using a strain of *E. coli*, involves the production of proinsulin completely whole, instead of separate fragments of insulin synthesis. Proinsulin consists of chains A and B, connected with chain C [16]. The gene encoding proinsulin cloned to *E. coli* cells is also expressed there. The resulting hormone is cleared and then the chain C is proteolytically removed [28, 43].

Insulin Lispro was the first recombinant fast-acting insulin analogue to gain marketing approval. It displays an amino acid sequence identical to native human insulin, with one alteration – an inversion of the natural proline-lysine sequence found at positions 28 and 29 of the insulin B-chain. This simple alteration significantly decreased the propensity of individual insulin molecules to self-associate when stored at therapeutic dose concentrations. The dimerization constant for Insulin Lispro is 300 times lower than that exhibited by unmodified human insulin. Structurally, this appears to occur as the change in sequence which disrupts the formation of inter-chain hydrophobic interactions critical to self-association. Insulin Lispro was developed by scientists at Eli Lilly (which, along with Novo Nordisk, are the world's largest producers of therapeutic insulin) [22]. The rationale underlying the sequence alteration was rooted in studies, not of insulin, but of the insulin-like growth factor-1. The latter displays a strong structural resemblance to proinsulin, with up to 50% of amino acid residues within the IGF-1 A- and B-domains being identical to those found in comparable positions in the insulin A- and B-chains. When compared to insulin, IGF-1 molecules display a significantly decreased propensity to self-association. Sequencing studies ear-

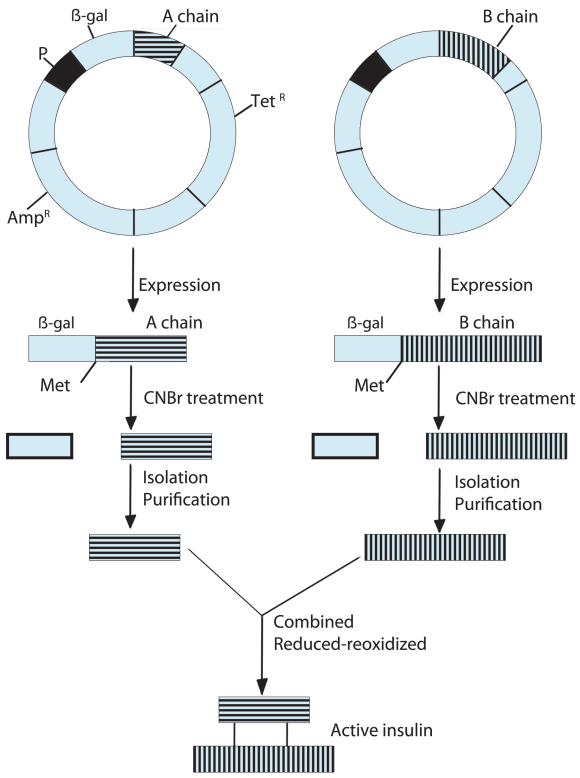

Fig. 2. Production of insulin

lier revealed that the ProlineB28-LysineB29 sequence characteristic of insulin is reversed in IGF-1. It was suggested that, if this sequence difference was responsible for the differences in the propensity for self-association, then inversion of the ProlineB28-LysineB29 sequence in insulin would result in its decreased self-association. Direct experimentation proved this hypothesis accurate. Insulin Lispro is manufactured commercially in a manner quite similar to the 'proinsulin' route used to manufacture native recombinant human insulin. A synthetic gene coding for LysB28-ProB29 proinsulin is expressed in *E. coli*. Following fermentation and recovery, the proinsulin is treated with trypsin and carboxypeptidase B, resulting in the proteolytic excision of the engineered insulin molecule. It is then purified to homogeneity by a number of high-resolution chromatographic steps. The final product formulation also contains *m*-cresol (preservative and stabilizer), zinc oxide (stabilizer), glycerol (tonicity modifier) and a phosphate-based buffer. The commercial product has a shelf life of 2 years when stored at 2–88°C [18, 48].

Production of the medicine against blood clot – ACTIVASE®

Activase® is a drug produced by the Genentech Inc. This pharmaceutical is used in the treatment of myocardial necrosis and pulmonary embolism and is administered to patients within three hours of stroke symptoms. Activase® dissolves clots in the blood vessels.

Activase® is an alteplase that is a tissue plasminogen activator (t-PA) and is produced on a global scale by recombinant DNA technology. The tissue plasminogen activator is glycoprotein, which is composed of 527 amino acids and belongs to a system regulating the blood flow [39, 41]. The t-PA is an enzyme activating the inactive plasminogen to its catalytic form – plasmin. Plasmin is responsible for fibrinolysis, which is a decomposition of thromboses [4, 43]. The production of biotechnological methods uses cDNA corresponding to the natural, human t-PA derived from melanoma cells. cDNA has been introduced into the ovary cells genome of a Chinese hamster (CHO, Chinese hamster ovary), and then carried out in cell culture medium containing gentamicin at a concentration of 100 mg/l. During the breeding, alteplase is excreted into the medium. For leveling the pH a phosphoric acid or a sodium hydroxide is added, while the protein synthesis is carried out by freeze-drying to yield a sterile white powder [19].

