Proteomic profiling of recombinant cells from large-scale mammalian cell culture processes

Paula Meleady

Received: 18 December 2006 / Accepted: 25 January 2007 / Published online: 24 February 2007
© Springer Science+Business Media B.V. 2007

Abstract Global expression profiling of mammalian cells used for the production of biopharmaceuticals will allow greater insights into the molecular mechanisms that result in a high producing cellular phenotype. These studies may give insights for genetic intervention to possibly create better host cell lines or even to provide clues to more rational strategies for cell line and process development. In this review I will focus on the contribution of proteomic technologies to a greater understanding of the biology of Chinese hamster ovary cells and other producing cell lines such as NS0 mouse cells.

Keywords Chinese hamster ovary · NS0 · Proteomics · High cell productivity

Abbreviations

CHO Chinese hamster ovary
MS mass spectrometry
PAGE Polyacrylamide Gel Electrophoresis
Mab monoclonal antibody

Introduction

Mammalian cell culture is widely used for the manufacture of many biopharmaceuticals for therapeutic, diagnostic and research uses. These recombinant proteins include the classical products such as erythropoietin, factor VIII and tissue plasminogen activator, and more recently there has been a surge in the discovery and manufacture of antibody-based therapeutics.

Many strategies are being used to improve productivity, including improvements in vector design (Fussenegger et al. 1998), optimisation of media formulation (Carvalho et al. 2003) supplementation of stimulatory agents (Liu et al. 2001a, b; Arts et al. 1995) and also a huge improvement in the design of bioprocess reactors with sophisticated control mechanisms.

Chinese hamster ovary (CHO) cells are currently the most important cells used for the production of biopharmaceuticals from mammalian cells and will probably remain so for the foreseeable future given their high productivity, their robust nature, their track record in industry, and their safety record (Chu and Robinson 2001). There has been considerable success in developing high producing CHO cell lines using such approaches as outlined above. However there is still a very poor fundamental understanding of the molecular mechanisms governing such phenotypes, and if there are to be considerable

---

P. Meleady (✉)
National Institute for Cellular Biotechnology,
Dublin City University,
Glasnevin, Dublin 9, Ireland
e-mail: Paula.Meleady@dcu.ie

improvements in achieving >10 g/l of product a basic understanding of the biology of these cells is required.

The global expression profiling methods such as expression DNA microarrays and proteomic analysis are been used extensively to gain insights into the biology of CHO cells and other producing cell lines such as NS0 mouse cells (Seth et al. 2006; Nissom et al. 2006). The relationship between transcriptome and proteome expression is not linear (Gygi et al. 1999a, de Nobel et al. 2001), therefore a combination of both transcriptome and proteome analyses should be performed for the comprehension of cellular mechanisms in large-scale mammalian cell culture processes. These studies may give insights for genetic intervention to possibly create better host cell lines or even to provide clues to more rational strategies for cell line and process development. This review will focus on the contribution of proteomic profiling studies to a better understanding of mammalian cell culture processes to date.

Proteomic technologies

There are several proteomics technologies used for protein expression profiling including Two-dimensional Polyacrylamide Gel Electrophoresis (2D-PAGE), Surface Enhanced Laser Desorption/Ionisation Time of Flight (SELDI-ToF), Protein Arrays, isobaric tag labelling technologies such as ICAT (Isotope Coded Affinity Tags) and iTRAQ^{TM} (Isobaric Tagging for Relative and Absolute Protein Quantitation), and Multidimensional Protein Identification Technology (MudPIT). To date most of the studies relating to large-scale mammalian cell culture have used 2D-PAGE and mass spectrometry based approaches, though isobaric tag labelling approaches are becoming more popular as the technology develops.

2D-PAGE and mass spectrometry

Two-dimensional Polyacrylamide Gel Electrophoresis (2D-PAGE) is perhaps the most widely used proteomics technique to study the proteome (Görg et al. 2004). 2D-PAGE remains challenging

mainly because of its low sensitivity and reproducibility. There are also many limitations inherent to the technique including difficulties in separating low abundant proteins, hydrophobic proteins (e.g. membrane), high/low molecular weight proteins, and also proteins with extremes of pI. Differential gel electrophoresis (DIGE), a modified version of 2D-PAGE, which uses differential labelling of protein samples with different fluorescent tags, offers increased throughput, ease of use, reproducibility, and accurate quantitation of protein expression differences (Unlu et al. 1997). This system enables the separation of two or three fluorescently labelled protein samples (Cy2, Cy3 and Cy5) on the same gel. Differential analysis software such as Decyder^{TM} identifies differentially expressed proteins of interest and these can then be enzymatically digested with trypsin and readily identified using mass spectrometry. Mass Spectrometry techniques such as Matrix Assisted Laser Desorption Ionisation Time-of-Flight Mass Spectrometry (MALDI-ToF MS) can identify proteins by generating peptide mass fingerprints (PMF) and subsequent database searching. Additionally electrospray ionisation (ESI-MS/MS) techniques are capable of providing amino acid sequence information on peptide fragments of the parent protein (Mann et al. 2001). A number of 2D electrophoresis maps containing the location of identified proteins have been generated for CHO cells (Naryzhny and Lee 2001; Hayduk et al. 2004; Champion et al. 1999) and NS0 cells (Smales et al. 2004).

Isobaric tag labelling technology

ICAT

Isotope-coded affinity tags (ICAT) use stable isotope labelling to perform quantitative analysis of paired protein samples. They contain a protein-reactive group, an ethylene glycol linker and a biotin tag (Gygi et al. 1999b). Two different isotope tags are generated by using linkers that contain either 8 hydrogen atoms (d0, light reagent) or 8 deuterium atoms (d8, heavy reagent) which bind covalently to cysteine moieties of amino acid within protein(s). Both samples

are mixed, digested with trypsin, fractionated by avidin affinity chromatography and then these differentially tagged peptides are scanned on the mass spectrometer. Spectral peak analysis in single mass spectrometric (MS) mode of the isotopically resolved peptides from the two different sources enables quantitation of the relative amounts of the peptide and hence the protein levels. Differentially expressed proteins can then be identified by tandem MS (MS/MS) sequencing. One weakness of ICAT is that only cysteine-containing peptides can be labelled. Approximately 10% of proteins do not have cysteine, therefore they will not be detected by ICAT.

iTRAQ

A similar isobaric labelling technology to ICAT, called iTRAQ, that labels amine residues in peptides has been recently developed (Ross et al. 2004). iTRAQ contains a set of four isobaric reagents and therefore can analyze up to four protein samples at the one time. After trypsin digestion, samples are labelled with 4 independent iTRAQ reagents. The reporter groups of the iTRAQ reagents will split from the peptide and generate small fragments for each sample with mass to charge ratios (m/z) of 114, 115, 116, and 117. The intensity of each of these peaks represents the quantity of small reporter group fragment and thus represents the quantity of a peptide sample. Peaks in the spectrum graph are used to identify peptide sequences and therefore identify the proteins of interest. By comparing the amounts of peptides labelled with each iTRAQ reagent, quantitative differences can be readily measured. Two recent reports have demonstrated the use of iTRAQ technology for profiling high producing recombinant CHO and NS0 cells (Seth et al. 2006; Nissom et al. 2006).

Other proteomic technologies

Other proteomic profiling technologies such as SELDI-ToF MS, protein arrays and MudPIT have been extensively used in biomedical applications such as cancer and other diseases (Seibert et al. 2005; Borrebaeck 2006; Kislinger et al. 2005).

To date there have been limited reports using these approaches in profiling recombinant mammalian cell lines for understanding phenotypic characteristics of relevance to large scale biopharmaceutical production. SELDI-ToF MS allows proteins/peptides to be profiled from different biological samples such as serum, on a variety of chemically (anionic, cationic, hydrophobic, hydrophilic, metal affinity capture, etc.) or bio-chemically (immobilised antibody, receptor, DNA, enzyme, etc.) defined chromatographic surfaces. The ToF reader records the time of flight and calculates the accurate molecular weight of proteins/peptides in the form of a spectral map containing mass to charge ratios (m/z) and intensities corresponding to each bound proteins/peptides. Software analysis of the spectral map allows the detection of differentially expressed protein/peptides with statistical significance (Koopman et al. 2004; Petricoin et al. 2002). MUDPIT is a non-gel approach that uses multidimensional high-pressure liquid chromatography (LC/LC) separation, tandem mass spectrometry, and database searching to identify differentially regulated proteins of interest (McDonald and Yates 2002). The complex protein mixture is digested with a specific protease, then peptide fragments allowed to separate in parallel with two-dimensional liquid chromatography using a strong cation exchange (SCX) column that separate peptides based on charge, and then by a reverse phase (RP) column based on hydrophobicity. Eluted peptides can then be identified using mass spectrometry. Protein arrays, which are similar in thinking to DNA microarrays are being used for drug discovery, biomarker identification and molecular profiling of cellular material. They can be generated by spotting antibodies or other affinity reagents, such as aptamers, purified proteins/peptides or fractionated proteins onto some sort of matrix to generate a protein array (Cretich et al. 2006).

