Recombinant DNA expression products for human therapeutic use

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Recombinant DNA (rDNA) technology has made a revolutionary impact in the area of human healthcare by enabling mass production of safe, pure and effective rDNA expression products. Currently, several categories of rDNA products, viz. hormones of therapeutic interest, haemopoietic growth factors, blood coagulation products, thrombolytic agents, anticoagulants, interferons, interleukins and therapeutic enzymes are being produced using rDNA technology for human use. The present article provides information on different aspects, viz. expression systems, production, purification, analysis and therapeutic applications of rDNA products. Further, future of rDNA products and Indian scenario are also highlighted. This article should be beneficial to biotechnology scientists and manufacturers of biopharmaceuticals.

Keywords: Biopharmaceuticals, expression host systems, production, purification and analysis of rDNA, recombinant therapeutic proteins.

THE advent of recombinant DNA (rDNA) technology and its application in the pharmaceutical industry has brought about a rapid growth of biotechnology companies and a number of therapeutic rDNA products are available for human use[1]. In view of increasing importance of rDNA products for human healthcare, this article provides general/recent information on different aspects, viz. expression systems, production, purification, analysis and therapeutic applications of selected rDNA products, especially hormones of therapeutic interest, haemopoietic growth factors, human blood coagulation products, thrombolytic agents, anticoagulants, human interferons, human interleukins and therapeutic enzymes. Further, future of rDNA products and Indian scenario are also highlighted.

Expression systems

Prokaryotic (bacteria) or eukaryotic (yeast, mammalian cell culture) systems are generally used as a host for the production of usable quantities of the desired rDNA product[2–4]. Most of the rDNA products approved by FDA are being produced using these systems. Bacteria such as *Escherichia coli* are widely used for the expression of rDNA products. They offer several advantages due to high level of recombinant protein expression, rapid growth of cell and simple media requirement. However, there are some limitations such as intracellular accumulation of heterologous proteins, the potential for product degradation due to trace of protease impurities and production of endotoxin. Yeast such as *Saccharomyces cerevisiae*, *Hansenulla polymorpha* and *Pichia pastoris* are among the simplest eukaryotic organisms. They grow relatively quickly and are highly adaptable to large-scale production. These organisms do not produce endotoxin. They are capable of glycosylating proteins up to a certain extent like mammalian cells. Mammalian systems such as Chinese hamster ovary (CHO) cell and baby hamster kidney (BHK) cell systems are often the choice for production of human therapeutic proteins. The CHO and BHK cell systems are an ideal choice as these are capable of glycosylating the protein at the correct sites. However, cost of production of the products using these systems is high because of slow growth and expensive nutrient media. The choice of expression system can influence the character, quantity and cost of a final product.

Recent advances have been made in producing therapeutic proteins by using transgenic animals[5]. Transgenic milk production is currently most feasible[6]. The advantages of this system are high expression levels and volume output, low capital investment, low operational costs and reproducible production facility, i.e. inbreeding could pass an animal's ability to produce transgenic protein to its offspring. Despite the attractiveness of this system, a number of issues remain to be solved before it is broadly accepted by the industries and regulatory authorities alike. These include, variability of expression levels and characterization of the exact nature of the post-translational modification in the mammary systems.

The use of genetically engineered plants to produce valuable proteins is increasing slowly[7,8]. The system has potential advantages of economy and scalability. However, variation

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in product yield, contamination with agrochemical and fertilizer, impact of pest and disease, and variable cultivation conditions should also be considered. Plant cell culture system combines the advantages of whole plant system as well as animal cell culture[9–11]. Although no recombinant products have yet been produced commercially using plant cell culture, several companies are investigating the commercial feasibility of such a production system.

Production and purification of rDNA products

A general process for the production of rDNA products is illustrated in Figure 1. The first step is isolation of the identified gene that is responsible for expression of the desired product. After isolation and characterization of the human gene, it is inserted into small circular pieces of DNA called plasmid. The recombinant plasmid is inserted into a bacterial yeast or cultured animal cell. Clones of transformed host cell are isolated and those that produced the protein of interest in the desired quantities are preserved under suitable condition as a master cell bank. The cell banks are characterized and properly maintained for use in subsequent transformation procedures. The cell bank should be periodically tested for cell viability, genetic and phenotypic stability. As manufacturing needs arise, cells from working cell can be scaled up to produce the product in roller bottles and/or fermentors.

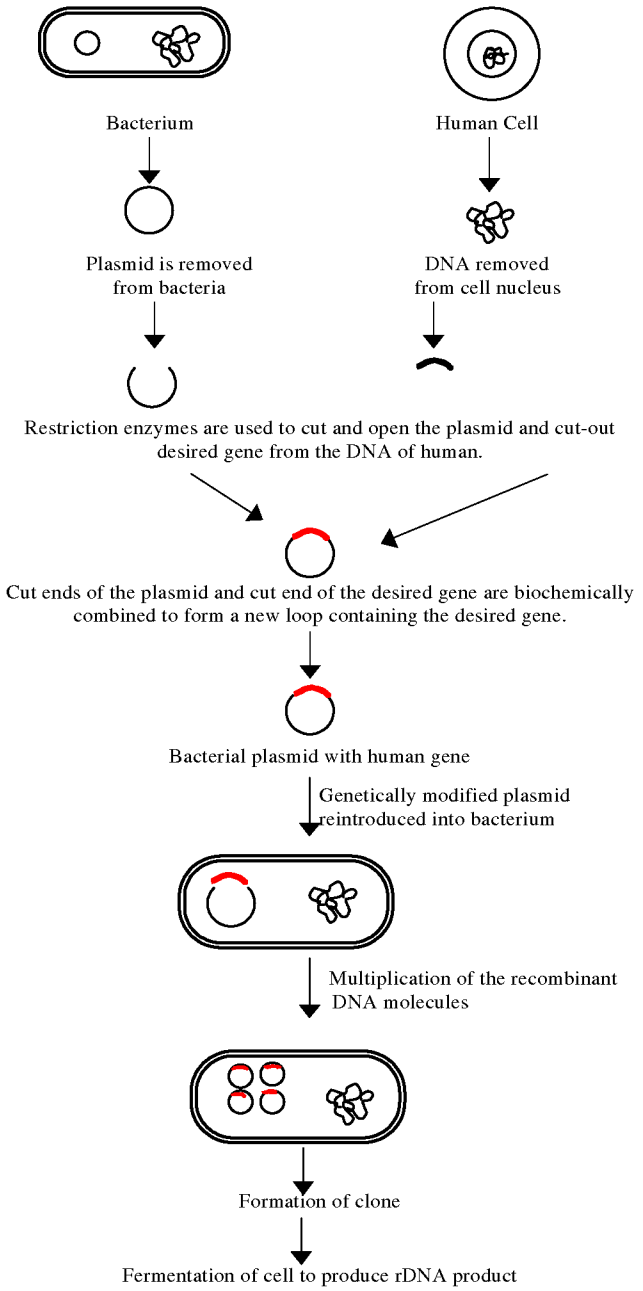


Figure 1. Recombinant DNA technology for production of human therapeutics.

Fermentors are generally used for growth of *E. coli* or yeast. Mammalian cells are often grown in roller bottles. Inoculation of host cells that contained an expression vector is added to defined volume of medium in either fermentors or roller bottles. The cells are allowed to grow until the nutrients in the medium are depleted or excreted by the products reaching inhibitory levels. By providing a balanced mixture of nutrients and/or chemicals to neutralize accumulating growth inhibitor, product yield in the medium or cell density can be improved. At the end of the run, the host cells are harvested and the recombinant product is isolated from culture medium or cells.

The following points should be considered for production of rDNA products.

(1) Plasmid instability is a major problem in continuous and large-scale fermentation, since these cultures go through many generations. The resulting effects are lower productivity and increase in production cost, because of the build-up of non-productive plasmid free cells. Mathematically structured and unstructured kinetic models of plasmid stability have been developed, which are ultimately useful for design of recombinant processes.

(2) High level expression of rDNA products in different host systems can often result in aggregation and accumulation of inclusion bodies. Under appropriate conditions, the rDNA products may get deposited in inclusion bodies in approximately 50% or more of the total cell protein. The rDNA protein is highly pure, stable and compact. Recovery of soluble, active rDNA protein from the inclusion body requires the following steps to be carried out.

(a) Isolation of the inclusion body from cell–cell disruption and centrifugation.

(b) Solubilization of the inclusion body protein using a chactrope (denaturant).

(c) Under reducing conditions (to separate all disulphide bonds).

