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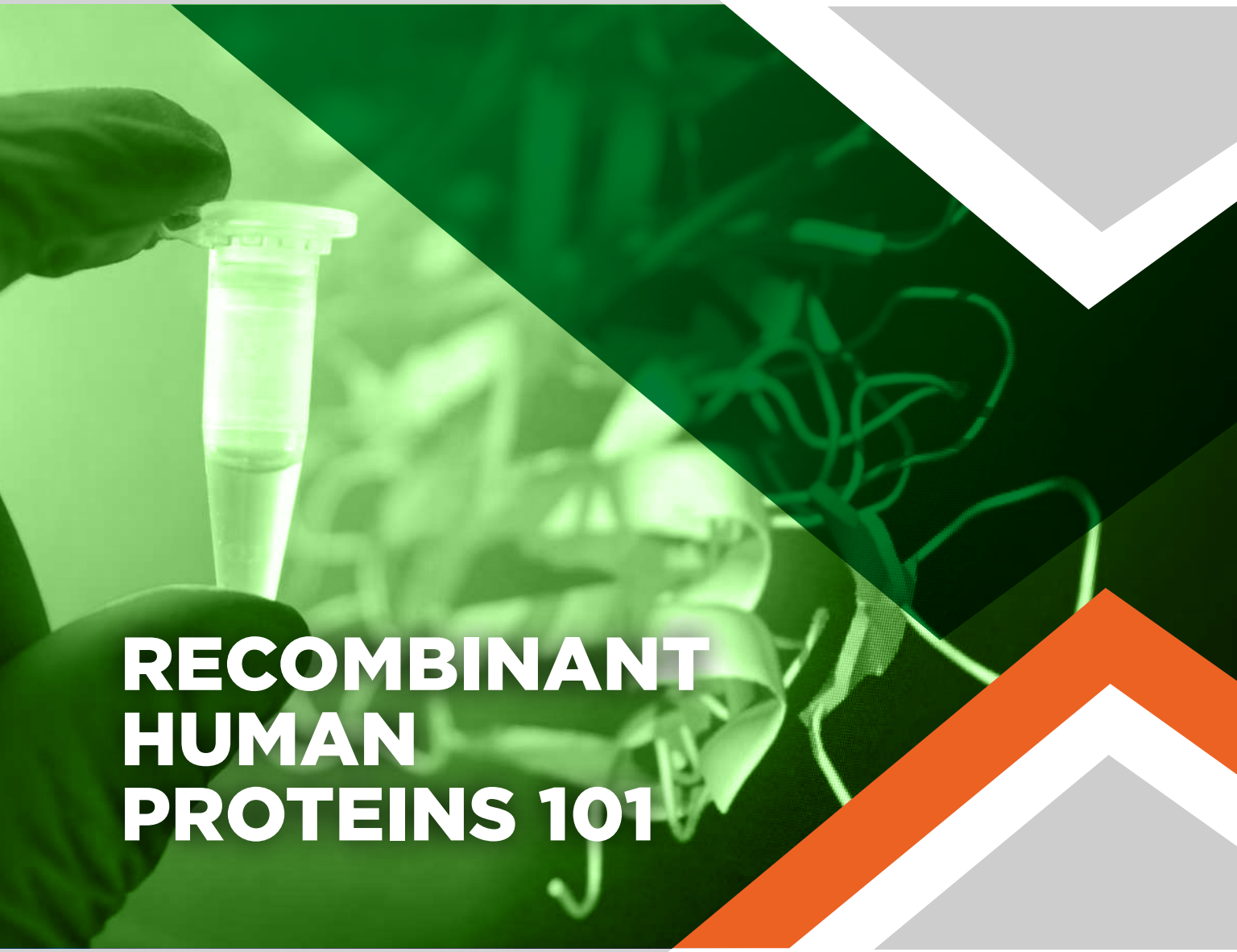
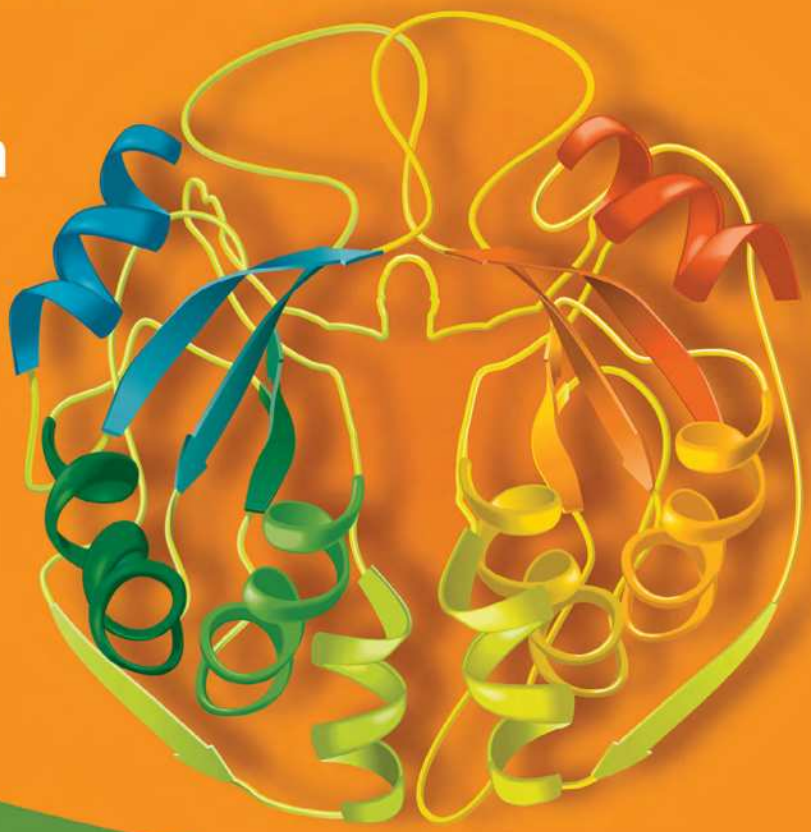