Proteomics applied to large-scale mammalian cell culture

There have been a number of studies published to date using proteomic technologies to gain insights

into the biology of mammalian cell lines used for biopharmaceutical production. Some of the studies have directly compared different clonal cell lines producing various levels of recombinant protein (from 'low' to 'high' producers), while other studies have used proteomic tools to gain an understanding to why media supplementation (e.g. butyrate, DMSO), metabolic shift, and temperature shift, for example, cause this increase in productivity of recombinant proteins. The ultimate aim from all of these studies is to potentially develop a 'hyper-producing' cell line.

High Productivity

A recent publication by Nissom et al. (2006) compared recombinant CHO cells expressing differing amounts of green fluorescent protein (GFP) using a combination of transcriptome and proteome analysis. High producers were selected using FACS analysis. Proteomic profiling was performed using iTRAQ labelling of samples, followed by nano LC and mass spectrometry for protein quantitation and identification. A large number of proteins were identified that were involved in processes related to protein biosynthesis and folding, including EIF2S3 (eukaryotic translation initiation factor 2 subunit 3) and Hspd1 (60 kDa heat shock protein, mitochondrial precursor), and are among a list of 20 differentially regulated proteins provided in this publication. A number of proteins involved in chromatin modification (Hmgb1 and Histone H1.2), cellular growth (S100a11), carbohydrate metabolism (Mdh2—malate dehydrogenase), signal transduction (Annexin A1) and transport (vimentin) were also found to be altered in the high producing CHO cell line.

Smales et al. (2004) used 2D electrophoresis and mass spectrometry to compare NS0 cells with varying levels of recombinant monoclonal antibody productivity. Four recombinant NS0 cell lines were selected that had approximately equal growth rates but varying Mab specific production rates. A number of proteins found to be up-regulated with increasing levels of Mab productivity included the ER luminal chaperones involved in Mab folding, (ENPL (endoplasmic

and BiP (GRP78)), the non-ER cytosolic and mitochondrial chaperones (HSC7 and HSP60), proteins involved in mRNA translation (TCTP, translationally controlled tumour protein), nucleoside metabolism (NDKA), and oxidative stress (PDX1). Down regulated proteins included two glycolytic enzymes (ALFA and KPYc). Protein Disulphide Isomerase (PDI) was also found to increase with lower statistical significance than set as criteria for the other protein changes in these experiments. The ER luminal chaperones ENPL, BiP and PDI are known to be involved in Mab synthesis and assembly (Meunier et al. 2002). HSP60 and HSC7 are both thought to have anti-apoptotic functions (Kirchhoff et al. 2002; Mosser et al. 2000). In a related study (Alete et al. 2005), the microsomal component was isolated from the same set of NS0 cells and proteomic analyses was performed, and again PDI and BiP were found to be increased with increased levels of Mab productivity. Other proteins altered were from diverse functional groups such as cellular metabolism, cytoskeletal organisation, protein turnover and mAb folding/assembly. One of the more interesting protein changes was the cytoskeletal protein, cofilin that was increased with increased specific productivity. This protein is involved in the reversible control of actin polymerisation and depolymerisation, which are both crucial components of cytoskeletal organisation. It is becoming clear that the cellular cytoskeletal apparatus interacts functionally with the translational apparatus and that interference may lead to a reduction in global protein synthesis (Stapulionis et al. 1997). In a more recent study on the same set of samples from this group, a set of 79 proteins identified in NS0 cells were analysed in abundance across all the four NS0 cell lines with varying levels of specific monoclonal antibody productivity, to statistically determine if particular cell lines have distinct functional capabilities that facilitate production of monoclonal antibodies (Dinnis et al. 2006). They found that the relative abundance of proteins in ER chaperone, non-ER chaperone, cytoskeletal, cell signalling, metabolic and mitochondrial categories were significantly increased with increased productivity of Mab. They suggest that individual cells within parental populations are more functionally

equipped for high-level recombinant protein production than others, and that this bias could be used to select cells that are more likely to achieve high productivity.