(d) Recovery of active protein by removal of denaturant under conditions that allow the protein to adapt its native configuration.

(e) Undesired disulphide bonds, if present in the structure, need to be re-oxidised under controlled condition, achieving their correct configuration present in native structure.

(3) The cell bank should be periodically tested for cell viability, genetic and phenotype stability. When eukaryotic cells are used for production, distinguishing genetic, phenotypic and immunological markers of the cell will be useful in establishing the identity of the cells. Likewise, when microbial cultures are used, specific phenotypic features which form a basis for identification should be described.

(4) Traditional small-scale fermentors are used for expansion of cells in suspension culture. The presence, extent and nature of any microbial contamination in the culture vessels must be thoroughly examined at suitable stages during production. All the systems associated with fermentors must be validated before being routinely used. In India validation studies should be carried out with follow-up validation studies at appropriate time intervals.

(5) Unintended variability in culture during production may lead to changes which cause alteration in the product. Such variations might result in differing yield of the products.

(6) The steps in a production process should be validated to ensure that the process intermediates are within specifications. Any assay used during process validation must itself be validated, before the process validation is commenced.

Purification is an important aspect in the production of rDNA products. The overall goal of purification is to bring as much product with as little loss as possible. A general purification process is presented in Figure 2. In case the desired product is intracellular, fermentation is followed by harvesting of cells. This step is normally achieved by centrifugation or filtration. The cells are disrupted or lysed and cell debris is removed by centrifugation, leaving behind a dilute solution of crude desired product. If mammalian or yeast system is used, the desired product can be obtained directly from the medium. Nowadays, ultrafiltration has become the method of choice of concentration of products[12].

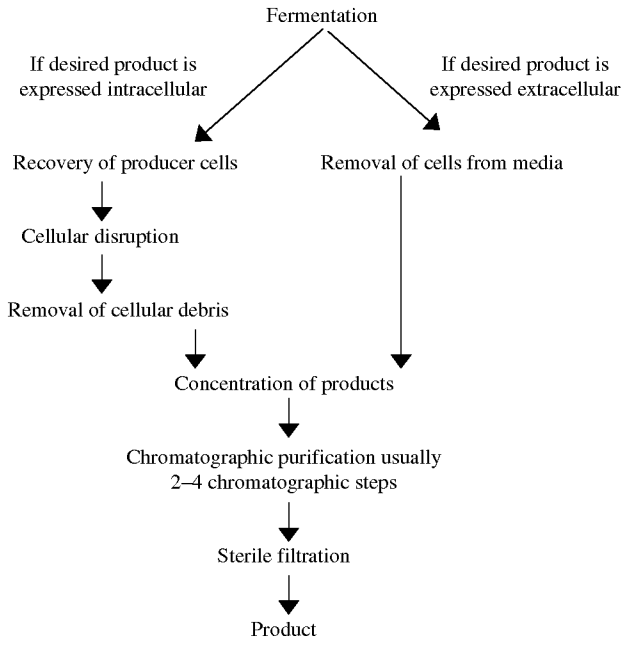


Figure 2. A general process for purification of rDNA products.

There are several different methods that can be used for purification of rDNA products, but only chromatographic purification methods are generally used (Table 1)[13–15]. A combination of two to four different chromatographic techniques is generally employed in a typical downstream processing procedure. Gel filtration and ion exchange chromatography are the most common. Affinity chromatography is employed wherever possible, as it has high biospecificity and one can achieve a high degree of purification.

