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Enhancement of production of protein biopharmaceuticals by mammalian cell cultures: the metabolomics perspective

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The new era of metabolic study is enabling a molecular rationalisation of cellular processes that support high production and quality of recombinant protein in bioprocessing. The completeness of, and confidence in, our understanding of culture metabolism has permitted the development of metabolic models for recombinant cell lines that will facilitate enhancement of bioprocess performance. For Chinese Hamster ovary (CHO) cell lines, key indicators of metabolic events linked to productivity have become clear in the past 2–3 years. In particular, the balance between glycolysis and the citric acid cycle appears as a determinant/indicator of both growth and productivity. The profiles observed offer potential to direct CHO cell function during culture through medium design and/or cell engineering.

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Introduction

Omics approaches are being used widely to decipher the processes that underlie cell function and the knowledge that comes from each approach, individually or in combination, of host cell status (phenotype) in relation to recombinant biopharmaceutical yield (via enhanced host cell biomass and/or cellular productivity) and product quality offers predictive rationalisation for bioprocessing. Perceived potential includes the selection of the 'best' recombinant clones, the development of the most effective bioprocessing environment (medium, feeds, culture process), the enhancement of selection of cell/bioprocess for distinct types of biopharmaceutical process and, potentially, for generation of engineered host cell lines to match the needs for future bioprocessing.

This review will focus on how knowledge of cellular metabolism illuminates bioprocessing design. Changes to metabolites (within cells or in the surrounding medium) reflect an integrative outcome of gene expression, and in total is a read-out of multiple processes controlling gene expression through to protein function (Figure 1). Whilst supplying intermediates and the energy currency to drive biosynthetic reactions (cell biomass, desired recombinant protein), intracellular metabolites are linked to signalling roles within the cell as exemplified by metabolite-level control of mTOR [1] and AMP-activated protein kinase [2]. Concentrations and changes to amounts of (specific) metabolites have profound significance in prediction of the effectiveness of cell cultures towards production of biopharmaceuticals [3,4]. With the current pre-eminence of mammalian cells (and Chinese Hamster ovary, CHO cells, in particular) as host cell platforms for production of biopharmaceuticals as therapeutics, this review will largely focus on developments in relation to CHO cell systems.

Metabolite determination: sample generation, analytics and data analysis/interpretation

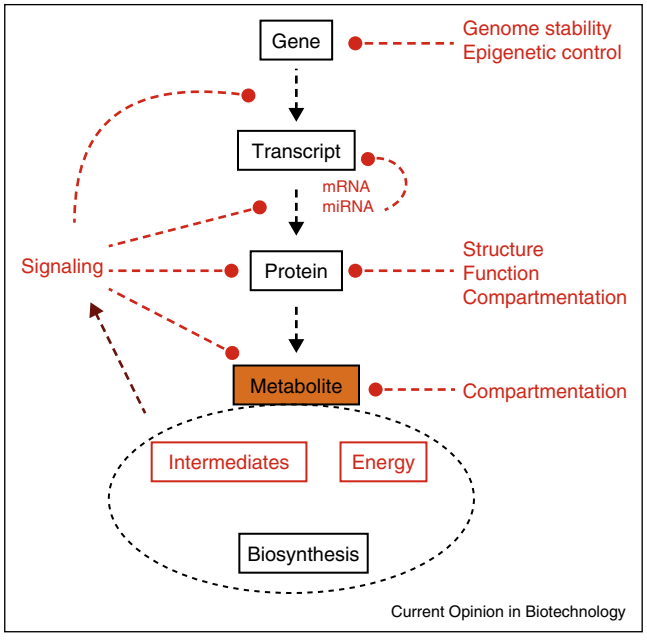
Extracellular (medium) and intracellular analyses

Whilst the analysis of metabolites in the medium alone may be sufficient to provide information for process improvement, by enabling process understanding from metabolic flux analysis [5] or development of feed optimisation [6], intracellular metabolite assessment can inform of metabolic profiles or interactions within cells responsible for desirable (or undesirable) phenotype. As well as indicating approaches (chemical or genetic) for cell line engineering to improve processes, this may also generate metabolic indicators to enhance cell line selection and predictive understanding of matches between cell line, product and medium/feed selection. Figure 2 gives a general overview of considerations to be taken in account and experimental/data handling required to generate meaningful interpretation of metabolomics-based approaches.

