WHAT DOES A CELL NEED FOR EFFICIENT PROTEIN SECRETION: DECIPHERING, MODELING, AND AUGMENTING THE CHO MACHINERY

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In biomanufacturing, we harness the natural machinery used by mammalian cells to synthesize and secrete thousands of diverse proteins in the human body. However, it remains unclear what truly governs the production of each protein and influences the amount of protein that is successfully secreted, including general features influencing all secreted proteins and features that are specific to individual protein products.

To quantify the general features driving protein secretion, we aimed to produce 2165 human-secreted proteins in CHO cells and quantified their expression. Using machine learning, we quantified different protein features and found specific biophysical and sequence features associated with higher productivity; however, protein features could only explain up to 15% of the variation in protein secretion. We further conducted RNA-Seq on cells producing 95 of the different human secreted proteins and found host cell gene expression features could explain up to 75% of the variation in protein secretion. Key pathways associated with higher titers included reactive oxygen species metabolism, N-linked glycosylation, and various processes in the secretory pathway, such as expression of the derlin family of proteins. To further identify proteins to co-express to boost titer, we used HEK293 cells to express proteins that express particularly poorly in CHO cells. A comparison of gene expression of the secretory pathway in the CHO and HEK cells helped to identify host cell factors more highly expressed in HEK293 cells that could be expressed in CHO cells to boost productivity. Thus general factors supporting protein secretion could be found and implemented to boost productivity.

While identifying general modulators of protein secretion has great value, many secreted proteins have unique needs for their proper synthesis, folding, post-translational modification, and transport. Thus, to identify the product-specific machinery, we deployed proximity biotinylation proteomics to quantify the protein interactions supporting the expression of specific therapeutic proteins. Applying this to a panel of Rituximab-producing CHO clones, and using a novel concept from queuing theory, machine learning, and a network reconstruction of the CHO secretory pathway, we deployed a systems biology approach to identify which steps in the secretory pathway act as bottlenecks for clones with lower secretion levels.

Thus, through a mix of omics assays and computational modeling, we are identifying more global regulators of protein secretion and product-specific requirements. Through this we are gaining a clearer picture of the mammalian secretory pathway and how it functions to secrete diverse recombinant biotherapeutics of interest.