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Trends and approaches in N-Glycosylation engineering in Chinese hamster ovary cell culture

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Summary

Chinese hamster ovary (CHO) cells have become the preferred expression system for the production of complex recombinant glycoproteins. It has been historically successful in industrial scale-up application and in generating human-like protein glycosylation. N-glycosylation of recombinant proteins, in particular, of those as drug substances, is extremely concerned in drug development and approval, as it will largely affect their stability, efficacy, clearance rate and immunogenicity. Therefore to engineering N-glycosylation of CHO cell-derived recombinant proteins are extremely important. Here, we will summarize a group of recent strategies and approaches and come up with case studies for N-glycosylation engineering in CHO cells and show several examples of relevant study cases from our research: 1) media and feed design, 2) culture process optimization, 3) substrate addition, 4) genetic engineering, 5) omics-based characterization, 6) mathematical modelling.

1. Medium and feed design [1]
- The balance of glucose and amino acid concentrations in the culture is important for cell growth, IgG, titre and fucose expression.
- Amino acids with the highest consumption (Ser, Leu) rates correlate with the most abundant amino acids present in the produced IgG, and thus require sufficient availability during cultivation.
- Higher specific glucose consumption rate is better for cell growth and maturation of IgGs.
- Extracellular glucose consumption and its uptake rate were positively correlated with extracellular UDP-Gal availability, which, in turn, resulted in higher galactosylation levels on the complex sugars present on the recombinant product.

2. Culture process optimization
- Initial medium: B+FB, final medium: aB + aA
- Overexpression of Amino Galactose Glycosylation mathematic modeling could help in the early stages of bioprocess development
- Higher Clone specific effect may affect the average specific productivity of mAbs and it is critical for glycosylation.

3. Substrate addition [2]
- Different substrate additives (glycosylation precursors, including mannose, galactose, fucose, GlcNAc, ManNAc, NeuNAc, uridine, and cysteine) were used as feed additives in fed-batch culture run in triplicates in well-controlled bioreactor systems.
- Depletion of the additives caused statistically significant changes to cell growth and IgG productivity.
- Galactose addition increased galactosylation by 21% to GlcNAc addition reduced galactosylation by 4%.
- ManNAc addition slightly reduced GlcNAc occupancy.
- ManNAc addition slightly increased fucosylation.

4. Genetic engineering
- Studied overexpression of either GlcT or UDP-GlcNAc transporter in two different IgG producing cell lines aA and aB.
- Western blot quantification.
- Localization confirmation using immunostaining.
- Downregulated GlcT, HA and UDP-GlcNAc transporter, FucG (Red), Golgi Marker (Green), Co-localization (yellow).

5. Omics-based characterization [3]
- Proteinomics analysis on day 2 and day 9 of replicate fed-batch culture.
- Day 2 up-regulated genes: transcription, cell cycle, nucleotide metabolism.
- Day 9 up-regulated genes: Glucose (Urid), and nucleotide sugar metabolism.
- Extracellular sensing and signal transduction.
- Protein trafficking and secretion.
- Glycosylation, aggregation.

- Glycosylation mathematical modeling could aid in cell line selection and engineering during the early stages of bioprocess development.
- In-silico prediction of dynamic distribution, kinetics and concentration of glycosylation enzymes along the Golgi space.
- In-silico glycoengineering prediction of GlcT overexpression.

References: