METABOLIC PATHWAY ENGINEERING IN MAMMALIAN CELLS THROUGH KINETIC MODEL OPTIMIZATION

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Excessive lactate production and accumulation in cell culture can result in reduced culture productivity and altered product quality. Engineering cell metabolism to eliminate or reduce lactate production can lead to higher performing, more robust processes. However, prior efforts to engineer cell lines with low lactate production have been met with limited success, often also resulting in reduced cell growth rate. Given the complex roles of energy metabolism in sustaining anabolism and redox homeostasis, it is likely that successful suppression of lactate production in proliferating cells will require simultaneous alteration of multiple genes in energy metabolism. Considering the nonlinear nature of the energy metabolism reactions and the number of reactions involved, we have taken a systems approach to devise a strategy to alter cellular metabolic behavior to advance cell culture bioprocessing.

In this study, we develop a method using optimization of a nonlinear kinetic model of cell metabolism designed to rewire glycolysis to have reduced lactate production while maintaining a high growth rate. The model encompasses glycolysis, the pentose phosphate pathway, and the citric acid cycle, and also includes the known allosteric regulations. The large number of possible enzyme combinations necessitates a method to search the parameter space intelligently for key changes that can be made to manipulate metabolism.

The model was solved using a local nonlinear optimizer embedded in General Algebraic Modeling System (GAMS). A multi-objective optimization was formulated, demonstrating the relationship between lactate production and glucose consumption. Constraints were chosen to maintain cellular requirements for growth including energy production and precursors for biosynthesis. The primary objective of this optimization was to minimize the rate of lactate production for cells in culture, but the objective function was modified with a penalty term to also reduce the number of genetic alterations in an optimal state to ease experimental design. The resulting solutions to the optimization problem contain small sets of recommended changes to enzyme expression and activity that can be tested in an engineered cell line.

We have demonstrated a method for rationally guiding cellular engineering through kinetic model optimization. The findings from this optimization are being evaluated experimentally, generating new cell lines with altered metabolic behavior. By identifying sets of parameter changes that yield desirable outcomes, optimization of kinetic models can greatly reduce the effort required to engineer cell metabolism, providing multiple metabolic engineering strategies with different enzyme combinations as well as deep insight into the reaction networks and their behavior.