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What can mathematical modelling say about CHO metabolism and protein glycosylation?

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ABSTRACT

Chinese hamster ovary cells have been in the spotlight for process optimization in recent years, due to being the major, long established cell factory for the production of recombinant proteins. A deep, quantitative understanding of CHO metabolism and mechanisms involved in protein glycosylation has proven to be attainable through the development of high throughput technologies. Here we review the most notable accomplishments in the field of modelling CHO metabolism and protein glycosylation.

1. Introduction

Mammalian cells, more specifically immortalized Chinese hamster ovary (CHO) cells, are the dominant biological platform for the production of many therapeutic recombinant proteins [1]. CHO cells are not only able to correctly fold these proteins, but they are also capable of performing human-compatible post-translational modifications (e.g. glycosylation) [2,3]. This is important for the correct functioning of the proteins and to prevent immunogenic responses in humans. In addition, CHO cells show high and stable expression of heterologous proteins and they easily adapt to growth in suspension. Both features are essential for industrial-scale production [4]. Furthermore, CHO cells are considered to be "safe", since most human pathogenic viruses do not replicate in CHO [5]. All of these characteristics have contributed to a steep increase in the number of approvals for products expressed in this system compared to those produced in non-mammalian cells [6].

Due to their major role in the biopharmaceutical industry, several efforts have been focused on optimizing the culture process [7,8]. In the past two decades, these efforts were mainly based on experimental observations of the metabolic profiles during cell culture [9,10]. However, the advent of -omics technologies and associated modelling approaches facilitated a better and more detailed understanding of cell behaviour and intercellular processes. In particular, the development of constraint-based modelling techniques contributed tremendously to our understanding of metabolic processes, pathways and networks, so that these techniques have become one of the most (if not the most) successful modelling approaches in systems biology. Key to this success is the analysis of genome-scale metabolic reconstructions (GSMR). Combined with constraint-based modelling approaches, these models provide a mechanistic basis to investigate and elucidate genotype-phenotype relationships [11,12].

Here we will review recent progress in the computational modelling of CHO cells. Specifically, we will focus on and analyze

Abbreviations: CHO, Chinese hamster ovary; FBA, Flux Balance Analysis; GSMR, genome-scale metabolic reconstruction; MFA, metabolic flux analysis; PPP, pentose phosphate pathway; TCA, tricarboxylic acid.

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two main issues associated with recombinant protein production: (i) metabolic burdens affecting growth and thus protein yield and (ii) understanding of the correct glycosylation process of the protein of interest, which is one of the major criteria for product quality.

2. CHO metabolism

The cultivation of CHO cells in bio-reactors is characterized by fast consumption of the main carbon and energy sources, glucose and glutamine, with the concomitant production of ammonia and lactate. The production of lactate not only indicates inefficient metabolisation of the carbon sources [two molecules of ATP compared to 36 if glucose was completely oxidized in the tricarboxylic acid (TCA) cycle], but also has a negative effect on pH and osmolarity [13], which reduces the specific growth rate [14,15] and protein yield [16]. High ammonia concentration in the medium has similar adverse effects on cell growth, productivity and glycosylation [17–20]. Several strategies have been devised to overcome the accumulation of these by-products: rational supplementation of glucose and glutamine in fed-batch cultures [21,22], use of alternative carbon sources [7] or cell engineering [23,24], among others. These approaches were, however, based on trial and error and lack deterministic, quantitative justification.

2.1. Modelling CHO metabolism

To gain mechanistic understanding of these processes, appropriate metabolic models are required that allow one to estimate cellular flux distributions. This can be done in two ways: (i) in a time-dependent or dynamic manner (kinetic analysis) or (ii) in a constraint-based, steady-state analysis. The former approach aims to assess the evolution of the concentrations of metabolites over time and requires a large number of kinetic parameters. Due to the lack of accurate, quantitative data, this approach is currently not feasible on a genome-scale level, but restricted to small-scale models that consider several tens of selected reactions and interactions. The latter approach, on the other hand, avoids the need for detailed kinetic information by focusing on the steady-state behaviour inside the cell. Disregarding dynamic processes makes this approach, called metabolic flux analysis (MFA), scalable and suitable for genome-wide analysis. For better understanding the modelling approaches are briefly reviewed in Box 1.

