Increase in the translation rate of transgenes in CHO-cells

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Introduction

Chinese Hamster Ovary (CHO) cells are the most commonly used mammalian cells for the production of recombinant proteins, for example antibodies. These cells are capable of posttranslational modifications and a human-like protein assembly which makes the produced biotherapeutics compatible with the human immune system. The demand for recombinant proteins as active components of biotherapeutics has strongly increased in the last years but it is still a challenging process to establish a high producing CHO cell line.

A limiting step in recombinant protein production is the transport of secretory proteins into the lumen of the endoplasmic reticulum (ER). This translocation is mediated by signal peptides. Recently it has been demonstrated that usage of alternative signal peptides can enhance recombinant protein production in CHO cells.

In order to increase the productivity of a IgG antibody in the Fraunhofer ITEM proprietary HIT-CHO cell line alternative signal peptides for light and heavy chain were applied.

Experimental approach

HIT-CHO cells were stably transfected with plasmids expressing the light chain (LC) of a monoclonal antibody. Various alternative signal peptides (Table 1) were fused to the light chain cDNA beforehand. A control cell pool with a reference signal peptide was also stably transfected. Batch cultivations were performed with stably transfected cell pools. The relative quantity of the produced light chain was estimated via SDS-PAGE with image lab software from bio-rad.

In the second approach stably transfected LC cell pools were additionally transfected with plasmids expressing a IgG heavy chain (HC). Signal peptides 2 and 7 or the control signal peptide were each fused to the heavy chain cDNA (Table 1) in advance. Batch cultivations were performed with stably transfected cell pools expressing the IgG antibody. The relative quantity of the produced antibody was assessed via SDS-PAGE with image lab software from bio-rad.

Table 1: Overview of all applied signal peptides

Signal peptide	Protein	Organism
2	Serum albumin preprotein	*Homo sapiens*
4	Immunoglobulin heavy chain variable region	*Homo sapiens*
5	Immunoglobulin gamma-4 chain C region	*Homo sapiens*
6	Immunoglobulin light chain variable region	*Homo sapiens*
7	Immunoglobulin lambda light chain VLJ region	*Homo sapiens*
8	Ribonuclease pancreatic	*Cricetulus griseus*
9	Integrin alpha-3	*Cricetulus griseus*

Results

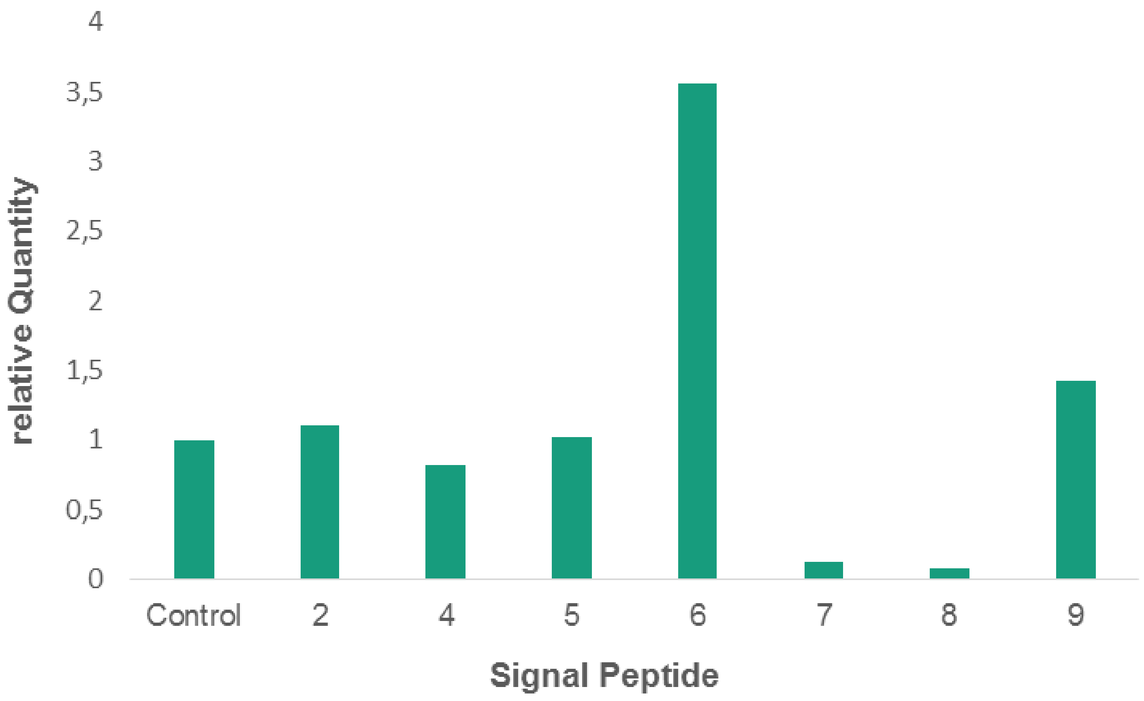

Figure 1: **Relative quantity of recombinant antibody LC in stably transfected CHO cell pools**. Various alternative signal peptides were cloned in front of the LC cDNA. Batch cultivations were performed after selection. All values represent the averages of double approaches and are normalized to the control signal peptide which is set to 1.