RECOMBINANT HUMAN PROTEINS 101

10,000 Human Proteins Made from Human Cells

Better Preservation of PTMs and Functions



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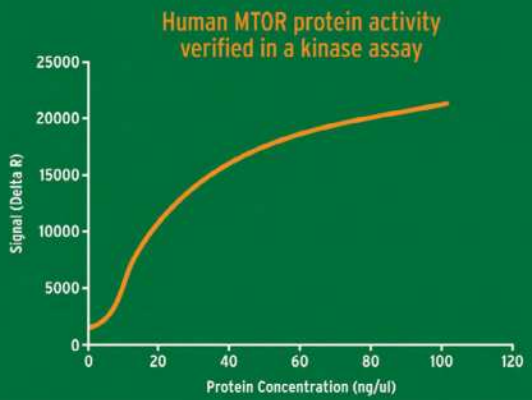

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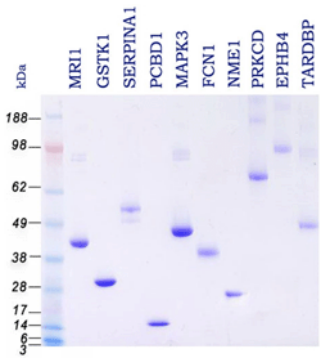


Why Do We Need Recombinant Proteins?

Proteins are fundamental drivers of biological systems. They provide structure, catalyze metabolic reactions, instigate responses to stimuli, and facilitate transport and communications. But to study proteins, scientists need access to them. In this article, we explore why recombinant proteins are needed for experimental observation and manipulation. Please note that while this article focuses on recombinant human proteins, much of the information applies to all recombinant proteins.

The Limitations of Native Proteins

Ideally, scientists would use purified native proteins for their experiments. However, it's not always possible to access proteins produced under native environments, in natural models, and by natural mechanisms. There are several possible reasons for this:

1. A low abundance of native source material (e.g., a rare cellular subset)
2. Naturally low expression levels (Researchers can pool experimental subjects together to obtain sufficient quantities, but this increases costs and logistical demands.)
3. The lack of an effective purification method (Separating a pathogenic protein from its wildtype counterpart, for example, can be very challenging technically.)

Recombinant Proteins to the Rescue?

Recombinant systems provide scientists with a simple, rapid, reproducible, and scalable supply of purified material for their investigations.

To make recombinant proteins, researchers introduce custom engineered DNA into a cell, usually with the help of a vector. The cell's natural machinery then takes over to produce the protein. This means that, theoretically, a cell can make any protein with a known coding sequence.

