MECHANISTIC INSIGHTS INTO N-GLYCOSYLATION OF RECOMBINANT PROTEINS PRODUCED IN CHO CELL FED-BATCH CULTURES THROUGH SYSTEMS BIOLOGY AND COMPUTATIONAL MODELING

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N-glycosylation is a critical quality attribute for several therapeutic proteins. Precision control of N-glycan heterogeneity is not only essential for developing biosimilars, but also provides leverage for dialing in wide ranges of glycan profiles in novel modalities. A robust control strategy is therefore needed during cell culture processes to accurately control desired glycoforms on the recombinant protein at harvest. However, it remains unclear how intra-cellular metabolic regulation and bottlenecks affect the production of these glycoforms. Some of the key factors include glycosyltransferases, nucleotide sugar donors (NSDs), and co-factors for enzymes involved in N-glycosylation processes. A systems approach was taken to analyze time course transcriptomic and metabolomic data from Chinese Hamster Ovary (CHO) cells cultivated in fed-batch bioreactors to dissect key factors and their dynamics. The systems approach provided the following insights: (1) major glycosylated species exhibit temporal dynamics during fedbatch processes, (2) key metabolic pathways linked to N-glycosylation exhibit significant temporal dynamics, (3) intracellular NSD dynamics directly influence glycoform heterogeneity, and (4) glycoform heterogeneity might be mitigated by supplementing NSD biosynthetic precursors.

Subsequently, knowledge acquired from the systems analysis were incorporated to develop a mechanistic mathematical model. The model was utilized to assess the influence of a few key factors on the kinetics of the glycan chain extension. The model provided several key insights into the non-linear regulation and bottlenecks in the N-glycosylation process. These regulatory mechanisms were exploited to modulation the N-glycosylation heterogeneity in cell culture processes. For example, the model suggested that the observed reduction in galactosylation could be due to decreased transport of UDP-Gal into the Golgi, potentially due to competitive inhibition of UDP-Gal transport by UDP-GlcNAc. This was tested by supplementation of GlcNAc, which is a precursor for UDP-GlcNAc. The supplementation resulted in significant reduction in terminally galactosylated (term-gal) species in fed-batch processes. Second, the model suggested that high specific productivity (q_P) can result in decrease in residence time of proteins through Golgi, potentially leading to less maturation of glycan species. We tested this hypothesis in two different ways: using high and low q_P clones, and by supplementing q_P enhancers. Experimental results validated the negative correlation between q_P and matured glycan species in both the cases. Thus, variability in q_P at clonal level or at process level can also impact N-glycoform heterogeneity.