Seth et al. (2006) profiled NS0 cells with high productivity levels using 2D gels and iTRAQ labelling in conjunction with their microarray profiling of the same sample set. Using these combined approaches they found that a large number of genes/proteins related to protein synthesis pathways and functions related to cell growth and death were altered in the high producing cell lines. The 22 protein changes were identified using 2D-PAGE and MS, while a further 30 proteins were identified using iTRAQ labelling and mass spectrometry identification of differentially regulated proteins, and are listed in this manuscript. It is also worth noting that the functional categories differ somewhat between the transcriptomic and proteomic results, for example they found that genes altered from the cell growth/death categories were more prominent at the transcript level. They also compared their data set to that of Smales et al. (2004), and found some commonly regulated proteins such as up regulated expression of PDI and endoplasmic, but opposite regulation was shown for 60-kDa heat shock protein, mitochondrial precursor, and Stress-70 protein mitochondrial precursor.

Metabolic Shift

Mammalian cells produce large quantities of lactate and ammonia that can adversely affect cell growth resulting in low cell densities. By maintaining nutrients at low concentrations in a fed-batch culture with feeding in response to the estimated metabolic demand, cellular metabolism can be altered such that lactate and ammonia accumulation is reduced whereas cell and product concentration is increased (Zhou et al. 1997). The shift in metabolism is reflected in the molar ratio of lactate produced to glucose consumed ($\Delta L/\Delta G$). At a metabolically shifted state, cells have markedly reduced glucose and other nutrient consumption and lactate production compared to cells in batch culture or in continuous culture without a metabolic shift. Korke et al. (2004) investigated the molecular mechanisms of

metabolic shift using two continuous cultures with different steady states with a glucose consumption to lactate production molar ratio of ($\Delta L/\Delta G$) of 0.08 and 1.4. The cells used were mouse–mouse hybridoma cell line MAK producing IgG monoclonal antibody. Proteomic analysis using 2D-PAGE and MS revealed a large number of proteins differentially regulated between the two sets of comparators including proteins involved in metabolism (e.g. glucose-6-phosphate isomerase, alpha enolase and L-lactate dehydrogenase), protein processing (proteasome activator complex subunit 1) and in cellular architecture (e.g. the cytoskeletal proteins, beta-actin and gamma-actin). In an earlier related study, Seow et al. (2001) identified 8 differentially regulated proteins related to metabolic shift including metabolic enzymes such as the glycolytic enzyme, phosphoglycerate mutase, and proteins involved in cellular architecture, such as beta-actin and gamma-actin. These two studies would appear to suggest that the actin content might be changing due to some cellular structure reorganisation during metabolic shift. It has been reported previously that an important mechanism that controls glycolysis is the reversible binding of glycolytic enzymes to the actin cytoskeleton (Knull and Walsh 1992; Bereiter-Hahn et al. 1995).

Temperature Shift

Low temperature cultivation has been shown to be an important step, depending on the cell type and expression system used, to significantly improve the specific productivity of recombinant proteins (Yoon et al. 2003; Fox et al. 2004). The cellular and molecular mechanisms underlying the effects of low temperature on growth and productivity in mammalian cells is poorly understood. Using a combination of 2D PAGE and western blotting, Kaufmann et al. (1999) investigated the effect of low-cultivation temperature on productivity of recombinant CHO cells following a temperature shift from 37 to 30°C. They also looked at changes in tyrosine phosphorylation and its role in the cold shock response of CHO cells. They used CHO-K1 cells engineered to produce SEAP (secreted alkaline phosphatase),

and found that the growth rate significantly decreased following temperature shift to 30°C but there was an associated 3.5-fold increase in production of SEAP protein. 2D gel electrophoresis showed 10 spots that had significantly higher or lower intensities in the 30°C gels compared to the 37°C standard gels, and western blotting showed altered levels of two tyrosine-phosphorylated proteins following time shift to 30°C.

