Proteomic Analysis of CHO Cells during Recombinant Protein Production in High-Density Culture

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Abstract

A proteomics approach was used to study protein expression changes in a recombinant IgG-producing CHO cell line throughout a fed-batch culture. Protein expression profiles were analyzed by 2-dimensional gel electrophoresis at four time points representing mid exponential, late exponential, mid and late stationary phases of growth. Differentially expressed proteins were selected using Phoretix™ 2D Expression software and identified using in-gel tryptic digest and LC-MS (MS). Molecules involved in cellular metabolism, protein synthesis and folding, anti-oxidation and cytoskeletal functions were identified to be upregulated during the fed-batch bioreactor run. These studies provided insight in the intracellular mechanisms that correlate with high-level recombinant production and may shed some light on the significance of protein folding and secretion in recombinant protein production. These results may also provide potential candidate genes for future cell line and metabolic engineering work.

Material and Methods

Bioreactor Culture: Stock culture of a recombinant IgG producing cell line was maintained in animal component-free suspension culture under G418 selection. A 5L bioreactor (B. Braun) was inoculated at 7.5 × 10^4cells/mL and maintained as fed-batch cultures until viability dropped below 80%. The culture was sampled daily and monitored for growth, viability, IgG productivity, and metabolic profile. Glucose and glutamine were fed to 3 g/L and 4 mM respectively when needed. A concentrated CHO feed (Cat. No. C1615) was delivered every other day beginning on day 5. Production of IgG was determined by an HPLC Protein G affinity assay.

Sample preparation: Cells (5 × 10^7) were extracted in 1 mL of extraction solution. The extraction solution was cellular and organelle membrane solubilizing reagent (Cat. No. C0356) with the addition of 10 mM spermine and 5 mM TCEP. The solutions were incubated for 1 hour with mixing at room temperature. Samples were centrifuged for 30 minutes at 16,000 RCF. Samples were reduced with tributylphosphine and alkylated with iodoacetamide using the ProteoPrep Reduction and Alkylation Kit (Cat. No. PROT-RA). Total protein concentration was determined using Bradford reagent (Cat. No. B6916). Samples were TCA precipitated using the ProteoPrep Protein Precipitation Kit (Cat. No. PROT-PR). Pellets were re-suspended in the extraction solution.

Separation by 2-DE: IPG strips (11 cm, pH 3–10, Cat. No. I3406) were rehydrated with the samples and were focused at 8,000 Volts for 85,000 Volt hours. The IPG strips were equilibrated for 20 minutes with IPG equilibration buffer (Cat. No. I7281) and SDS-PAGE was performed using 4-20% Tris-HCl precast gels (BioRad). The gels were electrophoresed for 10 minutes at 80 Volts followed by 70 minutes at 170 Volts. Two sets of gels were run for each time point in the culture. Protein bands were visualized in the gels using EZBlue gel stain (Cat. No. G1041). The gels were imaged using a Flour-S® MultiImager (BioRad) and analysis of the gels was performed using Phoretix™ 2D Expression Software (Nonlinear Dynamics).

LC-MS (MS) analysis: Spots of interest were excised and digested using Trypsin Profile IGD Kit (Cat. No. PP0100). The digests were dried in a vacuum centrifuge and dissolved with 40 µL 0.1% TFA. Digested samples (5 µL) were separated by reverse phase on a 150 × 2.1 mm, 5 µm Supelco Discovery HS C18 column. The separation was performed over 30 minutes with a gradient of formic acidified water and acetonitrile. Tandem mass spectrometric data was obtained on a Thermo Finnigan LTQ using a Data Dependent Acquisition (DDA) method with dynamic exclusion. Data sets were searched using the SEQUEST algorithm on Bioworks 3.2. Filtration for the proteins identified was achieved using high confidence filters[1].

