Simplifying Progress

Evaluation of Signal Peptides for Enhanced Production Levels In CHO DG44 Cells

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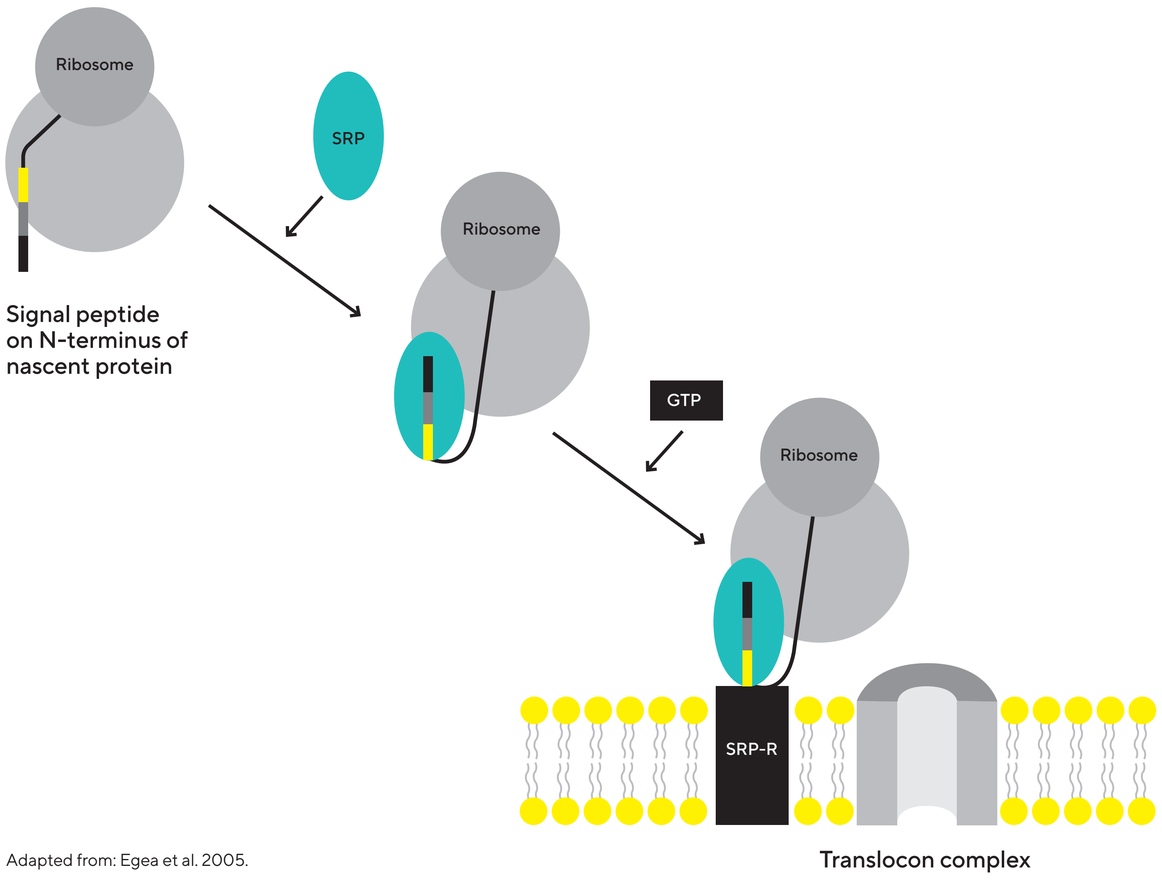
Abstract

The high-titer production of biopharmaceuticals using "Chinese hamster ovary" (CHO) cells is an important pillar of the pharmaceutical industry. Nevertheless, a potential bottleneck for profitable production is the successful secretion of the synthesized proteins through the endoplasmatic reticulum (ER) and the Golgi apparatus into the culture medium directed by the signal peptide (SP). Since natural SP sequences and efficiencies vary between proteins and species, we demonstrated that the utilization of different SPs can lead to enhanced protein expression in already existing production systems.

