Pharmaceutical BIOPROCESSING

Productivity: a minor consideration when looking at mammalian cell culture performance from the systems perspective

"...it is crucial to increase the number of cell lines under investigation to accurately capture both directional and random expression patterns associated with cell line identity and diversity."

Keywords: Chinese hamster ovary cells • DHFR • expression • monoclonal antibodies • proteomics • product quality • productivity • selective pressure • systems biology • transcriptomics

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In contrast to small molecule drugs which can be synthesized chemically, recombinant protein-based drugs are produced from living cells that are genetically modified to generate the desired products. While some biologics can be produced using prokaryotic or lower eukaryotic expression hosts such as yeast or insect cells [1,2], mammalian expression hosts are often used for the production of therapeutic glycoproteins such as monoclonal antibodies due to similar glycosylation patterns as those found in humans [3]. Chinese hamster ovary (CHO) cells remain to be the preferred choice of the biopharmaceutical industry for the manufacturing of recombinant glycoproteins, with more than 70% of therapeutic products reported to have been generated using CHO cells as the production host [4,5].

For each therapeutic product, a new production cell line needs to be developed from a host cell-line [6,7]. The process of making a producer cell line with high production capacity and desired product quality for each of the therapeutic recombinant proteins is highly demanding and requires time and labor-intensive steps [6–8]. Traditional methods involve introducing the product and selection genes into the host via transfection, followed by gene amplification and single-cell cloning. Due to rare and random events of genome integration and further alterations mediated by the amplification process, surviving populations in the presence of selection agent during the cell-line development process are highly heterogeneous. Thus, limiting dilution or single-cell cloning procedures are performed to obtain a single progenitor origin, followed by progressive expansion into sufficient number of cells to assess productivity and other attributes. Therefore, the entire duration of cell-line development typically spans several months and involves extensive screening of multiple pools and hundreds of clones before the final clonal production cell line is selected.

Despite the same host origin, the extent of phenotypic variability among different recombinant cell lines can be dramatic. Cell line-specific variations in functional attributes such as proliferation rate, productivity, cell size and metabolic profiles, as well as product glycosylation patterns, are frequently observed, and these variations can, in turn, affect process optimization parameters. During multiple rounds of screening and clonal expansion in the presence of selection agent(s), production cell lines acquire survival advantage to withstand selective pressure. During this microevolutionary process, different types of genetic as well as nongenetic alterations are expected to accu-

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mulate and contribute toward phenotypic variability observed among different recombinant cell lines. With the recent advances in CHO genome sequencing [9,10] and 'omics technology [5,11–13], these 'evolutionarily' derived recombinant cell lines can be examined in depth for their intrinsic properties defined by genetic composition and expression profiles.

From the perspective of industrial bioprocessing, productivity and product quality are the two most important criteria considered during clone selection and process optimization. It is thus of great interest to obtain fundamental biological understanding around these functional parameters; such endeavor could help to identify prediction markers and bottlenecks associated with these functional attributes to further improve process conditions across different scales. One of the specific approaches would include taking advantage of the phenotypic diversity observed among various production cell lines and compare them to genotypic diversity. Various cell lines with previously determined stability, productivity and product-quality profiles can be utilized as study models and compared directly against each other for the assessment of intrinsic properties.

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The recent evaluation of 14 such recombinant cell lines expressing various monoclonal antibody sequences showed rather unexpected results at the global level of expression architecture [14]. Although each of these cell lines were derived from the same clonal CHO host, expression segregation into two distinct clusters was clearly depicted by principal component analysis of transcriptomics and proteomics data. The observed gene-expression polarity was the result of a substantial number of genes that were upregulated in one group of cell lines while downregulated in the other, and *vice versa*. Differential expression patterns observed between the two groups of cell lines appears to exemplify the gene network dynamics model which illustrates gene expression changes occurring as a whole, rather than independently, due to the regulatory interactions and the resulting constraints generated by these interactions [15,16]. The network dynamics model also points out that substantial excess of expression for one set of genes over another can lead to stable equilibrium state, while having both sets of genes equally expressed leads to a highly unstable state due to the opposing effect created by two sets of genes inhibiting each other. It is possible that this type of model also applies to CHO cells as they differentiate from the parental host to the recombinant production cell lines. The pressure to efficiently amplify and express the exogenous genes of interest can result in a major rewiring or shifting of genetic expression structure as these cells struggle for survival under selective pressure. The expression polarity observed between the two clusters of cell lines suggests the existence of two distinct equilibrium states toward which differentiating recombinant cell lines are converting. The specific contributing factor(s) responsible for determining to which of the two equilibrium states a particular recombinant cell line assimilates is yet to be identified. The possibility of transcription factors and other upstream regulators playing a role as a 'molecular switch(es)' that controls expression directions for myriads of downstream genes and subsequently defining the specific equilibrium state is currently being explored.

Interestingly, the expression polarization detected between the two groups of cell lines did not correlate with productivity or any other major functional attribute of recombinant antibody production [14]. The observation that productivity is not the functional determinant associated with any of the predominant components of expression structure implies that productivity constitutes a rather minor status in the context of global transcription or protein expression space. It is yet to be determined whether the prominent layer of expression architecture displaying bimodal expression direction reflects the mode of survival or just simply illustrates the shift in the expression equilibrium. It has previously been reported that in the yeast model, genes that are essential for viability tend to have lower levels of expression fluctuations, because too much variability in essential genes could be detrimental for the organism [17]. If a similar trend also applies to CHO cells, we can anticipate that the genes displaying high levels of expression polarization, as depicted by the primary component of the expression architecture, are likely to be nonessential, and thus survival would not be considered a predominant functional attribute associated with expression polarity.

In theory, the DHFR/methotrexate (MTX) expression system is designed to take advantage of a close dependency between survival and productivity for effective screening. However, decoupling of these two parameters is rather easily achieved by those cell lines exhibiting low productivity. The global expression analysis of various recombinant cell lines constituting a wide range of productivity provides valuable insight into how such decoupling between survival and productivity can be established to enable the survival of

low producers in the presence of high-selection stringency. Such investigation could lead to identification of potential 'leakage' in the expression system often created by low-productivity cell lines in their attempt to overcome the selection pressure without having to produce high amount of recombinant proteins. For example, the reduced levels of DHFR to MTHFD2 protein ratio detected in low-productivity cell lines is likely to reflect a compensatory mechanism for survival for the cells with insufficient levels of recombinant DHFR by enhancing tetrahydrofolate (THF) production in the mitochondria to reduce the stringency for DHFR expression [14].

To a certain extent, low-productivity cell lines share similarity to cancer cells exhibiting drug resistance. During tumor progression, drug resistant clones acquire either activating mutation or alterations in the protein expression levels which would allow the cells to become resistant to drug-induced apoptosis [15]. Similarly, such genetic or nongenetic modifications can also take place in the recombinant CHO cell lines as they become subjected to selection pressure for survival under the absence of key nutrients (e.g., hypoxanthine and thymidine) and/or high concentrations of MTX, a chemotherapeutic drug known to possess teratogenic activity [18]. Although ideally the DHFR/MTX selection system is designed to eliminate the clones with insufficient levels of recombinant DHFR expression, a small fraction of cells may acquire resistance by obtaining an alternative means to survive without having to express a large amount of recombinant proteins. If these resistant variants gain proliferation advantage over other populations of cells, within several rounds of expansion a shift in the phenotype (i.e., lower productivity) could be detected. Considering the inherent genomic plasticity of CHO cells [19,20], heterogeneity of phenotype in a clonal cell population would be unavoidable as the round of expansion increases. As such, a decrease in productivity and increase in proliferation rate could be detected over time as these cells age and accumulate population doubling time. It would be beneficial to track genome-wide changes in the transcript and protein-expression profiles as the clonal populations mature over an extended period of time to assess whether progressive changes in the gene network dynamics and shift in expression equilibrium can be observed in the context of productivity and other functional traits of interest.

As we embrace the emerging era of systems biology for mammalian cell culture process, the intrinsic robustness and fragility of the recombinant production cell lines under the influence of time and external factors can be assessed at the systems level using advanced analytical and computational tools. It is, however, important to acknowledge and address the existence of a multilayered structure of genomic and expression space across different cell lines. Thus, it is crucial to increase the number of cell lines under investigation to accurately capture both directional and random expression patterns associated with cell line identity and diversity. This approach would help us to avoid making erroneous association and allow us to wisely distinguish the 'driver' from 'passenger' genes associated with cellular phenotypes of interest, including productivity in spite of its seemingly minor status in the context of an overall expression architecture. The ability to examine both the systems level properties and functional correlation is a powerful tool we can take advantage of as we strive toward gaining additional insights into regulatory and functional components of productivity and product quality attributes of therapeutic recombinant protein production.

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