EMERGENT RECOMBINANT PROTEINS IN CLINICAL DIAGNOSTICS

Abstract

Recombinant proteins are made into expression vectors that can be used to clone recombinant DNA. which enables messenger RNA to be translated and the gene to be expressed. A gene can be altered using recombinant DNA technology, which might cause a mutant protein to be produced. A modified form of natural protein is called recombinant protein, which is created in a number of ways to increase protein output, change gene sequences, and make useful commercial commodities. Production of recombinant proteins starts at the genetic level with the extraction and cloning of the desired protein's coding sequence into an expression plasmid vector. Although they are expressed in cultivated bacteria, yeast, animal cells, and plants, the bulk of therapeutically beneficial recombinant proteins are made by humans. Recombinant proteins have benefits for clinical diagnostics, including uniform production, scalability, and controlled quality, which result in more reliable and consistent test findings. They play a crucial role in tests such as immunoassays, molecular diagnostic methods, and others that rely on how precisely antigens and antibodies interact with other biomolecules. Recombinant technology has been used to make biopharmaceuticals like insulin, interferons, monoclonal antibodies, and cytokines effectively. With the development of more specialized and efficient medicines, these proteins have transformed the treatment of numerous illnesses, including genetic diseases, autoimmune disorders, and cancer.

Keywords: Recombinant Protein, Diseases, Expression System, Clinical Diagnostic, Biopharmaceuticals

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I. INTRODUCTION

Clinical diagnostics has made significant strides in recent years thanks to the fusion of biotechnology and recombinant DNA technology. Recombinant proteins, created by altering an organism's genetic code, have transformed the precision, responsiveness, and specificity of diagnostic assays. This chapter examines the use of emerging recombinant proteins in clinical diagnostics, emphasizing their function in the early diagnosis, ongoing monitoring, and individualized therapy of diseases. [1]

The majority of biological operations in a cell are made possible by proteins, including gene expression, cell development, proliferation, food uptake, intercellular communication, and death, which are the workhorses of biological systems. The blueprint for making proteins is found in DNA. It also functions as a model for the highly regulated transcriptional processes that give rise to messenger RNA (mRNA). A protein is created by translating the message encoded in the mRNA into certain amino acid sequences. The two-step process used by all organisms to generate proteins involves the translation of DNA first into RNA and then into proteins.

Humulin was the first recombinant protein-based medication to receive FDA approval in 1982 [2]. There are hundreds of candidates approved and undergoing clinical trials for protein-based therapies today, which represent a market worth close to $400 billion [3]. The first medical Nobel Prize was awarded for the discovery of an antibody-based therapy for diphtheria in 1891 [4]. Horse serum that had been exposed to an attenuated strain was used to remove the antibodies of the bacterium that causes diphtheria. Twenty years later, another important development—taking insulin from the pig pancreas to treat diabetes mellitus—is an example of using an exogenous protein to address an internal shortage [5, 6]. But to create eight ounces of protein, two tons of pig pancreas were needed [7]. Additionally, using proteins derived from animals may cause patients to develop an immunological reaction and expose them to animal infections. In 1982, insulin was produced using recombinant DNA technology in a bacterial host (E. coli), marking a significant development [8]. Recombinant DNA technology was successfully used to get around problems with animal-derived protein scale-up and immunogenicity. Given that any protein with a known linked gene could theoretically be expressed, tiny molecules, the mainstay of therapy at the time, faced real competition. Since proteins are naturally prone to denaturation, aggregation, and the subsequent decrease of activity, there have been inherent problems that have required being overcome [9]. Additionally, non-specific distribution, immunogenicity, toxicity, and late clearance from the body also presented pertinent issues [10].

II. WHAT ARE RECOMBINANT PROTEINS, AND HOW ARE THEY MADE?

Recombinant proteins are made by an expression vector that can be used to clone recombinant DNA, which enables messenger RNA to be translated and the gene to be expressed. A gene can be altered via recombinant DNA technology, which could lead to the generation of a mutant protein. Recombinant protein is a type of natural protein that has undergone various modifications in order to produce more protein, alter gene sequences, and produce valuable products for the market [11].

Genetics is where recombinant protein synthesis begins with the extraction and cloning of the desired protein's coding sequence into an expression plasmid vector. Even if they are produced in bacteria that have been grown in yeast or animal cells, the bulk of therapeutically beneficial recombinant proteins are made by humans. The incredibly complex human genes frequently contain introns, which are non-coding DNA segments. In order to produce a gene without introns, cDNA is produced from mRNA. The promoter, ribosome-binding site, and terminator sequences are provided by the expression vectors because the cDNA lacks regulatory regions. The main variables affecting the synthesis of recombinant proteins for research are the production process's effectiveness, speed, and cost-effectiveness, as well as adequate product yields. Co-expressed proteins in bacteria wouldn't have phosphorylation and glycosylation because they need eukaryotic expression systems for such changes.

