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Fan, Yuzhou; Kildegaard, Helene Fastrup; Andersen, Mikael Rørdam

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

Yuzhou Fan1, 2, Helene Fastrup Kildegaard2, and Mikael Roldam Andersen1
1Department of Systems Biology, Technical University of Denmark, 2800 Kgs. Lyngby, Denmark
2The Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, 2970 Hørsholm, Denmark
Email: yufen@bio.dtu.dk

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 concentration in the culture is important for cell growth, IgG titer and Fc glycosylation.
- Amino acids with the highest consumption (Ser, Thr) rates correlate with the most abundant amino acids present in the produced IgG, and thus require sufficient availability during growth.
- Higher specific glucose consumption rate is better for cell growth and maturation of IgG.
- Extracellular glucose consumption and its uptake rate were positively correlated with intracellular UDP-Gal availability, which, in turn, resulted in higher galactosylation levels on the complex sugars present on the recombinant product.

2. Culture process optimization

- The correlation of glucose uptake rate and UDP-Gal concentration.
- Extracellular glucose consumption and its uptake rate were positively correlated with intracellular UDP-Gal availability, which, in turn, resulted in higher galactosylation levels on the complex sugars present on the recombinant product.

3. Substrate addition [2]

- None of the additives caused statistically significant changes to cell growth and IgG productivity.
- Galactose addition increased galactosylation by 11%.
- ManNAc addition reduced galactosylation by 4%.
- Mannose addition slightly reduced GlcNAc occupancy.
- ManNAc addition slightly increased GlcNAc occupancy.

4. Genetic engineering

- Stably overexpress either GlcT or UDP-GlcNAc transporter in two different IgG-producing cell lines A and B.

5. Omics-based characterization [3]

- RT-qPCR analysis on day 2 and day 9 of replicate fed-batch culture
- Day 2 up-regulated genes: 
  - Transcription
  - Cell cycle
  - Nucleotide metabolism
- Day 2 up-regulated genes: 
  - Glucose (5p), and nucleotide sugar metabolism
  - Extracellular sensing and signal transduction
  - Protein trafficking and secretion
  - Glycosylation
  - Apoptosis
- The overall capabilities of protein secretion machinery from growth phase to stationary phase in the cells gradually exceed the capability of protein glycoprotein machinery that is particularly responsible for glycan maturation.

References: