Transcriptional Regulation of the Production of a Humanized Monoclonal Antibody by CHO Cells

by

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Monoclonal antibodies (mAbs) have drawn extensive interests especially due to their biopharmaceutical applications. The expanding market of therapeutic mAbs, due to high doses and extended treatment regimens, has demanded high productivity by cultured mammalian cells.

This study focused on understanding the regulation of mAb production in Chinese hamster ovary (CHO) cells. The work started with characterization of a mini-bank of CHO cell clones that were developed by dihydrofolate reductase (DHFR) mediated gene amplification and produce a humanized monoclonal antibody. A correlation between heavy chain (HC) mRNA levels and mAb specific productivity was observed, indicating that efficient HC transcription is critical for mAb productivity. The transcriptional efficiency of transgenes varies between cell clones, between amplified and corresponding parental clones, as well as between independent parental clones.

Further, epigenetic characteristics, in terms of how transgenes are integrated into the host genome, were investigated to study the mechanisms responsible for transcriptional regulation in our particular expression system. Transgene placement and accessibility were determined to be different between distinct parental clones, which might relate to their transcriptional rates. However, no apparent epigenetic difference between parental clones and their amplified progeny was observed, suggesting one or more undiscovered regulatory mechanisms.

The study was continued by examining the responses to sodium butyrate treatment. Sodium butyrate is an inhibitor of histone deacetylase (HDAC), and particularly augments transgene transcription. Clonal variations in response to butyrate were observed both in gene transcription and mAb productivity. The low producing clones are typically more sensitive to butyrate and some, when stimulated, reached comparable productivity as high producers. Therefore, sodium butyrate treatment could be an alternative to gene amplification for the purpose of generating high producing expression systems. Increases in qAb by butyrate mirror the increases in HC mRNA, confirming transcriptional regulation caused by butyrate. The HC and LC gene accessibility was probed using DNase I foot printing. The results show improved gene

accessibility due to butyrate treatment, demonstrating that sodium butyrate regulates gene transcription by improving gene accessibility.

Finally, a linear function between qAb and HC mRNA copy number was derived based on a structured model for mAb under two assumptions. This function successfully described the experimental data of qAb and HC mRNA copy number in cell clones that had undergone gene amplification. However, for an expanded experimental dataset, which included cell clones treated with sodium butyrate, a second-order polynomial model seemed to be better in describing the functional relation between qAb and HC mRNA copy number.