Comparative transcriptome analysis of a Trichoplusia ni cell line in response to recombinant baculoviral infections

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Insect cells are among the most important animal cells used for recombinant protein expression in biotechnology. Due to the infection with a lytic virus that takes over the cellular synthesis machinery after the onset of the infectious cycle, these cells hold a special place among the established production systems. The viral infection and the simultaneous overexpression of secreted proteins triggers the unfolded protein response (UPR) in the host as a result of the accumulation of unfolded or misfolded proteins in the lumen of the endoplasmic reticulum. Our hypothesis suggests that the overexpression of complex proteins with different localization should induce differential gene expression as part of various cellular pathways that should be detectable on the transcriptome. In order to identify potential marker genes for process monitoring, as well as targets for process optimization, comparative transcriptome analysis was conducted. The alphaherpesvirus-free Tn42 cell line was used to reveal the differences in the specific host response to the production of either a complex, glycosylated protein – the secreted variant of influenza A virus derived hemagglutinin H1 – or an intracellular model protein, the mCherry.

Introduction

Differentially expressed genes

Figure 3. Overview of the total number of up- and downregulated unigenes throughout the experiment. The number of significantly regulated unigenes (p < 0.05) is higher in the datasets of both viruses expressing either mCherry (MC) or hemagglutinin (HA) at all time points compared to the empty vector control (EVC).

Table 1. Overview of the sum of significantly up- and downregulated genes (p < 0.05) among the viral datasets at the different time points. Empty vector control (EVC), mCherry (MC), hemagglutinin (HA).

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>EVC vs. MC</th>
<th>EVC vs. HA</th>
<th>MC vs. HA</th>
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<tr>
<td>48</td>
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Biological interpretation

Figure 4. Gene ontology (GO) term enrichment results of the 319 mCherry-hemagglutinin comparison gene set. The top 15 GO categories (FDR < 0.05) show an increasing p value from left to right along the horizontal axis. The most highly enriched GO categories include those involved in the stress response to unfolded proteins and protein folding, supporting the theory that the host cells are under stress due to the parallel infection and secreted protein production. Additionally, when the gene set is further analyzed with the KEGG pathway mapping tool, “Protein processing in endoplasmic reticulum” is among the top ranked pathways.

Conclusions

A comparative transcriptome analysis was conducted using the insect cell line Tn42 to reveal the specific changes in the host transcriptome in response to a viral infection inducing the production of either an intracellular (mCherry) or an extracellular (hemagglutinin) protein. The results imply that the cells specifically react to the stress caused by the viral infection and clearly separated from that, they respond to secreted protein production, by upregulating various host factors responsible for the correct folding, glycosylation and proper disulfide bridge forming.

Experimental setup

Figure 1. Suspension cultures of Tn42 cells were infected in triplicates at MOI 5 with recombinant baculoviruses encoding the intracellular mCherry protein or the secreted version of the influenza A virus hemagglutinin 1. The control samples derived from the triplicate flasks infected by the empty vector control virus and the non-infected control culture. (h p.i.) hours post infection.

Recombinant protein detection

Figure 2. Western blot analysis of the samples obtained from the recombinant virus infected cultures between 0 and 48 h.p.i. (hours post infection). Immunoblotting of the recombinant baculovirus infected Tn42 cells verified that the intracellular mCherry protein (A) is present mainly in the cell pellet. The band at 48 h.p.i. in the supernatant presumably results from cell lysis. (B) Analysis of the secreted hemagglutinin glycoprotein revealed that the majority remains in the cells leaving only reduced amounts of the properly folded protein detectable in the supernatant. (M) marker.

pellet

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