Results and Discussion

Bioreactor Culture

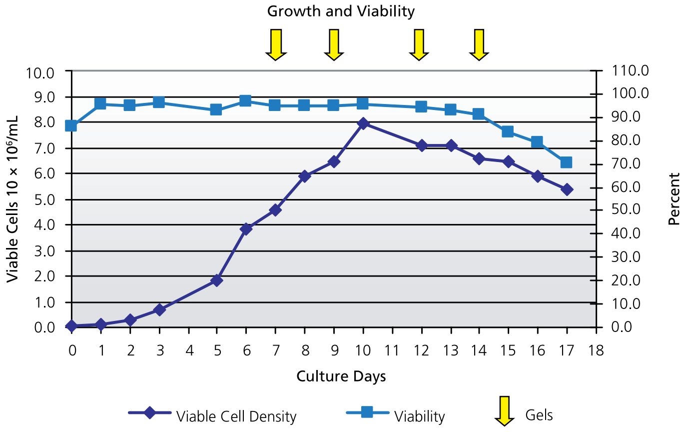

Figure 1: The bioreactor culture reached peak cell density of 8.0 × 10^6 cells/mL on day 10. Percent of viable cells remained above 90% through day 14. Culture was ended on day 18 when viability dropped below 70%. Harvested cells from days 7, 9, 12, and 14 were analyzed.

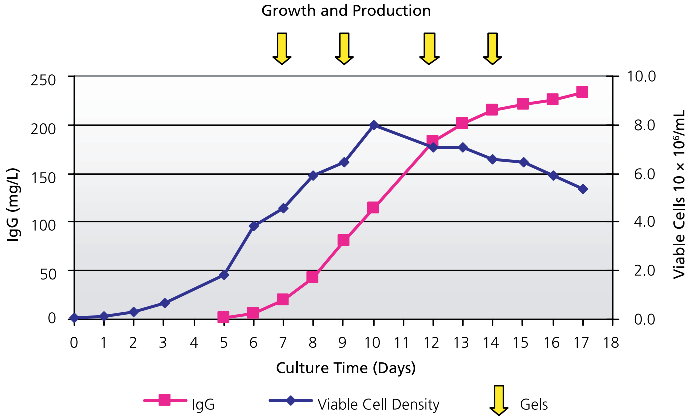

Figure 2: Recombinant IgG production became quantifiable around day 5 and increased rapidly through day 12. The rate of production then decreased sharply, although the culture maintained stationary phase for several more days. The days chosen for 2-DE analysis represent beginning, middle and end of IgG production for this fed-batch culture.