By adding various transcriptional and translational controls, researchers can increase the expression level of the target protein. After production, they can harvest recombinant proteins by collecting and lysing the production cells, or by extracting the culture media if the protein of interest is secreted. Vectors often contain expression tags to facilitate simple and cost-effective protein purification through affinity binding.

Because scientists design the DNA template sequences introduced into the cells, they can craft and modify the protein of interest to suit their needs. This opens countless new possibilities for investigating specific mutations, domains, interactions, or modifications.

The Possible Pitfalls of Using Recombinant Proteins

Recombinant proteins vastly expand research possibilities, but they are not a perfect replacement for native proteins. Prokaryotic cells such as *E. coli* are popular production models because they are easy to culture and transform. However, natural prokaryotic cells lack the machinery necessary to produce the full range of eukaryotic post-translational modifications, resulting in structural differences in the finished proteins they produce[1]. These differences can lead to functional differences between recombinant and native proteins. Because of this, eukaryotic production models are becoming more popular, despite their limits regarding yield and scaling (please see page 5 for more details).

Quality In, Quality Out

Ultimately, the relevance of any data obtained using recombinant proteins to natural situations depends on how similar the recombinant protein is to its native counterpart. How similar a recombinant and native protein are in terms of structure, conformation, and function depends on the production environment. Therefore, scientists need to exercise caution and diligence when setting up a recombinant protein production workflow or when obtaining purified recombinant proteins from third parties.

References

1. W.H. Brondyk, "Selecting an appropriate method for expressing a recombinant protein," *Methods Enzymol*, 463:131-147, 2009.

Producing Human Recombinant Proteins

Recombinant proteins have demonstrated their value both in the laboratory and the clinic. In the lab, they help researchers characterize individual proteins and determine how proteins respond to stimuli and interact with each other. In the clinic, recombinant protein production provides millions of patients with life-saving peptides and proteins such as insulin, factor VIII, and growth hormone.

Recombinant proteins are even bridging the gap between the lab and clinic, with designer antigens that stimulate custom antibodies. These facilitate development of antibody-based laboratory assays and immune-based therapeutic avenues.

Planning Ahead: Expression Vector Production

Recombinant protein production begins at the genetic level, where scientists must first identify the coding sequence for the protein of interest. Next, they place the coding sequences into plasmid or phage vectors for transport into the production cell. Vector selection and design depends on the selected expression system and the downstream applications of the recombinant proteins.

In addition to the coding sequence, vectors can also contain the following:

- Elements that support host cell translational and transcriptional machinery such as promoters, translational initiators, and transcriptional terminators
- Additional tags and markers enabling downstream selection (e.g., antibiotic resistance genes) or purification (e.g., His, FLAG tags)

Home Sweet Home: Selecting the Expression and Production Host

Selecting a production host is arguably the single most important decision when producing recombinant proteins.

Prokaryotic Hosts - Prokaryotic hosts remain the most popular expression models, with *E. coli* the most common. However, proteins produced using prokaryotic models do not match the 3D structures and post-translational modification profiles found in native proteins[1]. As a result, using eukaryotic expression models has become more common in recent years, especially for producing biopharmaceuticals[1].

Mammalian Cell Lines - Mammalian cell lines historically produced lower yields[2]. However, recent advances such as novel targeted transfection protocols and lentiviral vectors have largely removed these obstacles. It is now possible to achieve yields in the two most popular mammalian cell lines—human embryonic kidney 293 (HEK293) and Chinese hamster ovary (CHO) cells—that meet or exceed those achieved using traditional plasmid transfection.[1,2]

Yeast and Insect Cells - Yeast and insect cell models also produce recombinant proteins. These offer greater post-translational modification capabilities than prokaryotic cell models. Yeast is particularly attractive because it can grow in cell densities similar to bacteria, thereby offering increased yields.

Cell-Free Systems - Finally, cell-free systems, where cell-derived lysates produce proteins from genetic material, are also available. These offer a rapid and simple method for producing small amounts of functional protein without the need for traditional transfection, culture, and purification requirements[1].

Quality over Quantity: Protein Purification

A number of factors influence protein yield. Usually, *E. coli* yield more protein than mammalian cell lines such as CHO and HEK293. However, *E. coli*-produced proteins lack post-translational modifications or disulfide bonds. As a result, they tend to fold improperly, potentially impairing protein function.

