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Review

Recent advances in mammalian protein production

Ashok D. Bandaranayake [a,*], Steven C. Almo [a,b]

[a] Departments of Biochemistry, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461, United States
[b] Physiology and Biophysics, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461, United States

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ABSTRACT

Mammalian protein production platforms have had a profound impact in many areas of basic and applied research, and an increasing number of blockbuster drugs are recombinant mammalian proteins. With global sales of these drugs exceeding US$120 billion per year, both industry and academic research groups continue to develop cost effective methods for producing mammalian proteins to support pre-clinical and clinical evaluations of potential therapeutics. While a wide range of platforms have been successfully exploited for laboratory use, the bulk of recent biologics have been produced in mammalian cell lines due to the requirement for post translational modification and the biosynthetic complexity of the target proteins. In this review we highlight the range of mammalian expression platforms available for recombinant protein production, as well as advances in technologies for the rapid and efficient selection of highly productive clones.

1. Introduction

The ability to produce mammalian proteins in recombinant systems has had a profound impact in many areas of basic and applied research, as well as the biotech sector. While academic research groups require recombinant mammalian proteins for functional analysis (e.g. cellular signaling pathways) and high resolution structure determination, the biotech sector has heavily invested in the production of protein therapeutics (i.e. biologics), as a relatively new and transformative approach to treating human diseases. An increasing number of blockbuster drugs are recombinant mammalian proteins and the United States Food and Drug Administration (FDA) has approved over 100 recombinant protein therapeutics to date [1]. With this success, both industry and academic research groups continue to develop cost effective methods for producing mammalian proteins to support pre-clinical and clinical evaluations of potential therapeutics.

The need for multi-milligram quantities of recombinant mammalian protein in the academic sector is, in part, driven by focused and team-based structural biology initiatives, such as the New York Structural Genomics Research Consortium (NYSGRC) [2] [http://www.nysgrc.org/psi3-cgi/index.cgi], as well as pre-clinical research. The screening of 10–20 constructs may be required to obtain the multi-milligrams yields needed for successful structure determination [3]. As our understanding of cellular signaling pathways grow, so does the need for structural characterization of the signaling components and more importantly, structures of the relevant multi-component protein assemblies. As the tools for producing these mammalian proteins improve so does the complexity and value of the structures determined; a recent report describing the highly sought after ternary complex of the Wnt pathway proteins, R-spondin 1, RNF43 and LGR5, highlights this point [4].

By far the greatest demand for recombinant mammalian proteins is for therapeutic development and applications. The production of recombinant proteins for biopharmaceutical use is a multi-billion dollar industry, with global sales over US$120 billion per year and an anticipated increase to US$150 billion by 2015 [5]. Of the top nine classes of biologic drugs sold in 2011, monoclonal antibodies (mAbs) command the highest sales (Fig. 1) and this trend is growing [6].

While *Escherichia coli* (*E. coli*), yeast and insect cell lines are still the preferred platforms for many protein expression groups, mammalian proteins often require mammalian cells for optimum yields and activity. While a wide range of platforms have been successfully exploited for laboratory use, the bulk of recent biologics have been produced in mammalian cell lines due to the requirement for post translational modification and the biosynthetic complexity of the target proteins [1]. In the case of monoclonal antibodies, Chinese Hamster Ovary (CHO) and NS0 (mouse myeloma) cells are the most commonly used lines [7,8]. With the substantial growth being realized in the area of biologics, there is a continuing demand for new and enhanced mammalian expression platforms. The goal of this review is to highlight the range of mammalian platforms available for recombinant protein production as well as

* Corresponding author.
E-mail address: ashok.bandaranayake@einstein.yu.edu (A.D. Bandaranayake).

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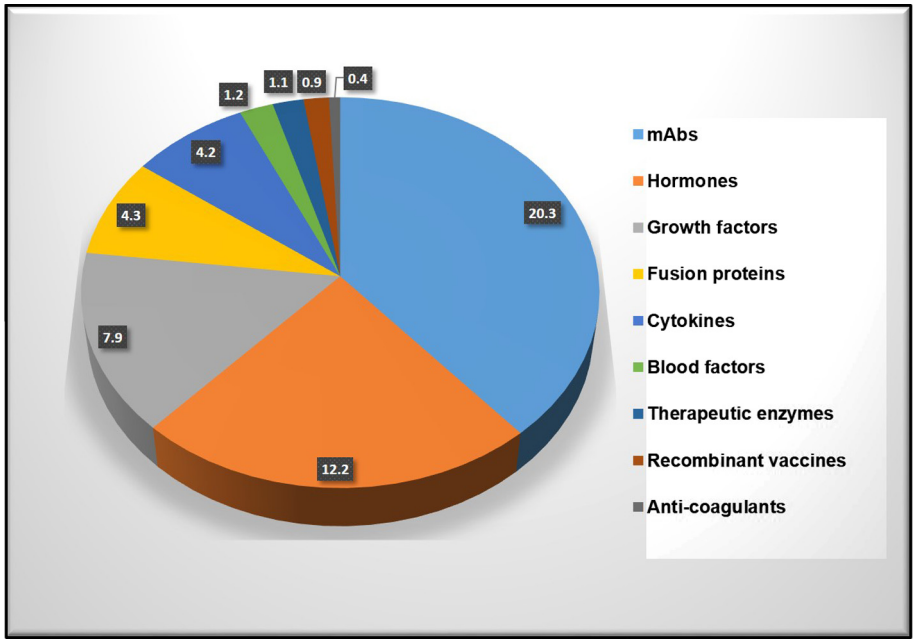