Introduction

Until today, the market for therapeutic proteins, especially monoclonal antibodies, is gaining more and more importance in the pharmaceutical field. To meet the increasing demand for these products, the industry made tremendous efforts to generate highly efficient production systems. One of the pharmaceutical industry's research focuses is the improvement of the secretion process in eukaryotic cells. In mammalian cells, the efficiency of protein transportation strongly depends on the translocation of a nascent protein into the ER, which is mostly conducted by the signal peptide (SP) coupled to the N-terminus (Figure 1). Through the interchangeability of signal peptides between products and even species, a large variety can be used to enhance protein expression in already existing production systems.



Adapted from: Egea et al. 2005.

Figure 1: Signal peptide recognition and translocation of the protein into the endoplasmatic reticulum (ER) in the secretory pathway of mammalian cells. The signal peptide located at the N-terminus of a nascent protein is bound by the signal recognition particle (SRP). The whole complex is moved to the ER and passes the membrane through interaction with the translocon pore complex. Finally, the signal peptide is cleaved and degraded by peptidases.

Experimental Approach

At first we compared the influence of four different natural SPs (SP7, 8, 9 and 10) on the secreted amount of an IgG4 model antibody (product A) in fed batches using a CHO DG44 host cell line. The second part, one promising candidate showing improved secretion (SP9) was identified and the influence of this SP on four additional antibody products (B to E, Table 1), which vary in their expressibility from good to mid/bad, was investigated. In both approaches, the standard SP was implemented for comparison reasons.

For these purposes, Mini Pools with 4000 cells/well and MTX concentrations of 0, 2.5, 5 and 10 nM were generated expressing the respective products. After 3 weeks, 90 MPs were transferred to 12 well and thereof 30 MPs were selected based on titers and expanded up to shake flask level. In a fed batch process, the performance of the top 10 pools (titer selection) was evaluated (Figure 2).

Table 1: Information of antibody products implemented into signal peptide screening and evaluation.

Signal peptide screening and evaluation was performed with CHO DG44 cells expressing the listed five IgG4 or IgG2 antibody products. These show different capability of expression ranging from good to mid/bad.

Product	Type of product	Expressibility
A	IgG4 antibody	0 / -
B	IgG4 antibody	+
C	IgG4 antibody	+
D	IgG4 antibody	-
E	IgG4 antibody	0 / -

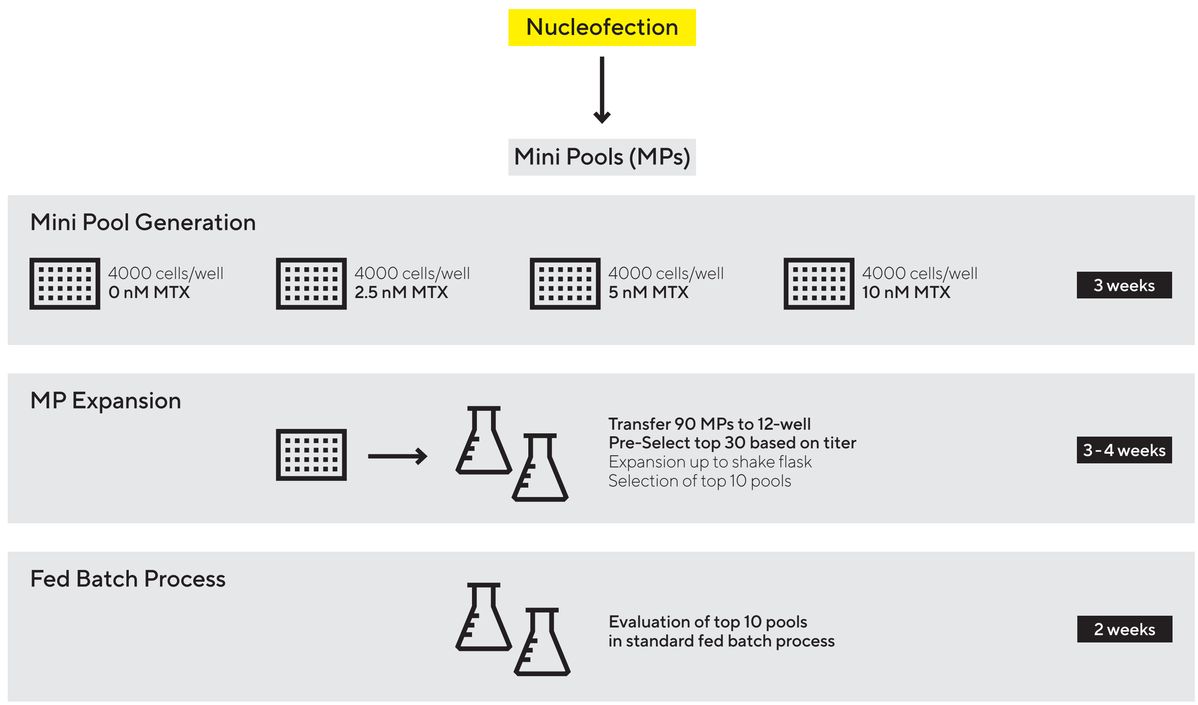


Figure 2: Schematic procedure of pool generation, expansion and evaluation in fed batch process utilizing a CHO DG44 platform.

Results

In the first approach, four signal peptides SP(7), SP(8), SP(9) and SP(10) were screened for their potential to improve the product secretion of CHO DG44 cells expressing a model antibody (product A).

The results revealed a 2.4-fold increase in average final fed-batch antibody titer of SP(9) when compared to the standard SP approach (standard SP = 0.44 g/L; SP(9) = 1.50 g/L) (Figure 3).

In the second approach, the enhancing capacity of SP(9) on secretion of four other IgG products (Table 1) was further evaluated. An improved performance was observed for all products when comparing SP(9) and the standard SP in a fed batch process. With an increase in average final fed-batch titers ranging from 28 to 354% and up to 290% in cell-specific productivities (Figure 4).

Taken together, with a positive influence on the final concentrations of all tested products, the results obtained with SP(9) contribute to the optimization of Sartorius Stedim Cellca´s standard cell line development process.

MP fed batch final titers of product A expressed with different signal peptides

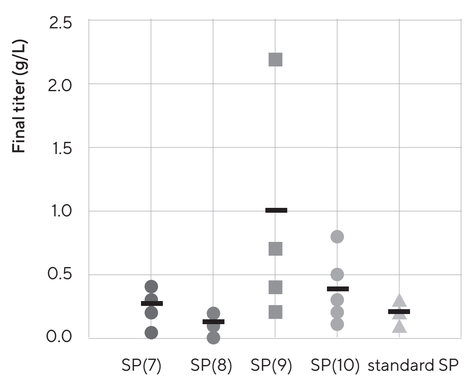


Figure 3: Final titers of the fed batch process of CHO DG44 Mini Pools expressing IgG4 antibody product A with different signal peptides compared to the standard SP.

Resulting final titers of the model antibody product A were compared to the secretion performance of the standard SP revealing a 2.4-fold increase in average final fed batch titers of SP(9).

Increase in final fed batch titer and cell-specific productivity of four products expressed with SP(9) relative to standard SP

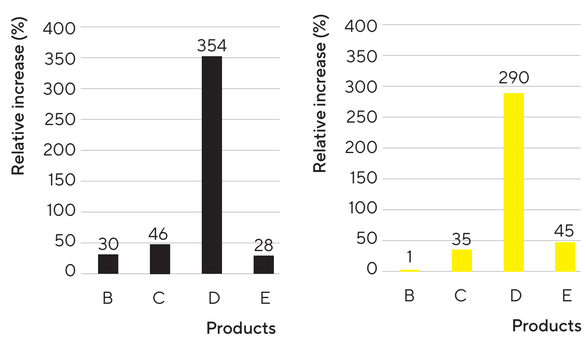


Figure 4: Percentage increase in final fed batch titers and cell-specific productivity of CHO DG44 Mini Pools with SP(9) expressing product B to E. in comparison to the standard SP.

The secretion performance of SP(9) was further evaluated in a fed batch process of four IgG model antibodies and put into comparison to the standard SP. Final average fed batch titers (left graph) of SP(9) showed an increase ranging from 28 to 354% and cell-specific productivities were improved up to 290%.

Conclusion

- Signal peptide SP(9) was identified as a promising candidate with an average 2.4-fold titer increase during screening of four signal peptides
- SP(9) was able to improve production titers up to 354% compared to standard SP
- SP(9) was able to improve cell-specific productivities up to 290% compared to standard SP
- Future usage of SP(9) contributes to the further optimization of Sartorius Stedim Cellca´s standard cell line development process