Additionally, *E. coli*-produced proteins frequently aggregate to form inclusion bodies, requiring denaturing and re-folding. These additional experimental steps can compromise the structure and function of the recombinant protein product.

On the other hand, mammalian cells produce recombinant proteins in an environment that more closely resembles the native protein environment. Mammalian cells can mimic human cell machinery, offer native chaperones to assist with proper folding, and replicate native post-translational modifications. Naturally, mammalian cells are the hosts of choice when protein structural or functional integrity is critical.

Mammalian cell culture is much more complex and expensive than prokaryotic cell culture, however. Scaling-up is also more complicated, as expensive fermentors are needed for large-scale mammalian cell-based protein production.

References

1. N. Ferrer-Miralles et al., "General introduction: recombinant protein production and purification of insoluble proteins," *Methods Mol Biol,* 1258:1-24, 2015.
2. D.L. Hacker, S. Balasubramanian, "Recombinant protein production from stable mammalian cell lines and pools," *Curr Opin Struct Biol,* 38:129-136, 2016.

PROS & CONS

Choosing the right expression host for producing recombinant human protein requires balancing yield, authenticity, cost, and bioactivity.

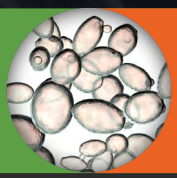
Bacteria

PROS
- Simple vector construction
- Straightforward culture methods
- High yields
- Amenable to scaling

CONS
- No post-translational modifications
- Improperly folded product
- Susceptible to aggregation
- Potential endotoxin contamination

Yeast

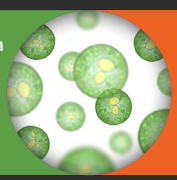
PROS
- Capable of eukaryotic post-translational modifications
- High yields possible
- Simple to culture



CONS
- Fermentation required for elevated yields
- Hyperglycosylation can occur

Algae

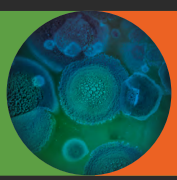
PROS
- Ideal for industrial enzyme/biofuel production
- Excellent for studying plant biology
- Easy to scale and high yield potential
- Not susceptible to virus/prion contamination



CONS
- Nascent technology
- Less-developed process

Fungi

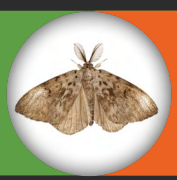
PROS
- Exceptionally high yield potential
- Easy to cultivate
- Product is secreted, making harvest simple
- Commonly used in industrial settings



CONS
- Glycosylation is different than in mammalian cells
- Internal quality control eliminates significant portion of expressed protein

Insect cell-baculovirus

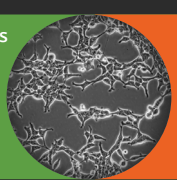
PROS
- Post-translational modification profile is similar to mammalian cells
- Effective protein folding
- More scalable than mammalian cells



CONS
- Virus production can be time consuming
- Tricky to culture
- Infection can induce premature lysis

Mammalian cells

PROS
- Most authentic products for human proteins
- Best preservation of native structure, function, and post-translational modifications
- Stable production cell lines can be created

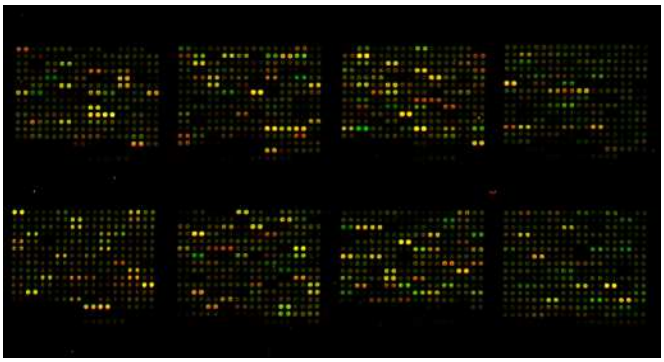


CONS
- Complex culture conditions
- Costly set-up and maintenance
- Low yields
- Scaling difficulties

Cell-free systems

PROS
- Fast and simple
- Highly malleable system
- Can produce cytotoxic, truncated, or otherwise modified proteins

CONS
- Difficult to scale
- Potentially expensive
- Susceptible to contamination/degradation



Recombinant Human Protein Applications

Recombinant protein production technology has enabled researchers to identify basic properties of uncharacterized proteins, investigate protein-protein interactions, and explore how certain proteins drive biological systems. It has also helped uncover potential therapeutic targets, study pathological mechanisms, and develop novel approaches for combating disease.

