ENGINEERING CHO CELLS FOR THE PRODUCTION OF "HARD-TO-PRODUCE" PROTEINS

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Over the past decades, the CHO cell has become increasingly popular as the favorite host cell line for the production of protein based therapeutic drugs. In comparison with the popularity of the CHO cells and the frequent use of these cells to produce a large part of the bestselling blockbuster drugs, less intensive efforts have been done to understand the machinery used by the CHO cells during growth and production. The main approach has (broadly speaking) been to approach the CHO cell as a "black box" where one could insert the gene of interest, perform a number of amplifying steps, like gene amplification, selection for stable clones, intense screening for stably expressing high producers, and massive efforts to optimize a specific bioprocess for the selected cell line(s).

Since 2013, the Novo Nordisk Foundation Center for Biosustainablity at the Technical University of Denmark has embarked on a large CHO program to open up the "black box", to get a deeper understanding of the available machinery inside the protein producing "cell factory" that is CHO cells. We are using this understanding to engineer new CHO cell lines having significantly improved features for the production of therapeutic proteins. We are not only doing this by improving the titer, quality, downstream processing and speed of development for already well-known proteins (e.g. Ab), but also for the production of therapeutic proteins that cannot be produced in CHO cells today, due low titer, wrong post translational modifications, and/or low activity.

By combining the competences embedded in the CHO program, we are able to exploit the combination of genome scale modelling, high throughput protein expression, deep understanding of both the glycosylation machinery as well as the secretory and metabolic pathways involved in the expression of secreted proteins. This knowledge is being used as input to a high throughput CHO cell line engineering pipeline, able to engineer up to 10 cell lines and 25 gene targets in parallel.

This has resulted in a large number of new CHO cell lines enabling the production of proteins with specific tailor-made glycoprofiles, higher quality, less degradation, improved bioprocess, higher viable cell density and better cell viability.

We have made a cell lines where we have removed a number of naturally expressed host cell proteins (HCP) from CHO, which has resulted in higher titer and higher VCD, cell lines showing increased resistance to viral infections, cell lines displaying homogenous glycoprofiles, reduced degradation, and drastically changed cell lines that does not produce lactate. These features are currently being combined to engineer CHO cells able to produce proteins that have not been possible to produce with adequate product quality and titer using CHO cells to date.