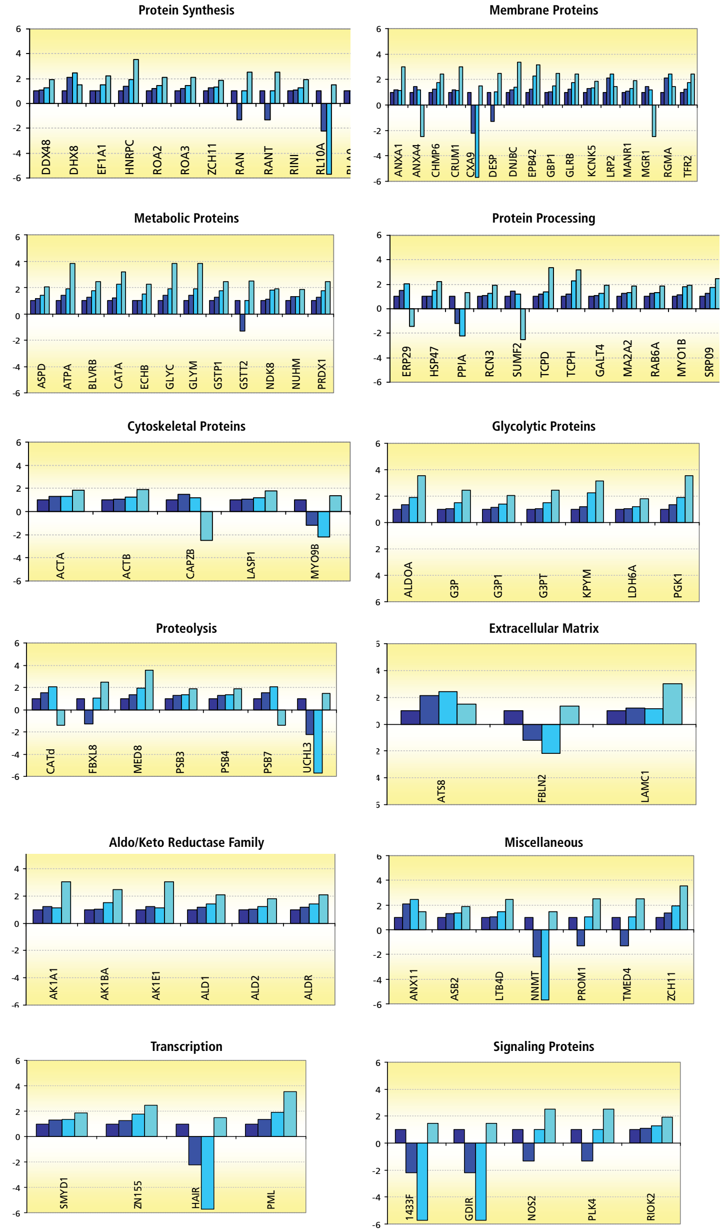

Figure 3: Expression profiles for identified protein spots. Stained gels were analyzed using the Phoretix™ 2D Expression Software. Protein spots with normalized volume change of 1.8 fold or greater were selected for analysis. Thirty spots were excised from gels, digested using Trypsin Profile IGD Kit and submitted for identification by LC-MS (MS). Mass spectrometry was utilized to successfully identify 96 proteins from these samples. The expression profiles for the 96 proteins identified are shown in **Figure 3**. These proteins were grouped based on their functions and/or localizations. Spot volumes are normalized to day 7 to demonstrate the relative change during the course of the bioreactor culture.

Conclusions

- Proteomic analysis was successfully utilized to study changes in protein expression during a recombinant CHO bioreactor culture.
- 96 proteins with altered expression levels were identified. These proteins could be categorized by function and/or localization.

  o Protein synthesis, processing, and proteolysis
  o Transcription-related proteins
  o ER proteins
  o Signaling proteins
  o Metabolic proteins
  o Glycolytic proteins
  o Aldo-keto reductase family proteins
  o Cytoskeletal proteins
  o Membrane proteins
  o Extracellular matrix
  o Miscellaneous
  o The protein expression changes seen over the time course of this culture may indicate alterations in biosynthetic pathways to support increased synthesis and processing of protein in this cell line.

  o The proteins identified in this experiment represent potential targets for cell engineering to generate higher producing cell lines.

Summary of Protein Identifications

Protein Synthesis

Gene Name	Species	Description	Accession
DDX48	Human	Probable ATP-dependent helicase DDX48 (DEAD-box protein 48)	P38919
DHX8	Human	ATP-dependent helicase DHX8	Q14562
EF1A1	Chinese hamster	Elongation factor 1-alpha 1	P62629
HNRPC	Rat	Heterogeneous nuclear ribonucleoprotein C	P17132
ROA2	Human	Heterogeneous nuclear ribonucleoproteins A2/B1	P22626
ROA3	Human	Heterogeneous nuclear ribonucleoprotein A3	P51991
ZCH11	Human	Zinc finger CCHC domain containing protein 11	Q5TAX3
RAN	Human	GTP-binding nuclear protein Ran	P62826
RANT	Mouse, Rat	GTP-binding nuclear protein Ran, testis-specific isoform	Q8K586
RINI	Rat	Ribonuclease inhibitor	P29315
RL10A	Human	60S ribosomal protein L10a (CSA-19)	P62906
RLA0	Human, rat	60S acidic ribosomal protein P0	P05388

