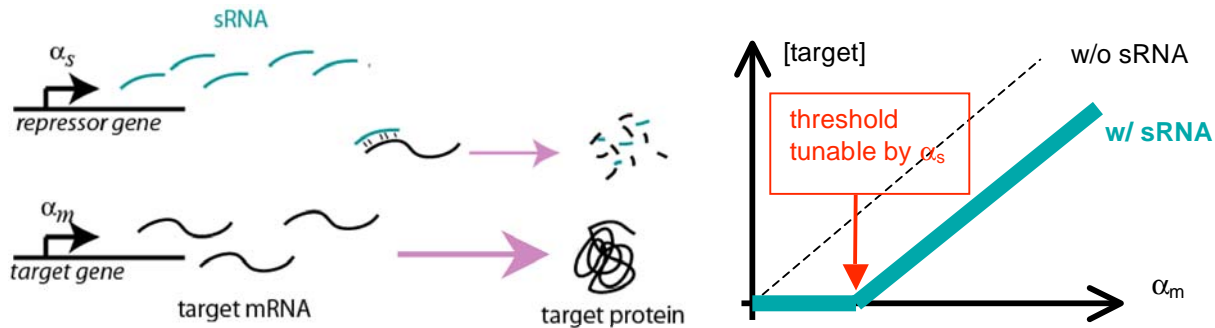


# Gene Regulation Mediated by Small RNA

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According to the “Central Dogma” of molecular biology, RNA is a mere messenger between genetic information and protein expression. In the last few years however, an increasing number of small RNAs (sRNA) have been discovered to play regulatory roles, e.g., silencing gene expression, in both prokaryotes and eukaryotes. Interestingly, targets of small RNA regulation tend to be critical regulatory genes themselves. In bacteria, sRNA regulation is predominantly involved in coordinating intricate stress responses, and in eukaryotes sRNA regulation has been implicated in processes such as differentiation in late development and programmed cell death.

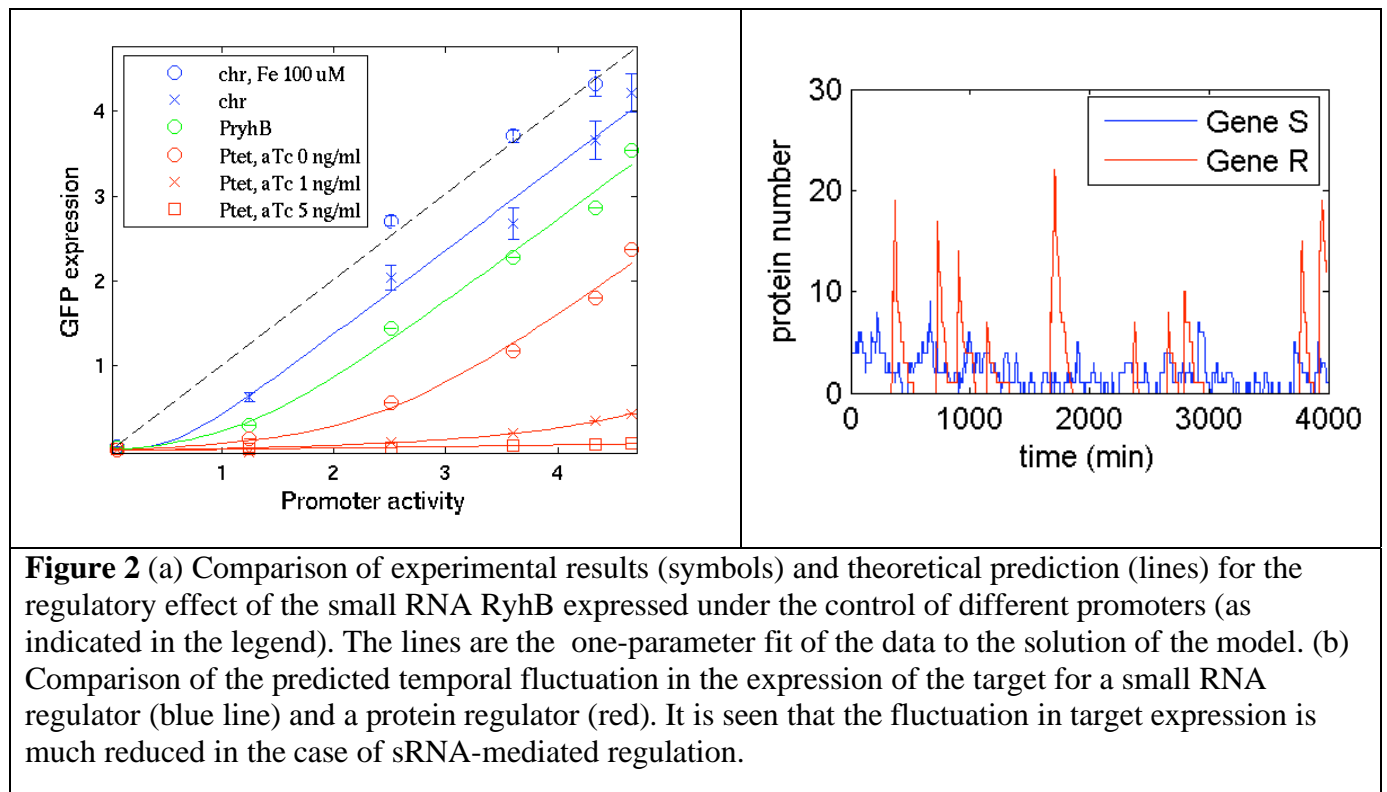
To see whether there might be special features of sRNA-mediated regulation that make it different from protein-mediated regulation, we performed a quantitative study of the most abundant class of bacterial sRNAs in *Escherichia coli* using a combination of experimental and theoretical approaches. We note that a key feature of sRNA regulation in bacteria, the stoichiometric nature of the regulation (Fig. 1a), suggests a novel threshold-linear mode of action, by which the expression of, e.g., a stress-response gene would be silenced below a *threshold*, and gradually activated above it. As illustrated by the thick teal line in Fig. 1b, if the transcription rate of the target mRNA ( $\alpha_m$ ) is below that of the sRNA ( $\alpha_s$ , indicated by the red arrow), then most of the targets are expected to pair with the sRNAs and be rapidly degraded. Conversely, if the transcription rate of the mRNA exceeds that of the sRNA, then most of the sRNAs are expected to turnover while the unconsumed mRNAs are free to express into proteins. In this case, the expressed protein level would reflect the difference between the two transcription rates.



