PRODUCTION OF IMPORTANT BIOPHARMACEUTICALS

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Introduction. The proteins produced in the body control and mediate the metabolic processes and help in its routine functioning. Any kind of impairment in protein production, such as production of mutated protein or misfolded protein, leads to disruption of the pathway controlled by that protein. This may manifest in the form of the disease. However, these diseases can be treated, by supplying the protein from outside or exogenously. The supply of active exogenous protein requires its production on large scale to fulfill the growing demand. The process is complex, requiring higher protein expression, purification, and processing. Each product needs unique settings or standardizations for large-scale production and purification. As only large-scale production can fulfill the growing demand, thus it needs to be cost-effective. The tools of genetic engineering are utilized to produce the proteins of human origin in bacteria, fungi, insect, or mammalian host. Usage of recombinant DNA technology for large-scale production of proteins requires ample amount of time, labor, and resources, but it also offers many opportunities for economic growth.

The recombinant therapeutic product used for clinical applications was produced from mammalian cells. Mammalian cells were maintained in serum-/blood-/plasma-based medium; therefore, the presence of any infectious agent in the product might be deleterious if not properly removed. Infections may range from HIV, coronavirus (severe acute respiratory syndrome (SARS), non-lipid-enveloped (NLE) viruses as circoviruses (Torque tenovirus (TTV) and Torque tenominivirus (TTMV)), HBV, HCV, HTLV (human T-cell lymphotropic virus), to West Nile virus. Prions that are self-replicating infectious proteins may also be present which may lead to variant Creutzfeldt-Jakob disease (vCJD).

The aim of the study. To study the basics about production of recombinant proteins in various hosts along with the advantages and limitations of each host system and properties and production of some of the important pharmaceutical compounds and growth factors.

Methods of research. Materials of literary and experimental data on the production of biopharmaceuticals were used, which include expression of foreign gene, inducible promoters, general considerations for protein production, microbial system for production of therapeutic protein, glycosylation, recombinant protein in fungal hosts, protein folding and molecular chaperons, recombinant protein in insect cell, recombinant protein in mammalian cell, using human cells for protein production and transgenics for protein production (transgenic animals, plants).

Main results. *Tissue Plasminogen Activator (tPA).* Plasminogen activator has great clinical relevance for the management of stroke and myocardial infarctions. For production of t-PA, *E. coli* and yeast system did not work properly due to lack of posttranslational modification and over glycosylation, respectively. A novel truncated form of t-PA with an improved fibrin affinity and an increased resistance to PAI-1 was expressed in a CHO DG44 expression system. Therapeutic protein was produced in stably transfected CHO DG44 cell lines. These cell lines were maintained in serum-free medium, with glutamine, hypoxanthine, and thymine in stirred tank

bioreactor. The cells were grown at 37°C, 140 rpm with 5 % CO_2 and 85% humidity. The protein was then purified, with higher yield [1].

Factor VIII. Transfection of HEK 293 cell cultures in serum-free suspension is being tried for optimal yield. Recombinant factor VIII (rFVIII) is produced by culturing mammalian cells as baby hamster kidney (BHK) or Chinese hamster ovary cells (CHO), using large-scale bioreactors. Standardizations are done to maximize yields.

Insulin. In the beginning, efforts were made to isolate mRNA for pre- and proinsulin from rat islets of Langerhans of pancreas and to synthesize cDNA. Thereafter, it was inserted into a plasmid. The recombinant plasmids were transferred into the *E. coli* cells, which secreted proinsulin [2, 3, 4, 5]. Scientists have chemically synthesized DNA sequences for two chains, A and B, of insulin and separately inserted into two pBR322 plasmids by the side of β-galactosidase gene. The recombinant plasmids were separately transferred into *E. coli* cells which secreted fused β-galactosidase-A chain and β-galactosidase-B chain separately. These chains were isolated in pure form by detaching from β-galactosidase with yields of about 10 mg/24 g of healthy and transformed cells.

Human Growth Hormone (HGH). Earlier pituitary-derived hGH was used but later on it was prohibited when found associated with Creutzfeldt–Jakob disease. Because of recombinant DNA technology, safe and abundant recombinant hGH was produced in various heterologous systems. As the non-glycosylated human growth hormone was biologically active, thus, the preferred system for its production is *E. coli*, which allows its rapid and economical production in large amounts. For optimal productivity, strong inducible promotersare preferred as IPL, IPR, trc, and T7 in *E. coli.* They are advantageous as they drive overproduction of recombinant proteins. Apart from *E. coli*, human somatotropin (hST) expression was tried in a biologically active, disulfide-bonded form in tobacco chloroplasts. The hormone is used for the treatment of hypopituitary dwarfism in children; additional indications are in treatment of Turner syndrome, chronic renal failure, HIV wasting syndrome, and possibly treatment of the elderly. Growth hormonedeficiency in human occurs both in children and adults [6, 7].