Extraction of intracellular metabolites

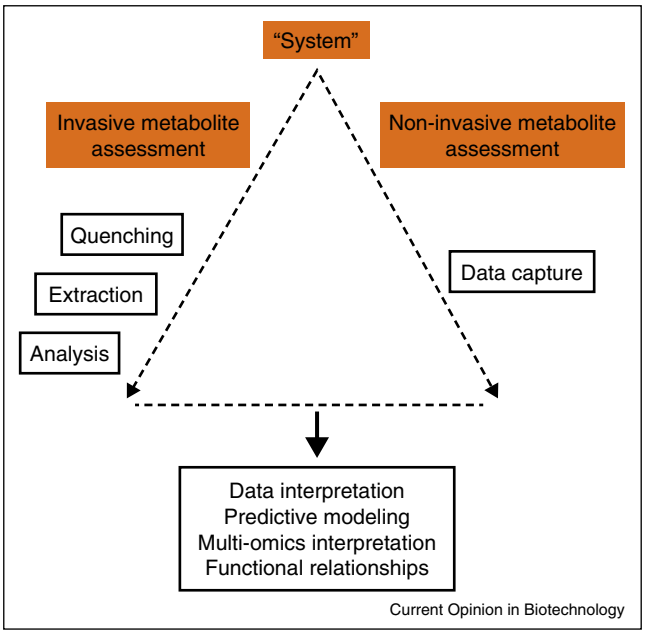
Generation of physiologically relevant intracellular metabolite samples in mammalian cell cultures is complicated by the large numbers (often at high concentration) of metabolites in medium used for mammalian cell culture. When combined with the relatively low density of mammalian cells in culture, separation of

Figure 1



Metabolites provide a read-out of cellular function. This figure illustrates that the concentration of metabolites is determined by the upstream regulatory events that control the flow from gene to active enzymes. As well as supporting ATP formation and producing intermediates (which together support synthesis of more cells and formation of functionally modified recombinant proteins) metabolites can operate as intracellular signals to regulate cell function at multiple sites. Specifically, control can be exerted by metabolites on genome stability and epigenetic regulation and on protein structure (via binding interactions) with subsequent effects on interactions, compartmentation and activity (function). Changes to protein activity may also influence metabolite compartmentation and target proteins (red dotted lines represent potential generic regulatory actions).

Figure 2



From system to interpretation/use: the metabolomics approach. Identification of the techniques and analysis that may be applied to generate meaningful data towards assessment of functional relationships between information on metabolites and cell function/ phenotype.

cellular material (and associated metabolites) with minimal contamination from the rich extracellular culture medium has presented a challenge that has to be optimised for each cell and medium combination. This has been a particular focus for suspension-adapted CHO cells and has led to a number of protocols for quenching and extraction to retain a physiological intracellular metabolite profile [7–18]. Some methods may prove more relevant to cells of specific phenotype (suspension or adherent) or may be more suitable to the subsequent analytical procedure. Developments towards high throughout methods will provide greater support for metabolome interpretation.

Analytical approaches for metabolites

Several analytical approaches may be used for determination of metabolites of cells in culture [19••,20]. Approach selection is based in several parameters, for example if there is prior knowledge, or not, of metabolites linked to the phenotype targets (focusing to measurement of a relatively small number of metabolites — metabolite profiling — in the first case or a 'measure everything possible' approach, in the latter situation — metabolomics). No single technique is likely to capture information about all potential cell culture metabolites. For example, varied extraction processes suitable for mass spectrometry (MS)-based analysis will extract subsets of metabolites [8] and derivatisation for MS analysis may destroy/modify specific metabolites [21]. Whichever analytical approach is used, replicate samples (frequently of cells taken from a time series under different conditions) generate large amounts of data. The management, analysis and interpretation of complex datasets — chemometrics and associated statistical approaches (e.g. multivariate analysis, partial least squares regression, principal components analysis, cluster analysis) — is a major undertaking, especially when handling the interpretation of the large number of metabolites in the metabolomics approach [22].

Metabolic flux analysis (MFA)

The use of stable isotopes (usually glucose or glutamine, labelled universally or on specific atoms) [5,23–27,28•,29] has been incorporated into several studies to define the steady-state activity of metabolic pathways of cells under different conditions. The application and interpretation of MFA is discussed in detail in a separate review in this issue [5].