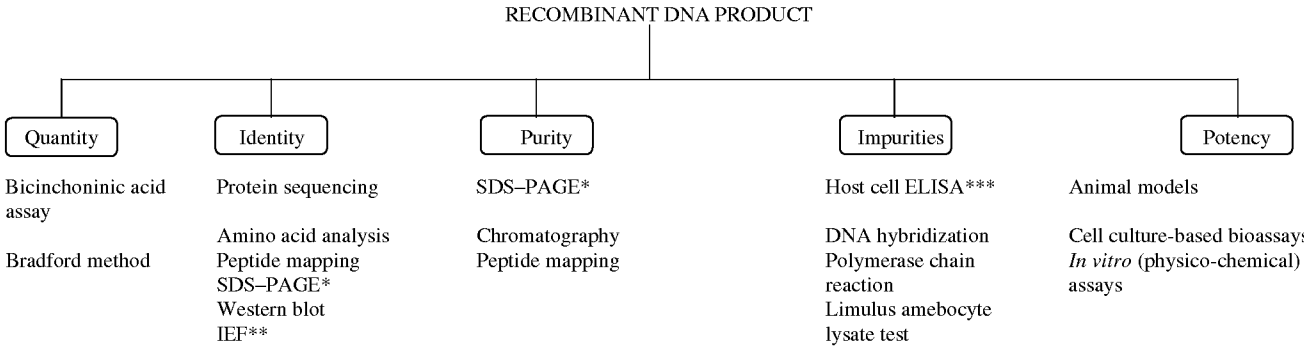
Appropriate attention needs to be given to validation of the purification process such as column loading capacity and column regeneration. The purification process must be validated to ensure that it is adequate to remove extraneous substances such as chemicals used in purification, column contaminations, endotoxin, residual cellular proteins and viruses. The reproducibility of the purification process with respect to its ability to remove specific contaminants should also be determined. Further, columns should also be validated regarding leaching of legends (e.g. dye, affinity legends, etc.) throughout the expected lifespan of the column. The evidence of purity of the purified product should be established. Physico-chemical, biological and immunological characterizations of purified product should also be obtained using a wide range of analytical tests. Characterization tests of products may include mainly amino acid composition, peptide mapping, electrophoretic assays (SDS–PAGE, Western blot, isoelectric focusing), HPLC, immunoassay, endotoxin test, potency determination test, etc.

Analysis of rDNA products

Analytical methods play a vital role in the determination of identity, purity and potency of rDNA products with respect

Table 1. Chromatographic purification methods used for rDNA products

Reversed phase chromatography
Size exclusion chromatography
Hydrophobic interaction chromatography
Charge transfer chromatography
Ion exchange chromatography
Anion
Cation
Affinity chromatography
Chemical
Dye/ligand
Monoclonal antibodies
Metal chelate



*Sodium dodecylsulphate polyacrylamide gel electrophoresis; **Isoelectric focusing; ***Enzyme linked immunosorbent assay.

Figure 3. Important analytical methods used for the analysis of rDNA products.

to safe and efficacious medicine for human use. A summary of the analytical methods used in analysis of products is illustrated in Figure 3 and described briefly in the following section. The principal and detailed procedures are available for the interested readers[16–19].

Bicinchoninic acid and Bradford method are generally used for the determination of protein concentration in rDNA product. These methods are important as they give the protein concentration of the product which is used in other assays. Protein sequencing provides structure information. Amino acid analysis is used to identify rDNA products based on their amino acid composition. Peptide mapping is used to compare protein structure of the product with that of the reference standard and to confirm lot-to-lot consistency of primary structure. Electrophoretic assays (sodium dodecylsulphate polyacrylamide gel electrophoresis, Western blot and isoelectric focusing) are most powerful to evaluate rDNA product purity, identity, homogeneity and stability. More recently, capillary electrophoresis has generated considerable interest as a complementary technique in the analysis of rDNA products. These assays can also be used for detecting molecular or chemical changes in the molecule due to denaturation, aggregation, oxidation, deamidation, etc.

Chromatographic method, viz. reverse phase-HPLC, size exclusion chromatography and hydrophobic interaction chromatography are used in the determination of purity of the product as well as the level of known impurities or degradation of product. Immunoassay and DNA hybridization are used for determination of host cell impurities. Polymerase chain reaction, which involves DNA amplification, may prove useful in detection and identification of contaminant DNA. The limulus amebocyte lysate (LAL) test is the most sensitive and specific test to detect and measure endotoxin. A comparative study demonstrated that LAL test is more sensitive than rabbit pyrogen test.

The potency of rDNA product is generally assessed by specific techniques. The choice of assay is often determined by the nature of the product and its intended therapeutic application. Animal model assay, cell culture-based assay and *in vitro* (physico-chemical) assay are used for determination of potency of rDNA product. These assays require reference standard (calibrated in international unit) and statistical support to correctly interpret the resultant data.

In addition to the afore-mentioned tests for protein analysis, carbohydrate analysis for glycoprotein plays a major role in characterizing rDNA products. In many products, changes in carbohydrate structures have affected biological activity.