Numerous recombinant proteins call for changes, including glycosylation, that can only be carried out in eukaryotic cells. In yeast, insect cells, and mammalian cell culture techniques, these post-translational alterations can be observed.

The last ten years have presumably seen the development of efficient transient transfection techniques. Proteins are produced momentarily using cell lines derived from HEK293 cells. Mammalian cells are now used to synthesize the bulk of recombinant therapeutic proteins due to their capacity to produce high-quality proteins that are comparable to those found naturally. With its rapid expansion, thoroughly studied genetics, and enormous output, E. coli also produces a significant amount of approved recombinant therapeutic proteins [12]. Two of the top causes of death worldwide are cancer and infectious diseases. Mass production of therapeutic proteins for the treatment of diseases in millions of people is one of humanity's fundamental needs. Recombinant DNA technologies have recently advanced, enabling recombinant protein synthesis for the creation of pharmaceuticals, vaccines, and diagnostic instruments. Systems for expressing prokaryotic and eukaryotic organisms, such as bacteria, mammalian cells, yeast, insect cells, and transgenic plants, are frequently used in both commercial and scientific settings to create recombinant proteins for these purposes [13]. These proteins are used as tools to identify and quantify certain macromolecules or antigens in patient samples and support illness diagnosis [14].

III. THE CONTRIBUTIONS OF EACH SYSTEM TO CLINICAL DIAGNOSIS ARE SUMMARIZED AS FOLLOWS

Biotechnology frequently uses prokaryotic and eukaryotic expression systems to create recombinant proteins for a variety of uses. The decision between each system depends on various elements, including the complexity of the protein, post-translational changes, yield requirements, and intended usage. Each approach has pros and cons.

1. **Prokaryotic Expression Systems:**Escherichia coli and other prokaryotic bacteria are frequently utilized as expression hosts for recombinant proteins. Prokaryotic systems have the following benefits:

   Prokaryotic systems are very easy and affordable to work with because of their quick growth rates and minimal culture requirements.

Prokaryotes may swiftly create high protein yields because of their rapid rates of translation and replication. However, in prokaryotic systems, some proteins can organize into inclusion bodies (aggregates).

Plasmid-based cloning is effective in bacteria, making it simple to introduce and work with recombinant DNA there.

- **Prokaryotic Expression Systems have Some Drawbacks:** Lack of Post-Translational Changes: Prokaryotes are unable to carry out various post-translational changes, including glycosylation, phosphorylation, and the creation of disulfide bonds, which are essential for the correct folding and operation of several proteins.

- **Protein Misfolding:** In prokaryotic systems, certain complicated or structurally intricate proteins may not fold properly, resulting in the development of insoluble clumps.Limited Secretion: Since the majority of bacterial proteins are expressed in the cytoplasm, it is difficult to create membrane-bound or secreted proteins.

2. **Eukaryotic Expression Systems**: Higher organisms like yeast, mammalian cells (such as those from Chinese hamster ovaries), and insect cells (like those used in the baculovirus system) serve as hosts for eukaryotic expression systems. Eukaryotic systems also have a number of benefits:

Eukaryotic cells are equipped with the machinery to carry out a variety of post-translational modifications, resulting in the synthesis of correctly folded and functioning proteins.

- **Correct Folding:** In eukaryotic systems, complex and physically intricate proteins are more likely to fold properly, producing physiologically active byproducts.

- **Membrane Proteins and Secretion:** Eukaryotic systems enable the generation of membrane-bound and secreted proteins with the proper modifications.

- **Mammalian Cell Systems:** Because they closely resemble human cells, mammalian cells can be used to make recombinant proteins that have therapeutic uses. Mammalian cells frequently synthesize proteins with human-like glycosylation patterns.

- **Eukaryotic Systems, however, can have Several Disadvantages:** Cost and Complexity: Working with eukaryotic systems is more difficult and expensive than working with prokaryotic systems.

  Lower Expression Levels: Compared to prokaryotic systems, eukaryotic systems typically express proteins at lower levels and with longer manufacturing durations.

Considerations Specific to Each Cell Line: Within eukaryotic systems, many cell lines have variable capacities for protein expression and post-translational modifications.

In conclusion, the decision among prokaryotic and eukaryotic expression systems is based on the unique needs of the recombinant protein, particularly its structure, function, and intended use. [15,16]

3. **Bacterial Recombinant Proteins:** In order to produce recombinant proteins, bacterial systems like Escherichia coli (E. coli) are frequently utilized because of their quick growth and ease of genetic manipulation. Recombinant proteins from bacteria are utilized in clinical diagnostics for a number of reasons, including Recombinant Enzymes: Bacterial enzymes like alkaline phosphatase and horseradish peroxidase are utilized in diagnostic assays to enhance signals, improving the detection of target molecules [17].