Protein Processing

Gene Name	Species	Description	Accession
ERP29	Mouse	Endoplasmic reticulum protein ERp29 precursor	P57759
HSP47	Human	47 kDa heat shock protein precursor	P29043
PPIA	Chinese hamster	Peptidyl-prolyl cis-trans isomerase A (Rotamase)	P14851
RCN3	Human, Mouse	Reticulocalbin-3 precursor	Q8BH97
SUMF2	Mouse	Sulfatase-modifying factor 2 precursor	Q8BPG6
TCPD	Human	T-complex protein 1, delta subunit	P50991
TCPH	Human	T-complex protein 1, eta subunit	Q99832
GALT4	Human	Polypeptide N-acetylgalactosaminyltransferase 4	Q8N4A0
MA2A2	Human	Alpha-mannosidase IIx	P49641
RAB6A	Human	Ras-related protein Rab-6A	P20340
MYO1B	Human	Myosin Ib	O43795
SRP09	Human	Signal recognition particle 9 kDa protein	P49458

Proteolysis

Gene Name	Species	Description	Accession
CATd	Rat	Description	P24268
FBXL8	Mouse	F-box/LRR-repeat protein 8	Q8CIG9
MED8	Human	Mediator of RNA polymerase II transcription subunit 8 homolog	Q96G25
PSB3	Human	Proteasome subunit beta type 3	P49720
PSB4	Human, Rat	Proteasome subunit beta type 4 precursor	P28070
PSB7	Human, Mouse, Rat	Proteasome subunit beta type 7 precursor	Q99436
UCHL3	Human	Ubiquitin carboxyl-terminal hydrolase isozyme L3	P15374

Cytoskeleton

Gene Name	Species	Description	Accession
ACTA	Human	Actin, aortic smooth muscle	P62736
ACTB	Chinese hamster	Actin, cytoplasmic 1 (Beta-actin)	P48975
CAPZB	Human	F-actin capping protein beta subunit	P47756
LASP1	Human	LIM and SH3 domain protein 1	Q14847
MYO9B	Human	Myosin-9B	Q13459

Plasma Membrane

Gene Name	Species	Description	Accession
ANXA1	Mouse	Annexin A1	P10107
ANXA4	Bovine	Annexin A4	P13214
CHMP6	Mouse	Charged multivesicular body protein 6	P0C0A3
CRUM1	Human	Crumbs protein homolog 1 precursor	P82279
CXA9	Mouse	Gap junction alpha-9 protein (Connexin 36)	O54851
DESP	Human	Desmoplakin	P15924
DNJBC	Human	DnaJ homolog subfamily B member 12	Q9NXW2
EPB42	Mouse	Erythrocyte membrane protein band 4.2	P49222
GBP1	Human	Interferon-induced guanylate-binding protein 1	P32455
GLRB	Human	Glycine receptor beta chain precursor	P48167
KCNK5	Human	Potassium channel subfamily K member 5	O95279
LRP2	Rat	Low-density lipoprotein receptor-related protein 2 precursor	P98158
MANR1	Human	Macrophage mannose receptor precursor	P22897
MGR1	Human	Metabotropic glutamate receptor 1 precursor	Q13255
RGMA	Human	Repulsive guidance molecule A precursor	Q96B86
TFR2	Human	Transferrin receptor protein 2	Q9UP52

Extra cellular Matrix

Gene Name	Species	Description	Accession
ATS8	Human	ADAMTS-8 precursor	Q9UP79
FBLN2	Mouse	Fibulin-2 precursor	P37889
LAMC1	Mouse	Laminin gamma-1 chain precursor	P02468

