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From media to mitochondria–rewiring cellular energy metabolism of Chinese hamster ovary cells for the enhanced production of biopharmaceuticals

Paul S Kelly[1], Antonio Alarcon Miguez[3], Christina Alves[4] and Niall Barron[1,2]

Meeting the metabolic demands of Chinese hamster ovary (CHO) cells has been an area of intense investigation over the last 3 decades as a means to improve these cell factories as producers of high quality recombinant therapeutic proteins. Metabolically, the cultivation of CHO cells is characterised by the rapid consumption of the primary carbon and energy sources, glucose and glutamine, with lactate and ammonia produced as by-products, respectively. In the context of bioprocess-relevant CHO cell phenotypes, glycolytic metabolism predominates during exponential cell growth culminating in lactate production while glucose channelling through the tri-carboxylic acid (TCA) cycle supports high-specific productivity. The genetic diversity inherent among CHO cell lineages (CHO-K1, CHO-S, and CHO-DG44), in addition to clonal isolates, makes media development a complex task which must often be performed on a clone by clone basis. However, designing tailored media formulations and sophisticated feeding regimens based on empirical observation has been one of the main driving forces behind the enhancements seen today in volumetric titres. To add to this complexity, CHO mitochondrial genetics have recently been shown to be heterogeneous resulting in an additional level of genetic pre-programming at the epicentre of cellular energy production. Cajoling CHO cells to utilise resources more efficiently through cell line development strategies, hypothermic adaptation or genetic engineering are areas of considerable interest within the biopharmaceutical community. Genetic re-programming of cellular metabolism through the manipulation of desirable metabolic pathways using microRNAs, siRNAs or gene overexpression have yielded some success. With the advent of sophisticated gene editing tools such as CRISPR-Cas9, a better understanding of CHO cell metabolism should drive knowledge-based multi-faceted cell line development pipelines combining both genetic engineering, selection of innately superior clones as well as tailored media formulations to improve the performance of this important therapeutic protein-producing cell line.

Addresses
[1] The National Institute for Bioprocessing Research and Training, Fosters Avenue, Mount Merrion, Blackrock, Co. Dublin, Ireland
[2] School of Chemical and Bioprocess Engineering, University College Dublin, Co. Dublin, Ireland
[3] The National Institute for Cellular Biotechnology, Dublin City University, Glasnevin, Dublin 9, Ireland
[4] Cell Culture Development, Biogen, Inc., Cambridge, MA, United States

Corresponding author: Barron, Niall (Niall.Barron@nibrt.ie)

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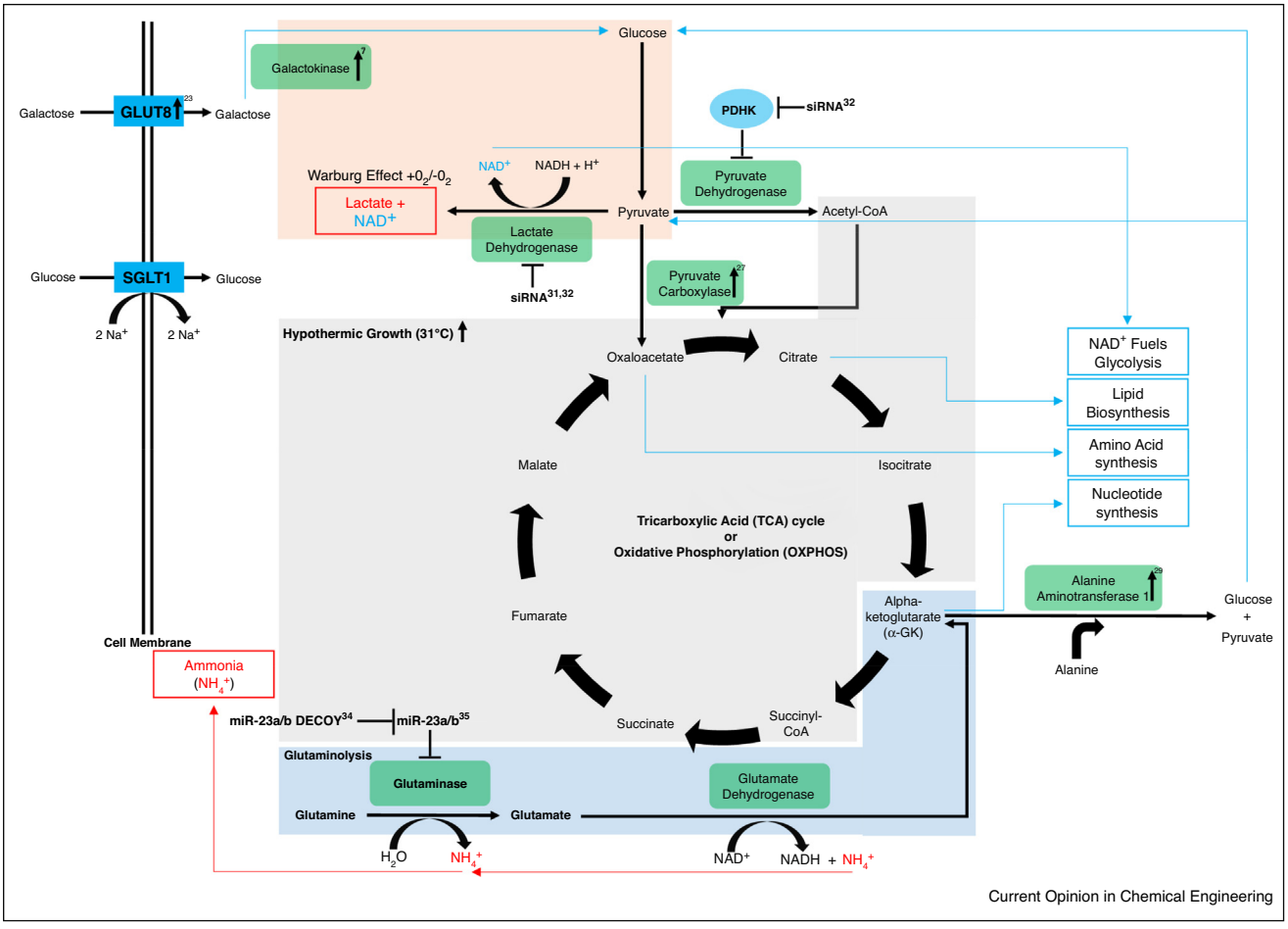
Introduction

