CELLULAR DEMANDS OF SECRETED PROTEIN PRODUCTS - SYSTEMS AND SYNTHETIC BIOLOGY IMPROVE QUALITY AND TITER

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Recombinant protein production can cause severe stress on cellular metabolism, resulting in limited titer and product quality. To investigate cellular and metabolic characteristics associated with these limitations on eukaryotic cells, a panel of human secreted and non-secreted proteins, and bispecific antibodies were explored as stable or transient cultures in CHO and/or HEK293 and analysed by transcriptomics to define targetable pathways for engineering to improve yield, quality and activity. In short we report on:

1.Tuned energy supply improve productivity. Transcriptomic and functional analyses showed a significantly higher metabolism and oxidative phosphorylation for top producing HEK293 clones versus low producers of erythropoietin (EPO) (secretory) a pattern not seen for GFP (non-secretory) clones of various productivity. The increase in energy demand, supported by upregulation of several protein complexes in the electron transport chain, was validated by flux analysis (Seahorse). In addition, ribosomal genes exhibited specific expression patterns depending on the nature of recombinant protein and its production rate, with indication of conserved shift of ribosomal structure for secreted proteins. In a clone displaying a dramatically increased EPO secretion, we detect higher gene expression related to negative regulation of endoplasmic reticulum (ER) stress, including upregulation of ATF6B, which we also could show to aid EPO production after upregulation in less producing clones. This further strengthens the proposed pseudo-synaptic link between ER and mitochondria.
2. A systemsbiology comparison of HEK293 and CHO as hosts for difficult to express proteins. Here we identify metabolic engineering targets limiting expression of human proteins through a systems biology analysis of the transcriptomes of CHO and HEK293 during recombinant expression. In an expression comparison of 24 difficult to express proteins, one third of the challenging human proteins displayed improved secretion upon host cell swapping from CHO to HEK293. The analysis guided us key differences between the hosts, especially highlighting differences in secretory pathway utilization, a co-expression screening of 21 secretory pathway components validated ATF4, SRP9, JUN, PDIA3 and HSPA8 as productivity boosters in CHO for a panel of challenging secreted human proteins.

3. Aggregation of bispecific antibodies during stable expression in CHO can be reduced by helper genes
Comparative transcriptomics of Ambr cultures of CHO producing various degree of aggregated bi-specific mAb defined several stress-responses including need for adequate proteasomal and autophagy function to increase soluble yield. Further specific chaperones were identified improving soluble yield upon upregulation in a validation experiment of stable clones producing challenging bi-specific antibodies.
4. Tuning of secretory helper proteins in CHO increase level of active sulfatase 150-fold
Transcriptome analysis of two CHO clones with varying cell specific productivity of active sulfatase were compared in Ambr cultures, leading to a final candidate set of three different genes related to sulfatase production. Co-transfections of identified genes increased specific sulfatase activity up to 150-fold. In addition, a better utility of the endogenous levels of the CHO support machinery for the sulfatase secretion and activation was shown to be possible by lowering transcriptional burden of product gene by synthetic biology.

In conclusion CHO cells may improve their ability to produce human or artificial proteins, such as bi-specific antibodies, by balancing expression of necessary helper genes and adjustment of transgene abundance.

References
1. Malm M et al., 2022, Metabolic engineering 72, 171-187
2. Saghaleyni R, Malm M et al., 2022, Cell Reports 39 (11), 110936
3. Barzadd MM, et al 2022 N Biotechnology 68, 68-76
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