   Recombinant Antigens: Recombinant proteins made by bacteria are designed to resemble particular pathogen antigens. These antigens are useful for diagnosing diseases like bacterial or viral infections by detecting antibodies against infectious agents in serological assays like ELISA and Western blot [14].Protein Markers: Recombinant bacterial proteins can serve as reference markers for quality control and standardization in diagnostic assays [18].

4. **Yeast Recombinant Proteins:** Eukaryotic protein expression is possible in yeast systems, enabling the generation of complicated proteins with suitable post-translational modifications and folding. Saccharomyces cerevisiae is particularly useful for producing more complicated proteins with suitable post-translational modifications and folding. One of their functions in clinical diagnosis is [19]. Recombinant Antigens: Serological techniques to find antibodies against fungi like Candida or Aspergillus use yeast-expressed recombinant antigens.

   Diagnostic Enzymes: To assist in diagnostic enzymology, yeast-produced enzymes, such as galactosidase, are utilized in colorimetric assays that identify particular substrates [20].

5. **Mammalian Recombinant Proteins:** Diagnostic uses for mammalian cell systems include [21]. These systems are frequently employed for more complicated proteins that need appropriate folding and post-translational modifications.

   Recombinant Monoclonal Antibodies: Mammalian cell systems are essential for the production of monoclonal antibodies used in immunoassays such as ELISA and flow cytometry, which allow the identification of certain antigens or markers in patient samples [22].

   Recombinant Hormones and Cytokines: Recombinant cytokines and hormones from mammalian cells play a key role in diagnostic assays for endocrine disorders, autoimmune illnesses, and immune response profiling [23].

6. **Plant-Based Recombinant Proteins:** A special platform for protein production is provided by plant systems, which may also be more scalable and less likely to harbor human pathogens. [24]

   Recombinant Antigens: In point-of-care testing for identifying antibodies against viral or bacterial infections, plant-expressed recombinant antigens are utilized. These tests provide quick and economical diagnostics in resource-constrained settings.

   Recombinant allergens created by plants are employed in IgE antibody testing to determine a person's sensitivity to a particular allergen [25].

7. **Insect Cell-Based Recombinant Proteins:** Insect cell systems are useful for creating complicated proteins that need appropriate folding and are frequently used in conjunction with the baculovirus expression system. Their diagnostic responsibilities include [26].

   Recombinant Glycoproteins: To create glycoproteins with the proper post-translational modifications, which are required for some diagnostic procedures, insect cell systems are used. [27].

   Viral Antigens for Serology: To find antibodies to viruses like dengue, Zika, or influenza, serological assays use viral antigens made by insect cells. [28].

   Recombinant proteins have benefits for clinical diagnostics, including uniform production, scalability, and controlled quality, which result in more reliable and consistent test findings. They play a crucial role in tests such as immunoassays, molecular diagnostic methods, and others that depend on the precise interaction between antigens and antibodies or other biomolecules.

IV. ADVANTAGES OF RECOMBINANT PROTEINS

It is possible to produce intricate and complicated proteins with suitable post-translational modifications and folding. In the field of clinical diagnosis, recombinant proteins are the preferred substitute if performance is homologous between the two, as they have numerous advantages over native proteins. The sustainability of the raw materials used to create recombinant proteins is one of their key benefits. Recombinants are produced from enlarged cell lines and collected as needed, making the pure protein accessible as required. Because it depends on the availability of certain donors, the procurement of native tissue is unpredictable. In recent years, it has been more challenging to obtain these raw materials, which has caused a reduction in supplies and an increase in demand for some native proteins. These issues already existed prior to COVID-19, but the pandemic and unanticipated occurrences like the Ukraine War have made them worse. As a result of the impact on the diagnostic market, many native proteins are currently in short supply.

The cost of generating native proteins is unstable and has been rising continuously over the past few years. There are no signs that this tendency will soon change during the next few years, as was already indicated. On the other hand, the price of making recombinant proteins is significantly more predictable. The cost of cell culture and fermentation reagents is far more stable than that of native tissues and is widely accessible. Numerous native

proteins have been purified from raw materials, which also include proteins that mimic the protein that is being purified, so there are no protein contaminants present. These contaminated proteins can interfere with immunoassays and lengthen purification cycles, which makes them problematic. It's improbable that recombinant proteins will contain proteins that physiologically or physically resemble the purified protein. This expedites the purifying procedure and reduces the possibility of assay interference from contaminating sources. Since native human proteins are isolated from materials that may include pathogens that cause infectious diseases, no disease-state testing is done. As a result, proteins extracted from natural sources may pose regulatory difficulties for companies that produce diagnostic test kits. Recombinant proteins are extracted from non-human, non-biohazardous cell cultures or fermentation methods [12].

