Understanding Transcriptional regulation in recombinant monoclonal producing Chinese hamster ovary cells

by

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ABSTRACT

The ever increasing demand for monoclonal antibodies has led to an interest in understanding productivity rates in CHO cells. As part of an ongoing collaboration with Biogen Idec, previous work has characterized two families of cell lines. For the cell lines A0 and its progeny A1 as well as C0 and its progeny C1: gene copy numbers, mRNA levels, and mAb productivities of CHO cells were determined. Previously, our results indicate that differences in specific productivity rates are directly proportional to the elevated levels of transcripts in higher producing cell lines, and that these elevated levels of transcription extend beyond the increase in transcript that would be expected simply from gene amplification. To investigate the reasons behind the transcriptional enhancement, we compared the RNA stability between the cell clones and studied whether the epigenetic mechanisms were responsible for the differential transcriptional rates in the cell clones.

Using actinomycin D treatment, RNA synthesis was arrested in the cell clones. After various incubation times, total RNA was extracted from the cells and the decreasing mRNA abundance was quantified and compared in the parental and progeny clones to illustrate the relative stability. Our results did not show any difference in the RNA stability of the cell lines.

To investigate whether differential transcriptional rates in parental and progeny cells is the result of altered interactions of transcriptional machinery with the CMV promoters, well characterized and commonly occurring transcription factors interacting with CMV promoter were selected. Chromatin immunoprecipitation was performed using antibodies to RNA pol II, cAMP response element binding protein (CREB), Activator protein (AP-2), and Specificity protein (Sp-1). Real time quantitative PCR (RT-qPCR) was used to quantitate the number of CMV promoter copies bound to these transcription factors. Our results indicate that there is an increased DNA-protein interaction in the higher producing cell lines. AP-2, NFkB and CREB show 2-3 fold increased association with CMV promoter in higher producing cell lines. Improved accessibility of the transgene inserts to the transcriptional machinery may explain the enhancement transcriptional rates.

In most industrial applications, the strong viral CMV promoter is used to drive recombinant protein expression. De-methylation of CMV promoters is known to improve accessibility to transcription. We demonstrated the loss of methylation along the promoter sequence of transgenes in amplified cell lines using methylated immunoprecipitation. Based upon our observations, we subjected the cells to known methyltransferase inhibitors and were able to increase the productivity of parental cell clones to the same level achieved by repeated rounds of MTX amplification.

Identification of these factors indicative of higher producers will help develop selection methods and strategies for cell design that will bring down costs, reduce timelines for development, and help realize the conversion of candidate molecules to therapeutics benefitting patients.