Baik et al. (2005) performed a transcriptome and proteome analysis on CHO cells producing recombinant erythropoietin (EPO) to investigate and understand the effects of low temperature cultivation on increased productivity of EPO (Yoon et al. 2003). The expression levels of 7 proteins (PDI, vimentin, NDK B, ERp57, RIKEN cDNA, phosphoglycerate kinase, and heat shock cognate 71 kDa) were increased by over 2-fold at 33°C and two proteins (HSP90-beta and EF2) were decreased over 2-fold, compared to the reference temperature of 37°C. These proteins have a number of roles in cellular activities such as protein folding (PDI, ERp57, and heat shock cognate 71 kDa), metabolism (phosphoglycerate kinase, NDK B), protection from cell stress (HSP90-beta), and protein synthesis (EF2). EF2 is known to specifically promote the GTP-dependent translocation of the nascent protein chain from the a-site to the p-site of the ribosome and a decrease of cellular EF2 may cause an inhibition of protein synthesis (Elo et al. 2005) possibly resulting in decreased growth rate at low temperatures.

Media Supplementation

Hyperosmotic pressure, which can be induced by adding salts or sugars to culture media, has been suggested as an economical solution to increase the protein productivity in recombinant CHO cells (Chen et al. 1998; Kim et al. 2000). Recombinant CHO cells expressing a monoclonal antibody were subjected to hyperosmotic pressure in order to understand intracellular responses of these cells to hyperosmotic pressure (Lee et al. 2003). Proteome profiling experiments using 2D-PAGE and MS resulted in the identification of 3 proteins that were differentially regulated between cells cultured in standard

media (300 mOsm/l) compared to hyperosmolar media at 450 mOsm/l (obtained through addition of NaCl). Two glycolytic enzymes, glyceraldehyde-3-phosphate dehydrogenase and pyruvate kinase were found to be up-regulated in the hyperosmolar media, and this may be due to an increased metabolic energy for antibody synthesis in these cells where they found that the hyperosmotic pressure enhanced specific glucose consumption rates, thus agreeing with the up-regulation of these enzymes. Specific antibody productivity was also increased by 139% compared with that obtained at 300 mOsm/l. Tubulin, a major component of microtubules, was down regulated in the hyperosmolar medium, and this may be due to alterations in cellular architecture as a result of increased productivity following hyperosmolar media conditions (Lee et al. 2003).

Van Dyk et al. (2003) investigated proteins potentially altered following elevated levels of human growth hormone (hGH) in recombinant CHO cells. Sodium butyrate is well known to enhance cellular productivity of recombinant proteins (Palermo et al. 1991), and appears to cause a complex series of changes in CHO cells that relate to longevity of culture and specific productivity. In this report, recombinant CHO cells expressing hGH were exposed to the media additives zinc and butyrate, and a number of cellular proteins were altered (i.e. increased expression) in response to these additives, resulting in a higher productivity of hGH. These were identified as GRP75 (mitochondrial member of the heat shock 70 family), enolase (glycolytic enzyme) and thioredoxin (involved in the biosynthetic reduction of ribonucleotides to deoxyribonucleotides) using a 2D-PAGE and mass spectrometry-based approach.

DMSO has previously been shown to increase specific productivity of a fusion protein in CHO cells (Liu et al. 2001a) but it is not clear how DMSO exerts this effect to improve productivity. Li et al. (2006) investigated the effect of DMSO on increased productivity of hepatitis B surface antigen expression in recombinant CHO cells using differential protein profiling using a 2D-PAGE and MS-based approach. They identified seven proteins whose expression was

significantly altered following exposure to DMSO and an associated increase in productivity. A number of proteins involved in metabolism, including 4 glycolytic enzymes (triosephosphate isomerase, GAPDH, aldolase and phosphoglycerate kinase) were shown to have down regulated expression.

Proteomic studies have also been performed to gain a better understanding of the molecular mechanisms that regulate cholesterol-dependent growth in NS0 cells (Seth et al. 2005). NS0 cells by their nature are cholesterol-dependent, thus their growth is dependent on the provision of cholesterol to the medium; however this presents difficulties because of its poor solubility. NS0 cells are capable of cholesterol-free growth and can be isolated by selecting mutant clones (Birch et al. 1994). Adaptation generally involves passaging cells over time during which the cholesterol concentration is gradually reduced (Sinacore et al. 1996). In this study by Seth et al. (2005), proteomic tools were used to compare the protein profiles of regular 'wild-type' NS0 cells and a 'revertant' cell line that can grow without cholesterol. 2D-PAGE and mass spectrometry identified 23 proteins that were differentially regulated in the revertant cells.