Interferons. Interferons are produced by either an established cell line (lymphoblastoid) or fresh cells isolated from blood. The production involves induction with virus and priming (incubation with some interferon) with interferon, resulting in better yield. The affinity chromatography with monoclonal antibodies packed in the column has helped in purification of interferons. But before the clinical usage, the removal or inactivation of virus is very important. The interferon therapy is used for cancers and viral infections (INF-α), multiple sclerosis (INF-β), and chronic granulomatous disease (INF-γ). Multiferon is natural interferon-α, which consists of several subtypes. In some of the cancers like Merkel cell carcinoma, type I interferons (multiferon, which is a mix of various INF-α subtypes and INF-β) are highly effective. Interferons are mentioned in protein therapeutics. Interferon is marketed as Roferon-A, Infergen, Intron A, and so on.

Erythropoietin. As supply is limited from the natural source that is kidney cells, thus a recombinant human erythropoietin EPOGEN® which is Amgen’s trade name for epoetin alfa is marketed for anemic condition involving erythropoietin. The

human gene encoding erythropoietin was cloned into the Chinese hamster ovary cell linefor production of the human protein. This cell linecontinues to be used today for the production of EPOGEN®. The half-life of erythropoietin can be increased by incorporating the glycosylation of the protein growth factor. Thus, Darbepoetin-α is an analog which is engineered for two extra amino acids which are substrates for glycosylation. Thus, production is done in CHO cell lines; the product has five N-linked sugar chains and has almost three times longer life than erythropoietin.

Platelet-Derived Growth Factor (PDGF). PDGF shows considerable heterogeneity with sizes of 27–31 kDa; however, purified PDGF is cationic protein of 30 kDa. Recombinant human platelet-derived growth factor (rh-PDGF) was the first recombinant protein to be approved by the US Food and Drug Administration for treatment of chronic foot ulcers in diabetic patients (Regranex, Ethicon Inc. Somerville, NJ). It has the potential for use in bone regeneration and increasing bone density in long bones and spine. PDGF is commercially produced by using *E. coli* and mammalian cells.

Epidermal Growth Factor (EGF). EGF is produced commercially by engineered *E. coli.* The other systems are also being explored for optimum EGF production [8].

Fibroblast Growth Factor (FGF). FGF19 is produced by intestinal cells but acts on FGFR4-expressing liver cells to downregulate key genes in the bile acid synthase pathway; FGF23 is produced by the bone but acts on FGFR1-expressing kidney cells to regulate the synthesis of vitamin D and in turn affect calcium homeostasis. FGF may be synthesized using *E. coli* as host system.

Nerve Growth Factor (NGF). Nerve growth factor enhances early regeneration of severed exons and is also important in maintaining the biochemical and morphological phenotype of mature basal forebrain cholinergic neurons (BFCNLs) after lesions or injury of the central nervous system (CNS). Thus, NGF may provide therapeutic option for preventing death of cholinergic neurons and other clinical conditions and is produced using *E. coli.*

Transforming Growth Factor Alpha (TGF-α). Ectodomain of TGF-α is cleaved in a highly regulated manner, releasing soluble TGF-α which activates paracrine signaling. The receptor is EGF/TGF alpha receptor; therefore, the focus is on understanding the important roles of TGF-α and EGF receptor signaling in carcinoma development.

Transforming Growth Factor Beta (TGF-β). It has a role in the control of embryonic development, cellular differentiation, hormonesecretion, and immune function. Its role as mesenchymal differentiation factor, with focus on the muscle, fat, and bone cell, might provide insights into its deregulation in skeletal and developmental diseases and is the area of active research. It is produced using CHO cell lines.

Conclusions. The system of choice is dependent upon the total cost of production and obtaining fully functional and appropriately modified (PTMs) (glycosylation, phosphorylation, or properly folded) protein. All these are essential for the biological activity of the protein. There is huge potential for future therapies using proteins as therapeutic agents. The recombinant proteins are not only beneficial, but the researchers can further engineer them to improve their activity and

prolonged stay in the body, for example, engineering of monoclonal antibodies to have toxin or radioisotope or generation of bi-specific antibody. Still the technology is struggling hard to make the diseases completely a text of books and having a society free of diseases and the pathogens. The production of proteins for therapeutic purpose is very important not only because of their specific action with minimum side effects but also due to their unique form and functions. Nowadays therapeutics (pancreatic enzymes from hog and pig pancreas or a-1-proteinase inhibitor from pooled human plasma) are not extracted from their native sources (from humans, animals) due to risk of transmission of pathogens. Other problems faced for extraction from native sources were the scarce availability of animal tissue for production, high cost of purification with less yield, and immunological reactions in the recipients.

Majority of the biological therapeutic agents are produced by using various advance tools and technologies as cloning, selection, purification, and stability monitoring. It is also very important to monitor risks and side effects of therapeutics obtained from a wide range of cells.

The various biological systems are available for production of recombinant protein as bacteria, fungal system (yeast), insect cell, mammalian cell, human cell, and transgenic plants and animals.

The protein products are having important therapeutic role. They are approved for marketing and use by various government agencies like the Food and Drug Administration (FDA) and European Union (EU). The approved recombinant biotechnology medicines include replacement products, monoclonal antibodies, interferons, vaccines, hormones, modified natural products, and many others. Many of them are in usage and helping to alleviate the symptoms or cause of the diseases.

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