V. NEW RECOMBINANT PROTEINS

Since the late 1980s and early 1990s, reproductive scientists have had access to recombinant versions of the hormones follicle-stimulating hormone, human chorionic gonadotropin, and luteinizing hormone. These three hormones are challenging recombinant targets because of their structural heterodimers. Recombinant hCG, FSH, and LH have not been utilized frequently in the diagnostic field, which is expected. For the evaluation of pregnancy, fertility, and ovulation, Scripps Laboratories created antibody-reactive, recombinant hCG, FSH, and LH using the aforementioned standards. Recombinant hCG is now receiving favorable evaluations. Recombinant FSH and LH are allowed to be used in many clinical immunoassays. The iron-accumulating protein ferritin, which is used as a biomarker for anemia, is one of the trickiest proteins utilized in medical diagnosis. The 24-subunit protein ferritin, which has heavy- and light-chain subunits, is released by the liver and spleen. Because the heavy and light chains have different compositions, it is known that there are several isoforms of ferritin. This presents an additional challenge for those working to produce recombinant proteins. For more than 30 years, recombinant ferritin has been produced, yet due to its intricate structural makeup, the diagnostic sector has only just begun to employ it. A multisubunit ferritin recombinant form has been created by Scripps for the diagnosis of iron-deficient anemia and other disorders. It functions incredibly well in a range of antibody-based test techniques and is composed of both heavy and light chains. The use of this recombinant version of ferritin in clinical immunoassays all around the world is now being evaluated [29, 30].

One of the main illnesses posing problems for public health around the world is dengue. Dengue virus is thought to cause 390 million infections annually, despite scientific advancements in the creation of vaccines against all of its serotypes. The key to the proper management and prevention of this condition has been laboratory diagnosis. The production of the non-structured 1 (NS1) antigen, which is utilized to capture the antibody seen in the serum of infected individuals, on a large scale is currently limiting the advancement of dengue diagnostic kits. In this study, we demonstrate that the non-structural proteins 1 (NS1-DENV1, NS1-DENV2, and NS1-DENV3) and NS1-DENV4 of the dengue virus (DENV) serotypes 1-4 may be produced by the methylotrophic yeast Pichia pastoris KM71H. Affinity chromatography was used to separate the secreted recombinant protein, and SDS-PAGE and ELISA were used to confirm its identity. Due to their efficient production in P. pastoris, the NS1 recombinant proteins have a substantial potential for use in diagnostic tests for dengue virus infections. In industrial-sized bioreactors, the altered yeast can be employed to make

commodities. This study demonstrated a viable approach that could aid in the development of an efficient diagnostic test for upcoming investments: employing yeast to manufacture antigens to identify anti-dengue antibodies. The recombinant proteins NS1DENV1-4 thereby produced are intriguing test candidates because of their high yield, antigenic integrity, and inexpensive cost for industrial-scale production [31].

Animal and human populations have been devastated by coronaviruses, which have had a negative impact on both economics and public health. Humans must employ science and evidence-based methods to combat the economic and health dangers posed by coronaviruses as they continue to evolve and re-emerge. Biotechnology, namely the heterologous synthesis of therapeutic proteins, is one of these methods. Insect cell/insect expression systems and mammalian expression systems are the two most efficient means of delivering recombinant proteins utilized as protein subunit vaccines, particularly for COVID-19. They have a great secretion capacity, a high product yield, and the ability to create complex and high-quality proteins, which may be the reason for their success. As more proteins are created and evaluated for therapeutic, preventive, and diagnostic uses in human clinical trials, recombinant protein technology using diverse expression systems advances and becomes more complex. This is especially true when fighting viral diseases like coronaviruses, which globally pose severe dangers to human health [32].

VI. REVIEW OF LITERATURE

Recombinant protein manufacturing and expression systems are frequently discussed in the literature. To manufacture recombinant proteins with enhanced productivity, quality, and functionality, researchers have looked into a variety of host organisms, including bacteria (Escherichia coli), yeast, insect cells, and mammalian cells. For recombinant proteins to be highly soluble and appropriately folded, expression system optimization is essential.

