METABOLIC ENGINEERING OF HIGH-PRODUCTIVITY CHO HOST LINES FOR BIOMANUFACTURING

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Industrial bioprocesses place extraordinary demands on the intermediary metabolism of host cells to meet the biosynthetic requirements for maximal growth and product formation. Therefore, engineering cell metabolism to promote high yield and specific productivity (qP) is a major goal of biomanufacturing research. ^{13}C metabolic flux analysis (MFA) provides a comprehensive approach to quantify host metabolic phenotypes by applying stable isotopes to trace the flow of carbon through intracellular biochemical pathways. Our previous ^{13}C MFA studies have discovered that high-producing CHO cell cultures possess a different metabolic phenotype compared to low-producing cultures. In particular, it was found that high mAb production is correlated to lactate consumption and elevated mitochondrial TCA cycle flux during stationary growth phase. Based on these findings, we have engineered multiple gene targets to enhance mitochondrial metabolism and overall bioenergetics of CHO host lines. First, we overexpressed a mitochondrial regulator to enhance TCA cycle flux and oxidative phosphorylation, which significantly improved final titers and qP compared to the parental line. Second, we engineered a cell line with reduced glutamine overflow and increased mAb production by attenuating the promoter driving expression of the glutamine synthetase (GS) selection marker. We have applied ^{13}C MFA to understand how these gene alterations impact intracellular metabolic fluxes and mAb biosynthesis. Finally, we expanded our metabolic flux models to include pathways for synthesis of nucleotide-sugars that supply precursors for product glycosylation, and have validated these models in cultures fed ^{13}C-glucose or ^{13}C-galactose. This presentation will highlight how host cell engineering guided by ^{13}C MFA can be applied to improve metabolic efficiency and product biosynthesis, which has broad implications for biomanufacturing research.