Microbial Cell Factories

Poster Presentation

Regulation of the secretion pathway of CHO cells for altered recombinant Mab production rates during the course of MTX amplification

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Background

Monoclonal antibody (Mab) production by mammalian cells is a complex, multiple-step process which is regulated at transcriptional, translational, and post-translational levels. A detailed understanding of how cells regulate this pathway is a prerequisite for designing genetic strategies for increasing antibody production [1]. Methotrexate (MTX), which is widely used in the creation of high-producing stable cell lines by amplification of gene copy number, provides an effective means to alter Mab production rates for mechanistic studies of the regulation of this pathway [2].

Results

In this work, stable CHO DG44 cell lines expressing a human anti-D Mab were created and single-cell clones were amplified to obtain a series of cultures with varying production rates. During the course of amplification, changes in the Mab gene copy numbers, transcriptional levels of Mab mRNAs, and accumulated intracellular Mab peptides were examined for each clone. In addition, changes in expression levels of representative genes with function in translation, folding, assembly, and degradation were determined. Gene copy number and transcription level were quantified by quantitative real time PCR, and the intracellular Mab peptides were quantified by western blotting and ELISA.

Conclusion

Results obtained in this work could help identify the rate-limiting steps and factors that are significant in limiting production rate for high-producing clones.

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