# A Better Understanding of Bacterial RNA Turnover Developed Through RNA Sequencing

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**Project Goals:**

For this project, we use RNA-sequencing techniques to study mRNA turnover and stability in *Escherichia coli* and *Synechococcus* sp. PCC7002, a cyanobacteria with promising industrial traits that could be used as a renewable route for producing fuels and chemicals. We use RNA sequencing to study how various RNA processing enzymes affect decay of mRNA from a global perspective. We use the knowledge gained from our studies of mRNA turnover to develop design rules for (de)stabilizing transcripts and apply these rules to ongoing metabolic engineering projects in PCC 7002 for use in photosynthetic biorefineries to sequester $CO_2$ that would otherwise contribute to climate change.

Our specific objectives are:

(1) Quantify decay rates for each nucleotide in bacterial transcriptome with RNA sequencing
(2) Design and test strategies for (de)stabilizing transcripts in PCC 7002
(3) Apply design rules to improving biofuel production in PCC 7002

Messenger RNA (mRNA) is a labile intermediate that affects protein expression levels. For metabolic engineering purposes we would ideally be able to predict and precisely control protein expression based on a given DNA sequence. Unfortunately there is insufficient foundational knowledge about RNA stability to be able to predict the half-life of a given transcript. This arises due to the numerous and complex ribonucleases (RNases) that facilitate mRNA degradation. We are particularly interested in RNase III, a ribonuclease that cleaves long double-stranded regions of RNA, because of its potential use to alter transcript stability and process structured RNAs (e.g. CRISPR guide RNAs). RNase III recognizes mRNA secondary structure and not a conserved nucleotide sequence, but it is still more amenable to engineering than other RNases that have less selectivity. Using RNA-seq we have identified novel targets of RNase III in *Escherichia coli*, and we have shown that RNase III regulates protein expression of important metabolic enzymes, processes read-through transcripts, and is involved in the turnover of mRNA of leader peptides. With our greatly expanded list of RNase III sites and sequences, we will test if these sites can be placed next to genes of interest to alter their stability and subsequent protein expression.

The genome of the cyanobacterium *Synechococcus sp.* strain PCC 7002 encodes three homologs of RNase III, two full-length and one mini-III, that are not essential even when deleted in combination. To discern if each enzyme had distinct responsibilities, we collected and sequenced global RNA samples from the wild type strain, the single, double, and triple RNase III mutants. Approximately 20% of genes were differentially expressed in various mutants with some operons and regulons showing complex changes in expression levels between mutants. Two RNase III's had a role in 23S rRNA maturation and one of those was also involved in copy number regulation one of six native plasmids. *In vitro*, purified RNase III enzymes were capable of cleaving some of the known *E. coli* RNase III target sequences, highlighting the remarkably conserved substrate specificity between organisms yet complex regulation of gene expression.

**Publications from the project**

1. Cameron JC, Gordon GC, Pfleger BF. Genetic and genomic analysis of RNases in model cyanobacteria. *Photosynthesis Research.* Oct;126(1):171-83 (**2015**). DOI: [10.1007/s11120-015-0076-2](#)

2. Markley AL, Begemann MB, Gordon GC, **Pfleger BF**. A synthetic biology toolbox for controlling gene expression in the cyanobacterium *Synechococcus* sp. PCC 7002. *ACS Synthetic Biology*. May 15;4(5):595-603 (**2015**). DOI: [10.1021/sb500260k](#)

3. Gordon GC, Korosh TC, Cameron JC, Markley AL, Begemann MB, Pfleger BF. CRISPR interference as a titratable, trans-acting regulatory tool for metabolic engineering in the cyanobacterium *Synechococcus* sp. strain PCC 7002. *Metabolic Engineering*. Jul 29. pii: S1096-7176(16)30062-3 (**2016**) DOI: [10.1016/j.ymben.2016.07.007](#)

4. Pfleger BF. Microbes Paired for Biological Gas to Liquids (Bio-GTL) Process. Proceedings of the National Academy of Science USA. Apr 5;113(14):3717-9. (**2016**). DOI: [10.1073/pnas.1601926113](#)

5. Gordon GC, Cameron JC, Pfleger BF. RNA-sequencing identifies new RNase III cleavage sites in Escherichia coli and reveals increased regulation of mRNA. *mBio*. Mar 28;8(2). pii: e00128-17. (**2017**) DOI: [10.1128/mBio.00128-17](#)

6. Wang C, Pfleger BF, Kim SW. Reassessing *Escherichia coli* as a cell factory for biofuel production. *Current Opinion in Biotechnology*. Mar 11;45:92-103. (**2017**) DOI: [10.1016/j.copbio.2017.02.010](#)

7. Gordon GC, Cameron JC, Pfleger BF. Distinct and redundant functions of three homologs of RNase III in the Cyanobacterium *Synechococcus* sp. PCC 7002 (*Revision submitted*)

8. Clark RL, Gordon GC, Bennett NR, Lyu H, Root TW, Pfleger BF. High-CO2 Requirement as a Mechanism for the Containment of Genetically Modified Cyanobacteria. (*Revision submitted*)

9. Gordon GC, Pfleger BF. Regulatory tools for controlling gene expression in cyanobacteria. (*invited book chapter – Submitted*)

10. Gordon GC, Cameron JC, Pfleger BF. Analysis of global RNA turnover in cyanobacteria. (*in preparation*)