Directed mutagenesis

This method allows for making of specific changes in the sequence of the protein recombinant, so that the peptide gains new and beneficial properties. Mutations are most often implemented in the essential areas for the function of the proteins, such as the active centre. Changes can concern even only one amino acid [25]. Accordingly, directed mutagenesis is a part of protein engineering [29, 36].

Mutations can be introduced: insertion, deletion or replacement of one amino acid or the whole significant fragment. There are lots of varieties of mutagenesis, but all of these are based on the fundamental mechanisms.

Mutagenesis using oligonucleotide

This method is used to obtain a point mutation. An interesting gene in the form of a single-stranded oligonucleotide is introduced into the vector – the single-stranded M13 phage [2]. The gene is cloned, then purified and sequenced [11]. Oligonucleotide complementary to the original is created, yet containing a given mutation. This is introduced again into phage M13. The DNA fragment is not perfectly complementary to the vector DNA. Polymerase builds on a complementary chain. The chain creates a double helix and contains the cloned gene, which is a primer for the enzyme at that stage. Phages are introduced into bacteria and their replication occurs [21]. Half of the resulting particles correspond to the original DNA, and the other half contains the mutations. Following the release of phage from the cell, also 50% of them also have a mutated single-stranded molecule. Then, the phages are plated on a plate with bacteria to produce plaques and the original oligonucleotides are used as a probe for hybridization with the mutant gene. The gene can now be transferred to the vector bacteria to produce a modified protein [8, 20, 22, 47].

Synthesis of the artificial gene

If the gene encodes a protein with a short and known sequence we can easily create it in an artificial form. It is then possible to introduce many changes within the sequence, besides the point mutation, as in the above description. This gene is formed in a tube and the mutation can be conducted anywhere. At first, they are coming into existence overlapping oligonucleotides for lengths of 150 bases. Sequences will form appropriately amended gene. These are then folded, and the gaps are filled by DNA polymerase. Ligase creates the missing phosphodiester bonds, forming a complete, double-stranded helix. If the ends of the chain sequences are restrictive, after splitting the corresponding enzyme get sticky ends after it is split and the fragment of the vector is introduced [11, 32].

Polymerase Chain Reaction

Another method, very fast and easy to carry out is the PCR (Fig. 3). As in the case of mutagenesis for using oligonucleotide [17], PCR can make only a single change in the sequence. Two reactions are carried out in each of the two types of primers; one is perfectly complementary to the template, and the second one's principle is unmatched, so that it is a specific mutation. These particles are mixed and subjected to another PCR reaction, in which complementary sequences are combined and extended by polymerase. The disadvantage of this method, however, is that the Taq polymerase makes errors and new, unplanned mutations can appear. PCR products must therefore be sequenced to determine which molecules carry the correct change [11]. More simply said, there are three major steps in PCR, which are repeated for 30 or 40 cycles. This is done on an automated cycler, which can heat and cool the tubes with the reaction mixture in a very short time.

1. Denaturation at 94°C

During the denaturation, the double strand melts openly to single stranded DNA, and all enzymatic reactions stop (for example: the extension from a previous cycle).

2. Annealing at 54°C

The primers are jiggle around, caused by the Brownian motions. Ionic bonds are constantly formed and broken between the single stranded primer and the single stranded template. The more stable bonds last a little bit longer (primers that fit exactly) and on that little piece of double stranded DNA (template and primer), the polymerase can attach and starts copying the template. Once there are a few bases built in, the ionic bond is so strong between the template and the primer, that it does not break anymore.

3. Extension at 72°C

This is the ideal working temperature for the polymerase. The primers, where there are a few bases built in, already have a stronger ionic attraction to the template than the forces breaking these attractions. Primers that are on positions with no exact match, become loose again (because of the higher temperature) and don't provide an extension of the fragment. The bases (complementary to the template) are coupled to the primer on the 3' side (the polymerase adds dNTP's from 5' to 3', reading the template from 3' to 5' side, bases are added complementary to the template) (Fig. 4).