A few points should be considered for analysis of rDNA products. Tests for identity usually require a combination of methods. These tests should be highly specific for unique properties of the products. A combination of methods should be used to assess purity of the product; the methods should be quantitative and capable of distinguishing the desired protein from product-related impurities. Process-related impurities should be minimized. Limits for product-related impurities should be set and quantitative methods employed to assure that these limits are met. Potency assays should have wide confidence limits and may include animal-based bioassay, *in vitro* cell culture assays or biochemical assay.

The FDA currently regulates certain rDNA products as drugs. All rDNA drugs undergo rigorous quality control testing in order to conform to predetermined specifications. The fundamental difference between quality control system for rDNA drugs and traditional pharmaceuticals lies in types of method used to determine drug identity, purity and potency. The use of suitable reference standard from internationally recognized sources such as WHO, NIH, etc. is important for identification and purity of rDNA-derived drugs. Potency testing is of obvious importance, ensuring that the drug will be efficacious when administered to the patients.

Therapeutic applications of rDNA products

The use of therapeutic proteins to replace or supplement endogenous protein molecules has been a long-established treatment for disease. Applications of some important rDNA products (Table 2) approved by FDA for human therapeutic use are described briefly in the following section.

Table 2. Important FDA approved recombinant products for human therapeutic use

Therapeutic category	Product	Expression host	Abbreviated indication
Hormone of therapeutic interest	Human insulin	*E. coli*/*S. cerevisiae*	Treatment of diabetes
	Insulin aspart	*S. cerevisiae*	Treatment of diabetes
	Insulin glargine	*E. coli*	Treatment of diabetes
	Insulin lispro	*E. coli*	Treatment of diabetes
	Insulin glulisine	*E. coli*	Treatment of diabetes
	Human choriogonadotropin	CHO cells	Treatment of women undergoing superovulation prior to assisted reproductive techniques such as *in vitro* fertilization
	Follicle-stimulating hormone	CHO cells	Treatment of infertility
	Luteinizing hormone	CHO cells	Induction of ovulation
	Somatotrophin	*E. coli*	Treatment of deficiency of growth failure
Haemopoietic growth factors	Erythropoietin alpha	CHO cells	Treatment of anaemia associated with renal failure, HIV infection, cancer
	Erythropoietin beta	CHO cells	Treatment of anaemia associated with renal failure
	Erythropoietin omega	BHK cells	Treatment of anaemia associated with renal failure and cancer
	Darbepoetin	CHO cells	Treatment of anaemia associated with renal failure and cancer
	Filgrastim	*E. coli*	Reduction in duration of neutropenia and incidence of febrile neutropenia in patients treated with cytotoxic chemotherapy for malignancy
	Surgramostim	*S. cerevisiae*	Treatment of neutrophil recovery
Human blood coagulation factors	Factor VIII	CHO cells	Treatment of haemophilia A
	Factor IX	CHO cells	Treatment of haemophilia B
	Factor VII A	BHK cells	Treatment of haemophilia A and B
Thrombolytic agents	Alteplase	CHO cells	Treatment of acute myocardial infarction
	Reteplase	*E. coli*	Treatment of acute myocardial infarction
	Tenecteplase	CHO cells	Treatment of acute myocardial infarction
	Saruplase	*E. coli*	Thrombolytic therapy for acute myocardial infarction
Anticoagulants	Lepirudin	*S. cerevisiae*	Anticoagulation therapy for heparin associated thrombocytopenia
	Desirudin	*S. cerevisiae*	Prevention of venous thrombosis
Human interferons	Interferon alpha-2b	*E. coli*	Treatment of hairy cell leukaemia, chronic hepatitis B and C, AIDS, cancer
	Interferon beta-1b	*E. coli*	Treatment of multiple sclerosis
	Interferon gamma	*E. coli*	Chronic granulomatous disease
Human interleukins	Interleukin-2	*E. coli*	Renal cell carcinoma
	Interleukin-11	*E. coli*	Treatment of thrombocytopenia
Therapeutic enzymes	Dorsase alpha	CHO cells	Cystic fibrosis
	Glucocerebrosidase	CHO cells	Replacement therapy in patients with Gaucher disease

