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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 consumption in the culture is important for cell growth, IgG titer and fucose incorporation.
- 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 feeding.
- Higher specific glucose consumption rate is better for cell growth and maturation of CHO.
- Extracellular glucose concentration and its uptake rate were positively correlated with intracellular UDP-Gal availability, which, in turn, resulted in higher glycosylation levels on the complex sugars present on the recombinant product.

2. Culture process optimization

- Strong positive correlation
- Strong negative correlation
- No clear correlation
- Weak positive correlation
- Weak negative correlation

Work flow in N-glycosylation engineering

3. Substrate addition [2]

- None of the additives caused statistically significant changes to cell growth and IgG productivity.
- Galactose addition increased galactosylation by 15%.
- Mannose addition slightly reduced GlcNAc occupancy.
- ManNAc addition slightly increased fucosylation.

4. Genetic engineering

- Stably overexpress either GnT1 or UDP-Gal:ManNAc transporter in two different IgG producing cell lines A and B.
- Western blot quantification
- Localization confirmation using immunostaining: Degrimented GnT1: HA and UDP-Gal:ManNAc transporter: FucA (Red), Dolip Marker (Green), Co-localization (yellow)

5. Omics-based characterization [3]

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- Glycosylation mathematical modelling could aid in cell line selection and engineering during the early stages of bioprocess development
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- In-silico prediction of dynamic distribution, kinetics and concentration of glycosylation enzymes along the Golgi space
- In-silico glycosylation engineering prediction of GnT1 overexpression

References: