Shifting the Balance

Transient transfection has the unique ability of facilitating progression of superior candidates through development, as well as accelerating the generation of quality stable cell lines. This can help to mitigate early risk, while bridging the gap with manufacturing to decrease time to market

Karen Donato, Krista Steger and Meg Duskin at MaxCyte, Inc

Successful biopharmaceutical companies must moderate development risks by reducing early-stage expenditures and lowering late-stage attrition rates. As a result, companies have looked to transient gene expression (TGE), rather than developing stable cell lines early in the development process, as a means to rapidly screen large numbers of antibodies or antibody-like molecules in order to identify promising candidates for further evaluation (1-2). At what point, however, does the balance shift between reduced risk via cell line development and the increased productivity of a stable cell line?

Stable cell lines offer consistent product quality, regulatory familiarity and lower costs for large-scale manufacturing. In addition, they produce the milligram to gram quantities of proteins required for full product development and biomanufacturing. For these reasons, stable cell lines have been the standard for protein production for more than two decades.

Yet stable cell line generation is an extended process that takes many months, even with improved cloning and expression technologies, and is costly in terms of labour and materials. These costs are particularly onerous during early-stage development of therapeutic antibodies, when stable cell lines must be created for hundreds of candidate antibodies. Furthermore, stable cell lines may not be possible to generate for all applications.

In recent years, researchers have turned to TGE as an alternative to stable cell line development for early-stage protein production when lower quantities of antibody are needed. With transient transfection, a variety of proteins – including antibodies, biochemical targets of interest, vaccines, viral vectors and virus-like particles – can be rapidly expressed. Using TGE as part of these activities is a sound economic decision if the system of choice produces antibodies: firstly, in sufficient quantities to effectively delay cell line generation until later in the development pipeline; and secondly, with similar biophysical properties to those produced by stable cell lines to ensure identification of relevant candidates. Unfortunately, early TGE methods have struggled in fulfilling both of these qualifiers.

Transfection Methods

A variety of transfection methods are viable options in the production of low milligram quantities of antibodies, which are sufficient for very early-stage candidate identification. However, high milligram to gram quantities are needed for mid- to late-stage development activities such as pharmacology, stability and manufacturability studies. Therefore, the performance and scalability of the transfection method greatly affects the ability to delay stable cell line production.

Transient transfection technologies have evolved from simple chemical carriers – such as DEAE-dextran, calcium phosphate and polyethylenimine (PEI) – into sophisticated, highly engineered methodologies – such as lipid-based reagents, viral-mediated delivery and high-throughput electroporation (3).

Chemical carriers like PEI, while inexpensive, often yield inconsistent results, are challenging to scale-up and are inefficient for many cells types. Virus delivery, lipid-mediated transfection and electroporation generally provide enhanced performance and are more reproducible, but differ significantly in scalability, time requirements and cost.

Viral delivery methods are scalable and achieve a high level of transfection efficiency – however, the creation of viral vectors and production of viral stocks requires extensive time and labour commitments, in addition to specialised technical expertise. Moreover, viral vectors have limited cell type flexibility, can impact protein yield due to purification challenges, and face further regulatory hurdles when used to produce proteins for clinical use.

Newer lipid-based technologies and electroporation have relatively high transfection efficiencies and an ability to transfect a range of cells – including mammalian cells, which are of great interest for biotherapeutic development. Lipid-mediated transfection can be scaled with reoptimisation, but can quickly become cost-prohibitive.

Electroporation has broad cell type flexibility and is much simpler than viral-based gene delivery methods. While cuvette-based electroporation is logistically difficult to scale, large-scale flow electroporation is available to overcome this limitation. As a result, flow electroporation provides a practical solution to the time, labour and cost challenges of stable cell lines, while overcoming the difficulties associated with other transient transfection methods.

Host Cells and TGE

Equally important to the method or platform used for the expression of recombinant proteins is the host cell. Mammalian cells – in particular,

Chinese hamster ovary (CHO) cells – remain the most commonly used system in the biomanufacturing of clinical grade antibodies. CHO cells have the capacity for high-level production of heterologous proteins with post-translational modifications similar to those of humans; can be cultured at high cell densities in chemically defined, protein-free media; and have a strong regulatory track record (4).

Initial CHO-based TGE activities were limited by poor transfection efficiencies and production of inadequate quantities of antibodies. This led to the use of transient expression systems based on human embryonic kidney (HEK) cells, which are easily cultured in suspension and generally have higher transfection efficiencies and antibody production capabilities (5). Literature shows, however, that there are differences in post-translational modifications of antibodies produced in HEK cells compared to those produced by CHO cells, thereby increasing the risk of later-stage developmental failures due to alterations in biophysical properties upon migration to CHO-based stable expression (6-7). In response, there has been a strong interest in developing large-capacity, CHO-based TGE systems for improved identification of viable therapeutic antibody candidates.

There are a number of reported TGE transfection protocols for CHO antibody expression using electroporation, PEI and lipid-based reagents. These methods vary widely in transfection performance, reproducibility and scalability – all features central to large-scale antibody production. PEI and lipid-based transfection result in optimised antibody titers ranging from 10 to 250mg/L (7-12).

Although these antibody yields represent improvements over the historic single digit milligram per litre titers of transient systems, they remain substantially less than the 3-10g/L productivity of stable CHO cell lines (13). Due to this disparity, during mid-stage antibody development, researchers are faced with balancing production needs and the expenditure of resources for cell line generation. In contrast to PEI and lipid-based transfection, flow electroporation has reported titers exceeding 1g/L within two weeks of transfection (14). The simple scalability of this technology enables the production of over 50g of antibody from a 50L culture following a single transfection. This level of productivity and scalability can significantly defer migration to stable expression systems to later stages of antibody development.

CHO Cell Engineering

An additional approach for increasing CHO productivity relies on genetic engineering of CHO cell lines and expression vectors through the over-expression of host genes or the introduction of viral elements. For example, researchers have engineered cells to increase transgene expression, reduce protein misfolding, or promote specific post-translational modifications (15-16). Others have focused on prolonging CHO cell viability and augmenting antibody production by regulating cellular metabolism and reducing the induction of apoptosis (17). Several recent papers report CHO antibody titers between 800 and 1,000mg/L following the creation and optimisation of modified CHO cell lines (18-19).

However, engineered CHO cell lines such as these are generally not commercially available, or their use is restricted through patents. Most frequently, researchers must generate, characterise and, subsequently, optimise engineered CHO cells from the ground up, prior to use as vehicles for TGE. Gram per litre productivity levels may ultimately be reached with the newly engineered cells for expression of a specific antibody candidate, but the universality of the engineered CHO cells for the expression of a wide array of antibodies is not ensured. The time and labour costs associated with development of engineered CHO cells for early-stage TGE must be evaluated against the anticipated gains in productivity compared with non-engineered cells, particularly in light of breakthroughs in universal CHO TGE systems.

The Balance Shift

During later stages of product development, the balance in antibody production needs shifts, favouring the use of stable expression systems. The stable cell line development process generally involves the introduction of genetic material via (co)transfection, creation and expansion of stable pools using selective pressure, single cell cloning, identification of high-performers through screening, characterisation of the final clone and production of the master cell bank.

Enhancements to methods early in the process, particularly transfection, can have substantial impacts on downstream procedures and outcomes. Several decades ago, the cell line development process often resulted in stable clones yielding less than 1g/L protein titers (20). New recombinant technologies and vast process improvements have reduced stable cell line development timelines to 20-30 weeks and increased clone productivity, with typical yields ranging from 3 to10g/L for cell lines used in biopharmaceutical manufacturing (13,20).

Historically, lipid- and electroporation-based transfection methods were used for (co)transfection of expression plasmids or cassettes during cell line generation (20). While not all electroporation methods perform equally well, electroporation as a whole has increased CHO transfection efficiencies, higher post-transfection cell viability, and the ability to empirically control plasmid DNA concentrations and/or ratios when compared with chemical- or lipid-based transfection methods (14). As a result, electroporation-based methods are more likely to:

- Produce a large pool of high expressing cells
- Allow for more stringent selection pressure
- Reduce cell recovery times post-selection
- Abrogate the need for selective cloning methods such as fluorescence activated cell sorting (FACS) or automated clone picking
- Minimise the number of clones that must be screened to identify high producers

Advanced electroporation methods with increased transfection efficiencies and cell viabilities have more significant impacts on these positive attributes.

A recent publication, which documented >1g/L antibody titers via TGE using advanced electroporation, also reported the generation and identification of a high-yield stable CHO clone within two months using the same transfection technology (14). The top-performing clone produced 3g/L of antibody and was identified upon screening of fewer than 500 clones.

Stable Impact of TGE

Approved manufacturing processes for human therapeutic antibodies have historically been based on stable cell clones. In the near term, at least, this will continue to be the case due to regulatory hurdles necessitating the eventual generation of stable cell lines. Nevertheless, transient transfection is a unifying method with impacts on both biotherapeutic development and manufacturing. Transient expression using advanced transfection methods has the capacity to produce gram to multi-gram quantities of therapeutic proteins, enabling more rapid and cost-effective identification and mid-stage development of quality candidates. Separately or in parallel, high-performance transient transfection systems can generate quality stable pools that can rapidly be screened to identify high-yield stable clones.