Finding Answers: Recombinant Proteins in Immunoassays

Researchers often use antibody-based laboratory assays to detect and study proteins. These assays measure signals from antibodies bound to a specific antigen. Purified recombinant proteins have improved the specificity, precision, and trustworthiness of these assays by serving as controls and standards.

- Recombinant protein controls and protein ladders can help confirm the identity of unknown bands in Western blotting.
- Purified protein samples with known concentrations are used to generate standard curves for colorimetric assays such as ELISA.
- Recombinant proteins can be used to establish and confirm antibody specificity, as long as the recombinant protein exhibits epitope affinity comparable to its native analog.

Untangling the Web: Using Recombinant Proteins to Examine Protein-Protein Interactions

Recombinant proteins can also be used to study protein-protein interactions. Historically, this was done through in vitro protein binding assays. These assays involved placing two proteins within the same environment and monitoring any interactions or binding that occurred. Recently, recombinant protein microarrays for examining protein-protein interactions have become popular. For this approach, researchers seed a slide with numerous immobilized proteins, which they then treat with a variety of molecules to examine how the two agents interact with one another. This allows for much higher throughputs when it comes to studying protein-protein interactions.

Using this system, scientists have studied protein interactions with other proteins or peptides, enzymes, small molecules, lipids, and nucleic acids[1]. Numerous new assays using microarray systems have been designed. These assays can rapidly and accurately identify enzyme substrates, characterize post-translational modifications, and probe antibody specificity[2].

The Next Level: Using Recombinant Proteins to Study Disease

Proteins mediate numerous cellular and systemic responses in vivo. Therefore, recombinant proteins give scientists tools for investigating natural systems and how they respond during homeostatic, stress, and disease situations. Cell and animal models are commonly treated with recombinant proteins and peptides to

- Induce acute responses (e.g., cell migration, angiogenesis, inflammation)
- Modulate cell differentiation or phenotype shifts (e.g., macrophages, stem cells)
- Elicit chronic disease states (e.g., angiotensin II infusion-induced abdominal aortic aneurysms, collagen-induced arthritis)

Similarly, recombinant proteins and peptides administered to animal models of disease can assist researchers in identifying novel potential therapeutic candidates.

Recombinant Proteins in Bioproduction for Biotherapeutics

Last but not least, the ability to produce large amounts of recombinant proteins is responsible for modern biotherapeutic production. For example, biosynthetic recombinant analogs for insulin have largely replaced animal- or human-sourced native peptides in both the laboratory and the clinic[3]. Other well-known mass-produced recombinant proteins include recombinant hormones, interferons, interleukins, hematopoietic growth factors (e.g., erythropoietin), tumor necrosis factors, blood-clotting factors, thrombolytic drugs, enzymes, monoclonal antibodies, and vaccines.

Recombinant protein production is also shaping new therapeutic fields. For example, custom-designed recombinant antibodies feature greater specificity than native antibodies, and do not require animal hosts for production[4,5]. They currently play a role in combatting cancer (e.g., CAR T-cell receptors), as well as HIV and other viral diseases. The importance of recombinant human proteins has increased rapidly for basic life science research, diagnostic reagents, and therapeutic drugs.

References

1. C.S. Chen, H. Zhu, "Protein microarrays," *Biotechniques,* 40(4):423, 425, 427 passim, 2006.
2. F.X. Reymond Sutandy et al., "Overview of protein microarrays," *Curr Protoc Protein Sci,* 0 27: Unit–27.1, 2013.
3. J.C. Mbanya et al., "Recombinant human insulin in global diabetes management - focus on clinical efficacy," *Eur Endocrinol,* 13(1):21-25, 2017.
4. G.A. Pietersz et al., "Therapeutic targeting in nanomedicine: the future lies in recombinant antibodies," *Nanomedicine (Lond),* 12(15):1873-1889, 2017.
5. A. Frenzel et al., "Expression of recombinant antibodies," *Front Immunol,* 4:217, 2013.

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