**Figure 1** (a) The stoichiometric nature of sRNA-mediated gene regulation. Each sRNA molecule can bind to a target mRNA molecule and trigger the degradation of the complex (target and the sRNA itself.) (b) Qualitative form of sRNA-mediated control is characterized by an expression threshold which is tunable by the degree of sRNA expression.

We developed a quantitative model of sRNA-mediated gene regulation based upon the qualitative model and quantitative parameters obtained from detailed experiments on RyhB, a small RNA involved in the regulation of iron homeostasis in *E. coli*. Solution of the model yielded forms analogous to the cartoon illustration of Fig. 1b, but with some rounding-off of the

threshold. The predicted behavior was tested in our laboratory. In order to circumvent the complex regulation of the endogenous system, we constructed a synthetic target gene, consisting of the 5' control-region of *sodB*, the strongest natural target of RyhB, transcriptionally fused to the coding sequence of the reporter *gfp*. The target gene, *crsodB-gfp*, was driven by an inducible *lac* promoter<sup>20</sup>. The experimental results (symbols in Fig. 2a) compare very well with the predicted form (solid lines) for a variety of sRNA sources using a single fitting parameter, the magnitude of the sRNA expression.



**Figure 2** (a) Comparison of experimental results (symbols) and theoretical prediction (lines) for the regulatory effect of the small RNA RyhB expressed under the control of different promoters (as indicated in the legend). The lines are the one-parameter fit of the data to the solution of the model. (b) Comparison of the predicted temporal fluctuation in the expression of the target for a small RNA regulator (blue line) and a protein regulator (red). It is seen that the fluctuation in target expression is much reduced in the case of sRNA-mediated regulation.

In addition to the tuneable expression threshold, we find sRNA mediated regulation to possess a robust noise resistance characteristic (see Fig. 2b), and a built-in capability for hierarchical cross talk. These properties are described in detail in a recent preprint which can be accessed at <http://matisse.ucsd.edu/~hwa/sRNA.pdf>.