For the past three decades, the biopharmaceutical industry have used mammalian cells, predominantly the Chinese hamster ovary (CHO) cell, to produce high quality recombinant therapeutic proteins with Human-like glycosylation patterns [1]. A large portion of approved recombinant proteins are glycosylated (78 out of 212) with CHO cells being the expression host of choice, representing 61.5% of these glycoproteins [2]. Although the correct post-translational modifications (PTMs) are critical for drug stability and potency, the process of developing a CHO cell line capable of thriving within the bioreactor environment is a complex and time-consuming task [3]. During cell line development (CLD), a variety of intrinsic (genetic) and external (environmental) factors are considered, that promote and encourage 'good' cell growth, prolong culture viability and enhance-specific productivity by supporting efficient cellular metabolism. These environmental factors include supplying key metabolites such as amino acids, carbohydrates and hydrolysates, all of which support biomass accumulation and meet internal cellular energy demands. However, providing these substrates in abundance fuels the generation and accumulation of inhibitory metabolic waste products such as lactate and ammonia (Figure 1). Below the surface, the genetic diversity of CHO cells both at the nuclear [4] and the mitochondrial genome level [5••] contribute a unique layer of metabolic programming. Not only can certain cell lineages be inherently suited to particular culture platforms or product types [1] but the genetic instability of CHO cells themselves contributes to the phenotypic heterogeneity [6] seen in the 100s of clones carried

Figure 1



This metabolic scheme follows the primary carbon source, glucose, through it's two dominant metabolic fates, glycolysis/Warburg effect or oxidative phosphorylation. Other metabolic programmes such as Glutaminolysis demonstrates how the TCA cycle can be supplemented from glutamine metabolism. Lastly, a series of genetic engineering strategies employed in Chinese hamster ovary cells are indicated and which metabolic pathway is influenced.

through CLD. As a result, the genetic diversity apparent in CHO cell lines after their isolation in the 1950s has given rise to the term 'Quasi-Species', meaning that no one cell line or clone is the same and therefore can and will have uniquely different metabolic demands [7•] which must be considered and understood when developing a production host.

Historically, discoveries in the field of cancer biology have paralleled that of observations in continuous cell lines, like CHO, in relation to the exclusive metabolism of glucose in support of exponential cell growth [8]. This phenomenon known as the Warburg effect or aerobic glycolysis results in lactate accumulation, a metabolic process that usually occurs under oxygen deprivation which ultimately contributes to increasing media acidity [9]. Glycolysis occurs at a rate 10–100 times faster than the complete oxidation of glucose in the mitochondria [10••] and although energetically inefficient, metabolic flux analysis (MFA) using ^{13}C labelling associates peak specific growth rate with this metabolic programme [11].

So why would active proliferation be associated with an apparently energetically inefficient metabolic process? One suggested explanation for this dynamic shift in cellular metabolism is that proliferating cells must repurpose their mitochondria from a predominantly catabolic engine producing ATP to an anabolic one, supplying biosynthetic intermediates to support biomass accumulation through, for example, citrate, oxaloacetate and alpha-ketoglutarate (α-KG) export thereby providing a carbon supply for lipid biosynthesis or raw materials for amino acid or nucleotide biosynthesis, respectively [8] (Figure 1). So although 'inefficient' from the perspective of