The results in figure 1 show that signal peptide 6 leads to the highest LC expression. The quantity is approximately 3.5 times higher compared to the control signal peptide. The *C. griseus* signal peptide 9 shows a slightly higher LC expression compared to the control. Interestingly usage of the second *C. griseus* signal peptide leads to the lowest LC expression.
Signal peptides 2, 4, 5 and 7 show less or similar LC quantities compared to the control. At the basis of this results cell pools containing LC signal peptides 2, 6, 9 and the control signal peptide were transfected with the HC expressing vector.

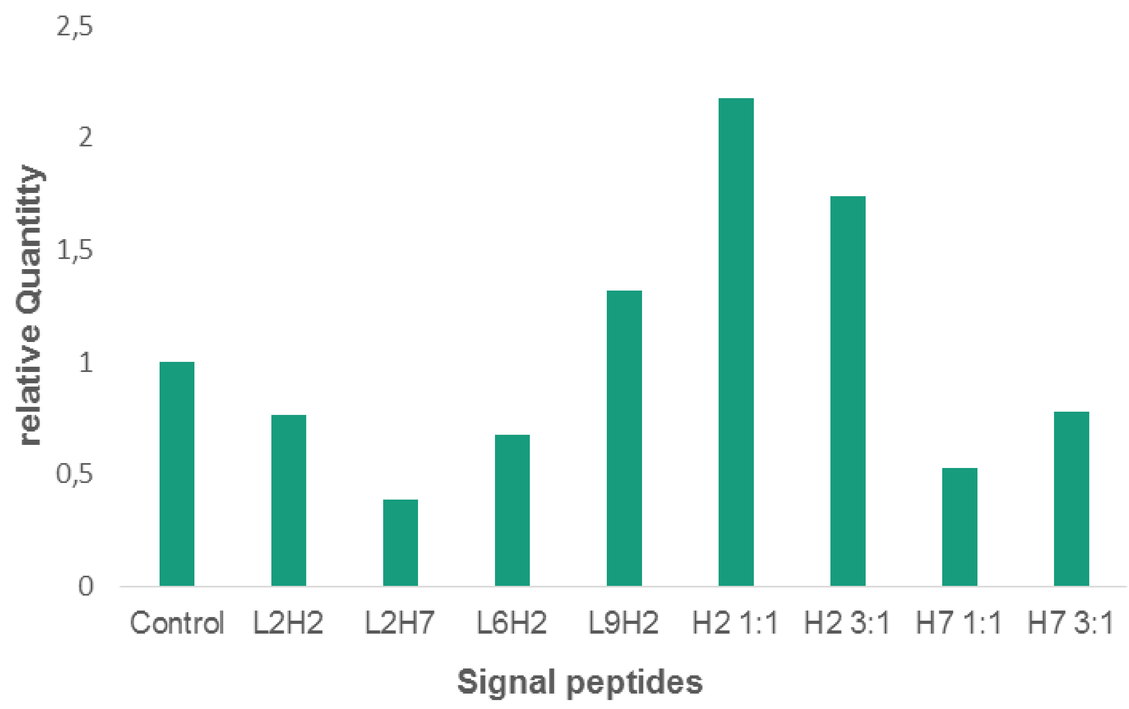

Figure 2: **Relative quantity of recombinant full size antibodies in stably transfected cell pools**. Stably LC transfected CHO cell pools were transfected with HC expressing plasmids. L means LC signal peptide and H means HC signal peptide. Cell pools named H2 and H7 exhibited the LC with the control signal peptide. Cell pools H2 and H7 were transfected with a different LC - HC ratio (1:1 or 3:1). After selection batch cultivations were performed. All values represent the averages of double approaches and are normalized to the control signal peptide which is set to 1.

Results of the full size antibody production shown in figure 2 indicate that the combination of control signal peptide for LC and signal peptide 2 for HC has a potential for overexpression of the full size antibody. An even higher expression can be achieved when the same amounts of LC and HC are transfected. Also a combination of signal peptide 9 for LC and signal peptide 2 for HC leads to a higher antibody expression compared to the control. However, transfection and selection of this cell pool was challenging due to a very low cell viability and cell growth during the selection phase. Cell pools transfected with HC signal peptide 7 did not survive selection in several approaches (data not shown). Interestingly usage of the signal peptide 6 for LC did not result in increased IgG production.

Conclusions

- Full size antibody LC control signal peptide and HC signal peptide 2 transfected in ratio 1:1 increase full size IgG expression by factor 2 compared to the control
- High expression of light chain does not stimulate high expression of full size antibody

Outlook

It is a challenge to identify alternative signal peptides which promote IgG overexpression. However, this work gives clear indications for the potential of signal peptides. As alternative or additional approaches chromatin function modifying elements like S/MARS and UCOEs will be applied. In contrary to signal peptides these elements do not directly interact with recombinant protein translation but serve distinctly as production enhancer through increased DNA accessibility.

References

Kober L, Zehe C, Bode B. (2013). Optimized Signal Peptides for the Development of High Expressing CHO Cell Lines. Biotechnology and Bioengineering (110) 1164-1173 DOI 10.1002/bit.24776. Haryadi R, Ho S, Kok YJ, Pu HX, Zheng L,Pereira NA, et al. (2015) Optimization of Heavy Chain and Light Chain Signal Peptides for High Level Expression of Therapeutic Antibodies in CHO Cells. PLoS ONE 10(2): e0116878. doi:10.1371/journal.pone.0116878. http://www.signalpeptide.de/