Hormones of therapeutic interest

Diabetes mellitus is one of the most common metabolic diseases in the world. It is characterized by hyperglycaemia resulting from defects in insulin secretion, insulin action or both. The disease can be treated by administration of human insulin produced using *S. cerevisae* or *E. coli* which is structurally identical to insulin produced by the pancreas in the human body[20]. Insulin aspart, is structurally identical to insulin human, except for the replacement of aspartic acid with proline at position 28 on the B-chain of the molecules. It provides rapid absorption than regular human insulin[21]. The main advantages are its long peakless action with better effects during down hours and a lower incidence of hypoglycaemia. Insulin glargine[22], a long-acting recombinant insulin, differs structurally from human insulin by the replacement of aspargine with glycine at position 21 of the A-chain and addition of two arginine groups to the C terminus of the B-chain. The structural modifications allow it to dissolve in an acidic solution, but it forms amorphous microprecipitate in subcutaneous tissue from which small amounts of insulin glargine are gradually released. Insulin lispro[23], produced in *E. coli*, is identical to human insulin except for the transposition of proline and lysine at positions 28 and 29 in the B-chain. The most prominent practical advantage is rapid onset of action. Insulin glulisine[24] produced by *E. coli* is a rapid, parenteral blood-glucose lowering agent. It differs from human insulin in that the

amino acid asparagine at position B3 is replaced by lysine and lysine in position B26 is replaced by glutamic acid. The availability of these new insulin analogues has the potential to significantly improve long-term control over blood glucose in diabetic patients.

The gonadotrophin hormones directly or indirectly regulate reproductive functions. Insufficient endogenous production of any gonadotrophin hormone adversely affects reproductive function, which can be treated by administration of recombinant hormones. Recombinant human choriogonadotropin (rhuCG), produced in CHO cells, is used to induce ovulation in the treatment of anovalatory infertility or as an adjunct to *in vitro* fertilization procedure[25]. Recombinant follicles stimulating hormone (rFSH), produced from CHO cells, is safe and effective in the treatment of fertility disorder[26]. It revealed more efficient ovarian response and better quality of oocysts than did urinary FSH[27]. Recombinant luteinizing hormone is likely to be recommended as a supplement to rFSH for ovulation induction in hypogonadotropic women[28]. It also benefits *in vitro* fertilization[29].

Growth hormone (GH) is required for normal growth and metabolic homeostasis in children. Deficiency of GH is characterized by an altered body composition, decreased exercise capacity and diminished quality of life. Somatotropin, a recombinant GH, produced in *E. coli* is identical to natural GH, except that it contains an additional methionine on the N-terminus of the molecule. It is used for the treatment of short stature resulting from GH deficiency[30]. It is also widely used as an adjunct in the treatment of other disorders such as intrauterine growth restriction, Turner syndrome, healthy children with short stature and skeletal dysphasia.

Haemopoietic growth factors

Recombinant human erythropoietin (rhuEPO) is widely used for the treatment of anaemia associated with renal failure, HIV infection, cancer and surgery. Three varieties of rhuEPO, i.e. epoetin alfa, epoetin beta and epoetin omega are available for clinical use[31–34]. Epoetin alfa and epoetin beta are produced in CHO cells, whereas epoetin omega is produced in BHK cells. All the three varieties of rhuEPO had the similar sequence of 165 amino acids, but differ in their carbohydrate content and site of glycosylation. Two internal disulphide bonds linking positions 7 and 161 and positions 29 and 33 are important for their biological activity. Recently, novel erythropoiesis stimulating protein, darbepoetin alfa has been developed for the treatment of anaemia[35]. Pharmacokinetic studies have demonstrated that darbepoetin has a longer plasma half-life compared to rhuEPO[36]. Therefore, treatment using the former requires less frequent administration.

Filgrastim, a recombinant human granulocyte colony stimulating factor (G-CSF) produced in *E. coli* principally affects the proliferation and differentiation of neutrophils within the bone marrow[37]. It is a single-chain polypeptide containing 175 amino acids, which differs from the endogenous glycoprotein by the addition of an N-terminal methionine. The addition of polyethylene glycol moiety to filgrastim results in the development of pegfilgrastim. Pegfilgrastim is a long-acting form of filgrastim, that requires less frequent administration for the management of chemotherapy-induced neutropenia[38].

Sargramostim (produced using *S. cerevisiae,* containing 126 amino acids) is used to treat and prevent neutropenia in patients receiving myelosuppressive cancer therapy. It is structurally identical to endogenous human GM–CSF, except for leucine instead of a proline at position 23. It stimulates the proliferation and differentiation of multiple hematopoietic progenitor cells in the myeloid lineage and activates or augments many of the functional activities of mature neutrophils, monocytes/macrophages and dendritic cells, enhancing host defences against a broad spectrum of invading microorganisms[39].

