Rational Control of Protein Expression using Small RNAs

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Background and Introduction

RNAs are not only the intermediates between the assembly of a protein and the DNA that encodes it, but also regulators of gene expression and protein production. Small RNAs (sRNAs) are short regulatory RNAs that bind to protein-encoding messenger RNAs (mRNAs) and either repress or activate production of the encoded protein. The goal of this project is to design a model to quantify the regulation of protein production by sRNAs and use that model to rationally construct synthetic sRNAs to control protein production rates.

Biological Model

A statistical thermodynamic model was designed to quantitatively predict the regulatory activity of a given sRNA on a specific mRNA. Protein expression level predictions are based upon the Gibbs Free Energy changes of the interactions between the sRNA, mRNA, and ribosome. The model takes into account three terms, as seen in Figure 1: \( \Delta G_s \), the interaction energy of the mRNA and ribosome in the absence of sRNA, \( \Delta G_m \), the interaction energy of the sRNA, mRNA, and ribosome. Using these three terms and the concentrations of mRNA and sRNA, the protein production rate (P) can be quantified as follows:

\[
P = \frac{[\text{mRNA}]_{\text{free}} \exp(-\beta \Delta G_m) \exp(-\beta \Delta G_a) + [\text{mRNA}]_{\text{E}} \exp(-\beta \Delta G_a)}{[\text{mRNA}]_{\text{E}} \exp(-\beta \Delta G_a)}
\]

Model Validation

The model was used to rationally design a sRNA to repress expression of mRF1 by at least 1000-fold. The predicted regulatory activity of the designed sRNA is plotted in Figure 3. The sRNA was then tested experimentally using the methods outlined above. The flow cytometry data in Figure 4 shows a reduction in fluorescence, and therefore of mRF1 expression, of at least 1000-fold for E. coli DH10B. The data shows a 2-fold reduction in fluorescence for E. coli pir+, the result of increased mRF1 mRNA concentrations from the higher pBAC copy number.

Experimental Approach

A BAC vector containing mRF1 with a strong ribosome binding site and a pColE1 vector containing the sRNA were double transformed into E. coli DH10B and E. coli pir+. BAC has a plasmid copy number of 1 in DH10B and 15 in pir+. ColE1 has a copy number of 60-100 in both strains. This varied the mRF1 mRNA levels while keeping the sRNA levels constant. Empty BAC and ColE1 plasmids were used as controls. Cells were maintained in exponential growth phase and fluorescence was measured using a TECAN M1000 spectrophotometer and BD Fortessa flow cytometer.

Improving Reaction Kinetics

Several nucleotide motifs were found to facilitate the kinetics of sRNA/mRNA interaction, facilitating the regulatory capability of the sRNAs. Franch et. al.\(^2\) found that YUNR motifs (\( Y = \) pyrimidine, \( R = \) purine; reverse complement = YNAR) present in loop structures and single stranded regions of RNA increase the binding kinetics of the RNA to complementary sequences. Small RNAs containing open reading frames (ORFs) were also found to enable sRNA/mRNA interaction, attracting ribosomes to the sRNA, which unfold secondary structures and protect the RNA from degradation.

Natural Regulatory Small RNAs

Natural sRNAs are versatile regulators of metabolism in response to environmental changes, and many have multiple regulatory targets. The biophysical model was used to analyze the interaction between sRNAs known to control key metabolic activities and their mRNA targets. One such well-studied sRNA, ryhB, is shown in Figure 6. Under low iron conditions, ryhB represses expression of iron-containing proteins, including enzymes involved in aerobic respiration and the TCA cycle.

Energy Applications

Natural metabolic processes, or natural processes coupled with engineered pathways, can produce small amounts of byproduct that are useful to harvest. For example, E. coli can produce fuels—such as ethanol, isobutanol, or biodiesel—and other useful materials as metabolic byproducts. By activating or repressing the expression of different proteins along a metabolic pathway, sRNAs can redirect metabolic fluids toward the generation of these useful byproducts. This technology is more tunable and simpler than knocking out genes; sRNAs have a wide range of regulatory strengths and can be easily transferred via plasmid.

References


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Figure 1: Visual representation of the regulation of mRNA in the presence of sRNA, showing the three Gibbs free energy interactions used in the biophysical model

A preliminary version of the small RNA calculator can be found online at http://salis.psu.edu/software

Figure 2: The pBAC and pColE1 two plasmid reporter system

Figure 3: Model-predicted regulatory activity of the synthetically designed sRNA on mRF1, depicted for both E. coli DH10B and pir+ strains

Figure 4: Flow cytometry data showing repression of mRF1 by at least 1000-fold in E. coli DH10B and 2-fold in E. coli pir+ in the presence of sRNA

Figure 5: Sequence analysis results show the number of natural sRNAs containing single-stranded YUNR and YNAR motifs as well as ORFs with ATG and GTG start codons

Figure 6: (Left) Schematic of the regulatory network of ryhB sRNA\(^1\); proteins regulated by ryhB are shown in green. (Right) Predicted fold repression of ryhB targets in the presence of ryhB, demonstrating the regulatory versatility of the sRNA