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APPLICATION OF ^{13}C FLUX ANALYSIS TO IDENTIFY HIGH-PRODUCTIVITY CHO METABOLIC PHENOTYPES

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Industrial bioprocesses place high demands on the intermediary metabolism of host cells to meet the biosynthetic requirements for maximal growth and protein expression. Identifying host cell metabolic phenotypes that promote high recombinant protein titer is a major goal of the biotech industry. ^{13}C metabolic flux analysis (MFA) provides a rigorous approach to quantify these metabolic phenotypes by applying isotope tracers to map the flow of carbon through intracellular metabolic pathways. We have conducted a series of ^{13}C MFA studies to examine the metabolic impacts of recombinant IgG expression using two common CHO expression systems, the glutamine synthetase (GS) and dihydrofolate reductase (DHFR) systems.

First, we performed ^{13}C MFA to characterize the metabolism of a IgG-expressing DHFR host (Amgen) during four separate phases of a fed-batch culture. We found that peak specific growth rate during early exponential phase was associated with high lactate production and minimal citric acid cycle (CAC) flux. Conversely, we found that lactate metabolism switched from net production to net consumption as the culture transitioned from peak growth to peak IgG production. During stationary phase when IgG production peaked, energy was primarily generated through CAC and oxidative phosphorylation.

Second, we examined nine CHOK1SV (Lonza) clones cultured in 3-liter fed-batch bioreactors, to assess their metabolism during stationary phase. Three of the clones did not express IgG. Six of the clones used the GS System™ to express one of three different IgGs. Four of the clones were genetically manipulated to be apoptosis-resistant by expressing Bcl-2Δ. Hierarchical clustering was performed to assess correlations amongst flux phenotypes of the nine clones. The six antibody-producing clones clustered together and were separated by host background (Bcl-2Δ or CHOK1SV). The lactate dehydrogenase (LDH) flux was most closely associated with specific IgG productivity: as IgG productivity increased, lactate production decreased. Additionally, elevated CAC fluxes corresponded strongly with increased specific productivity.

Taken together, these studies indicate that oxidative metabolism is enhanced in high-producing CHO cell lines. This presentation will discuss the central metabolic trends observed among both GS and DHFR expression systems as a means to provide potential metabolic engineering strategies to further enhance IgG productivity and titer of industrial CHO hosts.