TRANSCRIPTIONAL RESPONSE TO RECOMBINANT PROTEIN PRODUCTION IN ISOGENIC MULTI-COPY CHO CELLS

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While advances in CHO bioprocessing have led to significant improvements in protein titers over the past three decades, still many challenges remain in the field of cell line engineering[1]. The development of new CRISPR/Cas genome editing tools and the availability of genome sequencing data have allowed precise rational engineering of CHO cells[2]. However, cell productivity remains a complex trait to elucidate. Among different approaches, a holistic analysis of the cellular response to protein production through transcriptomic studies may facilitate our understanding and help to find new engineering targets.

To explore how the cells respond to increased protein production, we generated a panel of CHO cells by multi-copy targeted integration where one, two or four copies of our genes of interest (GOI) - Erythropoietin (EPO) or Etanercept (ETN) - were integrated into specific genomic sites supporting high heterologous expression (site A and T9). This platform allowed us to perform robust comparative studies, due to the low clonal variation. Using RNA-seq, we investigated both common and recombinant protein-specific patterns of differential gene expression.

Overall, this study unraveled bottlenecks that occur with an increase in gene dosage. Surprisingly, the bottleneck observed was at the transcript level rather than at the protein level (Figure 1). Transcriptomics analysis uncovered differentially expressed genes that were site-specific, EPO or ETN specific, and related to the increased gene dosage. Several cell line engineering strategies based on the transcriptomics analysis results are currently conducted to alleviate the transcriptional bottleneck. We will present the results of how these engineering strategies affect the transcription and production of our GOI in multiple copies.

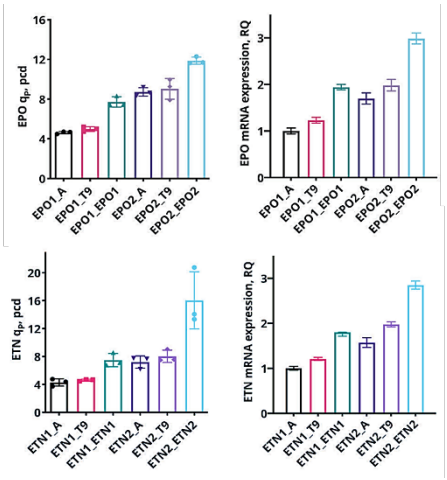

Figure 1 – Transcriptional bottleneck in both EPO and ETN clones in different sites (A and T9). From one to four copies, qPs (left) and correspondent transgene mRNA levels (right) increase of 2.6 and 3.2-fold, respectively, instead of 4.

(1) Templeton, N., and Young, J. D. (2018) Biochemical and metabolic engineering approaches to enhance production of therapeutic proteins in animal cell cultures. Biochemical Engineering Journal.
(2) Lee, J. S., Grav, L. M., Lewis, N. E., and Faustrup Kildegaard, H. (2015) CRISPR/Cas9- mediated genome engineering of CHO cell factories: Application and perspectives. Biotechnol. J. 10, 979–994.