Metabolic

Gene Name	Species	Description	Accession
ASPD	Mouse	Putative L-aspartate dehydrogenase	Q9DCQ2
ATPA	Human	ATP synthase alpha chain, mitochondrial precursor	P25705
BLVRB	Mouse	Flavin reductase	Q923D2
CATA	Human, Mouse	Catalase	P24270
ECHB	Human, Mouse, Rat	Trifunctional enzyme beta subunit, mitochondrial precursor	P55084
GLYC	Human	Serine hydroxymethyltransferase, cytosolic	P34896
GLYM	Human	Serine hydroxymethyltransferase, mitochondrial precursor	P34897
GSTP1	Long-tailed hamster	Glutathione S-transferase P	P46424
GSTT2	Mouse	Glutathione S-transferase theta 2	Q61133
NDKA	Human	Nucleoside diphosphate kinase A	P15531
NUHM	Mouse	NADH-ubiquinone oxidoreductase 24 kDa subunit	Q9D6J6
PRDX1	Human, Mouse, Rat	Peroxiredoxin 1	Q06830

Glycolysis

Gene Name	Species	Description	Accession
ALDOA	Human, Mouse	Fructose-bisphosphate aldolase A	P04075
G3P	Chinese hamster	Glyceraldehyde-3-phosphate dehydrogenase	P17244
G3P1	Human	Glyceraldehyde-3-phosphate dehydrogenase, muscle	P00354
G3PT	Human, Mouse	Glyceraldehyde-3-phosphate dehydrogenase, testis-specific	Q64467
KPYM	Human, Mouse, Rat	Pyruvate kinase, isozymes M1/M2	P14618
LDH6A	Human	L-lactate dehydrogenase A-like 6A	Q6ZMR3
PGK1	Chinese hamster	Phosphoglycerate kinase 1	P50310

Aldo/Keto Reductase Family

Gene Name	Species	Description	Accession
AK1A1	Chinese hamster	Alcohol dehydrogenase [NADP+]	O70473
AK1BA	Human	Aldo-keto reductase family 1 member B10	O60218
AK1E1	Mouse	Aldo-keto reductase family 1 member E1	Q9DCT1
ALD1	Mouse	Aldose reductase-related protein 1	P21300
ALD2	Chinese hamster	Aldose reductase-related protein 2	O08782
ALDR	Human, Mouse	Aldose reductase	P15121

Transcription

Gene Name	Species	Description	Accession
SMYD1	Human	SET and MYND domain containing protein 1	Q8NB12
ZN155	Human	Zinc finger protein 155	Q12901
HAIR	Mouse	Hairless protein	Q61645
PML	Human	Probable transcription factor PML	P29590

Miscellaneous

Gene Name	Species	Description	Accession
ANX11	Human, Mouse	Annexin A11	P97384
ASB2	Human	Ankyrin repeat and SOCS box protein 2	Q96Q27
LTB4D	Mouse, Rat	NADP-dependent leukotriene B4 12-hydroxydehydrogenase	P97584
NNMT	Human	Nicotinamide N-methyltransferase	P40261
PROM1	Human	Prominin 1 precursor	O43490
TMED4	Human	Transmembrane emp24 domain containing protein 4 precursor	Q7Z7H5
ZCH11	Human	Zinc finger CCHC domain containing protein 11	Q5TAX3

Signaling

Gene Name	Species	Description	Accession
1433F	Human	14-3-3 protein eta (Protein AS1)	Q04917
GDIR	Mouse, Rat	Rho GDP-dissociation inhibitor 1 (Rho GDI 1)	Q99PT1
NOS2	Rat	Nitric oxide synthase, inducible	Q06518
PLK4	Human	Serine/threonine-protein kinase PLK4	O00444
RIOK2	Mouse	Serine/threonine-protein kinase RIO2	Q9CQS5

Table 1: Summary of Protein Identifications. Tandem mass spectrometric data were searched and filtered as described in Materials and Methods.

Reference

[1] Proteomics 2005 (5): 3226-3245