EPIGENETIC REGULATION OF GENE EXPRESSION IN RESPONSE TO A CHANGING ENVIRONMENT IN CHO CELL BATCH CULTURE

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Chinese Hamster Ovary (CHO) cells have been the workhorse for industrial production of recombinant therapeutic proteins since 1987. Variations in cellular environment and phenotypes that occur throughout the bioprocess can bring about significant changes in productivity and quality of recombinant proteins. This can potentially lead to rejection of the production lot. Hence, there is interest in an in-depth understanding of cell-line behavior and control to achieve more predictable and reliable process performance. Biological systems undergo dynamic changes over time, where individual genes are turned “on”, “off” or “paused” as and when required. So far, there is very little information available for CHO cell lines, that elucidates the effect of dynamic epigenetic regulation on temporal expression of genes in response to altered substrate availability and culture conditions. While DNA methylation levels around TSS induce either expression or silencing of genes, transcriptional regulation is primarily considered to be an interplay of transcription factors and chromatin modifiers. On top of these, there is a rapid increase in indications that connects phase-specific long non-coding RNAs (lncRNAs) in transcriptional and post-transcriptional gene regulation. Unfortunately, the mechanism of interaction of these lncRNAs with coding genes have not been studied extensively. In this study, the gene transcription dynamics throughout a batch culture of CHO cells was examined by analyzing expression profiles and histone modifications in regular 12-24 hour intervals. Chromatin states and differential methylation profiles were used to understand the role of epigenetic modifiers in the regulation of gene expression. A good correlation between expression level and absence of DNA-methylation in the promoter regions was observed. Genes having all essential active chromatin marks - specific for promoter activity, genic enhancer and active transcription, also showed significantly high positive correlation between the changes in expression levels and histone marks. Both transcription and chromatin modifications during different growth phases were found to be highly dynamic. Clusters of genes showing similar trends of expression depict gradual and continuous adaptation to the changing substrate concentrations. Less narrowly spaced temporal analyses would have prevented detection of critical regulators involved in transient changes during the batch culture. Here, we also report a plausible mode of interaction of lncRNAs with the coding genes mediated by RNA-DNA-DNA triplex formations. Based on the identified interactions, we could predict possible gene targets and the target sites for the expressed lncRNAs and show high level of correlation of expression levels between interacting pairs. To the best of our knowledge this is the first and most comprehensive report of genome wide transcriptional regulation by epigenetic modifiers for CHO.

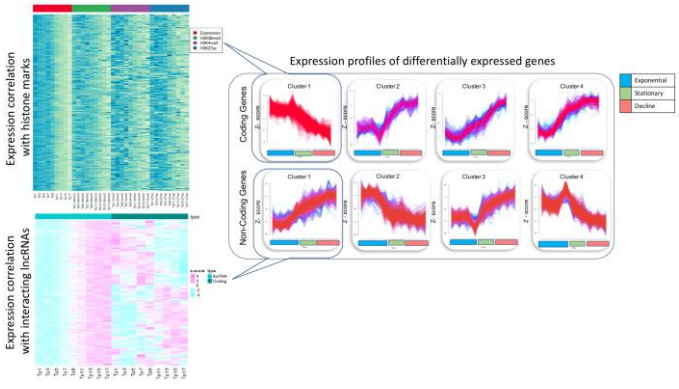

Figure 1: Epigenetic regulation of coding genes by histone modifications and lncRNA interactions. The figure shows clusters of co-regulated differentially expressed genes along-with correlation of cluster 1 expression levels (Red) with levels of histone marks (H3K36me3-green, H3K4me3-purple, H3K27ac - blue). It also reports the regulation of coding gene expression (dark cyan) by triplex mediated interactions with lncRNAs (light cyan) from cluster 1 of non-coding genes.