Epigenetic regulation of gene expression in response to changing environment in Chinese hamster ovary cell batch culture

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Introduction
Adaptations in cellular environment and phenotypes occurring throughout the bioprocess can bring about significant changes in quality and productivity of recombinant proteins. So far, very little information is available that can elucidate the effect of dynamic epigenetic regulation on temporal expression of genes in response to altered substrate availability and culture conditions in these highly variable Chinese Hamster Ovary (CHO) cell-lines. In this project, we aim to analyze the gene transcription dynamics throughout a batch culture of CHO cells by studying expression profiles, histone modifications and methylation patterns. We also investigated a plausible mode of IncRNA interaction with the coding genes mediated by RNA-DNA-DNA triplex formations. Such in-depth understanding of the cell-line will allow better process control and more reliable process performance.

DNA Methylation levels around TSS
Responsible factor for gene expression

DNA methylation was plotted for expressed and silent genes around TSS, including defined upstream and downstream regions. Both coding and non-coding genes show clear distinction of methylation levels around TSS being extremely low for expressed genes and completely methylated for the silent genes. Increase in methylation for non-coding genes and de-methylation for genes with active promoter states is also evident.

Adaptation of chromatin conformation in cells
For temporal regulation of gene expression

Out of 1,397 differentially expressed (DE) coding genes, 647 were found to have all essential active chromatin marks - Active transcription (H3K36me3) in gene body and promoter marks (H3K4me3, H3K27ac) around TSS. The effect of presence or absence of these marks was further checked in DE genes.

DNA methylation levels around TSS
Expressed
Non-Expressed
Expressed with active promoter states
Non-Expressed with active promoter states

Figure 2: Distinct patterns of DNA methylation around TSS for coding and non-coding genes. DNA methylation levels (a) 3kb upstream and downstream of TSS for coding genes (b) 1.5kb upstream and downstream of TSS for non-coding genes.

RNA-DNA-DNA triplex formations
IncrRNA mediated gene regulation

To investigate the probable interplay of IncRNA with various regulators in and around the coding genes, plausible triplex forming interaction sites were estimated using Triplexator. Temporal association of expression levels between the interacting gene pairs and the bias of IncRNA interactions within most and least differentially expressed genes was verified. Enrichment of TSSs was further analyzed within the chromatin states to investigate localization of interactions.

Figure 4: Regulation of coding gene expression by IncRNAs. (a) Correlation of expression levels within a subset of interacting IncRNA-coding gene pairs (b) Frequency distribution of percentage length covered by all TSSs within 1.5kb upstream TSS and 1.5 kb downstream TSS representing higher distribution of more interactions in genes with higher log2FoldChange (c) Comparison of density distribution for correlation coefficient of complete interactions with all IncRNAs (red) and only DE IncRNAs (blue) (d) Line plot reporting localization of interaction sites at all TPs throughout the batch culture, color coded for different chromatin states.

Conclusions

- DNA methylation levels around TSS indicate either expression or silencing for both coding and non-coding genes
- Chromatin modifications effect gene expression levels based on co-existence of active transcription mark in gene body and promoter marks around TSS
- RNA/DNA/DNA triplex formation is a possible mechanism for IncRNA interaction and subsequent regulation of coding genes.

References