4bd Exploring Physiological Landscapes for Cell and Metabolic Engineering

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The advent of genome sequencing and recombinant DNA technology ushered in a new era of opportunity for bio-based processes and products. With these technologies came prospects for eliminating harsh petroleum-based processes, developing new products, and uncovering disease states. For microbial engineering, these newfound capabilities open up the potential to engineer cells to produce natural and unnatural products, biofuels, pharmaceuticals and specialty chemicals. Yet, engineering metabolic pathways to reroute resources into modes favoring product formations is still a formidable task. Genome sequences and catalogues of bioreaction networks only provide a list of parts to be used in this endeavor. Beyond these, complexities and nonlinearities in the interactions of metabolic pathways and regulatory networks confound the process of metabolic engineering.

Cellular phenotype is a manifestation of gene expression levels, metabolic demand, resource availability, and cellular stresses. Above all, metabolic function is constrained by the stoichiometry and individual reaction kinetics of the reaction network. Due to the complex interaction of all these components, the optimization of metabolic phenotype often requires the simultaneous rerouting of metabolic intermediates and rewiring of regulatory networks. To accomplish these tasks in an efficient and comprehensive manner, a diverse set of molecular biology tools must accompany, and at times supplement, systematic analysis of pathways. These tools and methodologies must be both broad in effect (since different genes require different levels of modification) and in scope (since each pathway has a unique set of regulatory bounds). When collectively used, these advances in molecular biology and genetic engineering empower metabolic engineers with the increasing ability to create any desired cellular modification. However, a set of tools will only be as effective as the context in which they are used. As such, efficient phenotype optimization necessitates a robust, defined *search strategy to identify genetic targets* requiring modification. By exploring and probing the metabolic landscapes created by the underlying structure of genotype-phenotype interaction, lessons may be gained which can help guide future strain improvement programs aimed at the production of metabolites.

The subject of my thesis and this presentation focuses on the development and subsequent utilization of new tools and strategies for optimizing these cellular systems and traversing the metabolic landscape to obtain the desired phenotypes. Several new molecular biology tools have been developed which empower metabolic engineers with the ability to make new modifications at the genetic level. Finally, these tools are employed in the context of enhancing lycopene production in *E. coli*. In doing so, we have been able to, for the first time, visualize these complex metabolic landscapes. Through this endeavor, we have been able to optimize the lycopene production levels and, subsequently, suggest more general strategies for metabolic engineering.