Abstract

Transient transfection of mammalian cell lines is an effective approach for recombinant protein production, which can provide milligrams to grams of proteins in two weeks from cloning of the corresponding cDNA. Native glycosylated proteins prepared via this approach can be used for various purposes in molecular biology, immunology or pharmaceutical industry, i.e. initial phase of pre-clinical therapeutic protein research. One of the most used mammalian host cell lines is the human embryonic kidney cell line, that can be easily cultivated and chemically transfected. The amount of proteins produced by transiently transfected human embryonic kidney cells can be enhanced by a whole range of factors, i.e. co-expression or direct addition of acidic fibroblast growth factor to the culture medium, co-expression of cell cycle regulating proteins or anti-apoptotic proteins.

Expression plasmid pTW5 was prepared and further modified by gene insertion of aFGF, cell cycle regulator p18, p21 or p27 (cyclin-dependent kinase inhibitors) or apoptosis inhibitor bcl-2 or bcl-x. These plasmids were then used for optimization of HEK293T cell line expression system. The impact of every single regulator and their combinations, including hitherto undescribed effect of combination of cell cycle regulator and anti-apoptotic protein, was monitored via model proteins – green fluorescent protein and secreted alkaline phosphatase. Furthermore, the general conditions of cell line cultivation during protein production were optimized.

(The thesis is written in Czech.)