There may come a time when the balance will shift away from stable cell line development for antibody manufacturing, as it has for multiple, FDA-approved recombinant therapeutic vaccines that are produced using transient-based expression. Until a sufficient number of therapeutic antibodies manufactured via transient expression are approved by the FDA – thereby lowering the regulatory hurdles faced during the approval process – biopharma companies will have to continue fine-tuning the balance between transient versus stable expression systems.

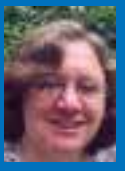
References

1. Strategies for rapid production of therapeutic proteins in mammalian cells, *BioProcess Int* 10(4): pp32-48, 2012
2. Recombinant protein production by large-scale transient gene expression in mammalian cells: State of the art and future perspectives, *Biotechnol Lett* 29(5): pp677-684, 2007
3. The inside scoop, evaluating gene delivery methods, *Nat Methods* 2: pp875-883, 2005
4. CHO cells in biotechnology for production of recombinant proteins: Current state and further potential, *Appl Microbiol Biotechnol* 93(3): pp917-930, 2012
5. Reflections on more than 10 years of TGE approaches, *Protein Expr Purif* 64(2): pp99-107, 2009
6. Differences in the glycosylation of recombinant proteins expressed in HEK and CHO cells, *J Biotechnol* 161: pp336-348, 2012
7. High-level protein expression in scalable CHO transient transfection, *Biotechnol Bioeng* 103(3): pp542-551, 2009
8. A high-yielding A CHO-K1-based transient transfection system, *BioProcess Int* 11(1): pp28-35, 2013
9. Design of experiment in CHO and HEK transient transfection condition optimization, *Protein Expression Purif* 78(1): pp61-68, 2011
10. Transient transfection factors for high-level recombinant protein production in suspension cultured mammalian cells, *Mol Biotechnol* 39(2): pp141-153, 2008
11. Scalable transient gene expression in Chinese hamster ovary cells in instrumented and non-instrumented cultivation systems, *Biotechnol Lett* 29(5): pp703-711, 2007
12. A simple high-yielding process for transient gene expression in CHO cells, *J Biotechnol* 153(1-2): pp22-26, 2011
13. Expression of antibody in mammalian cells, in Al-Rubeai M (ed), *Cell Engineering*, Springer Science and Business Media, Dordrecht, 2011
14. CHO-S antibody titers >1 gram/liter using flow electroporation-mediated transient gene expression followed by rapid migration to high-yield stable cell lines, *J Biomol Screen*, In review
15. Maximizing antibody production in suspension-cultured mammalian cell by the customized transient gene expression method, *Biosci Biotechnol Biochem* 77(6): pp1,207-1,213, 2013
16. Engineering the chaperone network of CHO cells for optimal recombinant protein production and authenticity, *Methods Mol Biol* 824: pp595-608, 2012
17. Use of an anti-apoptotic CHO cell line for transient gene expression, *Biotechnol Prog* 29(4): pp1,050-1,058, 2013
18. A high-yielding CHO transient system: Coexpression of genes encoding EBNA-1 and GS enhances transient protein expression, *Biotechnol Prog* 30(1): pp132-141, 2014
19. A CHO cell line engineered to express XBP1 and ERO1-Lα has increased levels of transient protein expression, *Biotechnol Prog* 29: pp697-706, 2013
20. Mammalian stable expression of biotherapeutics, in Voynov V and Caravella J (eds), *Therapeutic Proteins: Methods and Protocols, Methods in Molecular Biology*, Springer Science and Business Media, 2012

About the authors

Karen Donato is Executive Vice President, Global Business Development and Marketing at MaxCyte, Inc. She has a PhD in Chemical Engineering from Ohio University, US, and broad experience in new business development, R&D and operations in multinational, mid-size and startup companies. **Email: kdonato@maxcyte.com**

Krista Steger is President and founder of Forge4ward and a consultant to MaxCyte, Inc. She has over 15 years of experience in global scientific marketing at leading biotechnology companies and startups. She earned her PhD in Cell and Molecular Biology at the University of Wisconsin-Madison, US. **Email: ksteger@forge4ward.com**



Meg Duskin is the Marketing Communications Manager at MaxCyte, Inc. She has a background in biology, and more than 20 years of experience in marketing and communications, specialising in digital marketing. **Email: mduskin@maxcyte.com**