

117. Application of Next-Generation Sequencing to Engineering mRNA Turnover in Cyanobacteria

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Project Goals: The goal of our project is to understand mRNA stability in cyanobacteria such that tools can be developed to predictably control gene expression in these exciting phototrophs.

Demand for sustainable transportation fuels and commodity chemicals has motivated biotechnologists to investigate production processes that start from sunlight and CO₂. Biotechnology has long been used as a means to produce chemicals but the range of targets has dramatically increased since the advent of recombinant DNA technology. Modern biotechnology, and specifically synthetic biology, has advanced to the point where metabolic pathways can be designed from scratch and integrated into the genome of hosts with advantageous inherent traits. Furthermore, the dramatic increase in DNA sequencing and synthesis capabilities over the last decade has led to an explosion of systems biology and synthetic biology methods for analyzing cells and building complex regulatory circuits. The remaining fundamental challenge in assembling novel metabolic pathways is designing DNA sequences de novo that encode the necessary structural and regulatory components for optimizing pathway function.

Messenger RNA are key, labile intermediates in the path to synthesizing proteins inside cells. Among the many modes of regulating gene expression, mRNA turnover is the least understood process, particularly with respect to individual genes and sequence elements. Algorithms have been developed for identifying promoters, and predicting transcription and translation rates from genomic sequence, but a predictive model of mRNA turnover does not yet exist. This is in part because of the many modes by which mRNA molecules can be degraded and the multitude of RNA processing enzymes inside cells. Sequence and/or structural elements that can slow or accelerate mRNA processing by specific enzymes have been identified but their ability to confer the same traits to other gene sequences has not been straightforward. Instead, combinatorial methods of altering mRNA stability have been developed and shown to be a powerful strategy for optimizing metabolic pathways when facile screens or selections are available. In order to design optimal gene expression cassettes, additional knowledge of how a particular mRNA sequence decays is needed. Past efforts using DNA microarrays provided data that examined rates of mRNA decay on a global scale but lacked the spatial resolution to determine which parts of a transcript were recycled first, second, or last. We hypothesize that next-generation RNA sequencing protocols will provide this level of resolution and enable us to examine how each mRNA in a bacterium is turned over.

In this talk, we will describe our efforts to develop a genetic engineering toolbox for *Synechococcus* sp. PCC7002 and outline our plans to use RNA-sequencing techniques to study mRNA stability in this industrially promising cyanobacteria. Specifically, we will discuss a novel counterselection method (1) that has greatly facilitated segregation and enabled scarless mutagenesis in PCC 7002. We will also discuss transcriptional and translational control elements that have been characterized in the course of our NSF- sponsored “Cyanobacteria photobiorefineries” project.

References

1. Begemann MB, Zess EK, Walters EM, Schmitt EF, Markley AL, Pflieger BF. An Organic Acid Based Counter Selection System for Cyanobacteria. PLoS One. 2013 Oct 1;8(10):e76594.

Funding statement

The work presented in this talk was funded by DOE-BER through an Early Career award (DE-SC0010329) and by the National Science Foundation (EFRI-1240268, GEO-1215871).