CHAPTER 3

GENOMICS AND PROTEOMICS OF CHINESE HAMSTER OVARY (CHO) CELLS

Understanding CHO Cell Culture at the Molecular Level

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Abstract: This chapter provides an overview on the use of high throughput screening technologies like microarray and proteomics, to studying bioprocess issues in cell cultures. In bioprocessing, these tools may be used to study the genetic circuitry underlying cellular metabolism, growth, death and protein glycosylation during high cell density production processes and protein-free media adaptation. These studies can provide valuable insights into the crucial pathways involved in energy metabolism, product formation and cellular death as well as identify potential gene targets for engineering cell lines with desirable characteristics for biologics production.

Keywords: microarray, proteomics, proteome, CHO, Chinese hamster ovary

1. INTRODUCTION

Cell engineering of industrial cell lines, leading to enhanced traits such as improved viability, increased production of recombinant proteins, robustness and a host of other desirable characteristics, requires an understanding at the molecular level of how cells operate during cell culture. This requires a knowledge of which genes are expressed and subsequently translated into proteins. Gene expression is usually studied at the mRNA level using high throughput technologies like microarray while the protein levels are studied using proteomics. Gene expression studies measures message (mRNA) abundance but not actual protein levels. There may not be a good correlation between mRNA abundance and protein levels, as was shown in the study by Gygi et al on protein and mRNA abundance in yeast and Anderson and Seilhamer, with regards to the human liver. While there is a general lack of correlation at any given time point within the total proteome and transcriptome,
however, there may be correlation in functional subsets of genes/proteins. Subsets which do not correlate may represent pathways regulated by post-translational modification.³

In recent years, with advances in genomics and proteomics, genomic-scale analyses have become a more practical approach as opposed to traditional biochemical assays which focused on analyzing a single reaction at a time. Microarrays permit rapid, large scale analyses of samples. The ability to analyze many samples simultaneously is perhaps the most attractive feature of this technology. A very well established use of DNA microarrays is to create transcription profiles which is a measure of gene expression. Microarrays are patterns of cDNA sequences deposited on a substrate, usually glass. These arrays of DNA are then probed with fluorescently labeled mRNAs extracted from cells. The mRNAs will hybridize to their immobilized complementary DNA on the chip (Figure 1). The fluorescence intensities give a relative measure of gene expression.

The proteome is defined as the complement of proteins that is present in a cell at a given moment in time and under a particular set of environmental conditions. Whilst the genome is a relatively static entity the proteome, in contrast, is a highly dynamic system and constantly undergoing change. Proteomics, or the study of the proteome, is analogous to genomics but is much more complicated due to the greater number of proteins present than are the genes that code for them. This is due to the presence of protein isoforms as well as posttranslational modifications of proteins such as phosphorylation, glycosylation, acetylation and a myriad of other known chemical modifications – each giving rise to a different and distinguishable entity.

Figure 1. Microarray analysis workflow for a typical pairwise comparison of two samples. The total RNA or mRNA of a reference sample is reverse transcribed in the presence of a green fluorescent dye (Cyanine 3) into cDNA. Similarly, the total RNA or mRNA of a test sample reversed transcribed in the presence of a red fluorescent dye (Cyanine 5). The two samples of red and green labeled cDNAs (targets) are then pooled and hybridized onto cDNA probes arrayed onto microscope slides. The location and extent of hybridization of the probes to their targets is a quantitative measure of the identity and gene expression level of the gene in the samples.