METABOLIC ENGINEERING OF CHO CELLS TOWARDS CYSTEINE PROTOTROPHY

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Chinese hamster ovary (CHO) cells are currently the workhorses for recombinant therapeutic protein production. In culture, these cells require the supplementation of vitamins, trace elements cofactors, non-essential amino acids, as well as essential amino acids such as cysteine. Supplementation of cysteine comes with its own problems: cysteine/cystine is most soluble and stable in very basic or acidic conditions and is prone to precipitation as well as co-precipitation of other amino acids in chemically defined medias in neutral pH ranges. Excess extracellular cysteine can cause trisulfide formation and have other redox associated impacts like under disulfide bond (UDB) formation on a biologic product of interest (POI). On the other hand, cysteine depletion not only impacts protein synthesis but also causes cysteine misincorporation in POI, low viability, and cell death. For these reasons, enabling cells to biosynthesize cysteine intrinsically from other nutrients supplemented in culture would be beneficial. While the genes encoding enzymes in the biosynthesis pathway for cysteine including methionine catabolism and transsulfuration are present in genome of CHO cells, it has been reported that genes in the transsulfuration pathway, specifically, CTH and CBS, have negligible expression levels[1].

Genetic engineering methods were explored to increase carbon flux through the methionine catabolism and transsulfuration pathways, along with use of extracellular additives to enable engineered cells to produce sufficient cysteine for proliferation in cysteine free conditions. Mouse orthologs of multiple negligibly expressed genes in methionine catabolism and/or transsulfuration pathways were overexpressed in CHO cells. The presentation will go into recovery of cell pools overexpressing the above-mentioned genes, single cell cloning of cell pools and characterization of single cell clones.

1. Hefzi et al. (2017) A Consensus Genome-scale Reconstruction of Chinese Hamster Ovary Cell Metabolism. *Cell Systems* 3:434–443