Recombinant therapeutic proteins have received a lot of attention recently. Recombinant technology has been used to make biopharmaceuticals like insulin, interferons, monoclonal antibodies, and cytokines effectively. With the development of more specialized and efficient medicines, these proteins have transformed the treatment of many illnesses, such as cancer, autoimmune conditions, and hereditary diseases. [33,34]. Recombinant enzymes are used in a variety of industries, such as food processing, agriculture, and the creation of biofuels. Examples include proteases for laundry detergents and cellulases for the generation of bioethanol. [35] The creation of vaccines has greatly benefited from the appearance of recombinant proteins. It is possible to build vaccinations that are both safer and more effective by using recombinant antigens from diseases. This strategy has been effective for hepatitis B, human papillomavirus (HPV), and influenza vaccinations. [36] In structural biology investigations, recombinant proteins have been crucial tools because they have made it possible to discover protein structures in three dimensions (3D), employing techniques like X-ray crystallography and NMR spectroscopy. These structures offer useful information for target identification and logical drug design. [37, 38] Novel recombinant proteins with specialized functions and features have been made possible by advancements in protein engineering and synthetic biology. The creation of artificial proteins with particular binding properties, enzymatic activity, and medicinal uses is highlighted in the literature. [39]

The literature on emergent recombinant proteins emphasizes their significant influence across a range of fields. Recombinant proteins have revolutionized how we approach problems and possibilities in biology, medicine, and biotechnology, from medicines to industrial uses.

VII. FUTURE DIRECTIONS

Modern Protein Engineering: Protein engineering is a rapidly developing field. Methods for creating and engineering proteins with enhanced stability, specificity, and novel functionalities are being investigated by researchers. [40] Rapid protein synthesis, effective isotope labeling, and control over reaction conditions are all benefits of cell-free protein synthesis, which enables protein manufacture without the need for whole cells. [41]

Synthetic biology methods the development of new biological systems for protein synthesis is made possible by synthetic biology approaches, which also allow for the effective tuning of cellular machinery for the synthesis of recombinant proteins [42]. Recombinant proteins can be customized for personalized medicine, in which certain protein variations are created to correspond to a patient's genetic profile, enhancing therapy effectiveness and reducing side effects. [30] Integrating data from many "omics" methodologies, such as proteomics, genomics, and others, can shed light on how proteins are expressed, folded, and modified, which can be used to improve production methods. [43]

Novel Expression Hosts: Investigating non-traditional expression hosts, such as yeast, algae, and insect cells, can have benefits for scalability, post-translational modifications, and protein folding. Moving from batch to continuous bioprocessing can boost output, save costs, and improve the quality of recombinant protein production. [44]

CRISPR/Cas-Based Methods: By using CRISPR/Cas systems for targeted gene editing in host cells, one can precisely control the amounts and alterations of protein expression. [45]. Biosafety and biosecurity: As the usage of recombinant proteins grows, it is crucial to take precautions to avoid unintentional exposure of information or improper use. [46]

Consequently, despite the difficulties that emergent recombinant proteins face, recent developments in synthetic biology, cell-free systems, protein engineering, and other fields show promise for overcoming these difficulties and realizing the full potential of recombinant proteins in a variety of applications.

VIII. DISCUSSION

Therapeutic recombinant proteins are exogenous proteins produced by an organism that are utilized to treat or prevent illness in humans or other animals. Therapeutic recombinant proteins have emerged as the most significant advancement in pharmaceuticals since Escherichia coli produced human insulin for the first time in 1982 [47, 48]. Many recombinant proteins have been created, then medications with the potential to treat everything from cancer to arthritis have entered the market, and there are presently hundreds more being developed [49, 50, 51, 52, 53]. Recombinant proteins, in contrast to conventionally produced pharmaceuticals, are huge, complex, and able to behave in a variety of smart and specialized ways. Utilizing the protein production apparatus present in all cells,

these innovative drugs must be produced physiologically due to the difficulty of chemically creating proteins due to their size and complexity [54]. The pharmaceutical industry has undergone a "major paradigm shift" as a result of the efficiency and scalability of production using plant expression systems [49].

Monoclonal antibodies (mAbs), which were initially cloned from human immunoglobulin G1 (IgG1), are some of the most potent therapeutic recombinant proteins because of their ability to target epitopes with high specificity. Thanks to technological improvements, mAbs today have the ability to accomplish a number of goals as therapeutic agents. For instance, mAbs can present an antigen or imitate a signaling ligand; they may stop the activity of other proteins or inhibit enzymes; and they can stimulate the host immune system to attack a specific cancer cell [55]. Today, therapeutic recombinant proteins are being generated and developed for use as vaccinations; serum proteins, enzymes, growth factors, cytokines, hormones, and growth factors are only a few examples [49, 50, 52, 53].