Blood coagulation factors

Human coagulation factor VIII is a cofactor that serves as a critical component in the intrinsic blood coagulation pathway. Its deficiency causes haemophilia A, the most commonly inherited bleeding disorder[40]. Affected persons are unable to form blood clots normally and therefore risk serious and life-threatening bleeding episodes. Recombinant human factor VIII (produced in CHO cells containing 1438 amino acids) provides a temporary replacement to prevent or control bleeding episodes or to perform emergency of elective surgery in patients with haemophilia A[41]. Deficiency of human factor IX develops haemophilia B. Its clinical consequences are similar to that of deficiency of factor VIII. Recombinant factor IX (produced in CHO cells containing 415 amino acids)[42] is indicated for the control of bleeding events in haemophilia B patients. Recombinant factor VIIa (produced in BHK cells containing 406 amino acids) is a unique haemostatic agent with potential for broad application in bleeding patients with congenital and acquired bleeding abnormalities. It is approved for the treatment of haemophilia A and haemophilia B patients[43,44], who have acquired antibodies to factor VIII and IX.

Thrombolytic agents

Tissue plasminogen activator (TPA) is used to dissolve blood clots that have formed in the blood vessels of the heart and seriously lessen the flow of blood. Alteplase, a recombinant TPA, consists of 527 amino acids and stimulates the fibrinolysis of blood clots by converting plasminogen to plasmin. It is now a treatment choice in the management of acute myocardial infarction (AMI)[45]. In addition to AMI, it is also approved for the treatment of acute ischaemic stroke and pulmonary thromboembolism. Reteplase[46], produced in

E. coli, has prolonged half life and lower binding affinity to fibrin compared to alteplase. It is approved for the management of AMI, reduction in the incidence of congestive heart failure and reduction of mortality associated with AMI. Tenecteplase is an engineered variant of alteplase, designed to have increased fibrin specificity, greater efficacy and a longer half life[47,48]. It is produced using CHO cells which contain 527 amino acids. Saruplase is known as recombinant single-chain, urokinase-type plasminogen activator or prourokinase[49]. It is a single-chain polypeptide consisting of 411 amino acids.

Anticoagulants

Lepirudin, a recombinant derivative of the naturally occurring leech anticoagulant hirudin, is used as a thrombin inhibitor for the treatment of heparin-induced thrombocytopenia[50]. It is derived from yeast, which is composed of 65 amino acids. It is identical to natural hirudin, except for substitution of leucine for isoleucine at the N-terminal end of the molecule and the absence of a sulphate group on the tyrosine at position 69. Disirudin, a recombinant hirudin is used in the prevention and management of thromboembolic disease[51]. It is a thrombin inhibitor, which binds directly and with high affinity to clot-bound and fluid-phase thrombin. It is more effective than heparin in the prevention of deep vein thrombosis in patients undergoing elective hip-replacement.

Human interferons

Three recombinant human interferons (rhuIFN) alpha, beta and gamma, have a broad range of activities. rhuIFN alpha-2b is approved for the treatment of hairy cell leukaemia, AIDS-associated kaposi sarcoma, hepatitis B and C, malignant melanoma and renal cell carcinoma[52]. rhuIFN beta-1b (produced in *E. coli* containing 165 amino acids), is the first line therapeutics in relapsing, remitting and secondary progressive multiple sclerosis[53,54]. rhuIFN gamma is produced from *E. coli*, containing 139 amino acids. It is indicated in reducing the frequency and severity of serious infections associated with chronic granulomatous disease[55].

Human interleukins

The recombinant human interleukin (rhuIL)-2 produced in *E. coli* differs from native IL-2 in that it has no alanine at the N-terminal and serine is substituted for cysteine at amino acid 125. It is indicated for treatment of metastatic renal cell carcinoma and melanoma[56,57]. rhuIL-11 is a thrombopoietic growth factor that stimulates the production of hematopoietic stem cells and megakaryocytic progenitor cells resulting in platelet production[58]. It is produced in *E. coli* containing 179 amino acids. It differs from native IL-11 only in the absence of N-terminal proline residue.