In the following section we review current advances in metabolic modelling of CHO cells (listed chronologically in Fig. 1), focusing on those that investigate the accumulation of the two main metabolic by-products that are detrimental to cell growth, i.e. lactate and ammonia.

2.1.1. The metabolic fate of lactate

Altamirano et al. [31] investigated the metabolic fate of lactate on a metabolic network of CHO core metabolism. They argued that, when re-metabolized, lactate is not used as an energy source, as their experimentally measured low oxygen uptake rate was inconsistent with a full oxidation of lactate via the TCA cycle. Consequently, they proposed alternative pathways for the non-oxidative decarboxylation of pyruvate, which are known to exist in cancer cells [32], to be present in CHO cells too. Nevertheless, the accumulation of the end product of these pathways, i.e. acetoin, was not experimentally proven. In a more recent work, Martinez et al. [33] were able to refute this hypothesis. In their study, they analyzed the metabolic switch from lactate production to lactate uptake by means of FBA in a reduced mouse-derived metabolic model. Contrary to Altamirano et al., Martinez et al. showed that their oxygen uptake rate measurements were consistent with lactate oxidation in the TCA cycle. This suggests that the metabolic network of Altamirano et al. might have been too simplistic to capture the metabolic changes between the phases. Compared to Martinez, Altamirano's model lacked fatty acid, steroid and glycogen metabolism. In addition, the prediction of the ATP yield per mol carbon identified lactate consumption to be energetically more efficient than glucose consumption. Furthermore, they showed that the estimation of ranges for the metabolic fluxes (due to the insufficient amount of experimentally measured data in an underdetermined network) provides a valuable, semi-quantitative description of the changes between the two metabolic states. This concept was also supported by Zamorano et al. [34], who performed MFA in an under-determined network containing 100 reactions of the core metabolism and obtained narrow intervals for the fluxes with a relatively low amount of extracellular measurements.

FBA can be combined with isotopomer analysis to improve the accuracy of the predicted fluxes. Sengupta et al. [35] studied the main metabolic fluxes in a simplified network during the stationary phase of cell culture by ^{13}C MFA. This phase is typically characterized by reduced production of lactate and high protein yields. Likewise, Templeton et al. [36] performed ^{13}C MFA to understand the metabolic changes between growth and stationary phases in a producer CHO cell line. They found that, during the antibody production peak (stationary phase), fluxes through the TCA cycle were maximal while lactate was not produced. Moreover, this increased activity of the TCA cycle correlated with increased fluxes through the oxidative pentose phosphate pathway (PPP) when compared to the exponential phase, where high glycolytic fluxes predominate. They provide several explanations for the activation of the oxidative PPP: to regenerate NADPH/NADP$^+$, to compensate reduction during exponential growth, to suppress oxidative stress or to cover NADPH requirements during protein folding and secretion. Irrespective of the ultimate reason, these findings point towards metabolic engineering to increase oxidative TCA cycle (CO_2-producing reactions) and PPP fluxes which would help achieve higher protein yields.

Box 1
Common modelling approaches.

MFA (Metabolic Flux Analysis): pathway analysis method based on the stoichiometry of metabolic reactions and mass balances under pseudo-steady-state assumption [25]. It can be implemented in several ways. Among them:

- **FBA** (Flux Balance Analysis): an implementation of MFA based on the optimization of a cellular function (such as growth) under specific constraints (experimental metabolic uptake and secretion rates, thermodynamic data, etc.) [26,27].
- **^{13}C MFA**: isotope-labelled substrates are added to the culture media and, once the isotopic steady-state is reached, the distribution of the isotopes is measured via nuclear magnetic resonance or gas chromatography—mass spectrometry [28].

Markov chain Monte Carlo sampling: the glycosylation process is described as a series of states with transition probabilities from one state to the other. In the references reviewed herein, it is used to overcome the lack of kinetic parameters (metabolic and glycosylation enzymes) [29].
Artificial Neural Network models: aim to predict the behaviour of complex, non-linear systems by detecting and "learning" patterns and relationships within a training set which can be applied then to the input data [30].

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