A sizeable part of therapeutic proteins are also made in the yeast Saccharomyces cerevisiae and murine myeloma cells, although the majority are made in (CHO) Chinese hamster ovary cultured cells or E. coli fermentations. [56] These expression systems are the ones with the best production of protein identification, and each system has benefits and drawbacks of its own. Other expression systems, which have not been used as much, might be able to create novel therapeutic medications or enhance the synthesis of already existing proteins. More than 130 RPs have been given clinical use approval by the US FDA since 1982. The production and usage of more than 170 RPs in medicine are widespread, though. Recombinant human insulin was a very early example of how biotechnology was being used in the creation of pharmaceuticals. Recombinant proteins are more quickly developed than tiny compounds and are powerful medications free from unintended side effects. A multibillion-dollar market, all Big Pharma now develops RP as medications [57]. The bulk of human illnesses are caused, in whole or in part, by the malfunction of certain proteins. Diabetes, viral diseases, hemophilia, and anemia are just a few of the conditions that can be treated with therapeutic proteins. Antibodies, interleukins, enzymes, fc-fusion proteins, hormones, and anticoagulants are typical therapeutic proteins. The market for therapeutic pharmaceuticals is mainly reliant on genetically engineered human proteins. The Hepatitis B vaccination is one of the many RP vaccinations that the FDA has authorized, which guards against illness brought on by every known subtype of the Hepatitis B virus [58]. For the treatment of severe conditions such as cancer, asthma, rheumatoid arthritis, cerebral apoplexy, myocardial infarction, Crohn's disease, neutropenia, thrombocytopenia, anemia, hepatitis, and dwarfism, recombinant proteins are also used in the clinic [12]. Recombinant proteins are used in agriculture, bioengineering, and the manufacture of food. Enzymes, for instance, can be used in animal feed in the breeding business to improve animal performance, support animal gastrointestinal health, lower feed and waste management costs, and boost the nutritional content of feed materials. Furthermore, the manufacture of fermented foods has long been accomplished using lactic acid bacteria (LAB). Recently, LAB has been modified to express recombinant proteins, which have a variety of uses, including enhancing human and animal digestion and nutrition [59].

IX. CONCLUSION

The creation of recombinant proteins for various uses has increased and been facilitated by developments in the field of biotechnology. For the development of medicinal medications, diagnostic tools, and basic life science research, RP's significance has grown quickly. Their contribution to biotechnology is indispensable. Other scientists anticipate further advancements in the use of recombinant proteins to treat a variety of ailments. Continuous research improves production processes, broadens uses, and opens up new opportunities for these adaptable compounds.

REFERENCES

[1] Khan S, Ullah MW, Siddique R, Nabi G, Manan S, Yousaf M, Hou H. Role of recombinant DNA technology to improve life. International journal of genomics. 2016 Oct;2016.
[2] Chung YH, Church D, Koellhoffer EC, Osota E, Shukla S, Rybicki EP, Pokorski JK, Steinmetz NF. Integrating plant molecular farming and materials research for next-generation vaccines. Nature Reviews Materials. 2022 May;7(5):372-88.
[3] Kaufmann SH. Emil von Behring: translational medicine at the dawn of immunology. Nature Reviews Immunology. 2017 Jun;17(6):341-3.
[4] Wang MY, Chen L, Clark GO, Lee Y, Stevens RD, Ilkayeva OR, Wenner BR, Bain JR, Charron MJ, Newgard CB, Unger RH. Leptin therapy in insulin-deficient type I diabetes. Proceedings of the national academy of Sciences. 2010 Mar 16;107(11):4813-9.
[5] Banting FG, Best CH. The internal secretion of the pancreas. Indian Journal of Medical Research. 2007 Mar 1;125(3):L251.
[6] Gebel E. Making insulin. A behind-the-scenes look (and why you can't do it yourself at home). Diabetes Forecast. 2013 Jul 1;66(7):54-7.
[7] Johnson IS. Human insulin from recombinant DNA technology. Science. 1983 Feb 11;219(4585):632-7.
[8] Frokjaer S, Otzen DE. Protein drug stability: a formulation challenge. Nature reviews drug discovery. 2005 Apr;4(4):298-306.
[9] Zhang P, Sun F, Tsao C, Liu S, Jain P, Sinclair A, Hung HC, Bai T, Wu K, Jiang S. Zwitterionic gel encapsulation promotes protein stability, enhances pharmacokinetics, and reduces immunogenicity. Proceedings of the National Academy of Sciences. 2015 Sep 29;112(39):12046-51.
[10] Vugmeyster Y, Xu X, Theil FP, Khawli LA, Leach MW. Pharmacokinetics and toxicology of therapeutic proteins: advances and challenges. World journal of biological chemistry. 2012 Apr 4;3(4):73.
[11] What are recombinant proteins?Evitria https://www.evitria.com
[12] https://www.enzolifesciences.com/science-center/technotes/2020/january/why-do-we-need-recombinant-proteins?/#:~:text=Recombinant%20proteins%20are%20useful%20tools,important%20role%20in%20cellular%20processes.
[13] Tripathi NK, Shrivastava A. Recent developments in bioprocessing of recombinant proteins: expression hosts and process development. Frontiers in bioengineering and biotechnology. 2019 Dec 20;7:420.
[14] Spencer KA, Osorio FA, Hiscox JA. Recombinant viral proteins for use in diagnostic ELISAs to detect virus infection. Vaccine. 2007 Jul 26;25(30):5653-9.
[15] Kleiner- Grote GR, Risse JM, Friehs K. Secretion of recombinant proteins from E. coli. Engineering in Life Sciences. 2018 Aug;18(8):532-50.
[16] https://info.gbiosciences.com/blog/recombinant-protein-expression-systems-pros-cons
[17] Rosano GL, Ceccarelli EA. Recombinant protein expression in Escherichia coli: advances and challenges. Frontiers in microbiology. 2014 Apr 17;5:172.
[18] Pala L, Sirec T, Spitz U. Modified enzyme substrates for the detection of bacteria: A review. Molecules. 2020 Aug 13;25(16):3690.
[19] Karbalaei M, Rezaee SA, Farsiani H. Pichia pastoris: A highly successful expression system for optimal synthesis of heterologous proteins. Journal of cellular physiology. 2020 Sep;235(9):5867-81.
[20] He ZX, Chen J, Li W, Cheng Y, Zhang HP, Zhang LN, Hou TW. Serological response and diagnostic value of recombinant candida cell wall protein enolase, phosphoglycerate kinase, and β-glucosidase. Frontiers in Microbiology. 2015 Sep 10;6:920.
[21] Khan KH. Gene expression in mammalian cells and its applications. Advanced pharmaceutical bulletin. 2013 Dec;3(2):257.

