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Perspectives

Integration of systems biology in cell line and process development for biopharmaceutical manufacturing

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ABSTRACT

The evolution of cell line and bioprocess development for biopharmaceutical manufacturing using mammalian cell culture over the past few decades has been striking. Despite this success, the future presents new challenges that include productivity increase, product quality modulation for comparability and biosimilarity, and efficient manufacturing of novel modalities. While empirical process development techniques will continue to play an important role in addressing these challenges, approaches based on mechanistic understanding are likely to be more impactful for solving the more complex multidimensional problems by providing insights into the interplays between the cell line, bioprocess, and product quality. Systems biology is one such approach that provides information on cellular physiology at the molecular level, which, when rigorously interpreted, can provide targets for cell line and/or process modifications. We present a general framework for applying systems biology to biotherapeutic-producing mammalian cells and summarize published work that exemplifies successful applications of this technique. We highlight gaps in our current understanding that limit widespread application of systems biology to mammalian cell-based bioprocess development and propose remediation methods that can encourage increased adoption. More nuanced understanding of cellular physiology and the interplay between expressing novel proteins and product quality attributes is possible through systems biology and this understanding will better position the field to successfully engage with the challenges of the future.

1. Introduction

The contribution of biopharmaceuticals to recently approved therapeutic entities has been steadily increasing [1]. In 2004, biopharmaceuticals made up 12% of all prescription drugs that were approved, and this number is expected to double by 2020 [2]. Additionally, in 2020, more than half of the top 100 medications are expected to be biopharmaceuticals [2]. Biopharmaceuticals are manufactured using biological host organisms, and while a variety of hosts can be used, mammalian cells have been the host of choice over the last two decades [1,3]. Complex biologics, especially those that involve post translational modifications (PTMs), require a mammalian cell host to produce a molecule that has the desired *in vivo* activity [4]. Recognizing the dominant role of mammalian cells in biopharmaceutical manufacturing, substantial advances in improving protein productivity and product quality, both from cells line and bioprocess standpoints, have been made in the last decade and these efforts will continue into the future [5].

However, the landscape of biopharmaceutical discovery, development, and commercialization is rapidly changing. Development and commercialization costs continue to rise and there is increased competition even at the point of new biologics introduction [6]. Comparability criteria continue to be more rigorous and biosimilars approvals in Europe [1] and recently in the US [7] reduce originator product exclusivity while creating opportunities for those interested in developing biosimilars. With no exposure to the originator's cell line and process conditions, development of high quality biosimilar molecules is challenging. Sophisticated understanding of how cell lines respond to process variable changes and variations in incoming raw materials are essential to design a robust process to meet comparability and biosimilarity criteria. Furthermore, while monoclonal antibodies have been the dominant modality for currently licensed biologics, novel non-antibody modalities are likely to be a higher percentage of licensed commercial biologics in the future and present unique process development challenges [8,9].

While traditional, empirical approaches to cell line and process development will continue to play an important role as the indus-

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try embraces this changing landscape, the need to fundamentally understand the relationships between cells and bioprocesses and their impact on product quality will increase. Such fundamental understanding can be a differentiator when stringent comparability targets have to be met for late-stage processes, for biosimilars to robustly match originator product quality attributes, and for novel modality biologics to have the desired *in vivo* efficacy. Systems biology is one discipline that enables molecular-level understanding of observed cell line and bioprocess phenomena [10]. Recent advances in this field have made the approach more powerful and accessible which collectively enable the possibility to assign cause-effect relationships that are mechanism driven rather than empiricisms [11–13].

In this communication, we examine some impactful advances to date in the context of mammalian cell culture and articulate how systems biology can influence the next phase of mammalian cell culture process development. A summary of previous publications that highlight the cradle to grave journey of systems biology applications (mechanistic understanding followed by target identification and validation that ultimately resulted in cell line/process changes to obtain the desired outcome) will be provided to demonstrate the potential of systems biology to meaningfully influence mammalian cell culture process development. Existing gaps that limit widespread applications of systems biology in mammalian cell culture process development will be highlighted and suggestions to address them will be provided to enable more widespread utilization of this approach to advance biopharmaceuticals to patients.

2. Enablers for application of systems biology in mamamlian cell culture

Systems biology is a holistic approach that focuses on complex interactions of basic components within biological systems [14,15]. It allows exploration of the entire sequence of events starting from gene expression through protein and metabolite production. When experiments are carefully designed and the resulting high-density multi-dimensional data collectively gathered and analyzed, it is possible to have a deep understanding of the underlying mechanistic changes that take place inside the cell. Systems biology approaches accomplish this by employing a spectrum of approaches, including systems engineering, systems characterization, and systems analysis. Systems engineering tools are required to convert a complex hypothesis into a controllable and reproducible experiment. Systems characterization is necessary for monitoring the experiment and for generating a rich high-dimensional and/or high-throughput data set. Lastly, translating this comprehensive data set into knowledge and actionable items for cell line/process modifications requires both statistical and computational data analysis techniques. Technology advancements in all these dimensions have enabled successful applications of systems biology for bioprocess development. For example, genome editing tools (engineering), -omics techniques (characterization), and genome-scale modeling of cellular metabolism (modeling analysis) have greatly enhanced the application of systems biology to bioprocess development with microbial hosts [11,16,17]. While more challenging, the application of systems biology to more complex hosts such as mammalian cells, is also rapidly emerging [10,12,17,18]. The primary enablers for systems biology applications to Chinese hamster ovary (CHO) cells, the most prevalent mammalian cell lines in the biopharmaceutical industry, are summarized below.

2.1. Multi-omics technologies for systems characterization of CHO cells

The ability to characterize cellular machinery of CHO cells is a major enabler for CHO systems biology. This ability has been greatly enhanced by recent advances in multi-omics technologies in terms of speed, cost, and robustness. The release of the CHO-K1 genome in 2011 [19] provided a comprehensive part list for CHO cells for the first time. Since then, a series of genomes for CHO cell lines as well as the Chinese hamster species have been published [20,21]. The public availability of CHO genome sequences has supported the development and application of other -omics approaches such as transcriptomics and proteomics [12]. For CHO cells, comparative transcriptome analysis has enabled identification of gene expression differences between different cell lines or different cultivation conditions [22–24]. Increasing availability of transcriptome information has resulted in better annotation of CHO genomes. Proteomics identifies the global landscape of proteins both inside and outside the cells [25]. Successful applications of proteomics on CHO cells have been reported using a combination of approaches [26]. A public proteomics database consisting of both intracellular and secreted CHO proteins is now available [27]. Metabolomics provides global identification and quantification of both intra- and extra-cellular metabolites in CHO cells and is a good representation of CHO cell physiology because metabolites are the end products. Fluxomics provides functional assessment of CHO cell metabolism by characterizing the reaction rates of metabolites within the cell [12]. Methods such as flux balance analysis (FBA) and isotope-based metabolic flux analysis (MFA) have become powerful tools to study individual metabolic pathways in CHO cells [28,29]. Overall, high resolution and dynamic characterization of cellular components in CHO cells enabled by fast-advancing -omics technologies will provide a wealth of cellular-level knowledge of CHO cells, which can feed into the needs for mechanism-driven cell line development and process optimization.

2.2. Systems engineering of CHO cells enabled by gene editing tools

The ability to redesign CHO cells is another major enabler to realize the potential of systems biology. Engineering of CHO cells is an important avenue to improve productivity and product quality, especially when adjusting process parameters alone is not enough to achieve the desired goal. One major output of systems biology is the identification of targets for genetic engineering which need to be validated by making edits to the CHO cell genome. Rapid and precise modification of mammalian cell machinery has recently been enabled by emerging genome editing technologies such as transcription activator-like effector nucleases (TALENs), zinc-finger nucleases (ZFNs), and the clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 system [30]. The recent availability of CHO genome references [19,20] provides a genetic map for applying these techniques to CHO cells and this combination allows systems biology target validation in a fraction of the time and cost compared to what was typically needed before. Consequently, we can expect a shift in future systems biology applications where, unlike today, the scope will not be limited to the application of -omics technologies but will rather be extended to include target validation and ultimately proof of concept dimensions. When this transition happens, the pragmatic impact of systems biology on CHO cell process development will be substantially amplified and can result in more widespread adoption of systems biology approaches for process development.

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