Fig. 1. US sales ($ billions) of top nine classes of biologic drugs in 2011. Adapted from Aggarwal et al. [6].

advances in technologies for the rapid and efficient selection of highly productive clones.

2. Expression platforms

2.1. CHO

The workhorse of mammalian protein production (especially at industrial scale) is the CHO cell line, isolated by Theodore Puck in the late 1950's [9]. Initially selected for radiation cytogenetic studies due to their low chromosome number ($2n = 22$), CHO cells have proven to be a hardy and reliable cell line in culture. Since the commercial introduction of human tissue plasminogen activator (tPA) as the first recombinant therapeutic protein produced from mammalian cells [10], the annual global revenue of products from CHO cells has increased to more than US$100 billion and continues to grow [11]. The wide spread success of the CHO platform is due to its unparalleled adaptability allowing for growth of these cells at high densities in suspension cultures and ease of adaptation to serum free conditions. There has been a great deal of improvement in the quality and availability of chemically defined, serum free media that is devoid of animal-derived materials. These tailored media are more cost effective, as they do not require supplementation with fetal calf serum; safer, as there is less risk of viral and prion contamination from bovine serum; and have simplified downstream processing requirements, as they contain fewer protein contaminants. In addition, a study in 1989 tested 44 human pathogens (including human immunodeficiency virus (HIV), influenza, polio, herpes and measles), and found the majority of them do not replicate in CHO cells, thus making CHOs ideal from a regulatory standpoint [12]. However, the adaptability of the CHO line also has its drawbacks, as each production target requires the selection of clones that exhibit the necessary phenotypic properties, including product quality/uniformity, doubling time and long term viability, under bioprocess conditions. Even when an appropriate CHO production clone has been identified, phenotypic drift (i.e. changes in the previously selected characteristics) is not uncommon and remains a challenge [11].

While enhancing clonal stability (i.e. product uniformity) is an area of intense study, the most significant improvements to CHO culture have resulted from the optimization of media, feeding strategies and downstream processing. These improvements have resulted in yields ranging between 2 and 6 g/L for antibody products [13]. A particular focus of product optimization in CHO cells is glycosylation, as it has been shown that variation in glycosylation patterns affects product stability and function, and non-natural glycoforms can be immunogenic [14–16]. This is particularly true of the terminal galactose-α-1,3-galactose (α-Gal) epitope, which has been demonstrated to be added to proteins produced in murine cell lines, and is capable of inducing an immune response in humans [17]. The importance of glycoform profiles in therapeutics has been highlighted by the adverse clinical effects associated with an induced IgE-mediated anaphylaxis response in patients treated with the commercial antibody Erbitux (cetuximab), which was manufactured in a murine myeloma cell line and possessed α-Gal epitopes [17,18]. It was thought that CHO cells lacked the biosynthetic machinery to produce the α-Gal epitope, but this assumption has been brought into question by reports that CHO cells can add the α-Gal antigen to recombinant products, as has been observed for Orencia (abatacept) [17]. Therefore, it is essential that CHO production clones be carefully monitored for product uniformity and safety in terms of glycosylation profiles.

The genomic variability of CHO cells and the fact that they are functionally hemizygous for many genes [19,20] also has certain advantages, as it has allowed for the isolation of mutant lines with deficiencies in metabolic enzymes. These mutants are dependent on certain nutrients for survival, making them ideal for the generation of producer lines by using the deficiency as a selection marker. Mutants of the enzyme dihydrofolate reductase (DHFR) were generated in this manner [21] and will be discussed below. To support further development of CHOs for protein production, a genome sequencing project was initiated in 2002 as a collaboration between the University of Minnesota and the Bioprocessing Technology Institute of Singapore (A*STAR). This collaboration led to two cDNA libraries being constructed from three CHO cell lines, grown under different conditions, corresponding to over 4000 expressed sequence tags (ESTs) [22]. This initial study led to further sequencing efforts under the auspices of the Consortium on Chinese Hamster Ovary Cell Genomics in partnership with the Society