[22] Stravinskiene D, Sliziene A, Baranauskiene L, Petrikaite V, Zvirbliene A. Inhibitory monoclonal antibodies and their recombinant derivatives targeting surface-exposed carbonic anhydrase XII on cancer cells. International journal of molecular sciences. 2020 Dec 10;21(24):9411.
[23] Zhu MM, Mollet M, Hubert RS, Kyung YS, Zhang GG. Industrial production of therapeutic proteins: cell lines, cell culture, and purification. Handbook of industrial chemistry and biotechnology. 2017:1639-69.
[24] Chen Q, Davis KR. The potential of plants as a system for the development and production of human biologics. F1000Research. 2016;5.
[25] Ko K. Expression of recombinant vaccines and antibodies in plants. Monoclonal antibodies in immunodiagnosis and immunotherapy. 2014 Jun 1;33(3):192-8.
[26] Felberbaum RS. The baculovirus expression vector system: A commercial manufacturing platform for viral vaccines and gene therapy vectors. Biotechnology journal. 2015 May;10(5):702-14.
[27] Hong M, Li T, Xue W, Zhang S, Cui L, Wang H, Zhang Y, Zhou L, Gu Y, Xia N, Li S. Genetic engineering of baculovirus-insect cell system to improve protein production. Frontiers in bioengineering and biotechnology. 2022 Sep 20;10:994743.
[28] Vainionpää R, Waris M, Leinikki P. Diagnostic techniques: serological and molecular approaches. Reference module in biomedical sciences. 2015.
[29] George DA. Recombinant Proteins Benefit the Clinical Diagnostic Industry: Scripps Laboratories demonstrates how recombinant proteins offer advantages over native proteins in purity, performance, and sustainability. Genetic Engineering & Biotechnology News. 2022 Sep 1;42(9):20-2.
[30] Chromeextension://efaidnbmnnnibpcajpcglclefindmkaj/https://scrippslabs.com/content/thescientist-ebook-may-2023.pdf
[31] Xisto MF, Prates JW, Dias IM, Dias RS, da Silva CC, de Paula SO. NS1 recombinant proteins are efficiently produced in pichia pastoris and have great potential for use in diagnostic kits for dengue virus infections. Diagnostics. 2020 Jun 6;10(6):379.
[32] Cabal AB, Wu TY. Recombinant Protein Technology in the Challenging Era of Coronaviruses. Processes. 2022 May 10;10(5):946.
[33] Tripathi NK, Shrivastava A. Recent developments in bioprocessing of recombinant proteins: expression hosts and process development. Frontiers in bioengineering and biotechnology. 2019 Dec 20;7:420.
[34] Ebrahimi SB, Samanta D. Engineering protein-based therapeutics through structural and chemical design. Nature Communications. 2023 Apr 27;14(1):2411.
[35] Mesbah NM. Industrial biotechnology based on enzymes from extreme environments. Frontiers in Bioengineering and Biotechnology. 2022 Apr 5;10:870083.
[36] Nascimento IP, Leite L. Recombinant vaccines and the development of new vaccine strategies. Brazilian journal of medical and biological research. 2012;45:1102-11.
[37] Hu Y, Cheng K, He L, Zhang X, Jiang B, Jiang L, Li C, Wang G, Yang Y, Liu M. NMR-based methods for protein analysis. Analytical chemistry. 2021 Jan 13;93(4):1866-79.
[38] Zheng H, Handing KB, Zimmerman MD, Shabalin IG, Almo SC, Minor W. X-ray crystallography over the past decade for novel drug discovery–where are we heading next?. Expert opinion on drug discovery. 2015 Sep 2;10(9):975-89.
[39] Madhavan A, Arun KB, Binod P, Sirohi R, Tarafdar A, Reshmy R, Awasthi MK, Sindhu R. Design of novel enzyme biocatalysts for industrial bioprocess: Harnessing the power of protein engineering, high throughput screening and synthetic biology. Bioresource Technology. 2021 Apr 1;325:124617.
[40] Ebrahimi SB, Samanta D. Engineering protein-based therapeutics through structural and chemical design. Nature Communications. 2023 Apr 27;14(1):2411.
[41] Khambhati K, Bhattacharjee G, Gohil N, Braddick D, Kulkarni V, Singh V. Exploring the potential of cell-free protein synthesis for extending the abilities of biological systems. Frontiers in bioengineering and biotechnology. 2019 Oct 11;7:248.
[42] Yue K, Chen J, Li Y, Kai L. Advancing synthetic biology through cell-free protein synthesis. Computational and Structural Biotechnology Journal. 2023 May 4.
[43] Goetz LH, Schork NJ. Personalized medicine: motivation, challenges, and progress. Fertility and sterility. 2018 Jun 1;109(6):952-63.
[44] Santiago-Rodriguez TM, Hollister EB. Multi 'omic data integration: A review of concepts, considerations, and approaches. InSeminars in Perinatology 2021 Oct 1 (Vol. 45, No. 6, p. 151456). WB Saunders.
[45] Zhang H, Qin C, An C, Zheng X, Wen S, Chen W, Liu X, Lv Z, Yang P, Xu W, Gao W. Application of the CRISPR/Cas9-based gene editing technique in basic research, diagnosis, and therapy of cancer. Molecular Cancer. 2021 Dec;20:1-22.
[46] Wang F, Zhang W. Synthetic biology: recent progress, biosafety and biosecurity concerns, and possible solutions. Journal of Biosafety and Biosecurity. 2019 Mar 1;1(1):22-30.