Therapeutic enzymes

Recombinant dorsase alfa (rhudeoxyribonuclease 1)[59], an enzyme prepared from CHO cells, is developed specifically for cystic fibrosis. It contains 260 amino acids, which is identical to the amino acid sequence of endogenous human enzyme. It is used as an adjunctive therapy in patients with cystic fibrosis to reduce mucous viscosity.

Recombinant glucocerebrosidase[60] is intended to replace deficient endogenous enzyme in patients with Gaucher's disease. Treatment with it improves haematological abnormalities, hepatosplenomegalia and quality of life in a matter of few months.

Future of rDNA products

The future of rDNA products as a human therapeutic is looking good. More than 110 companies are involved in discovery, development and marketing of rDNA products. These companies have more than 80 therapeutics in clinical development and combined portfolio of 73-marketed products[61,62]. During 2004, two rDNA products, insulin gluline (Apidra, Sanofi Aventis, Strasbourg, France) and the fertility drug, Lutropin alfa (Luveris, Serono, Geneva, Switzerland) have been approved by USA FDA. Three products, insulin determir (Latus, Novo Nordisk, Begsvard, Denmark) for diabetes; calcitonin, for treating osteoporosis (Unigene Laboratories, Fairfield, NJ, USA) and palifermin, a keratinocyte growth factor used for treating mucosites (Amgen-woodland Hills, CA, USA) are undergoing FDA review.

Analysis of therapeutic market segmentation from 2001 to 2003 reveals that there are five therapy areas – haematology, diabetes and endocrinology, oncology, central nervous system disorders and infectious diseases – that are the key market shareholders. Products of haematology will continue to lead sales over the next 6 years (2010). Products of oncology will become the second most important revenue generator. As for the future market, erythropoietin will continue to lead sales, interferon will follow and insulin will maintain its third place.

Indian scenario on rDNA products

In India, human insulin, FSH, GH, EPO, GM–CSF, G–CSF, factor VIII, TPA, IFN-alfa and gamma, IL are already approved for human use[63]. These products were successfully developed by Indian companies either through foreign collaboration or through total indigenous efforts. The current market of approved recombinant therapeutics has been estimated to be about Rs 5357 million, which is approximately 1.6% of the world market. The market of the above-mentioned products is growing at the rate of 15% and is likely to be around Rs 9580 million by 2005.

In India, all the recombinant products are considered to be new according to the current Indian Drug Act, 1988 and

Table 3. Potential rDNA products facing patent expiration by 2006

Brand name	Generic name	US patent expiration
Humulin	Human insulin	2001
Intron A	Interferon alpha-2b	2002
Avonex	Interferon beta-1a	2003
Humatrope	Somatropin	2003
Epogen	Epoetin alfa	2004
Novolin	Human insulin	2005
Activase	Alteplase	2005
Neupogen	Filgrastim	2006

therefore require the permission of Drug Control Authorities for both import and local production for marketing purposes. According to the Biosafety Guidelines, research work related to recombinant technology is overseen by the Department of Biotechnology through Institutional Biosafety Committees and Review Committee on Genetic Manipulation, whereas commercial/large-scale applications are dealt with the Genetic Engineering Approval Committee of the Ministry of Environment and Forests.

Recent advances in biotechnology have created many legal issues, particularly under the patent system. Patent represents one of several types of intellectual properties; their ownership confers the right to exclude others from benefitting from the tangible products of a proprietary subject matter. The discovery and initial characterization of any rDNA product of potential therapeutics application are followed by its patenting. The normal duration of a patent is 20 years, which starts from the date of filing. Thus the timing assumes importance in terms of the duration for marketing of a product. If the patent is filed too early, the window of opportunity to market the product as well as the size of the market will be smaller. The *Biotech Industry Guide* would be useful for industrial approval, regulatory clearance to rDNA product and biosafety aspects[64]. It would also provide help to scientists in obtaining patents on their inventions in India.

More new rDNA products are being approved by FDA. Some of the blockbuster products are coming off patent (Table 3).

In view of high market potential for recombinant therapeutics, indigenous technology should be developed. This can be achieved by strengthening the linkages among various institutes having expertise in different disciplines related to rDNA technology and increased interaction with the industry.

Conclusion

rDNA technology has indeed made tremendous breakthrough in the discovery of various rDNA products. Besides the products approved by FDA for human use, several products are undergoing clinical trials. Products developed in the field of haematology, endocrinology and oncology will be most valuable for further development of rDNA products in the coming years.

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