LEVERAGING SINGLE-CELL AND BULK TRANSCRIPTOMICS TOWARDS IMPROVED INSECT CELL FACTORIES FOR BIOPHARMACEUTICALS

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The insect cell - baculovirus expression vector system (IC-BEVS) has emerged as a time- and cost-efficient alternative production platform for a variety of biopharmaceuticals. However, product titers and/or quality are still often below those achieved in other industrial-relevant cell lines and major improvements to IC-BEVS remain limited by poor understanding of the underlying biological mechanisms driving host cell response to baculovirus infection and efficient expression of recombinant genes. This work leverages single-cell RNA-sequencing (scRNA-seq) and bulk RNA-seq of baculovirus-infected Sf9 and High Five insect cells producing recombinant adeno-associated virus (AAV) vectors for gene therapy or influenza hemagglutinin (HA)-displaying virus-like particle (VLP)-based vaccines, respectively.

Bulk transcriptome analysis revealed the impact of viral infection on the host cell transcriptome (overtaking up to 90 % of the cell transcriptome in later infection stages), resulting in alterations of cell physiology (e.g., cell growth, viral response, and amino acid metabolism) as well as the protein expression machinery (e.g., protein folding). Additionally, the enhanced recombinant protein production capacity of pH-adapted insect cells[1] (using adapted laboratory evolution) was investigated, revealing a decreased susceptibility of adapted cells to baculovirus infection. Using scRNA-seq an increase in cell heterogeneity as infection progresses was shown in both cell lines, which was accompanied by a shift of cells towards clusters with higher expression of viral genes. Additionally, infected cells revealed varying transgene levels, highlighting limitations in recombinant gene expression using viral expression systems. This was further emphasized when evaluating a dual baculovirus, low MOI production process (as used for AAV), where as little as 30 % of all cells at 24 hours post infection expressed both transgenes. Gene expression changes along time of infection and between different cell states were revealed using trajectory- and cluster-based approaches. Identified genes were associated to biological processes such as cell cycle and metabolic pathways, showing great promise to assist genetic and metabolic engineering in insect cells.

Overall, the increased knowledge on the underlying biological mechanisms of different IC-BEVS production processes herein attained, shows great promise to support advancements in insect cell-based biopharmaceutical production and encourages further use of transcriptomics in IC-BEVS.

References:

[1]Correia, R., et al., 2020. Improving Influenza HA-Vlps Production in Insect High Five Cells via Adaptive Laboratory Evolution. Vaccines 8, 589. https://doi.org/10.3390/vaccines8040589