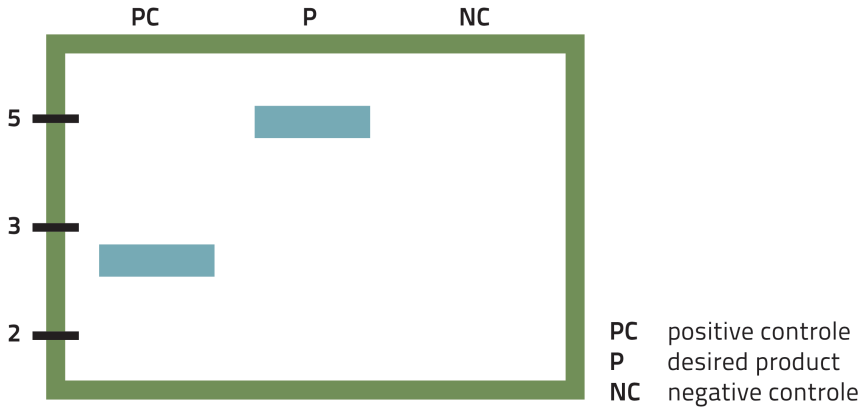

Fig. 3. PCR

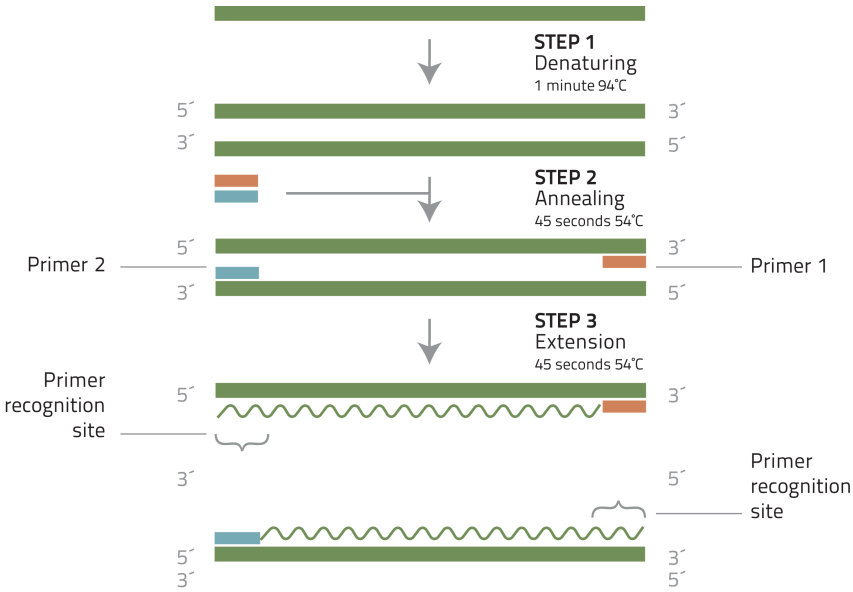

Fig. 4. Steps in PCR

Production of the human somatostatin hormone

Somatostatin inhibits the secretion of somatotropin (growth hormone) by the pituitary gland as well as some other hormones such as insulin and glucagon. It is used to treat acromegaly (excessive production of somatotropin) and its analogs are used as anticancer drugs. It consists of 14 amino acids.

For the production of somatostatin, a method of gene fusion was used, which produced a fusion mRNA. Cloning is conducted with the use of plasmids having the promoter lac and as such, the gene of the somatostatin will undergone to the expression along with β-galactosidase. Since the amino acid sequence of the protein is short and well known, it creates an artificial oligonucleotides [42].

It have however made some changes in order to facilitate cloning:

1. For the C-terminal cysteine codon two additional stop codons were included. This ensures that the translation will end after the somatostatin gene.

2. Before the N-terminal methionine there was added alanine codon, which will separate the fragment β-galactosidase from the desired protein and allows their separation by treating the CNBr.

3. The ends of the restriction fragment included sequences of EcoRI and BamHI, which create sticky ends.

Thus, the prepared oligonucleotide is introduced into the vector and is brought to transfection of

E. coli. After incubation, the recombinant fusion protein is selected and purified. CNBr dissects it and yields somatostatin [26, 27, 37].

Production of the ARANESP™ (DARBEPOETIN ALFA)

Darbepoetin alfa is a synthetic form of erythropoietin. It stimulates erythropoesis (increases red blood cell levels) and is used to treat anemia, commonly associated with chronic renal failure and cancer chemotherapy. Darbepoetin is marketed by Amgen under the trade name Aranesp.

Aranesp (darbepoetin alfa) is an erythropoiesis-stimulating protein that is produced in CHO cells by recombinant DNA technology. Aranesp is a 165-amino acid protein that differs from recombinant human erythropoietin in containing 5 N-linked oligosaccharide chains, whereas recombinant human erythropoietin contains 3 chains. The 2 additional *N*-glycosylation sites result from amino acid substitutions in the erythropoietin peptide backbone. The approximate molecular weight of darbepoetin alfa is 37,000 D. The finished product is given intravenously or subcutaneously [25].

Conclusion

The presented processes dominate in biotechnological production methods, therapeutics, and recombinant DNA technology as a basic tool for the majority of them, allowing their constant development and improvement. By genomics, the study of the genomes of organisms, and proteomics, plus studying proteins normally synthesized in the cell, produces a lot of information that serves as the basis for the design of new biopharmaceuticals [13, 38, 43, 48]. Drugs produced by biotechnology assume an increasing share among therapeutics. Most biopharmaceuticals are produced using *E. coli*, *S. cerevisiae* and animal cells (as CHO).

Biotechnology is thus a rapidly expanding field, showing the possibility for the effective treatment of diseases that today we may seem incurable.

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