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MULTI-OMIC PROFILING OF EPO PRODUCING CHO CELL PANEL REVEALS METABOLIC ADAPTATION TO HETEROLOGOUS PROTEIN PRODUCTION

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The Chinese hamster ovary (CHO) cell line is the predominant mammalian cell factory for production of therapeutic glycoproteins. In this work, we aimed to study bottlenecks in the secretory pathway associated with the production of human erythropoietin (EPO) in CHO cells. In connection to this, we discovered indications of metabolic adaptation of the amino acid catabolism in favor of heterologous protein production. We established a panel of stably EPO expressing CHO-K1 clones spanning a 25-fold productivity range and characterized the clones in batch and chemostat cultures. For this, we employed a multi-omic physiological characterization including metabolic footprinting of amino acids, metabolite fingerprinting of glycolytic intermediates, NAD(P)H-/NAD(P)+ and adenosine nucleotide phosphates. We used qPCR, qRT-PCR, western blots and Affymetrix CHO microarrays to assess EPO gene copy numbers, EPO gene expression, intracellular protein levels and genome-wide differential gene expression analysis of genes functionally related to secretory protein processing, respectively. Finally, we generated a network reconstruction of the amino acid catabolism in CHO cells. The reconstruction was utilized as a platform for interpretation of differential gene expression data in a biological meaningful manner. To identify bottlenecks in the protein secretory pathway, we compared EPO gene copy numbers, EPO gene expression levels, intracellular EPO retention and extracellular EPO levels for a high and low producing clone during chemostat culture. The EPO productivity levels were not reflected in EPO gene load, EPO gene expression or intracellular protein retention, indicating that these processes were not limiting EPO productivity. The global gene expression analysis did not identify significant differentially expressed genes related to secretory protein processing. However, when inspecting the gene expression landscape of the amino acid catabolism, we observed an apparent adaptation in favor of EPO production. That is, we discovered that the gene expression levels of amino acid catabolic genes had adapted to preserve the most abundant amino acids in EPO in the high producing clone relative to the low producing clone. Based on these data, we speculate that the amino acid metabolism in CHO cells may undergo adaptation in favor of heterologous protein production during long-term cultivation.