[47] Kamionka M. Engineering of therapeutic proteins production in Escherichia coli. Current pharmaceutical biotechnology. 2011 Feb 1;12(2):268-74.
[48] Pavlou AK, Reichert JM. Recombinant protein therapeutics—success rates, market trends and values to 2010. Nature biotechnology. 2004 Dec;22(12):1513-9.
[49] Margolin E, Chapman R, Williamson AL, Rybicki EP, Meyers AE. Production of complex viral glycoproteins in plants as vaccine immunogens. Plant biotechnology journal. 2018 Sep;16(9):1531-45.
[50] Marsian J, Lomonossoff GP. Molecular pharming—VLPs made in plants. Current opinion in biotechnology. 2016 Feb 1;37:201-6.
[51] Meyer HP, Brass J, Jungo C, Klein J, Wenger J, Mommers R. An emerging star for therapeutic and catalytic protein production. BioProcess International. 2008 Jun;6:10.
[52] Rader RA. FDA biopharmaceutical product approvals and trends in 2012. BioProcess Int. 2013 Mar;11(3):18-27.
[53] Shahid N, Daniell H. Plant- based oral vaccines against zoonotic and non- zoonotic diseases. Plant biotechnology journal. 2016 Nov;14(11):2079-99.
[54] Herdt RW. Biotechnology in agriculture. Annu. Rev. Environ. Resour.. 2006 Nov 21;31:265-95.
[55] Van Dijk MA, Van De Winkel JG. Human antibodies as next generation therapeutics. Current opinion in chemical biology. 2001 Aug 1;5(4):368-74.
[56] Rader RA. Expression systems for process and product improvement. BioProcess Int. 2008 Jun;6(Suppl 4):4-9.
[57] Li W, Li K, Wei W, Ding S. Chemical approaches to stem cell biology and therapeutics. Cell stem cell. 2013 Sep 5;13(3):270-83.
[58] Kishore K, Krishan P. Pharmacology of recombinant or genetically engineered drugs. Journal of Young Pharmacists. 2009;1(2):140
[59] 59. CUSABIO team. Applications of recombinant proteins. https://www.cusabio.com/c-20272.htmls