Conclusions

A number of studies have been performed to date profiling mammalian cells used for biopharmaceutical production to gain a better understanding of the biology of these cells, with the ultimate aim to generate a cellular phenotype capable of high productivity of recombinant protein. The data generated so far from these studies is suggesting that a wide range of cellular mechanisms are altered in high producing cells, especially from categories such as protein folding and secretion, protein synthesis, cellular architecture (cytoskeleton), cellular metabolism (e.g. glucose, protein, lipid, etc.), cellular growth/death, and signal transduction. Challenges lie ahead in trying to make sense of all these data that have been generated so far and will do so in the future, as proteomic technologies continue to develop. However, these studies will allow a greater insight into the biology

of these cells which have been poorly understood to date, and ultimately leading to the development of a hyper-producing cell line.

Acknowledgements This work is funded by Science Foundation Ireland (SFI).

References

- Alete DE, Racher AJ, Birch JR, Stansfield SH, James DC, Smales MC (2005) Proteomic analysis of enriched microsomal fractions from GS-NS0 murine myeloma cells with varying secreted recombinant monoclonal antibody productivities. *Proteomics* 5:4689–4704
- Arts J, Lansink M, Grimbergen J, Toet KH, Kooistra T (1995) Stimulation of tissue-type plasminogen activator gene expression by sodium butyrate and trichostatin A in human endothelial cells involved histone acetylation. *Biochem J* 310:171–6
- Baik JY, Lee MS, An SR, Yoon SK, Joo EJ, Kim YH, Park HW, Lee GM (2005) Initial transcriptome and proteome analyses of low culture temperature-induced expression in CHO cells producing erythropoietin. *Biotechnol Bioeng* 93:361–371
- Bereiter-Hahn J, Stubig C, Heymann V (1995) Cell cycle-related changes in F-actin distribution are correlated with glycolytic enzymes. *Exp Cell Res* 218:551–560
- Birch JR, Boranston RC, Metcalfe H, Brown ME, Bebbington CR, Field RP (1994) Selecting and designing cell lines for improved physiological characteristics. *Cytotechnology* 15:11–16
- Borrebaeck CA (2006) Antibody microarray-based oncoproteomics. *Expert Opin Biol Ther* 6:833–838
- Carvalho AV, Santos SS, Calado J, Haury M, Carrondo MJT (2003) Cell growth arrest by nucleotides, nucleosides and bases as a tool for improved production of recombinant proteins. *Biotechnol Prog* 19:69–83
- Champion KM, Arnott D, Henzel WJ, Hermes S, Weikert S, Stults J, Vanderlaan M, Krummen L (1999) A two-dimensional protein map of Chinese hamster ovary cells. *Electrophoresis* 20:944–1000
- Chen Z, Liu H, Wu B (1998) Hyperosmolality leads to an increase in tissue-type plasminogen activator production by a Chinese hamster ovary cell line. *Biotechnol Tech* 12:207–209
- Chu L, Robinson DK (2001) Industrial choices for protein production by large scale cell culture. *Curr Opin Biotechnol* 12:180–187
- Cretich M, Damin F, Pirri G, Chiari M (2006) Protein and peptide arrays: recent trends and new directions. *Biomol Eng* 23:77–88
- Dinnis DM, Stansfield SH, Schlatter S, Smales CM, Alete D, Birch JR, Racher AJ, Marshall CT, Nielsen LK, James DC (2006) Functional proteomic analysis of GS-NS0 murine myeloma cell lines with varying recombinant monoclonal antibody production rate. *Biotech Bioeng* 94:830–841

