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Strategies for recombinant production of human glycosylation enzymes: Comparison of mammalian and insect cell expression systems

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Glycan structures on cell surface and secreted glycoproteins play critical roles in biological recognition and targeting events in animal systems. The enzymes that synthesize these structures reside in membranes of the secretory pathway and modify glycoproteins in transit to the cell surface. These enzymes are generally poorly understood largely because they are challenging targets for functional expression even in eukaryotic hosts. We have established library of expression constructs encoding all human glycosylation enzymes (target gene list of ~350 coding regions) as secreted catalytic domain fusion proteins for recombinant production in HEK293 cells and baculovirus-infected insect cells. Comparison of expression levels in each host system indicates similar trends where most proteins are well-expressed but only a subset are efficiently secreted. However, many of the enzymes are more effectively produced and secreted in one recombinant host system or the other suggesting that differences in host secretory machinery can influence the yield of recombinant products. This presentation will summarize our strategies for expression and downstream workflows for biochemical and structural studies with goals to advance our understanding of the enzymatic machinery for glycan synthesis and modification.

Biography

Kelley W Moremen is a Distinguished Research Professor of Biochemistry and Molecular Biology, Complex Carbohydrate Research Center, University of Georgia. He has received his PhD at Vanderbilt University and Postdoctoral training at MIT. He is a leader in the structure, function, roles and regulation of enzymes involved in mammalian glycan biosynthesis and catabolism.

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