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DEEPENING KNOWLEDGE ON CHO CELLS METABOLISM USING MULTIPLE TRACER SUBSTRATES

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Key Words: GS-CHO cells metabolism; carbon and nitrogen sources; tracer studies; GC-MS analysis; Metabolic flux analysis.

Optimization of culture conditions through trial-and-error has led to much of the past progress on animal cell culture. Today, a renewed account of the underlying biologies through a “systems” view is providing a deeper knowledge of cellular physiology and laying the basis to reengineer desirable states of further increased culture performance. In this work, we took a comprehensive view of cellular metabolism by extensively profiling extracellular and intracellular metabolomes after ¹³C-label supplementation in parallel labelling cultures of [1,2-¹³C]Glucose, [U-¹³C,¹⁵N]Asn, [U-¹³C,¹⁵N]Ser and [1-¹³C]Pyruvate. This integrative approach was used to trace metabolic rearrangements in different scenarios of asparagine and serine availability in GS-CHO cells. The absence of asparagine in the medium caused growth arrest, and was associated with a dramatic increase in pyruvate uptake, a higher ratio of pyruvate carboxylation to dehydrogenation and an inability for *de novo* asparagine synthesis. The release of ammonia and amino acids such as aspartate, glutamate and alanine were deeply impacted. This confirms asparagine to be essential for GS-CHO cells as the main source of intracellular nitrogen as well as having an important anaplerotic role in TCA cycle activity. In turn, serine limitation also negatively affected culture growth while triggering its *de novo* synthesis, confirmed by label incorporation coming from pyruvate, and reduced glycine and formate secretion congruent with its role as a precursor in the metabolism of one-carbon units. The results obtained suggest that feeding schemes of asparagine or serine should be tightly tuned to minimize by-product formation while assuring biosynthetic needs. Ongoing is the contextualization of the data from the parallel labelling cultures into a metabolic network model for detailed mapping of cellular fluxomes in each scenario. Overall, this work contributes to unfold important insights into GS-CHO cells metabolism, and can be used as a basis for exploring bioprocess optimization strategies.

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