- Elo MA, Karjalainen HM, Sironen RK, Valmu L, Redpath NT, Browne GJ, Kalkinen N, Helminen HJ, Lammi MJ (2005) High hydrostatic pressure inhibits the biosynthesis of eukaryotic elongation factor-2. *J Cell Biochem* 94:497–507
- Fox SR, Patel UA, Yap MGS, Wang DIC (2004) Maximizing interferon- γ production by Chinese hamster ovary cells through temperature shift optimization: experimental and modelling. *Biotechnol Bioeng* 85:177–184
- Fussenegger M, Schlatter S, Dätwyler D, Mazur X, Bailey JE (1998) Controlled proliferation by multigene metabolic engineering enhances the productivity of Chinese hamster ovary cells. *Nat Biotechnol* 16:468–472
- Gygi SP, Rochon Y, Franz BR, Aebersold R (1999a) Correlation between protein and mRNA abundance in yeast. *Mol Cell Biol* 19:1720–1730
- Gygi SP, Rist B, Gerber SA, Turecek F, Gelb MH, Aebersold R (1999b) Quantitative analysis of complex protein mixtures using isotope-coded affinity tags. *Nat Biotechnol* 17:994–999
- Görg A, Weiss W, Dunn WJ (2004) Current two-dimensional electrophoresis technology for proteomics. *Proteomics* 4:3665–3685
- Hayduk EJ, Choe LH, Lee KH (2004) A two-dimensional electrophoresis map of Chinese hamster ovary cell proteins based on fluorescence staining. *Electrophoresis* 25:2545–2556
- Kaufmann H, Mazur X, Fussenegger M, Bailey JE (1999) Influence of low temperature on productivity, proteome and protein phosphorylation of CHO cells. *Biotechnol Bioeng* 63:573–582
- Kim TK, Ryu JS, Chung JY, Kim MS, Lee GM (2000) Osmoprotective effect of glycine betaine on thrombopoietin production in hyperosmotic Chinese hamster ovary cell culture: Clonal variations. *Biotechnol Prog* 16:775–781
- Kirchhoff SR, Gupta S, Knowlton AA (2002) Cytosolic heat shock protein 60, apoptosis, and myocardial injury. *Circulation* 105:2899–2904
- Kislinger T, Gramolini AO, MacLennan DH, Emili A (2005) Multidimensional protein technology (MudPIT): technical overview of a profiling method optimized for the comprehensive proteomic investigation of normal and diseased heart tissue. *J Am Soc Mass Spectrom* 16:1207–1220
- Knoll HR, Walsh JL (1992) Association of glycolytic enzymes with cytoskeleton. *Curr Top Cell Regul* 33:15–30
- Koopmann J, Zhang Z, White N, Rosenzweig J, Fedarko N, Jagannath S, Canto MI, Yeo CJ, Chan DW, Goggins M (2004) Serum diagnosis of pancreatic adenocarcinoma using surface-enhanced laser desorption and ionization mass spectrometry. *Clin Cancer Res* 10:860–868
- Korke R, Gatti MdL, Lau ALY, Lim JWE, Seow TK, Chung MCM, Hu WS (2004) Large scale gene expression profiling of metabolic shift of mammalian cells in culture. *J Biotechnol* 107:1–17
- Lee MS, Kim KW, Kim YH, Lee GM (2003) Proteome analysis of antibody-expressing CHO cells in response to hyperosmotic pressure. *Biotechnol Prog* 19:1734–1741
- Li J, Huang Z, Sun X, Yang P, Zhang Y (2006) Understanding the enhanced effect of dimethyl sulfoxide on hepatitis B surface antigen expression in the culture of Chinese hamster ovary cells on the basis of proteome analysis. *Enzyme Microb Technol* 38:372–380
- Liu CH, Chu IM, Hwang SM (2001a) Enhanced expression of various genes in recombinant Chinese hamster ovary cells in presence of dimethylsulfoxide. *Biotechnol Lett* 23:1641–1655
- Liu CH, Chu IM, Hwang SM (2001b) Pentanoic acid, a novel protein synthesis stimulant for Chinese hamster ovary (CHO) cells. *J Biosci Bioeng* 91:71–75
- Mann M, Hendrickson RC, Pandey A (2001) Analysis of proteins and proteomes by mass spectrometry. *Annu Rev Biochem* 70:437–473
- McDonald WH, Yates JR 3rd (2002) Shotgun proteomics and biomarker discovery. *Dis Markers* 18:99–105
- Meunier L, Usherwood YK, Chung KT, Hendershot LM (2002) A subset of chaperones and folding enzymes form multiprotein complexes in endoplasmic reticulum to bind nascent proteins. *Mol Biol Cell* 13:4456–4469
- Mosser DD, Caron AW, Bourget L, Meriin AB, Sherman MY, Morimoto RI, Massie B (2000) The chaperone function of hsp70 is required for protection against stress-induced apoptosis. *Mol Cell Biol* 20:7146–7159
- Naryzhny SN, Lee H (2001) Protein profiles of the Chinese hamster ovary cells in the resting and proliferating stages. *Electrophoresis* 22:1764–1775
- Nissom PM, Sanny A, Kok YJ, Hiang YT, Chuah SH, Shing TK, Lee YY, Wong KTK, Hu WS, Sim MYG, Philip R (2006) Transcriptome and proteome profiling to understanding the biology of high productivity CHO cells. *Mol Biotechnol* 34:125–140
- de Nobel H, Lawrie L, Brul S, Klis F, Davis M, Alloush H, Coote P (2001) Parallel and comparative analysis of the proteome and transcriptome of sorbic acid-stressed *Saccharomyces cerevisiae*. *Yeast* 18:1413–1428
- Palermo DP, DeGraaf ME, Marotti KR, Rehburg E, Post LE (1991) Production of analytical quantities of recombinant proteins in Chinese hamster ovary cells using sodium butyrate to elevate gene expression. *J Biotechnol* 19:35–47
- Petricoin EF 3rd, Ardekani AM, Hitt BA, Levine PJ, Fusaro VA, Steinberg SM, Mills GB, Simone C, Fishman DA, Kohn EC, Liotta LA (2002) Use of proteomic patterns in serum to identify ovarian cancer. *Lancet* 359:572–577
- Ross PL, Huang YN, Marchese JN, Williamson B, Parker K, Hattan S, Khainovski N, Pillai S, Dey S, Daniels S, Purkayastha S, Juhasz P, Martin S, Bartlett-Jones M, He F, Jacobson A, Pappin DJ (2004) Multiplexed protein quantitation in *Saccharomyces cerevisiae* using amine-reactive isobaric tagging reagents. *Mol Cell Proteomics* 3:1154–1169

- Seibert V, Ebert MP, Buschmann T (2005) Advances in clinical cancer proteomics: SELDI-ToF-mass spectrometry and biomarker discovery. *Brief Funct Genomic Proteomic* 4:16–26
- Seow TK, Korke R, Liang RCMY, Ong SE, Ou K, Wong K, Hu WS, Chung MCM (2001) Proteomic investigation of metabolic shift in mammalian cell culture. *Biotechnol Prog* 17:1137–1144
- Seth G, Philp RJ, Denoya CD, McGrath K, Stutzman-Engwall KJ, Yap M, Hu WS (2005) Large-scale gene expression analysis of cholesterol dependence in NS0 cells. *Biotechnol Bioeng* 90:552–567
- Seth G, Philp RJ, Lau A, Jiun KY, Yap M, Hu WS (2006) Molecular portrait of high productivity in recombinant NS0 cells. *Biotechnol Bioeng* DOI 10.1002/bit.21234
- Sinacore MS, Charlebois TS, Harrison S, Brennan S, Richards T, Hamilton M, Scott S, Brodeur S, Oakes P, Leonard M et al (1996) Cho Dukx cell lineages preadapted to growth in serum-free culture enable rapid development of cell culture processes for manufacture of recombinant proteins. *Biotechnol Bioeng* 52:518–528
- Smales CM, Dinnis DM, Stansfield SH, Alete D, Sage EA, Birch JR, Racher AJ, Marshall CT, James DC (2004) Comparative proteomic analysis of GS-NS0 murine myeloma cells lines with varying recombinant monoclonal antibody production rate. *Biotechnol Bioeng* 88:474–488
- Stapulionis R, Kolli S, Deutscher MP (1997) Efficient mammalian protein synthesis requires an intact F-actin system. *J Biol Chem* 272:24980–24986
- Unlu M, Morgan ME, Minden JS (1997) Difference gel electrophoresis: a single gel method for detecting changes in protein extracts. *Electrophoresis* 18:2071–2077
- Van Dyk DD, Misztal DR, Wilkins MR, Mackintosh JA, Poljak A, Varnai JC, Teber E, Walsh BJ, Gray PP (2003) Identification of cellular changes associated with increased production of human growth hormone in a recombinant Chinese hamster ovary cell line. *Proteomics* 3:147–156
- Yoon SK, Song JY, Lee GM (2003) Effect of low culture temperature on specific productivity, transcription level and heterogeneity of erythropoietin in Chinese hamster ovary cells. *Biotechnol Bioeng* 82:289–289
- Zhou WC, Rehm J, Europa A, Hu WS (1997) Alteration of mammalian cell metabolism by dynamic nutrient feeding. *Cytotechnology* 24:99–108