153f Metabolic Engineering of Escherichia Coli for Sugar Nucleotide and Oligosaccharides Synthesis

Zichao Mao, Anne Ruffing, Hyun-Dong Shin, and Rachel Ruizhen Chen

Carbohydrate moieties of glycoconjugates play key roles in cell adhesion, inflammation, cancer, metastasis, and many other disease-causing events. Carbohydrate-based molecules are being pursued by many pharmaceutical and biotech companies as anti-infective drugs and vaccines. However, the synthetic difficulties in generating these molecules with the required specific linkage and anomerity have long hampered the clinical development of carbohydrate-based therapy. Biotechnology-based strategies toward complex carbohydrate represent emerging technologies that have the potential to greatly simplify synthesis process. This requires significant metabolic engineering in both carbon and nitrogen metabolism pathways. The interactions of carbon and nitrogen metabolism, the interconnections with energy metabolism make the metabolic engineering effort particularly challenging.

In an effort to develop whole-cell catalysts for improved synthesis of oligosaccharide, we engineered the metabolic pathway leading up to the synthesis of UDP-Glucose by overexpressing several enzymes involved in its synthesis. By introducing to the modified strain additional enzymes required for the synthesis of the model compound, N-acetyllactosamine, the diasaccharide product was produced from glucose and N-acetylglucosamine and the product was accumulated up to 8 mM, indicating that the metabolically engineered cells were used to supply the needed sugar nucleotide synthesis. In this presentation, we detailed the metabolic engineering strategy, the analysis of the potential bottlenecks in the synthesis, the optimization of the synthesis, and discuss the complications brought by the interactions of multi-pathways and further strategies to delineate the interplay of nitrogen metabolism from carbon metabolism.